PROCEEDINGS

American Academy of Forensic Sciences

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PROCEEDINGS

of the American Academy of Forensic Sciences 69th Annual Scientific Meeting

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

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English is the official language of the AAFS and its meetings; neither oral nor written translations will be provided.

PROCEEDINGS

of the American Academy of Forensic Sciences **February 2017**
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SPECIAL SESSIONS

S1 Interdisciplinary Symposium: The Evolution of Daubert and Its Effects on the Forensic Sciences

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After attending this presentation, attendees will better understand how forensic science practitioners have addressed the evolution of *Daubert* and the reaction of the legal community as the standard has progressed. Attendees will be exposed to the challenges faced by forensic scientists and the research that is addressing criticisms. Furthermore, the Interdisciplinary Symposium will help attendees understand how the courts understand *Daubert* and how they address the admissibility of forensic practitioners and science.

This presentation will impact the forensic science community by discussing the past, present, and future ramifications of the *Daubert* Trilogy on the admissibility of forensic evidence from the viewpoint of practitioners, attorneys, educators, and judges.

Stephanie Domitrovich, JD, PhD; W. Milton Nuzum, JD - The American Bar Association (ABA) recently adopted a Resolution wherein the ABA urges the National Commission on Forensic Science to support the development of a model curriculum in the law and forensic science as well as training in that curriculum for federal, state, territorial, and tribal judges. The NAS recommended: "Better connections must be established and promoted between experts in the forensic science disciplines and law schools, legal scholars, and practitioners." Specifically, the NAS Report further indicated: "And judges need to be better educated in forensic science methodologies and practices." Consistent with these recommendations, presenters in this session will discuss the evolving developments in judicial education over the years in providing state and federal trial judges the necessary tools for their tool boxes to become better equipped to understand forensic scientific evidence issues in both *Daubert* and *Frye* jurisdictions. Judicial educators will discuss the design of such curricula to teach state and federal trial judges to become more competent gatekeepers when deciding scientific issues in all areas, for instance, civil, criminal, family, and orphans' court. Judicial educators will also discuss implementing needs assessments, curricula development, and adult education principles as their latest tools in education theories to accomplish these goals.

Stephen T. Goudge, LLB - This presentation will address the public inquiry Judge Goudge chaired into Pediatric Forensic Pathology in Ontario. The Inquiry was created by the government of Ontario because unreliable opinion evidence from this science had resulted in a number of wrongful convictions. In his report, Judge Goudge addressed the challenge of unreliable scientific opinion evidence and how the justice system could guard against it.

Neal H. Haskell, PhD - The science of Forensic Entomology (FE) has regularly entered the courtroom in the United States within the past three decades. At first, it was perceived as a "new and novel science" using insects to answer questions regarding a human death. During this time, the *Frye* test of acceptability in courts was the

standard method of reliability. Only a handful of states had actually utilized FE in courts. This led to numerous challenges of its use in courts as more states sought use of FE, the gatekeepers being the justices on the bench.

As was explained to each new state where testimony was given, whether *Frye* or *Daubert*, FE is nothing more than proven and reliable entomological scientific principles dating back to 1265 China in a murder case. Forensic application of entomology is one of the oldest sciences to be used in the courtroom. In short, FE is the use of biological aspects of entomology encompassing insect biology, insect behavior, insect growth and development, insect ecology, interacting with climatology and environmental biology, all of which have been studied for centuries and are fully accepted in the biological sciences.

Peter V. Tytell, BA - One practical change in post-*Daubert* courtrooms has been at the *voir dire* stage of an expert's appearance. Previously, opposing counsel would challenge the specifics of the education, training, and experience of the witness in an effort to disqualify that individual as an expert in a certain discipline (or at least lessen the witness's credibility in the eyes of the jury). Today, the challenge is often to the reliability of the discipline itself rather than the qualifications of the witness as a practitioner. It is increasingly likely that the *voir dire* will be conducted outside the presence of the jury, and that it will be essentially indistinguishable from a *Daubert* hearing in content and intent.

Today's expert witness must be prepared not just to present the results of an examination and to explain the specifics of each of the findings, but must also be prepared to present the basis for the reliability of the discipline with specifics relating to each of the *Daubert* factors and the requirements of Rule 702. Of equal importance, today's trial attorney must be prepared not just to present the facts of a case, but must also be prepared to ask the right questions of the expert and to present *Daubert*-appropriate references, citations, and arguments. This presentation will illustrate these changes as they relate to forensic document examiners with case examples highlighting problems that can arise in these situations.

Joseph J. Maltese, JD, PhD - The expansion of *Daubert* and *Kumho Tire* standards into the Federal Rules of Evidence, and their adoption by many state courts, have served as a catalyst for the criminal defense bar to challenge the reliability of the "specialized knowledge" utilized in most of the forensic disciplines. With greater scrutiny being applied by the courts, many forensic disciplines have attempted to validate or revalidate their methodology and procedures to withstand objections raised by attorneys and skeptical judges about the admissibility of such evidence, which was rarely subject to objection before the *Daubert* revolution. This presentation will address some of these issues.

Barry C. Scheck, JD - As the National Commission on Forensic Science recently acknowledged in a views document, protecting against cognitive bias through blind examinations and sequential unmasking is critical to conducting sound science. Forensic scientists, lawyers (prosecutors and defense), judges, and police officials must all take simple, systematic precautions to minimize the effects of cognitive bias in their investigations, scientific testing, and assessments of evidence. Fashioning judicial remedies to enforce best practices plays an important role in this process.

Mara Merlino, PhD - This presentation will discuss the issues faced by forensic practitioners as the various forensic disciplines work to articulate and standardize education, training, and other practices to ensure that valid and reliable evidence is produced in service to the justice system. The process of the social construction of scientific knowledge and the impact of legal requirements on scientific "facts" that cross the boundary of admissibility to become scientific "evidence" will be discussed in the context of *Daubert, Joiner,* and *Kumho* decisions.

John J. Lentini, BA - While *Daubert* challenges are becoming more and more common in all expert disciplines, fire investigation seems to attract a significant number of them. The first *Daubert* challenge occurred in 1997, in the case of *Michigan Millers Mutual Ins. v. Janelle R. Benfield*, when a fire investigator with 40 years of experience was precluded from testifying because he could not articulate the scientific method, even though he claimed to have used it. However, in the same case, a firefighter was allowed to render an opinion based on his "experience." Courts have ruled on hundreds of *Daubert* challenges, including the Supreme Court. Sometimes the court gets the science completely wrong, as the 10th circuit did in the case of *Truck Insurance Exchange, A Farmers Insurance Company v. Magnetek, Incorporated*. They arrived at the correct result, but in so doing, completely confused a lot of other courts.

The prevalence of *Daubert* challenges has led to a more general "Rule 702" challenge. In the past, challenges to fire investigators have focused mainly on methodology. There is a trend now toward challenging an investigator's qualifications. After being unable to answer simple fire chemistry questions, an investigator is likely to be withdrawn and the case is likely to settle. Such cases will not result in new case law.

In fire cases, the *Daubert* challenge is now as common as motions for summary judgment. This presentation will discuss several important fire-related *Daubert* cases and what they portend for the future of fire investigation.

Daubert, Evidence, Admissibility

S2 What Shapes Our Future?: Foundations and New Directions

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After attending this presentation, attendees will better understand the different forensic science disciplines as well as the current issues in the field of forensic science. Additionally, attendees will have the opportunity to listen to prominent scientists share their experiences, cases, and thoughts on the future directions of the field.

This presentation will impact the forensic science community by providing a forum for young scientists to learn more about the field, share their ideas, and network with other professionals.

The purpose of the Young Forensic Scientists Forum Special Session is to educate college students and young professionals about the exciting field of forensic science. In addition to the presentations given by Academy members, the session provides attendees with information regarding AAFS membership and mentorship opportunities. The Bring Your Own Slides and Bring Your Own Posters Sessions will give young scientists the opportunity to present their research, and the Breakfast Session will connect attendees with established professionals for résumé reviews and feedback.

The 2017 YFSF Special Session entitled, *What Shapes Our Future? Foundations and New Directions,* is a day-long session scheduled for Tuesday, February 14, that features speakers from many Academy sections. Topics will include current issues in various fields of forensic sciences, as well as forthcoming changes in these fields. Attendees will learn about Academy membership and will have the opportunity to build professional relationships through networking and mentorship. This special session will also showcase two Forensic Sciences Foundation, Inc. Emerging Forensic Scientist Award winners, who will be presenting their award-winning papers.

The Bring Your Own Posters (BYOP) and Bring Your Own Slides (BYOS) Sessions are two opportunities provided by the YFSF for students and young scientists to present their research. The BYOP Session will take place the evening of Tuesday, February 14, and the BYOS will be held on the evening of Wednesday, February 15. YFSF does not require presenters of YFSF BYOS and BYOP Sessions to be members of AAFS and does not require they attend the special session, but they are encouraged to do so. The program will conclude on Thursday, February 16, with the annual YFSF Breakfast Session which includes a résumé review panel. Attendees of the breakfast session must be registered for the YFSF Special Session.

The Breakfast Session on Thursday morning will feature two speakers, followed by a Question and Answer Session. The theme for the Breakfast Session is *Professionalism and Etiquette "Eggs-pectation*s" and will cover topics in courtroom conduct and testimony. Attendees will then have the opportunity to meet with established AAFS members and receive feedback on their résumés.

YFSF, Education, Mentorship

BS1 The Mummies of Central New York — A Unique Experience for a Medical Examiner's Office

Ronald Brunelli, Onondaga County Medical Examiner, 100 Elizabeth Blackwell Street, Syracuse, NY 13210; and Robert Stoppacher, MD*, 100 Elizabeth Blackwell Street, Syracuse, NY 13210*

After attending this presentation, attendees will learn about the unique opportunity the Onondaga County Medical Examiner's Office had in examining two Egyptian mummies. Participants will be able to understand how a multidisciplinary approach led to interesting historical findings as well as a cancer diagnosis. The cancer diagnosis discussion will ask the question, "Does this diagnosis change theories that cancer is a result of modern lifestyles and environment?"

This presentation will impact the forensic science community by explaining: (1) how the medical examiner's office examined two Egyptian mummies; (2) the collaborative efforts of other forensic science disciplines; and, (3) the modern forensic techniques that lead to interesting historical information and possible information for the future.

Medical examiner personnel often see the effects of human mummification in their daily operation. This postmortem change typically occurs on human bodies that have been left in a dry, hot environment for a period of time. This type of postmortem change is often referred to as natural mummification.

From 6000 B.C. to 600 A.D., ancient Egyptians were known to have been the first civilization to perform preservation of not only dead humans, but also non-human remains such as cats and birds. This preservation process was performed by removing the brain, evisceration, immersion, dehydration, and wrapping the body in cloth. Egypt's desert environment aided this mummification process. This is an example of artificial mummification.

The Onondaga County Medical Examiner's Office had the opportunity to examine two Egyptian mummies. One mummy was a mummified head that had been found during an estate auction of a World War II United States Army veteran. He had found the head in a castle while he was stationed in Germany and brought it home at the end of his tour. Research revealed that Napoleon stowed two mummy heads there during his raid of Egypt. After this invasion, Napoleon had been known to stay in this castle on his way back to France.

The other mummy was a full body that had been on display at a local library's Egyptian exhibit. This mummy had been brought to this community in the 1800s by a well-known wealthy citizen of this village. When he died, his Last Will and Testament was read in which this mummy and other Egyptian artifacts were donated to the library.

The forensic disciplines involved in these examinations were pathology, anthropology, trace evidence, chemistry, and radiology. Carbon dating was also performed. Information from these disciplines revealed information about the age at time of death, the possible eras in which these people lived, and their possible status in society. The full body mummy was determined to have had cancer. Does this cancer revelation provide humans with any information about future cancer research?

The routine day-to-day death investigations at a medical examiner's office often involve decedents who have been naturally mummified; however, it is a unique experience for a moderate-sized office to have the opportunity to examine two Egyptian mummies within a one-year time frame. This presentation will discuss this involvement.

Mummies, Radiology, History

BS2 Analysis of Black-and-White Documents — Seeing Beyond the Monochrome

Irina Geiman, MS, United States Secret Service, Criminal Investigative Division, 950 H Street, NW, Ste 5000, Washington, DC 20223; Julia M. Barker, MSFS*, USSS CID, 950 H Street, NW, Washington, DC 20223; and Amanda Moffett, MFS*, 9611 Laurel Oak Place, Fairfax Station, VA 22039*

After attending this presentation, attendees will understand the basic scientific methodology used in the forensic examination of black-and-white documents. Attendees will have an opportunity to learn how physical, optical, and chemical analyses are utilized in the examination of inks, toners, and papers.

This presentation will impact the forensic science community by demonstrating the importance of using the best analytical practices in the scientific analysis of questioned documents. This presentation will discuss the application of Thin-Layer Chromatography (TLC), Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy, and X-Ray Fluorescence (XRF) spectroscopy in the analysis of inks, toners, and papers. This presentation will also showcase how the evolution of forensic document examination techniques has improved sample discrimination through the expanded use of analytical chemistry and statistical analysis.

Forensic examination of documents commonly includes microscopic analysis utilizing white light and other energy sources such as Ultraviolet (UV) and Infrared (IR). Although this level of examination may be sufficient for the analysis of color inks and papers, in many instances it does not provide enough discriminating power to differentiate components of black-and-white documents. For example, black toners often appear similar microscopically with the possible difference of fusing patterns. Black inks may, to an extent, be distinguished based on their physical and optical characteristics and white papers may contain IR luminescent fibers or UV brighteners, allowing for a certain degree of discrimination. While optical examinations provide a wealth of information, it is prudent to recognize their limitations, such as false positive results from document contamination or alteration.

The best practice is to continue with chemical analyses upon completion of the physical and optical examinations. Optimally, a suite of analytical techniques should be used to evaluate various aspects of the sample, including colorants, resins, and elemental composition. For example, TLC is a well-established technique for the analysis of soluble colorants in inks and brighteners in papers. It is also successfully used to evaluate resins and waxes found in toner particles. FTIR spectroscopy is employed to characterize toner polymers and paper coatings. XRF spectroscopy is used to examine elemental composition of analytes and may allow discrimination of papers and toners based on concentrations of iron, silicon, sulfur, titanium, or other elements.

This presentation will demonstrate that chemical examination of black-and-white documents provides a significant amount of information that may be crucial in discriminating samples. As forensic examination of documents has progressed significantly over the past century and moved from penmanship teachers conducting physical examinations using a loupe to highly trained chemists performing analyses using sophisticated instrumentation, it is important to employ the current best practices, which include a full spectrum of techniques.

Ink Analysis, Toner Analysis, Paper Analysis

BS3 Reliving the Jeffrey Dahmer Case 25 Years Later

Jeffrey M. Jentzen, MD, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109; and Carl J. Schmidt, MD, Wayne County MEO, 1300 Warren, Detroit, MI 48207*

After attending this presentation, participants will recognize the characteristics of organized and disorganized serial killers.

This presentation will impact the forensic science community by providing a model death investigation of a serial killer and his motives.

On July 23, 1991, personnel from the Milwaukee County Medical Examiner Office and Milwaukee Police Department responded to an apartment where partially skeletonized remains of 11 individuals had been detected. The assailant, Jeffrey Dahmer, was quickly taken into custody. In this unique situation, Dahmer freely discussed his role in the murders with law enforcement and provided a valuable resource for collaboration of scene, anthropological, and autopsy findings to pathologists and law enforcement personnel during the investigation. Scene investigation and autopsy analysis of the bodies provided prosecutors with detailed evidence of motives and the psychological state of Dahmer during the course of a death spree that lasted nine months.

The evaluation of the scene allowed investigators to: (1) establish methods of death; (2) begin the preliminary identification process; and, (3) demonstrate the deteriorating mental capacity of the assailant. Forensic pathologists assisted with the identification, established the cause of death, and documented injuries that allowed investigators to question Dahmer on various injuries he inflicted upon his victims. An anthropological examination assisted with the identification and also resulted in the establishment of victim profiles.

A forensic psychiatrist constructed Dahmer's psychological profile, which was characterized by a destructive behavior in which his collection of fetishistic memorabilia provided an expression of his deep ambivalence and mixed hostility toward his victims. Frustrated with his sexual immaturity and continual rejection, Dahmer channeled his hostility into a sadistic sexual behavior characteristic of the psychopathology of a serial killer.

The multidisciplinary investigation that followed provided a number of conclusions that permitted the successful prosecution and conviction of Jeffrey Dahmer. Dahmer strangled his victims following his use of "chemical" restraints. He experimented with a method of injecting caustic material into the brains of his victims to sedate and pacify them into being helpless "zombies." Forensic autopsies demonstrated the methods of death, postmortem dissection, and disposition. Anthropological analysis suggested Dahmer was attracted to a certain anthropometric body type. Materials recovered from the scene confirmed the methods of an "organized" serial killer with souvenir-taking to enhance sexual pleasure.

The Jeffrey Dahmer case has captured and captivated the American consciousness for the past 25 years. This presentation will discuss pitfalls in the investigative process using the lessons learned in the Dahmer case. Participants will understand the complexity of the crime and relive the experience by confronting the difficulties encountered in real time by investigators at the scene, which played an important role in the judicial proceedings that followed.

Jeffrey Dahmer, Serial Killers, High-Profile Murders

BS4 Cold Cases: An Exploratory Study of the Status of Unresolved Homicides in the United States

Sarah L. Stein, PhD, The CRUC, 409 Belchertown Road, Ware, MA 01082; Erin H. Kimmerle, PhD*, University of South Florida, Dept of Anthropology, 4202 E Fowler, SOC 107, Tampa, FL 33820; James M. Adcock, PhD*, 4586 Mayfield Woods Circle, Unit 206, Collierville, TN 38017; and Sara A. Martin, MS*, 26 Hillock Drive, Wallingford, CT 06492*

After attending this presentation, attendees will have a better understanding of the status of unresolved homicides in the United States. More specifically, attendees will: (1) realize the total number of unresolved homicides in the country is far greater than previously believed and is increasing each year; (2) understand how each jurisdiction varies in their own definition of what constitutes a "cold" case; (3) see the varying degrees of perspectives as to what does and does not solve cold cases; and, (4) be more informed as to the status and influence of the unidentified dead and missing persons in our system and how this relates to open homicides.

This presentation will impact the forensic science community by providing information new to this forum that will hopefully guide future decision making as it relates to: (1) the funding and efforts put forth toward the investigation of not only the unresolved homicides, but also the "hot" cases that are presently being investigated; and, (2) how we can improve the manner in which we are investigating the unidentified dead.

In 2010, *ScrippsNews* reported that from 1980 to 2008, based on Uniform Crime Report (UCR) clearance data, the United States had accumulated nearly 185,000 unresolved homicides.[1] Until now, that figure had not been validated or refuted. In the fall of 2015, an exploratory study was initiated that consisted of three phases: Phase I — survey law enforcement agencies regarding the status of their unresolved homicides; Phase II — survey the coroners and medical examiners concerning the status of their unidentified dead; and, Phase III — perform an analysis of the UCR clearance data for homicides from 1980 to 2014.

Phase I: Utilizing Survey Monkey®, solicitations were emailed to 10,500 law enforcement agencies. After a 90-day solicitation period, approximately 1,230 responses were received. After culling out those with inconsistent or incomplete information, the survey concluded with 992 responses that were demographically balanced as to population, department sizes, and geographic location.

Some of the overall findings suggest that approximately 73% reported the "lack of investigative leads" as the primary reason for a case going cold; 56% of the agencies said they have cold cases, yet only 19% of those have a dedicated cold case team consisting primarily of one to two detectives; and only about 24% of the teams have a prosecutor assigned to the team, while very few utilize the services of an analyst.

In rating the importance of factors contributing to solving cold cases, DNA was first, followed by interviewing skills of the detectives, detective decisions, cooperation of witnesses, availability of witnesses, latent prints, and other physical evidence. It was not surprising to see DNA identified as the leading solvability factor for cold cases, but this is contrary to what the research tells us about ongoing homicide investigations where DNA's role is minimal compared to detective decisions.[2-4] A further review and analysis of this data with significant correlations will be provided.

Unresolved Homicides, Unidentified Dead, Death Investigation

L1 Murder on Campus — Understanding the Critical Incident and Forensic Response to a Targeted Murder on a College Campus

Matthew C. Wietbrock, BS, 629 N 6th Street, Lafayette, IN 47909; and Carrie Costello, BA*, 6329 Munsee Drive, West Lafayette, IN 47906*

After attending this presentation, attendees will gain a better understanding of the events surrounding a brutal targeted murder on the campus of Purdue University, including lessons learned from the initial response to the scene of a critical incident and the coordination of processing multiple scenes in separate jurisdictions.

This presentation will impact the forensic science community by detailing the facts surrounding the response to a critical incident, the communication challenges faced by first responders, safety concerns when responding to an active scene, and the forensic tasks employed to process multiple crime scenes properly. Subsequent laboratory analysis and court preparation will also be discussed.

In recent years, professional police agencies around the nation and world have trained to confront and to neutralize active shooter events. The Purdue University Police Department is among those agencies. While this incident did not claim multiple victims, as it was a targeted murder, lessons from the initial police response, crime scene investigation, and meeting the subsequent community expectations will be of great impact to the forensic community.

Tuesday, January 21, 2014, was a frigid winter day in West Lafayette. Students had recently returned to campus after the holiday break and were settling in to a new semester. Community members were making lunch plans without an inkling that tragedy was about to strike.

At 12:03 p.m., the Purdue University Police Department started receiving numerous Emergency 911 calls reporting that shots had been fired within the Electrical Engineering Building and that a killer was on the loose. As this report was dispatched via police radios, officers from three area agencies began to respond to the area. Responding officers made entry into the academic building, prepared to confront an active shooter. In accordance with their training, they rushed past the panicked students and faculty and made their way toward the basement. When the first officers made it to the scene, they found to their surprise, that the classroom laboratory was still full of students. Some of the students were even standing over their deceased teaching assistant, too shocked to move. Police quickly took control of the environment by ensuring that the student witnesses were taken care of and sequestered. Officers then began to search the area, looking for the killer. These searches quickly bore positive results, as the suspect surrendered himself outside of the building, not 100 yards from where he had claimed a life. Secondary searches of the building commenced as officers began to look for additional threats to the public. Investigators closest to the apprehension of the suspect were given strong reason to believe that the suspect had acted alone. To the rest of the responding officers, the "fog of war" had descended and situational awareness became the greatest of currency. Rumors of additional shooters abounded in the early minutes of the response. These rumors were compounded by confusing radio communication and the activation of the fire alarm, which triggered a full evacuation of the building.

Even while the scene remained chaotic, crime scene professionals from four separate agencies began to descend upon the scene. A true team effort would take place in the processing of the murder scene. This effort would include many specializations such as blood spatter analysis, photogrammetry, medicolegal death investigation, and computer forensics.

Murder, Critical Incident, Crime Scene Investigation

EVENING SESSION

E1 The Last Lecture

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After attending this presentation, attendees will gain insights relating to life and work from fellow Academy members.

This presentation will impact the forensic science community by providing personal insights into the speakers' journeys throughout their personal and professional lives.

Many times, professors are asked to give "last lectures" to provide guidance and advice to their students while inspiring them to achieve their dreams and take risks in both their personal and professional lives. Members of the Academy have been asked to share what they believe matters most in living a full and rewarding life, both personally and professionally.

In 2007, Randy Pausch famously presented "The Last Lecture" as the first in a lecture series entitled "Journeys." In these lectures, members of the Carnegie Melon University community shared "their reflections and insights on their personal and professional journeys." Dying of pancreatic cancer, Randy Pausch brought down the house with his talk, "Really Achieving Your Childhood Dreams." This lecture has become infamous for the inspiration it instilled in everyone who has heard it.

In the spirit of this legacy, members of the Academy have been asked to share what they believe matters most in living a full and rewarding life, both personally and professionally, including their childhood dreams and inspirations, taking risks, overcoming obstacles, and the adventures and lessons learned along their journeys. The speakers reflect varying experiences and different walks of life. Share and learn from their joys, challenges, integrity, and recognition during this entertaining and educational session.

Jan Garvaglia, MD, is the retired chief medical examiner for the District Nine Medical Examiner Office in Orlando, FL. She is best known as the reality TV star "Dr. G" on the Discovery Network. The show, *"Dr. G: Medical Examiner,"* won an International Health and Medical Media Award for "Best Health Series" in 2008. Dr. G will share her personal story about the trials of being a forensic pathologist along with the pearls of the profession, while highlighting interesting cases and discussing how the reality-based TV series has impacted her life.

John Lentini, BA, is one of a handful of people certified to conduct both fire scene investigations and fire debris analysis. He has personally conducted more than 2,000 fire scene inspections and has appeared as an expert witness on more than 200 occasions. Mr. Lentini is an independent consultant, working with both prosecution and defense in pending cases and in cases involving the wrongfully convicted.

Ken Melson, JD, has had a long and lively career within the forensic community, serving as a federal prosecutor for more than 20 years. As Director of the Office for United States Attorneys, he was responsible for the oversight of the 94 United States Attorney's Offices. He is a past president of the AAFS and has served on numerous committees. In April 2009, Mr. Melson was named Acting Director of the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) by President Obama, where he served until August 2011. Prior to retiring from the United States Department

of Justice in 2012, Mr. Melson was the Senior Advisor on Forensic Science in the Office of Legal Policy and served as co-chair on the Subcomittee on Forensic Science within the Executive Office of the President.

Katherine Ramsland, PhD, is the Director of the Master of Arts in Criminal Justice Program at DeSales University. She also teaches forensic psychology. Dr. Ramsland is a prolific writer, with most of her attention focused on crime, forensics, and serial killers. She recently completed *Confession of a Serial Killer: The Untold Story of Dennis Rader, the BTK Killer.* However, she also authors such inspiring works as *Snap: Seizing your Aha! Moments*, focusing on "the latest neuroscience findings on spontaneous thought processes, or "snaps."" Dr. Ramsland "describes how everyone—not just geniuses—can learn to improve the likelihood of their own "eureka" moments by adopting certain rewarding attitudes and habits."

Douglas Ubelaker, PhD, is a curator and senior scientist at the Smithsonian Institution's National Museum of Natural History in Washington, DC, where he serves as a consultant in forensic anthropology. Dr. Ubelaker has published extensively and has served on editorial boards of leading scientific journals. Dr. Ubelaker is regarded as one of the world's leading anthropologists, receiving many international and national honors and awards throughout his esteemed career in the analysis of human skeletal remains with an emphasis on forensic applications. Dr. Ubelaker served as the AAFS President from 2011-12. Dr. Ubelaker works tirelessly on behalf of human rights internationally. He currently serves as Chair of the AAFS Human & Humanitarian Rights Resource Center, which promotes forensic science principles and applications to global projects requiring special assistance.

Last Lecture, Mentoring, Forensic Science

W1 Behind the Curtain: Understanding the Basic Science and Testimony of Latent Prints

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After attending this presentation, attendees will be well-positioned to critically evaluate latent print reports and testimony. Prosecutors will be more aware of changing trends in testimony and will better understand how to prep their expert witnesses to make sure they aren't falling into "old school" traps and are well prepared to discuss the relevant literature. Defense attorneys will be able to spot unsupported statements and effectively challenge them in court. Judges will gain a basic understanding of the modern scientific research detailing the strengths and limitations of latent print comparisons, which will allow them to be more effective gatekeepers. All legal professionals will be equipped to spot the difference between the "old school" or "dogma" expert and the "transparent" expert and will be able to react accordingly.

This presentation will impact the forensic science community by assisting the legal community to better understand the basic scientific principles behind friction ridge comparisons. This workshop will provide the legal community with the tools to critically evaluate friction ridge testimony, which will in turn allow them to better advocate for the science, challenge the science, or fulfill a gatekeeping role, as their positions within the legal system dictate. These skills will improve the application of friction ridge comparison science in the legal system by helping to ensure that it is presented responsibly and used appropriately, increasing its value to the legal system.

Judges and attorneys have an important responsibility in cases involving forensic science evidence. While most judges and attorneys do not have scientific training, they nonetheless have the task of assessing forensic evidence for reliability. Attorneys have the additional responsibility of effectively challenging forensic evidence when appropriate. In recent years, there has been a growing spotlight on the idea of "junk science" in the courtroom and a growing pressure on legal professionals to better understand the science that is being presented. The prevalence of forensic evidence in criminal cases coupled with the pace of research and reform in the forensic community will cause this pressure to increase in the future.

The basics of forensic science disciplines to a legal audience will be explained in addition to tips on how legal professionals can evaluate the reliability of forensic evidence in criminal cases will be provided. An overview of the latent print discipline will be provided with a discussion of what latent print examiners do in their work and the foundational science behind latent print comparison. Reporting and testimony will also be presented detailing the traditional ways latent print examiners have expressed their conclusions, which parts are inappropriate, and why. It will also discuss the ways in which latent print testimony is shifting in the post-NAS environment. This discussion will be supplemented with discussion of some landmark studies in the discipline and how legal professionals can use these studies during litigation. What is valid and what is not in the discipline will be highlighted, providing legal professionals with some of the necessary tools and road maps to assess forensic evidence and litigate questionable forensic evidence in criminal cases.

Come take a peek behind the curtain into the science of latent print examinations.

Latent Prints, Testimony, Gatekeeping

W2 A Computational Framework for Skeletal Age-at-Death Estimation Using Laser Scans of the Adult Pubic Symphysis: Theory, Methods, and Software

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After attending this presentation, attendees will understand state-of-the-art skeletal age estimation, the problems forensic anthropologists face when estimating age by conventional methods, the needs of the medicolegal community, and the potential for advancing the field using new shape-based methods. Attendees will receive instruction in three new methods that apply numerical shape algorithms to laser scans of the skeletal age indicator. Attendees will also receive hands-on training in using the equipment, software, casts, and data.

This presentation will impact the forensic science community by delivering instruction on implementing three new fully computational methods for age-at-death estimation from skeletal laser scans that produce estimates that closely approximate true age, with minimal risk of subjectivity or low-method/observer-induced error.

The estimation of age-at-death in forensic anthropology represents an essential component of the biological profile, providing information on the individual that is key to medicolegal case identification. Skeletal indicators of age are widely used to estimate age-at-death from adult remains in this casework context. Of the pelvic, thoracic, and cranial features for which age-related change is known, the pubic symphysis remains the preferred, most frequently studied indicator. Common practice requires the macroscopic comparison of the bone surface morphology to a set of population-specific criteria that represent a series of pre-defined scores or phases. The case-specific age-at-death is then estimated from an age range previously associated with the assigned score or phase. While the simplicity of this approach is attractive, the limitations of this kind of visual analysis are well-documented across the field, not only for age-at-death estimation and but also for other parameters of interest to the biological profile. In general, this methodology is known to introduce a large degree of subjectivity and intra/inter observer-related error. For age estimation especially, these problems have been shown to variably impact the reliability and repeatability of results; therefore posing, significant challenges to meeting current medico-legal standards of evidence and successful forensic case identification.

In response to these concerns, an alternative, fully computational approach to the macromorphoscopic assessment methods traditionally applied to skeletal indicators of age and, specifically, to the pubic symphysis has been proposed. It is argued that accurate, precise, and objective age estimates can be obtained by sourcing three-dimensional coordinate data from laser scans of the pubic symphysis, subjecting these data to shape-analysis algorithms, and combining the resulting shape measures in multivariate regression models. In recent publications the value of this novel approach for contemporary skeletal analysis have been demonstrated. Using Bass Collection samples, forensic cases, and the Suchey-Brooks and McKern & Stewart casts, these methods produce estimates that differ from the exact age-at-death by $\approx 11.72 \pm 0.97$ years. To standardize implementation, a protocol for data collection and extraction has been formalized. The software, forAge, was developed to facilitate accurate and efficient method application among forensic practitioners, whose levels of familiarity with laser scanning technology, statistical computing, and the morphological characteristics of the pubic symphysis may vary.

This workshop will introduce forensic practitioners to the theory that underlies the methods and provide laboratory instruction on its implementation. It will also be broadly applicable to other scan and shape-related research. To contextualize the work, a review of the current state of the art of age-estimation, the demands that working within the medico-legal context places on forensic case analysis, and the advantages that these methods offer for estimation, evidence, ease of use, and data preservation or sharing. The anatomical properties of pubic symphyseal morphology that make this indicator well-suited to shape-based inference as well as the mathematical theory that supports the calculation of our shape measures will be explained. The appropriate use of these measures

and clarification of how an estimate of age is generated will be discussed. To provide practical instruction, protocols for laser scan collection using the NextEngine scanner, scan editing and manipulation using ScanStudio or Meshlab, extraction of shape information as three-dimensional coordinates, file storage of these data, and for standardization and processing of the coordinates prior to analysis will be demonstrated.

The detailed implementation of the following will be discussed: (1) the *SAH-Score* method that captures the variance on the symphyseal face to capture the gradual flattening of the surface associated with aging;[1] (2) the thin plate splines method that determines the bending energy required for transforming a perfectly flat, infinitely thin plate to match the surface of a pubic symphysis scan;[2] and, (3) the ventral curvature method that quantifies the progressive formation of a rim around the entire symphyseal surface and its later erosion.[3] A tutorial on the use of the forAge software for these analyses and to produce age-estimates via multivariate regression will be provided. With France Casting, the age-determination casts for calibration and validation will be discussed. Finally, recommendations for data collection in the field and laboratory will be offered. Workshop attendees will have the opportunity to train on the scanning equipment and software and produce age estimates directly from specimens and coordinate data in various stages of processing.

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3. Stoyanova D, Algee-Hewitt B, Kim J, Slice D. A Computational Framework for Age-at-Death Estimation from the Skeleton: Surface and Outline Analysis of 3D Laser Scans of the Adult Pubic Symphysis. J Forensic Sci 2016: in review (submitted April. 26, 2016).

Age-at-Death Estimation, 3D Laser Scans, Computational (Shape) Analysis

W3 Shooting Reconstruction

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After attending this presentation, attendees will learn some of the phenomena that must be taken into consideration when assessing a shooting scene. Several different types of ammunition will be discussed, along with their interactions with several different substrates commonly encountered. Attendees will also become familiar with evidence recognition, documentation, and recovery for laboratory analysis.

This presentation will impact the forensic science community by exploring some of the techniques used in shooting scene reconstruction and subsequent laboratory analysis of related evidence.

The complex nature of a shooting incident may generate a variety of firearm-related evidence, such as the firearm itself, cycled or discharged ammunition components, gunshot residue, trace evidence on a bullet, or impact sites with traces of the bullet's prior presence. Whether considered firearm evidence or trace evidence, this information may have to be integrated by the scientist to be most beneficial.

When a shooting incident takes place and firearm evidence is recovered at the scene, whether in the form of cartridge cases or bullets, it is likely that an examination of these ammunition components will ensue, using the well-established and proven methods of comparison microscopy. Recently, use of comparison microscopy has become the focus of criticism, but it nevertheless provides valuable information for both opaque samples using reflected light and for transparent samples using transmitted light. There are some occasions, however, where the question of which firearm was involved, or which bullet came from what firearm is not in dispute; but instead, questions arise about the specific path of a bullet, the relative positions of the shooter and the victim, the presence of an intervening object, or the sequence of the shots that were fired.

Pulling the trigger of a firearm initiates a series of events that culminates with the discharge of a bullet with considerable energy, along with primer and propellant resides as secondary ejecta. The bullet may not only impact its intended target; it may perforate an intermediate object or objects on its way to the target or it may pass completely through the target and retain sufficient energy to continue downrange and impact an unintended object.

These types of interactions and impacts invariably impart information about the event onto the bullet and onto the impacted substrates. If information from the inadvertent or intended impact is recognized, examined, and deciphered, it can be helpful in developing a more accurate shooting scene reconstruction. This workshop will consider the transfer of material from the substrate to the bullet, per the Locard Exchange Principle, the overall change to both the bullet and substrate from the energy exchange, the potential path the bullet followed, and the possibility of ricochet.

Determining the angle at which a bullet will successfully ricochet is essential information when a shooting investigation involves indirect fire. This information provides the forensic scientist with fundamental data required for the scientific reconstruction and assessment of a shooting scene. Depending upon the substrate, the bullet's design, velocity, construction, and its angle of impact, a bullet may fail to ricochet upon impact, or the bullet will successfully ricochet. Knowledge of bullet behavior with common substrates provides valuable information for scientific investigation of shooting scenes where bullets have impacted intermediate surfaces. A timely and accurate scene reconstruction is imperative in both the investigative and the adjudicative stages of a shooting incident.

Shooting, Reconstruction, Bullet Path

W4 Sex-Related Homicide and Death Investigations: Practical and Clinical Perspectives — The Significance of Pornography, Sexual Deviance, Autoerotic Fatalities, Signature and *Modus Operandi* (M.O.) in Serial Murder Investigation and Criminal Investigative Analysis

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After attending this presentation, attendees will be aware of common assumptions made by responders on scene, such as a death is an autoerotic asphyxia death prior to investigating if more than one person may have been involved. Attendees of this presentation will better understand the significance of sexual deviancy, fantasy, and pornography in sex-related events, as well as the investigative and behavioral analysis and medicolegal considerations involved in these types of cases. This presentation will provide attendees with practical, proven procedures for crime scene documentation, law enforcement investigation, and medicolegal investigations.

This presentation will impact the forensic science community by providing and familiarizing forensic scientists and investigators with the investigative expertise and medicolegal science involved in the professional examination of sex-related homicide and death investigations. There will be case examples that focus on determining whether or not the death is a paraphilic accident or a sex-related homicide.

Sex-related homicides and other deaths occurring during sexual activities have drastically increased over the years and claim victims from all walks of life: men or women; lovers or strangers; elders or children. These fatalities may occur from recreational misadventures or sex-related crimes, which are perpetrated by sex offenders and are among the most horrific crimes imaginable. The internet has certainly provided society with technological advances but has also resulted in the proliferation of pornography and easy access to sex-related materials to anyone with a computer or computer access. It is significant that the sex industry, which consists of commercial enterprises providing adult entertainment, earns over $13 billion annually in the United States and how that may influence the increase in sex-related events.

Sexual deviance, fantasy, and the significance of pornography perform major roles in the proliferation of sex-related homicides and unnatural deaths due to autoerotic activities, which expose the participants to danger as in cases involving some of the paraphilias. In DSM-5, the term paraphilia denotes any intense and persistent sexual interest, other than sexual interest in genital stimulation or preparatory fondling with phenotypically normal physically mature consenting adults. Some paraphilias may concern an individual's erotic activities while others primarily concern an individual's erotic targets. An example would be the voyeur, who satisfies himself sexually by observing an unsuspecting person naked, in the process of disrobing or engaging in sexual activity versus the sexual sadist who is aroused by the physical or psychological suffering of another person.

The applications of clinical criteria and abnormal psychology to the investigative process are an integral parts of criminal personality profiling and have been universally recognized and accepted as genuine and legitimate investigative techniques. Detectives and criminal investigators routinely employ these techniques in their investigation of violent crime on a case-by-case basis. From a practical standpoint, there are only so many ways to kill and only so many stories to tell as an offender attempts to explain the killings. After a while, a distinct pattern emerges, which encompasses a series of clusters of behavioral information and specific typologies of offenders. Human behavior is frequently repetitive. Certain actions engaged in at the scene by certain types of personalities will tend to repeat themselves.

Clinically speaking, there is a behavioral distinctiveness in human sexuality. This unique aspect of our sexual arousal and response system accounts for why individuals differ in their sexual behaviors and engage in a specific series of behavioral patterns. In sex-related criminal incidents, the offender is oftentimes subconsciously "acting out" a sexually significant behavioral pattern, which reflects the underlying personality, lifestyle, and developmental experiences and fantasies of the offender.

Sex-Related Deaths, Sexual Deviance, Signature and M.O.

W5 The Opioid Epidemic: Trends, Challenges, and a Path Forward

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After attending this presentation, attendees will be able to: (1) describe the current epidemiology of prescription and non-prescription opioid drugs in North America and around the world; (2) identify and distinguish between the various chemical categories of novel emerging opioid drugs; (3) implement a drug-screening protocol for novel emerging opioid drugs, looking for the most relevant drugs; and, (4) apply data from intoxication cases involving traditional and emerging opioid agonists in the interpretation of forensic toxicology and death investigation casework.

This presentation will impact the forensic science community by providing an update to the current state of the opioid epidemic in which emerging novel opioid drugs are challenging professionals in forensic chemistry, toxicology, emergency response, and death investigation.

In recent years, opioid use has increased dramatically due to the overprescribing of painkillers such as hydrocodone and oxycodone, the increased demand for heroin and other illicit drugs sold as either contaminants or substitutions, and the emergence of novel synthetic opioids, including repurposed pharmaceutical cast-offs (e.g., U-47700, AH-7921) and non-pharmaceutical fentanyls (e.g., furanyl fentanyl, β-hydroxythiofentanyl). Opioid use and abuse has garnered national attention; news stories vary from celebrity overdose deaths, to presidential campaign promises to address the opioid crisis, to the approval of nasal naloxone spray to reverse overdoses. Despite being a familiar drug class with a long history. These changes make it necessary for the forensic science community to revisit and understand the current state of the opioid problem.

The demand for heroin and other pharmaceutical painkillers has become so intense that it has created a dynamic marketplace for illicit drug manufacturers. Across the United States and Europe, users are able to buy these substances not only through a neighborhood dealer, but also through online cryptomarkets, making more substances accessible to more people. Besides the multiple ways in which these illicit drugs can be obtained, clandestine drug makers are also distributing a variety of substances that pose substantial risks to end users who do not know what, and how much, they are consuming. Perspectives regarding both the United States experience and the European experience with the current state of the opioid market will be discussed.

This workshop has been designed to provide an end-to-end update on the current opioid crisis including a discussion and review of the current trends in opioid deaths and intoxications in the United States, and a deeper look at some of the factors contributing to the increased popularity and the links between overprescribing and escalation to more intense illicit opioid abuse. The United States illicit opioids market will also be described and examine available knowledge about approaches to illicit opioid synthesis and distribution, including use of signature analysis to track the origins of illicit opioids. An emergency medical physician dealing with opioid intoxications will discuss some of the features of current intoxications with novel opioids and its impact on effectiveness of opioid reversals, and consequences for naloxone programs.

The opioid trade is now international and the experience of professionals in other parts of the world often presages our experience in the United States. Experts from the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) in Lisbon will share international intelligence on the opioid trade and what may be in store for the United States. The known and emerging knowledge about factors affecting the potency of opioids including their receptor binding and functional affect, as well as structure activity relationships will be reviewed in attempts to explain the relative potency of suspected agonists.

Among the more unusual trends is an increase in abuse of the mu-opioid agonist loperamide reviewing recent reports of toxicity and fatalities associated with abuse of this drug.

The session will conclude with a panel presentation of forensic toxicology case series involving the most recently identified compounds of concern, including acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, MT-45, AH-7921, U-47700, 3-methyl fentanyl, W-15, and W-18, the latest and most mysterious of the alleged novel opioids.

Opioids, Fentanyl Analogues, Heroin

W6 Taking a Bite Out of Crime and Other Hairy Situations

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After attending this presentation, attendees will understand how wrongful convictions and exonerations come to light through the post-conviction process and will be provided examples of how to respond when mistakes are uncovered.

This presentation will impact the forensic science community by highlighting what has been learned from two forensic disciplines under fire and how addressing these weaknesses has led and can lead to a stronger forensic future.

Wrongful convictions often happen for a multitude of reasons in any given case. They are also often the bellwether for change, making the need for reflection, reform, and growth within both the legal and forensic communities a priority. This workshop will delve into two specific exonerations. Each raises different issues, has sparked different responses, and is leading to the evolution of not only these disciplines but the forensic science community as a whole.

The workshop will begin by focusing on the stories of two men convicted for crimes they did not commit and the post-conviction journey that led to their ultimate exonerations. Kirk Odom was exonerated on July 13, 2012, after spending 22 years in prison and another nine years on parole as a registered sex offender for a rape he did not commit. The victim, a 27-year-old woman, who was attacked in Washington, DC at first could not identify her attacker. She would later identify Mr. Odom in a police line-up. This misidentification along with testimony from an FBI Special Agent that the hairs found on the victim's nightgown were microscopically similar to Odom's hair, "meaning that the samples were indistinguishable," contributed to his conviction. His post-conviction proceedings were handled by Sandra Levick who will speak about her experiences working on Mr. Odom's case; ultimately, leading to the certificate of actual innocence, which was signed on Mr. Odom's 50th birthday.

The second case involves a rape and murder from Virginia. In this case, Keith Allen Harward was convicted based at least in part on bitemark testimony presented at his trial. He spent 33 years of a life sentence in a Virginia prison. Mr. Harward will speak about his experience as someone wrongfully convicted of a crime and what the post-conviction process looks and feels like from the perspective of the accused. His post-conviction experience will also be discussed by the lawyers involved. In addition, two forensic odontologists will provide their perspectives into how the discipline has or is changing and what might be done in the future to avoid these kinds of errors.

Reflection alone is not enough, and in a number of ways, the forensic science community has responded to stories like those of Mr. Odom and Mr. Harward. Those responses will also be explored.

Lynn Garcia from the Texas Forensic Science Commission (TFSC) will focus on the experiences of the Commission in providing oversight, coordination, and advocacy for accredited crime laboratories in Texas. Discussion will include: (1) specific case examples of how shared expectations regarding laboratory self-disclosure can increase transparency and improve organizational culture will be discussed; (2) the importance of resisting external political agendas and adopting a collaborative approach with all affected stakeholders; and, (3) focus on the importance of a state commission's outreach to affected prosecutors, judges, and the defense bar to ensure they understand the implications of a particular forensic non-conformance. Attendees will learn about the commission's efforts to develop a statewide notice protocol to ensure potentially affected defendants receive notice in the wake of concerns in key, high-volume disciplines like DNA/forensic biology. Lessons learned from Texas may serve as a model for other states seeking to establish similar commissions to examine various aspects of the state of forensic science locally and globally.

Sherry Sabol, General Counsel from the FBI will talk about what happened in the wake of cases like Mr. Odom's. This includes a review of the testimony by other FBI analysts and what the FBI is doing in this area of comparative science not only to look back on old cases but to improve the quality of testimony for the future.

Finally, Judge Chris Plourd and Judge Pam King will discuss the challenges faced in the justice system, address the admissibility of forensic science evidence and opinions and the court's role as the gatekeeper and explore how the criminal justice system is changing and then provide some insight into the challenges to change built into the judicial system.

Post-Conviction, Hair Analysis, Bitemark Analysis

W7 How to Manage the Multidisciplinary Response to a Mass Fatality Event

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After attending this presentation, attendees will: (1) understand what to expect if they are involved in a mass fatality event; (2) understand who is involved; (3) understand what the expectations are of the different parties; (4) learn how to work with different government agencies, private parties, media, and families; (5) know how to provide leadership and direction; and, (6) understand the practical lessons learned from multiple events so they can review existing plans, update them if needed, and be better prepared for the variety of events in which they are likely to be involved.

This presentation will impact the forensic science community by providing practical, experienced guidance on what to expect, how to manage, and how to avoid the very common mistakes that medical examiners, coroners, law enforcement, and others who have the responsibility of preparing for and responding to mass fatality events will face when these events occur.

Mass fatalities from terrorist attacks, natural disasters, industrial accidents, and transportation losses are complex events for the forensic community to manage. Complexities include the challenges created by fragmented, potentially contaminated human remains and the collection and preservation of evidence. Events which might include the tragic loss of 100 people could easily result in a recovery scene of several square miles and over 10,000 human remains fragments. They also include the involvement of multiple local, national, and international governments at the forensic, family assistance, and political levels, often with competing interests. These factors, combined with the massive logistical requirements and the overwhelming media and public attention, can create a chaotic situation for forensic practitioners who are more accustomed to controlled environments.

Today's forensic practitioners are expected to successfully manage these events. This includes medical examiners, coroners, law enforcement, and justice officials. The goal of this workshop is to provide the practical, overall view of mass fatality event management strategy. This eight-hour workshop will cover: (1) an overview of who's involved, from occurrence of the event to final litigation/court, as well as managing the best practice teamwork that is needed for success; (2) an overview of what's involved – the functional areas beyond the morgue that need to be addressed, such as collecting antemortem information, call centers to register missing persons, and family briefs; (3) media and communications management - how and what to say; secure websites; family, media, and political briefings; (4) common mistakes in identification and release of human remains; (5) dealing with HAZMAT (including deliberately introduced) or infectious (public health) contaminated human remains and scenes; and, (6) managing the critical incident stress impact on yourself and staff.

This workshop won't cover how to do an autopsy, or how to take and compare fingerprints, hot to make an ID, or how to write a report. Rather, it teaches how to bring all areas of death investigations together in a cohesive and professional mass fatality response. Attendees will learn how to create a system to control all aspects of the response. This includes practical matters such as: quality assurance systems to manage the release of highly fragmented remains; how to develop checklists to help people who have not worked together; how to organize a morgue triage and process flow; and, how to manage and share the enormous amount of data that is both collected and requested by others.

Drawing on extensive, real-world, multi-event practical experience, participants will learn how to deal with the consequences of the event. No event is unpredictable and there are no new lessons, only new people learning lessons for their first time. This is an opportunity to learn and take the information back to your own organization to identify and fill in gaps in your own plans.

Mass Fatality Response, Multidisciplinary Approach, Mass Disasters

W8 Applications of Hair Testing in Toxicology: Legal, Technical, and Medical Challenges

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After attending this presentation, attendees will be able to evaluate the usefulness of hair testing in forensic casework by becoming more knowledgeable, which will allow attendees to: (1) describe and explain the existing consensus documents on hair analysis, recognize the importance of guidelines in hair testing, and evaluate their own laboratory routines in the light of the existing guidelines; (2) describe how Ethyl Glucuronide (EtG) is incorporated into hair and interpret the results of this type of analysis; (3) describe the differences between urine/blood testing and hair testing for doping agents and be more knowledgeable concerning sport regulations in cases of doping offenses in addition to relevant political issues in using hair for doping purposes; (4) realize the possible applications of hair analysis in forensic toxicology and the circumstances and challenges that must be considered for the correct interpretation of the results; and, (5) gain an overview of workplace drug testing and the challenges of hair testing in a production laboratory.

This presentation will impact the forensic science community by allowing attendees to gain insight into the broader scope of the analysis of hair for drugs and toxins in a range of fields applicable to forensic science. By including discussion on recent changes in legislation and standards, the usefulness of these tests in the greater context of the law and recent regulatory changes will be clearer to the forensic science community.

Hair is a unique and challenging matrix for forensic toxicological testing in that detailed information on historical use is recorded over time as compared to traditional blood and urine matrices. In order to make best use of hair testing in forensic casework, a thorough understanding of the advantages and limitations of this matrix is essential. An overview of the current status of hair testing in a variety of toxicological areas including reference to current standards and consensus agreements as set by the Society of Hair Testing (SoHT) will be provided. By providing both an overview and more detailed information in workplace testing, pediatric and maternal testing, doping and alcohol biomarkers, as well as forensic toxicological testing of hair, the workshop is not only amenable to toxicologists but also relevant to pathologists and criminalists with an interest in expanding their understanding of the importance of these test in the investigation of crime. Discussion will focus on the latest technologies used for the testing of hair within the different areas covered, from screening tests to confirmation. The effects of age, sex, health, and the environment on the interpretation of results will be explained. All presentations will be given in the context of the SoHT guidelines to ensure that attendees understand best practice and safeguard against over-interpretation of results. Case examples will be discussed for each testing type and attendees will be invited to share their own casework examples for discussion by the panel of experts.

Hair Testing, Drugs, Guidelines

W9 Staged Crime Scenes: Crime Scene Clues to Suspect Misdirection of the Investigation

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After attending this presentation, attendees will learn three new categories of staged scenes, with case examples of different types of crimes where staging is prevalent. Additionally, attendees will learn the "red flags" or common findings in staged scenes.

This presentation will impact the forensic science community by introducing the distinct categories of staging based on the intent of the offender's scene alteration. Ultimately, applying these categories may help in the quick recognition of scene alterations or staging.

In the course of their career, most detectives and forensic practitioners will come into contact with a staged crime scene; a scene that has been altered by the offender to either mislead a police investigation as to the true facts of the crime or for other reasons understood only by the offender. Staged scenes and "staging" are possible in nearly every type of criminal offense raging from property crimes, such as arson and burglary, to violent crimes such as homicide, child abuse, or sexual assaults.

To better understand the dynamics and the general nature of "staging," this workshop will introduce distinct categories of staged crime scenes based on the intent of the offender's scene alteration. The ultimate goal of this workshop is to understand that the offender's actions to stage a scene can actually be identified through common findings or "red flags" that are often found when scenes are altered. Further, that these same red flags may help in the quick recognition of scene alterations or staging, and based on new criteria may be divided into three separate and distinct categories. These categories are herein referred to as, primary, secondary, and tertiary.

The primary staged scene is intentionally altered or changed by the offender with criminal intent to misdirect a subsequent police investigation and can be further sub-categorized into two types: ad hoc, and premeditated. The ad hoc subtype is staged without forethought and planning, at the spur of the moment after the event has taken place, and is generally intended to deflect attention away from the offender and true facts of the crime. The scene is considered premeditated when the offender preplans the scene alterations in accordance with a preconceived scenario. Premeditated staging is often designed to focus attention onto the staging and false evidence. Ultimately, through primary staging the offender in effect creates a false reality that in his/her mind will successfully and with criminal intent, misdirect the police investigation.

Secondary staging involves the intentional alteration or manipulation of the crime scene or victim by an offender that is unrelated to misdirecting or diverting subsequent investigations. This is really a new category of staged scenes and would include such examples as posing the victim's body into sexual provocative positions as found in sexual homicides or other elements of what is more commonly known as "staging" such as covering the face or body in what is generally often described as depersonalization. The perpetrator's purpose in secondary staging is not to misdirect the investigation, rather it is often something that is psychologically "part and parcel" to the crime, such as demeaning the victim or demonstrating ultimate control over the victim.

The third category to be discussed and defined consists of noncriminal, accidental, or innocent alterations (i.e., changes to the original crime scene), generally by witnesses or family members, who find the victim and alter the scene without any criminal intent. An example would be a family member finding a loved one in an embarrassing position from an autoerotic misadventure and changing the scene to prevent embarrassment to the family. These types of alterations are better described as tertiary, and are best regarded as scene artifacts.

This workshop would have application to persons in forensic pathology, criminalistics, crime scene analysts, and criminal investigations. It introduces three new categories of staged scenes, provides case examples, and explains the "red flags" commonly encountered when confronted with a staged scene. The workshop culminates with case studies for each student to work through and identify the various "red flags" in real cases.

Primary Staging, Secondary Staging, Tertiary Staging

W10 Child Abuse: A Multidisciplinary Approach

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After attending this presentation, attendees will be able to: (1) identify the neuropathological findings of abusive head trauma, understand how the biomechanical mechanisms of inertial brain injury create these injuries, and recognize how the distinction between focal and diffuse brain injury determines the clinical presentation of timing of symptoms, such as level of consciousness; (2) properly perform a gross and microscopic examination of the eye and document the significant pathological features of retinal hemorrhages; (3) properly perform and document the evaluation of a child's fracture; and, (4) identify and exclude cases of abusive trauma to children using all relevant clinical and pathological data and information.

This presentation will impact the forensic science community by assisting in the evaluation of head injuries in infants and young children as well as assisting in the evaluation of traumatic injuries to the chest, abdomen, extremities, and bones of young children. Child abuse deaths are fairly common deaths seen by forensic pathologists in jurisdictions of all sizes. These cases are extremely complex and represent some of the most difficult cases handled by forensic pathologists. Forensic pathologists can be assisted in their work on these cases by input from other medical specialties and areas of interest, including child abuse pediatrics, pediatric ophthalmology, pediatric neurosurgery, neuroradiology, as well as a general knowledge of developmental pediatrics. Familiarity with these other areas of expertise will allow forensic pathologists to better decide upon cause and manner of death and to answer questions about forensic issues such as timing of injuries and onset of unconsciousness.

Head injury is the leading cause of death and disability in children. Of childhood deaths resulting from head injury, inflicted neurotrauma accounts for the greatest number. Sixty-four percent of head injuries serious enough to warrant admission to the hospital (excluding uncomplicated skull fractures) and 95% of serious intracranial injuries are the result of inflicted trauma. It is estimated that in the United States, 2,000 children die each year from abuse and neglect. Head injury is the leading cause of death from inflicted trauma and accounts for a large number of nonlethal abusive injuries. Falls are frequent occurrences in childhood and most head injuries in young children are caused by falls; yet, the great majority of these fall-related head injuries are trivial and only a few are lethal. Making the distinction between inflicted and accidental head injury is a common problem in the pediatric population and concerns pediatricians, forensic pathologists, neurosurgeons, and other medical specialties. The distinction between accident and inflicted is however an exceedingly important distinction to make. The features and issues necessary in distinguishing abusive head injury from accidental injury as well as from non-traumatic conditions such as genetic causes of subdural or retinal hemorrhages will be closely considered. Certain issues in abusive head injury have caused intense discussion among the various physicians and engineers who have examined these cases. Two areas of "controversy" include the debate over shaking as a mechanism of head injury in young children and the role that hypoxia might play in the causation of subdural bleeding. These topics will be considered in this workshop.

Traumatic brain injury can be classified into static and dynamic injuries depending upon the rate at which force is loaded onto the head. Static injuries occur over longer periods of time, usually greater than 200 milliseconds, and result in crushing head injuries. Crushing head injury refers to actual crushing of the facial skeleton and skull by a heavy weight. While relatively rare in the overall number of head injuries at all ages, it is an injury seen in some accidental childhood head injuries. At all ages, the greater number of head injuries result from dynamic forces that occur when force is rapidly loaded onto the head (in less than 200 milliseconds) and imparts an impulsive motion to

the head either as a result of impact to the head, which is free to move, or as a result of an action to the body, which causes the head to move such as the collision of two athletes or the violent shaking of an infant. Impulsive loading of significant degree may create inertial movement of the brain within the cranial cavity which causes differential movement between the brain (with its attached arachnoid) and the skull (with the attached dura). This inertial movement of the brain within the cranial cavity is the cause of bridging vein failure and results in subdural and subarachnoid hemorrhage and traumatic diffuse axonal injury. Inertial brain motion is also considered to be the cause of retinal hemorrhages of the type found in abusive head trauma. Brain injuries may also be classified as either focal or diffuse injuries. Focal injuries result from direct contact injury to the head and are visible to the naked eye. Focal injuries include scalp contusion and laceration, skull fracture, epidural hemorrhage, focal subdural hemorrhage, and brain contusions. Focal injuries become clinically symptomatic by causing increasing intracranial pressure which takes place over time and may have a fatal outcome from herniation. Focal brain injuries typically have a lucid interval. Accidental head injuries in young children with sufficient focal injury may lead to fatal outcomes and need to be recognized by all medical specialists. Diffuse injuries result from inertial forces and include interhemispheric subdural hemorrhage and traumatic diffuse axonal injury. Diffuse injuries may not be visible to the naked eye and these injuries may become clinically evident by the onset of immediate traumatic unconsciousness and tend not to have a lucid interval. Inflicted head trauma is most often of the diffuse brain injury form. Multiple medical specialists will discuss their unique approaches and perspectives in examining and evaluating head injuries in young children including how each specialty can assist the others in the interpretation and appreciation of the forensic and legal implications of these injuries. This workshop is intended to provide sufficient detail and depth to be of use to forensic pathologists in particular as well as other physicians who are concerned with child head injuries.

Fractures, Child Abuse, Subdural Hemorrhage

W11 Extreme Violence in the Military: Multiple Case Studies and the Exploration of Murder, Rape, and Cold Cases in the United States Military

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After attending this presentation, attendees will understand: (1) the processing of mass murder scenes; (2) the forensic roles of military criminal investigators; (3) the responsibilities of the Armed Forces Medical Examiner; (4) investigations of United States personnel in foreign countries; (5) use of digital media in death investigations; (6) linking child pornography to cold cases; (7) gunshot range determination in suspected suicides; (8) multimodal crime scene investigation methods of rape and death scenes; (9) post-blast analysis; and, (10) methods to solve an array of violent crimes.

This presentation will impact the forensic science community through the detailed accounts of multiple violent crime scenes observed around the world, as described by the 21 special agents and a medical examiner who solved the cases. All of the crimes were investigated by the military criminal investigative organizations. The investigations will present multimodal approaches elaborating on crime scene processing, evidence collection, interrogation methodology, post-black reconstruction, medicolegal death determinations, and judicial hurdles and findings.

The forensic science consultants of the Military Criminal Investigative Organizations (MCIO), which consists of the United States Army Criminal Investigation Division, the Air Force Office of Special Investigations, and the Naval Criminal Investigative Service, conduct felony investigations around the world. After an overview of each MCIO's capabilities, investigative presentations by 21 Special Agents and the Armed Forces Medical Examiner will include:

Case 1: A 20 year-old, local Japanese girl went missing. Security cameras led local authorities to interview an Air Force civilian who provided a partial confession and led investigators to the location of victim's body. Forensics agents teamed up with the local Japanese crime scene investigators to search for additional evidence; to conduct a FARO® scan of all crime scenes; and to conduct an analysis of waterways. The subject was charged with first degree murder, rape, and improper disposal of a corpse in the Japanese courts.

Case 2: The wife of a military officer observed her husband's sudden death while chatting with him over Skype during his deployment. Agents will provide an overview of the investigative techniques, the challenges of a deployed environment, and the psychological aspects of next-of-kin briefings.

Case 3: A victim's partially decomposed, nude body was found in a ravine. Investigators recognized the importance of electronic media in determining a timeline of the murder and who may have been responsible. Analysis of surveillance videos, cellular tracking, and credit card purchases solved this homicide.

Case 4: A disgruntled soldier began firing on unit personnel and continued his shooting spree at eight different locations. More than 20 were injured and four killed. The case presentation will discuss crime scene highlights and the cooperation of CID, FBI, Texas Rangers, Texas DPS, ATF, and local PDs.

The Armed Forces Medical Examiner will then explore the background and capabilities of the Armed Forces Medical Examiner System and provide further insight into the forensic pathology of cases.

Case 5: Two brothers reported they were sexually abused 30 years prior by a family friend. In January 2014, agents received a break when the subject attempted suicide and was hospitalized. He provided consent for a neighbor to access an iPad, which revealed nude images of adolescent children. A search warrant was obtained of the residence, which provided evidence of the subject molesting a young adolescent male as well as over five million images and videos of child pornography.

Case 6: A Navy victim was found dead in a closet in her barracks room. The victim had a pillow case over her head and no injuries to her body. The victim's cell phone and laptop were missing from her barrack's room. It was not until a local police officer made an observation that began to unravel a bizarre series of crimes that led to the fate of the deceased.

Case 7: In April 2014, a United States Army soldier suspected his wife of cheating on him. He kidnapped his wife and 3-year-old son and began driving around to look for the wife's suspected lover. The wife escaped and the soldier fled. Arriving back home, the soldier turned on the 3-year-old, stabbing him several times in the torso while the child laid in his crib. The soldier then attempted multiple methods of suicide – all failed.

Case 8: A victim suffered a suspected, self-inflicted gunshot wound to her face. A pistol was on the floor by her feet. The victim's husband told police he had left his wife in the living room while he went to the kitchen and heard a gunshot. The pathologist's findings would lead to a bizarre ending.

Case 9: An Air Force Cadet was found unresponsive in his dorm room with 28 sharp force injuries covering his body after having his wisdom teeth removed earlier. Bloodstain pattern analysis suggested the Cadet was alone at the time of the incident.

Case 10: A task force investigation involving the beating death of a child in Korea. The same child was a prior victim of a non-fatal aggravated assault perpetrated by the father. Prosecutors were hesitant to move forward with the case, but a month after correcting the disjointed investigative and reporting effort, charges were preferred for murder.

Case 11: A post-blast scene examination following the unexpected detonation of approximately 345 explosive rounds which killed four Explosive Ordnance Disposal (EOD) Marines during a clearing operation in California.

Homicide Investigation, Forensics, Pathology

W12 Forensic Digital Multimedia: Enhancement and Authentication

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After attending this presentation, attendees will understand the important components to digital audio and image/video enhancement and to digital audio and image/video authentication.

This presentation will impact the forensic science community by providing a short training opportunity on the concepts of multimedia enhancement and authentication in: (1) digital audio enhancement; (2) digital image and video enhancement; (3) digital audio authentication; and, (4) digital image and video authentication.

Media authentication has become more important than ever before. Often a crucial element discovered during investigations, digital media has become a commonly contested form of evidence. Even the trustworthiness of press photography has become questionable due to manipulation. With the proliferation of digital media manipulation tools, media manipulation is a dangerous reality in the modern digital society.

Digital media authentication is a growing field of research that seeks to determine the validity of digital multimedia by investigating known signatures within a file's data combined with signal analysis of coding and compression effects on audio or image data. This workshop will discuss the media authentication process providing the user with methods of authenticating both image and audio. It will also demonstrate the incorporation of multiple tools and techniques into unified frameworks appropriate in forensic examinations where reducing examiner bias and error is crucial.

The goal of this workshop is to provide an overall view of conducting comprehensive digital multimedia examinations which rely on the results of multiple analyses to enhance recordings or to formulate an ultimate finding or opinion. During the discussion of digital multimedia enhancement, common image and audio filters which can be useful in processing recordings to increase intelligibility or visual details will be discussed and presented within their respective order of operations. This order is based on the effects of sequential processing that have been researched and optimized into an order which provides optimal results.

Multimedia authentication analysis frameworks, focusing on different aspects of image/audio creation to determine both source and authenticity will be presented. Global analysis investigates the validity of multimedia as whole while local analysis determines temporal or pixel level manipulation. Demonstration of how both container and content analysis can be used to determine authenticity of recordings as well as the purported source. Container analysis exploits characteristics of the multimedia file format which can be used to establish media provenance. Content analysis will cover both global and local analysis.

Multimedia Authentication, Multimedia Enhancement, Digital Evidence

W13 Forensics and Social Media: The Intersection of Education, Personal Promotion, and Professionalism

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After attending this presentation, attendees will be able to harness the power of social media for professional promotion while learning to avoid potential pitfalls and classic mistakes. Social media platforms to be addressed include: Instagram™, Twitter™, LinkedIn®, ResearchGate, Snapchat™, Academia.edu, Facebook®, Hootsuite™, and YouTube®.

This presentation will impact the forensic science community by instructing attendees in the appropriate use, advantages, and pitfalls of social media engagement for the purposes of professionalism, education, and outreach. It is hoped that by defining and demystifying some common platforms, attendees will be encouraged to explore this brave new world of social connectivity.

According to the Pew Research Center, nearly three-quarters of Americans use social media in some form and engage with it on a daily basis. Despite the substantial social media user-base and the currently strong interest in the forensic sciences among the general public, the forensic science community has done little to nothing to address the phenomenon or integrate itself into the social media landscape. While many forensic scientists avoid social media for fear of appearing unprofessional or violating confidentiality, social media is a powerful tool for professional promotion, education of the general public, and recruitment of potential new scientists. This goal of this workshop is to introduce a range of social media platforms, their utility, and their potential use within the forensic science community.

The professional use of limited-content platforms will be addressed. Instagram™ is an image posting application in which users are encouraged to "tell a story" through captioned pictures. Twitter™ allows for images and video to be posted, but text is limited to 140 characters forcing the user to be exceptionally precise. Figure 1© is limited to healthcare professionals allowing them to communicate and receive feedback from colleagues using HIPAA-compliant messaging. For each of these services, their benefits and limitations as well as the means by which they can be harnessed to advance the forensic science community will be defined. Additionally, the pitfalls and responsibilities inherent in establishing oneself as an influential expert with a dedicated social media following will be discussed.

Platforms designed for professional promotion and career development will also be covered. LinkedIn® provides the ability to create an online CV that can be examined by other users. Other platforms that will be covered enable the professional to share publications, projects, presentations, and educational handouts. The user community and limitations of platforms such as Academia.edu, ResearchGate, and the Academic Room will be introduced to help participants determine which service best suits their needs.

The final platforms to be discussed are those suitable for promoting one's institution or agency such as YouTube, Twitter, and Periscope. The creation of mutually beneficial relationships between institutions through social media will be covered, as will goal-directed content and analysis metrics on multiple platforms. The workshop will finish with a review of how to generate and distribute content, original quality content vs. reposting, migrating to new platforms, cross-platform posting, boundaries and sensitivities, social media plagiarism, and legal issues surrounding content ownership.

Social Media, Public Outreach, Career Development

W14 Retinal Hemorrhages (RHs) Associated With Pediatric Abusive and Non-Abusive Head Injury — Systematic Reviews and Their Evidence Base: A Review

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After attending this presentation, attendees will be able to: (1) list the limitations of systematic reviews of retinal hemorrhages in childhood head injuries; (2) recognize observational studies employing imprecise case definition, circularity, selection or information bias, and misrepresentation of data; (3) discuss the use of confessions in case definition for abusive head trauma; and, (4) review issues with proposed statistical tools for the diagnosis of abusive head trauma.

This presentation will impact the forensic science community by exploring the value and pitfalls of systematic reviews and the selected observational studies focused on the diagnostic specificity of RHs in Abusive Head Trauma/Shaken Baby Syndrome (AHT/SBS) and of a proposed analytical tool intended to make the diagnosis of AHT/SBS more objective. This presentation will help physicians understand how to identify common problems in the underlying studies and the analyses, including logical fallacies, circularity, selection and information bias, imprecise case definition including confessions, and even misrepresentation of data.

The presumed mechanism of injury in AHT/SBS is thought to be severe rotational acceleration secondary to violent shaking, direct impact, or a combination of the two. Ocular findings are pivotal in cases of AHT/SBS because retinal hemorrhages (RHs) are often considered diagnostically specific for inflicted head injury. However, researchers are now questioning both the criteria used to identify AHT/SBS and the specificity of retinal findings.

Because the evidence base for the SBS theory relies on anecdote and opinion, proponents often point to confessions as proof that shaking an infant causes the brain and ocular findings that are commonly used to diagnose AHT/SBS. This effort fails to recognize that false confessions occur and pretends that confessions are obtained independent of medical information and psychological coercion.

Case-control studies, in contrast to cohort or cross-sectional studies, begin with subjects who have the outcome (cases) and compare them to individuals who do not have the outcome (controls). A crucial issue in evaluating a case-control study is control selection and the resulting comparability of cases and controls. The selection of controls is complex and often the most controversial facet of conducting a case-control study. Observational studies are assessed in terms of internal and external validity. Internal validity refers to the strength of the interpretation from the study. External validity is the ability to infer study results to a more general, broader population. Accordingly, internal validity is essential for external validity. A systematic review is an essential tool for summarizing evidence accurately and reliably. Unfortunately, critical information is often poorly reported in either the review or the primary studies; thus, diminishing the potential usefulness of a systematic review.

The four systematic reviews are by Togioka et al. in the Journal of Emergency Medicine (2009), Bhardwaj et al. in Ophthalmology (2010), Piteau et al. in Pediatrics (2012), and Maguire et al. in Eye (2013).[1-4] The four systematic reviews identified 19 comparative studies published between 1985 and 2010 that the reviewers considered high quality. Of the 19 comparative studies, 11 studies were only selected by only one of the four systematic reviews; 5 studies were selected by two of the four systematic reviews; 1 study was selected by three of the systematic reviews; and, only 2 comparative studies were selected by all four systematic reviews. A comparative study by Vinchon et al. published in Childs Nervous System (2010) was online in November 2009, so the systematic reviews by Togioka et al. and Bhardwaj et al. could not have included it in their systematic reviews.[5]

Attendees will participate with the workshop faculty in evaluating the four systematic reviews by examining the 19 primary comparative studies for case definition criteria and for fallacies of logic including circularity, selection and information bias, and data misrepresentation. The faculty will also discuss the validity of confessions for case definition and statistical problems caused by common flaws in study design affecting the scientific tool.

Observational studies that are well designed and carefully executed can provide useful and reliable results. However, observational studies can be misleading when they involve circular reasoning, systemic bias, and skewed age distribution between case and control groups; or, if data gatherers are not blinded for the cases and controls or the study's hypothesis. Researchers in any given field may be prejudiced because of their belief in a scientific theory and claimed research findings from observational studies with methodological flaws may be measuring only prevailing bias.

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Retinal Hemorrhages, Abusive Head Trauma, Systematic Reviews

W15 Considerations for Crime Scene Analysis When Utilizing Forensic Science Experts for Post-Scene Analysis

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After attending this presentation, attendees will understand how forensic science experts collaborate with crime scene personnel in evaluating physical evidence present and will be able to render expert opinions and testimony for the successful outcome of the investigation. Attendees will be better able to assess which items of physical evidence could generate more probative value from expert analysis. Methods to minimize evidence contamination prior to expert analysis will also be addressed.

This presentation will impact the forensic science community by challenging law enforcement personnel, crime scene investigators (death investigators), and forensic science specialists (entomologists and anthropologists) to recognize and increase their knowledge base on how a collaborative effort with experts can and will increase the successful outcome of a criminal investigation.

Our past tells us that we need to utilize an interdisciplinary approach to solving crimes to launch ourselves into the future of forensic science in post scene analysis. This workshop will present issues that have been present on crime scenes, difficulties in rendering expert testimony due to crime scene related issues, and discuss how to overcome such obstacles in hopes of assisting in such cases in the future.

Crime scene investigators know that in many situations, only one opportunity exists to completely document and collect all pertinent physical evidence relating to the crime being investigated. This workshop will focus on the importance of documenting crime scenes and collecting physical evidence in anticipation of utilizing forensic experts such as, entomologists and anthropologists in the successful prosecution of a crime. Most agencies cannot afford to have anthropologists, entomologists, and other such forensic experts on staff. With this in mind, many times these experts may be called in at a later date to assist in the investigation. Many times after a scene is processed, events that may or may not have occurred in the crime scene may prevent future analysis from forensic science experts to be able to provide valuable information. This could be a result of improper documentation, improper handling of the evidence, lack of experience and training, and/or an unwillingness to share information. We must keep in mind that our own knowledge base may be limited and the use of experts can provide a broader, better-educated outcome of an investigation, remembering that collectively this is a joint effort of multiple individuals from multi-disciplines to accomplish the ultimate goal.

Crime Scene, Forensic Entomology, Forensic Anthropology

W16 The AAFS Humanitarian and Human Rights Resource Center: Year Two

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After attending this presentation, attendees will understand the structure of the AAFS Humanitarian and Human Rights Resource Center (HHRRC or Center) and its impact on multiple global projects as well as understand the current applications of contemporary forensic science and forensic medicine principles and methodology to global issues relating to humanitarian and human rights and the impact of these efforts. Participants also will receive new information on the progress and products of the AAFS Humanitarian and Human Rights Resource Center created in 2015.

This presentation will impact the forensic science community by raising awareness of the structure and accomplishments of the AAFS HHRC and the important developments in the global application of humanitarian and human rights forensic science.

The value of the application of forensic science to humanitarian and human rights cases is growing as research expands and new global areas of need arise. This workshop will provide current information on the development, structure, and progress of the new AAFS Center and its subcommittees which promote the application of contemporary forensic science to humanitarian and human rights cases around the world. Participants will learn the difference between humanitarian applications and those relating to human rights in today's world. Specific methodology and case examples are provided on the forensic investigation and documentation of torture and ill-treatment. Capacity building represents a major application involving training and education. Detail is provided on techniques of capacity building and lessons learned from diverse global experiences. Recent years have witnessed global sharing of resources and assistance in casework, training, and expertise. Details are provided on the dynamics and successes of such global cooperation.

Casework in the humanitarian and human rights arena has defined specific problems in need of new research. With support from the AAFS HHRRC, new research is focusing on the uptake and detection of nerve agents in human bone and using stable isotope forensics as an investigative tool for geolocating unidentified border crossers from the Texas-Mexico border. Center support also has enabled training sessions to strengthen human exhumation and identification efforts in Mexico and to conserve and analyze human remains resulting from Khmer Rouge era mass violence in Krang Ta Chan, Cambodia. Principal investigators in these projects will provide information on how to formulate proposals for support and the complexities of defining, articulating, and reaching goals. Participants will gain an understanding of the challenges faced and the benefits to be attained from involvement in research and case applications in this area. Presenters from diverse research backgrounds will showcase how the new Center and its developments can facilitate progress in the application of forensic science to humanitarian and human rights issues in different contexts around the world.

Humanitarian, Human Rights, Resource Center

W17 Communication Strategies to Mitigate Bias and Strengthen Scientific Foundations in Forensic Science

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After attending this presentation, attendees will recognize the myriad ways in which cognitive and linguistic biases can inadvertently affect judgment and, with heightened awareness, attendees will be able to select from a variety of potential solutions for reducing bias.

This presentation will impact the forensic science community by providing immediate methods for reducing cognitive and confirmation biases in the forensic science workflow from initiation of forensic analysis to trial.

The workshop will present state-of-the-art strategies for mitigating bias and strengthening methodologies in the lab, workplace, and courtroom. Hands-on exercises will follow each talk to encourage interaction with the audience, enable attendees to practice each skill as soon as the discussion is concluded, as well as tailor the exercise to their own job-specific environments. These exercises will also create fodder for the discussion periods, when attendees will be able to ask questions and propose topics for further discussion across the represented fields.

The workshop will begin by describing how all humans create "mental maps," learning things from our families, culture, and subcultures that subtly skew our personal interpretations in productive and counterproductive ways, especially where our reasoning processes are concerned. After discussing the importance of credibility, clarity and closure, and their implications for the legal/investigative process, proven strategies for countering such biases will be addressed.

In linguistics, a well-documented phenomenon is "semantic priming" or the ability of certain words to prime the meaning of words that occur later. Semantic priming is the root cause of much bias in forensic science pattern-recognition techniques. Forensic linguistics provides an object lesson, for many forensic disciplines, in how to overcome cognitive bias in a forensic technique that classifies patterns. Currently, there are two different approaches to forensic linguistics: qualitative and quantitative. As in other forensic disciplines, qualitative work is prone to subjective biases, an overstatement of conclusions, and confirmation bias; while quantitative work has built-in safeguards that can be even further enhanced by employing a metalinguistic filter that controls semantic priming. This filter controls semantic priming by making the implicit assumptions or mental maps explicit so that speakers and hearers communicate with awareness of linguistic triggers of bias. Thus, having both a strong methodology and a strong filter helps to counteract the cognitive bias triggered by semantic priming and its harmful effects on judgment and decision-making at each step in the forensic science process.

Though cognitive biases are hardly news across forensic science disciplines, less attention is paid to the fact that all of these biases are conveyed via the language we use when communicating. Reflecting on and altering the way we communicate is one way of addressing bias. For instance, "Were there any matches for sample A?" and "Did sample A match the boyfriend?" on the face of things are two seemingly similar questions; but the presuppositions of these questions are not the same and have the potential to create confirmation bias. We review presupposition differences and present guidelines for improving communication.

The final presentation focuses on bias in the courtroom. Many methodological, organizational, professional, and even physical solutions have been suggested to combat the introduction of bias-inducing influences and information in the analytical, reporting, and testimonial parts of the forensic process. The tension between an adversarial legal system and the ideal for the forensic scientist to produce scientifically valid results for use in court, without regard to their legal context, must be resolved by the scientist against partisan influence or affiliation. Ways of using insulation for the analysts—protecting their independence from sources of bias and preserving their integrity in their reporting of results—need to be discussed and shared.

Bias is a natural human phenomenon that pervades all disciplines and can affect the expert and layperson alike. It is thus, imperative, that we become aware of its many forms and take action against its potentially harmful effects to protect the integrity of forensic sciences. By focusing on communication strategies to heighten metalinguistic awareness, the forensic science community—law enforcement, investigators, the forensic scientist, attorneys. and judges—can mitigate the harmful effects of cognitive bias.

Cognitive Bias, Linguistic Bias and Priming, Scientific Method

W18 The Opiate Crisis, Dirty Bombs, Big Data/Big Problems, and Driverless Cars: On the Leading Edge of Forensic Science — 2017 Theoretical Forensic Sciences "Think Tank"

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The goal of this presentation is to describe how new developments might impact forensic scientists in their work. Practical examples will be presented on lab automation, driverless cars, designer drugs, nuclear forensics, cybercrime, and big data.

A wide variety of developments that will soon impact forensic science have been identified within the Think Tank Committee of the Forensic Sciences Foundation, Inc. This presentation will impact the forensic science community by providing an overview of some of the new developments in forensic science and by opening a forum for the discussion of issues that arise regarding such developments.

The amount of data that is available from digital investigation and from sensors is rising each year and the question is if a statistical analysis of this data can be presented in court. Biometric algorithms are improving and analyzing large amounts of video and images in combination with location data and other data available provide the possibility to make summaries of the data which can be presented in court. When applying these methods, the users should also be aware of the limitations and the error rates of the algorithms used. In addition, the use of Bayesian conclusion scales are under discussion.

In this day with genetics, you can learn your ethic background, find your relatives, even learn about your predisposition to diseases right from your own home. Over two million people have paid to include their DNA information in a website's database. While many have concerns about law enforcement databases and the rights to privacy of their genetic information, others are willingly mailing their DNA to these private companies. What is the difference in these databases and how are they secured and monitored? There are numerous concerns and questions that should be addressed and discussed to inform the public about putting their genetic information "online."

We see the developments of UAVs and drones and the forensic issues with finding digital traces as one of the topics. The driverless car is also a reality and it is unknown how these cars and their drivers will perform in the real world.

The investigation within a Chemical, Biological, Radiological, and Nuclear (CBRN) crime scene and the interrogation of CBR agents present a variety of problems and are another important issue or development. Primary amongst those at the scene is an intense degree of political scrutiny and a high thermal burden. How do you accurately take high-value samples when you are in a Level A "spacesuit"? How do you know where they are and what should you prioritize in the 20 minutes of air that you will have on the scene? The European Commission Generic Integrated Forensic Toolbox (GIFT) is answering these questions and can share some of the data with you.

Another issue is designer drugs. Designer drugs are novel compounds selected for manufacture and distribution based on their pharmacological effect and questionable legal status. Starting in 2008, enterprising chemists began synthesizing and selling novel drugs whose structures were taken from or based on the pharmaceutical scientific literature and patents. For the most part, the drugs had never been evaluated or tested on humans or were never brought to market because of potentially dangerous or unknown side effects resulting in many intoxications and deaths. Testing for these drugs is often a challenge as analytical standards are not available and the substances are not in shared or commercial databases or libraries. Metabolic studies have not been performed, so frequently toxicologists do not know what metabolites to test for and fail to recognize them even when they are present. Additionally, the designer drug market turns over very quickly with many novel toxic substances appearing and

disappearing from online sales within six to nine months. To keep up, laboratories need be innovative in their approach to monitoring the market through drug user intelligence, peer-reviewed literature, building in house libraries and databases, and many other channels of drug intelligence in anticipation of possible new threats.

Forensic Science, New Developments, Cybercrime

W19 Women in the Sciences: Examining Systemic Barriers and Becoming Agents of Change

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After attending this presentation, attendees will become better acquainted with the state of female representation in the sciences. Attendees will also learn how this representation has shifted over the past several decades, the systemic inequalities in equity and parity between the sexes, and gender-specific micro- and macro-aggressions. Finally, attendees will discuss how these issues manifest within the forensic sciences and how to work collaboratively to conceive strategies for change.

This presentation will impact the forensic science community by demonstrating how gender-specific societal bias in the sciences creates barriers for the advancement of women, contributes to gender inequity in leadership positions, and undermines forensic science's ability to live up to its full potential.

Over the last two years, gender equality has made headlines in the sciences, beginning with the ill-judged "trouble with girls" comment by a British biochemist to more serious cases of endemic sexual misconduct in astronomy and biological anthropology. As more women populate the sciences, specifically forensic science, blatant discrimination is less common, but women face numerous invisible and unacknowledged obstacles. Evaluation criterion for hiring, promotion, invited talks, and awards has been shown to still be biased toward men. In our own Academy, from 2000-2016 eighty-eight percent of AAFS presidents have been men (15/17). The International Academy of Legal Medicine (IALM) has never had a female president (1936-present).

In 2005, the National Academies created the Committee on Maximizing the Potential of Women in Academic Science and Engineering. This committee was designed to provide recommendations on how to maximize female talent in science and engineering. In 2007, the committee reported its findings. Specifically, they found that despite women having the cognitive ability to succeed in science and engineering, women interested in such fields are lost at every educational transition. Moreover, once in these professions, women are more likely to experience discrimination from implicit biases as well as face disadvantageous subjective evaluation criteria and organization structure. In response to this report, the National Institutes of Health formed the Working Group of Women in Biomedical Careers to consider barriers to women in the sciences, to develop recruitment and advancement initiatives, and created $16.5 million in funding opportunities to support research on these topics. Nearly a decade after the initial report, the results of this funding still find significant gender inequalities in compensation, research funding, work-life integration, gender consciousness, and access to mentorship. These topics will be explored in this workshop through podium presentations, panel discussions, and break-out sessions.

An overview of the current trajectory of women in the sciences and conclude by discussing current approaches and best practice to capturing and capitalizing on female talent will be provided. Finally, the audience will be challenged to create action plans to limit systemic barriers and societal biases that deprive the American Academy of Forensic Sciences and their own institutions/organizations of talented and accomplished women across all levels of leadership.

Women, Science, Inequality

W20 Instrumental Chromatographic Separation Techniques for Forensic Analyses: Application to the Analysis of Emerging Drugs

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After attending this presentation, attendees will be better able to evaluate and select the proper chromatographic technique(s) for separating complex mixtures that are necessary for the analysis of emerging drugs present in seized drug-evidence submissions and toxicological samples.

This presentation will impact the forensic science community by providing attendees with a respectable and fundamental understanding of the different modern separation techniques and how they have been applied to the analysis of emerging drugs in forensic exhibits. This workshop has a strong interdisciplinary focus.

The expanded availability of designer drugs on the street has resulted in a confusing array of complex and difficult problems in the determination of drug identity. Many of these drugs are positional and stereoisomers, with concomitant legal issues, and in some cases significant pharmacological and toxicological differences. Isolation and identification of these materials results in numerous technical challenges, many of which cannot be resolved even by the most advanced mass spectrometric technology. Fortunately current advances manifested in separation techniques such as gas chromatography (GC), ultrahigh performance liquid chromatography (UHPLC) and ultrahigh performance supercritical fluid chromatography (UHPSFC) provides a solution to the problem. These techniques have exceptional selectivity and separation power, and in the future they will be increasingly needed in order to distinguish these compounds by retention time as other techniques fail.

The isolation problem becomes even more complex when you consider toxicological assays. Complexity increases with the need to consider drug metabolites and isolation of these materials from complex matrices. Thus we feel that the forensic community could benefit from a series of lectures on the basics of chromatographic separations and their application to problems in the separation of emerging, designer drugs.

The basic principles of chromatography will be presented. In addition, sample preparation techniques, which are integral to successful chromatographic separation will be discussed. These include liquid-liquid extraction, solid phase extraction, and vapor phase extraction techniques. The modern forensic practice of GC, LC, and SFC will be discussed including hardware, choice of columns and methods development. A comparison of GC, UHPLC, and UHPSFC for emerging drugs such as synthetic cathinones and synthetic cannabinoids will also be presented. In the field of forensic toxicology, sample preparation, and separation technology employed for the analysis of emerging drugs and or their metabolites in urine, blood, and hair will be reviewed as well as emerging drug stability *in vitro* and *in vivo*. A discussion on future directions in separation science and emerging drugs will be discussed to include questions from the attendees regarding material related to these chromatographic separations.

Separation Techniques, Chromatography, Emerging Drugs

W21 Forensic Photography and the Exposure Triangle: What Every Forensic Professional Should Know About ISO, Depth of Field (DOF), and Shutter Speed (SS)

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After attending this presentation, attendees will better understand the proper use of a Digital Single-Lens Reflex (DSLR) camera for crime scene, sexual assault, autopsy, and laboratory work. Specific focus will be placed on understanding the "exposure triangle," which includes settings for ISO, DOF, and SS, and the importance of knowing how to adjust each component in order to properly capture forensic evidence for presentation at a later date in a criminal or civil trial. In addition, best practices and legal standards will be presented. Compression levels and when to utilize which level of compression will be taught, so attendees can properly image crime scenes and other forensic evidence in the proper format. Attendees will learn how to utilize a DSLR camera in the *Manual* mode, instead of failing to properly capture forensic images in the *Automatic* mode. Attendees will be exposed to common crime scene photography practices, such as, "fill-flash", "back-lighting," "fill-the-frame," "film plane parallel," omitting blur, maximizing depth of field, and concepts of Painting With Light (PWL) for low-light situations. Attendees will understand how each of these principles should be applied to imaging crime scenes, physical evidence, deceased individuals, and living sexual assault victims and suspects. Forensic science professionals in all disciplines that are charged with taking forensic photographs, or those who are tasked with properly understanding the proper legal formatting of forensic images will benefit from this workshop.

This presentation will impact the forensic science community by providing professionals with a greater understanding of how photography is one of the three primary means of documenting crime scenes, autopsy findings, physical injury, and forensic evidence; yet, it remains the least-understood and least-trained discipline. This training will enhance the attendees' knowledge and competency when using a DSLR camera in the performance of their duties to properly document crime scenes and forensic evidence.

Despite law enforcement and forensic science professionals exposing a plethora of images each year in the performance of their duties; only a small number of these images are introduced into the courtroom. This is despite jurors wanting and even expecting, to see high-quality images of crime scenes, injuries, and other physical evidence. All too often, images exposed in an attempt to capture the various forensic evidence found at crime scenes and other forensic settings are lacking in quality or do not meet the basic legal standards required. In response to these recognized deficiencies, attendees will learn the basic legal requirements for introducing properly formatted images into the courtroom; including, when to utilize RAW uncompressed – lossless – settings. Informal surveys of forensic science and law enforcement professionals have shown repeatedly that most law enforcement and crime scene photographers do not understand the nuances in compression levels (such as when to utilize JPEG vs. RAW) in general crime scene work when capturing critical comparison or evidence quality images.

Forensic Photography, Crime Scene Photography, Sexual Assault Nurse Examiner

W22 Viewing Research Through Different Lenses: How to Achieve Success in Court

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After attending this presentation, attendees will better understand the value of scientific research in their courtroom practice. Participants will understand the value of scientific research and how to identify credible reference material, interpret information, and access such materials through the internet. Practitioners, such as forensic investigators, researchers, and lawyers, view scientific information through different lenses. Determining what is valuable and where to locate these resources is often challenging.

This presentation will impact the forensic science community by raising awareness of practitioners, scientists, and lawyers to high-quality, no-cost, as well as paid, resources available through the internet and online materials. Those in attendance will take such awareness back to their respective employers and potentially increase the use of such materials by associated individuals and their organizations. Improving participants' skills in evaluating and locating information sources could decrease their reliance on materials of questionable value.

Researchers, practitioners, and attorneys rely on published scientific information to interpret forensic evidence. Historically, individuals relied on their training within their given profession to locate such resources; however, given such training can vary depending on their profession (e.g., researcher versus attorney), the end result of these searches can be quite different which can lead to: (1) confusion; (2) reliance on low quality data; (3) conflicting terminology being applied; and, (4) frustration due to lack of training in locating resources of value. This workshop will utilize library and information science to provide participants with valuable tools for assessing published research to determine the most appropriate materials for use in court. Along with library scientists, experts in forensic entomology and microbiology will guide participants and lead discussions of discerning accurate and discriminative scientific and experimental details. Upon completion of this workshop, participants should be able to: (1) identify resources for locating scientific information on the internet; (2) identify scholarly information; and, (3) determine associated scientific merit, application, and limitations.

This "hands-on" workshop will focus on locating and evaluating information resources across the science and law spectrum through the internet. Awareness of practitioners, scientists, and lawyers to high quality, no-cost, as well as paid, resources available through the internet and online materials will be raised. Those in attendance will take such awareness back to their respective employers and potentially increase the use of such materials by associated individuals and their organizations. Improving participants' skills in evaluating and locating information sources could decrease their reliance on materials of questionable value. Entomology and microbiology will be discussed. What is thought to be known about these sciences within forensics (give a presentation making the sciences look like they are phenomenal tools for forensics) will be discussed, which will be followed by presentations demonstrating what really is known and where the challenges are located. Such contrasts are important to be emphasized to researchers, practitioners and attorneys. A review of forensic science-applicable science and legal information resources that are available for participants to use (i.e., American Academy of Forensic Sciences Reference Library, PubMed, Google Scholar) and how to potentially determine high- versus low-quality scientific research publications will be presented.

W23 Fundamentals of Printing and Graphic Arts Examinations

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After attending this presentation, attendees will: (1) learn about the various types of office and commercial printing processes as well as the basic microscopic characteristics used to differentiate printing technologies through discussion of the fundamental concepts associated with examination and comparison of various printing technologies; and, (2) gain experience regarding printing/graphic arts examinations by case studies and hands-on assessment of actual specimens.

This presentation will impact the forensic science community by providing forensic document examiners with the knowledge and introductory experience to conduct printing process examinations. Through participation in this workshop, attendees will: (1) gain a more thorough knowledge about the fundamentals of various printing processes; (2) acquire or improve upon their ability to properly classify and identify the printing technology used to directly produce a questioned document through observation of the physical and microscopic characteristics; (3) be able to provide investigators thorough instructions regarding the collection of proper printing standards; (4) gain competence in conducting printing source determination, while recognizing limitations and crafting proper conclusions; (5) gain useful contacts within the printing and forensic document community; and, (6) be introduced to reference materials that may assist participants with printing/graphic arts examinations.

A common request by investigators in forensic document examination cases is to determine whether a certain machine was used to print or produce a questioned document submitted for analysis. To answer this question, a multifaceted approach is required. One of the first steps in this process is the classification and recognition of various printing processes by their physical and microscopic characteristics. An overview of office technologies (e.g., ink jet and toner processes) and commercial technologies (e.g., lithographic and relief processes) will be discussed. The fundamental concepts associated with the examination and comparison of various print technologies will be the major focus of this workshop. Participants will gain an introduction to the requisite knowledge needed to classify various printing processes. Participants will work on the practical application of the information provided by conducting hands-on examinations in order to identify the printing technology used to produce various provided samples.

Recognition and classification of printing processes is just the first step in determining common source. For useful comparisons, adequate standards must be selected and submitted for examination. Procedures for the production and collection of proper standards will be discussed for various printing processes. Specifics of the process involved in printing source determination examinations will be discussed and reinforced through practical comparison exercises. Limitations regarding appropriate results and conclusions that can be rendered will be reviewed, to include suggested verbiage. While the number of cases involving definitive conclusions regarding source determination are often limited in number, these types of cases can have a significant impact on investigations. One such case study, where the association of trash marks proved pivotal in securing a plea deal in a high-profile case, will be highlighted during the workshop.

Print Process Identification, Printing Characteristics, Graphic Arts

W24 Navigating the Sea of Resources for Sexual Assault Programs

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After attending this presentation, attendees will understand best practices for seeking resources, including federal funding to improve policy, efficiency, and operational sustainability in the processing of Sexual Assault Kits (SAKs) and forensic evidence in their agencies and forensic laboratories. Furthermore, this workshop will bring an understanding of the benefits, comparative differences, and requirements to enable funding consideration and use.

This workshop will impact the forensic science community by highlighting how federal resources in the United States can advance victim-centered support programs, investigations, and forensic services that aid sexual assault programs, especially the processing of untested SAKs and forensic evidence, which bolster the criminal justice process.

In the United States, many jurisdictions are addressing the need to resolve the existence of unanalyzed sexual assault evidence, including sexual assault kits (SAKs). Annually, tens of millions of dollars are provided to criminal justice agencies, forensic laboratories, and researchers to support various sexual assault programs. In addition to economic support, there are many federal resources and programs that provide evidence-based research and technological developments to advance policy and practices; support survivors of sexual assault; establish effective and sustainable practices for collecting and processing forensic evidence; investigate and prosecute sexual assault cases; and, uphold justice by convicting the guilty or exonerating the innocent.

An overview of sexual assault investigations and United States support programs, specifically programs to reduce and process untested sexual assault kits and other forensic evidence more expeditiously and effectively, will be presented. Various federal funding programs will be discussed including research and evaluation (National Institute of Justice, NIJ), DNA capacity enhancement and backlog reduction (CEBR), cold case investigations (NIJ), national sexual assault kit initiative (Bureau of Justice Assistance, BJA), and sexual assault kit partnerships such as the joint venture established by the NIJ and the Federal Bureau of Investigation (FBI). A comparison of program components and requirements, their differences, and potential practitioner use of multiple resources will be included. Examples of a particular program or need within the community will be shared and an explanation of how it corresponds within a funding resource will provide further guidance. Additional resources such as NIJ's Sexual Assault Forensic Evidence Reporting (SAFER) Working Group and the Forensic Technology Center of Excellence will also be highlighted. Laboratory practitioners who have successfully utilized multiple federal programs to help reduce their SAKs, to introduce laboratory efficiencies, to inventory their forensic evidence, and to work with their jurisdictional sexual assault response teams will share their experiences.

Federal agency representatives, laboratory practitioners, researchers, and technology advocates will provide varying perspectives for resources of sexual assault programs, sharing how the programs they represent, or have used, continue to transform the landscape of sexual assault investigations and forensic services using evidenced-based practices to advance the latest technology, to improve program policy, and to reduce the number of untested sexual assault kits and other forensic evidence nationwide.

Sexual Assault, Forensic Evidence, Research and Technology

A1 Quantifying and Visualizing Skeletal Thoracic Trauma

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The goal of this presentation is to introduce attendees to ongoing research in thoracic trauma and fracture pattern trends in vehicular accidents and to describe how advanced imaging techniques can act as tools in trauma research.

This presentation will impact the forensic science community by contributing statistically substantiated results, which will directly affect the interpretation of fracture patterns and ultimately further baseline knowledge of the complex interaction of variables in the thoracic region.

Trauma analysis is largely heuristic; while there have been advancements in recent years, overall statistical substantiation of findings is lacking in comparison to other research areas within forensic anthropology. Thoracic blunt trauma, in particular, is difficult because of the complexity and variation in bone morphology and muscle attachment sites. Additionally, the number and magnitude of extrinsic variables is problematic when making interpretations. The goal of this presentation is to shed light on extrinsic and intrinsic variables that should be taken into consideration when interpreting fracture patterns in the thoracic area. Specifically, this presentation will explore the relationship between rib fractures and known variables in a vehicular crash setting.

Individuals over 18 years of age that were in a motor vehicle accident, hit from the front or side, with or without the use of a seat belt were included in the study. Computed Tomography (CT) images with associated medical records of injuries to the thoracic region of 180 individuals were evaluated. The two continuous variables, age and speed, were also categorized to better observe relationships among variables. Additional variables, such as presence/absence of fractures, side (left/right) of fractures, rib number, specific location on the rib (anterior, posterior, lateral), and number of fractures were scored for each individual. Chi-squared tests and logistic regression were conducted to explore the relationship between presence/absence of fractures to the above-mentioned variables. Density plots were used in order to visualize patterns of fracture location and provide insight to overall trends.

Only one variable, age, showed a statistically significant relationship with the presence of fractures using a chi-squared test. When age category was tested against the presence of fractures, the two variables appeared to be dependent ($\chi 2(2) = 15.902$, $p < 0.05$). The null hypothesis was accepted for all other variables, suggesting no relationship ($p > 0.05$). The age category was also the only variable to demonstrate a significant relationship in the logistic regression. A middle-aged individual is 2.61 times more likely to have fractures reported than an individual in the young-age category. An older-aged individual is 5.55 times more likely to have fractures present than an individual in the middle-age category. There was a slight trend that males, on average, incurred more fractures than women; however, women in the middle-aged category were 2.6 times more likely to fracture than males in the same age category. Counterintuitively, speed was not found to be a significant variable with the presence of fractures. A slight trend appeared in the data associating a slightly higher number of fractures when hit anteriorly. The density plots displayed a lack of differentiation of number of fractures between sides but the greatest density of fractures was located laterally.

Practicing forensic anthropologists should be aware that when observing fracture patterns in the thoracic region, age will play an important role in number of fractures, while all other recorded variables exhibited little impact on the presence and location of fractures. The current project exemplifies the evolution of forensic science because of the inter-disciplinary collaboration, the utilization of advanced imaging techniques, and the incorporation of

statistical analyses, which moves us away from experience-based trauma interpretation and toward scientifically sound interpretations.

Ribs, Vehicular, Blunt Trauma

A2 Improving Non-Metric Sex Classification for Hispanic Individuals

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After attending this presentation, attendees will understand how two commonly used non-metric sex estimation methods for the skull and pelvis perform when applied to Hispanic individuals.

This presentation will impact the forensic science community by providing new recalibrated equations that are population-specific and more appropriate than the original methods used with Hispanic individuals who are encountered in forensic contexts.

Forensic anthropologists are tasked with helping to identify Hispanic Undocumented Border Crossers (UBCs) that die during the journey into the United States; however, current techniques used by forensic anthropologists in the identification of unknown human skeletal remains have largely been created using United States Black and White samples. When applied to Hispanic individuals, these techniques often perform poorly.[1] The increase in migrant deaths at the United States-Mexico border in recent years has created the imperative need to validate methods using Hispanic samples or to create population-specific standards for Hispanic individuals. This research examines the classification accuracies obtained by the original Walker and Klales et al. methods using logistic regression equations for non-metric sex estimation and provides recalibrated population-specific equations.[2,3]

An experienced observer collected ordinal score data of the skull using the five Walker method traits and of the innominate using the three Klales et al. method traits. The sample consisted of 55 (28 females, 27 males) Hispanic individuals housed at the Forensic Anthropology Center at Texas State University. The majority of individuals are UBCs from the Center's on-going Operation Identification (OPID) project. The remaining individuals are from the Texas State University Donated Skeletal Collection. Individuals within the donated collection are positively identified and, therefore, have known demographic information. The demographic information from the OPID UBCs had to be inferred based on a number of variables, including DNA, FORDISC®, and associated artifacts.

Frequency distributions were calculated for each trait score by sex and a chi-square test was used to test for significant differences in score frequencies between the two sexes. The scores for each individual were then entered into the original logistic regression equations provided by the Walker and Klales et al. methods to test the validity of the original equations for this sample of modern Hispanic individuals. Next, the equations were recalibrated to generate population-specific regression formulas for Hispanics, as both original publications give equations developed using only White and Black individuals. Classification accuracies between the validation and recalibration tests were compared to determine if population-specific formulas are more appropriate for Hispanic individuals.

Males and females differed significantly in score frequencies for all traits at the $p > 0.05$ level. Using the fourth Walker method, combined classification accuracy was 81.8% (females 71.4%, males 92.6%). The disparity in classification between males and females regarding the skull indicates a high sex bias (21.2%) in favor of males. Overall accuracy for combined sexes using the Klales et al. method was 87.5% (females 91.7%, males 83.3%). The disparity in classification between males and females indicates a low sex bias in favor of females (-8.4%). Recalibration of the Klales et al. equation improved accuracy (87.5% vs. 91.7%), while recalibration of the Walker method equation decreased accuracy (81.8% vs. 74.5%), but greatly reduced sex bias (21.2% vs. -8.2%).

In conclusion, classification accuracy and sex bias improved for the innominate when using the recalibrated regression equations specifically for Hispanic individuals. These results are not surprising given that similar results were obtained in another study using modern South African, Thai, and United States samples. Interestingly, the skull recalibration decreased overall accuracy in the current study; however, sex bias was reduced by 13%, thereby making the recalibrated equation more appropriate for use with modern Hispanics. Although the recalibrated equation performed worse, the original equation achieved a classification accuracy of only 81.8%, which is much lower than accuracy achieved by Walker in his original study. This study recommends placing greater emphasis on the results of the innominate rather than the skull due to the higher level of sexual dimorphism in this skeletal region for Hispanics.

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Hispanic, Sex Estimation, Non-Metric

A3 Sex-Specific Patterns of Ossification in Macerated Thyroid Cartilages

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After attending this presentation, attendees will be aware that the sequence of ossification of the thyroid cartilage differs between the sexes.

This presentation will impact the forensic science community by introducing sequences of sex-specific patterns of ossification in the thyroid cartilage, providing a novel component for use in sex estimation in individuals.

Previous studies of the ossification process of the thyroid cartilage using radiographs, CT scans, and macerated samples focused on potential correlations between age-at-death and patterns of ossification.[1-8] Recent research reveals there is no correlation between age and ossification of the thyroid cartilage, but there does appear to be a difference in the pattern of ossification between the sexes.[8] This finding suggests implications for the use of this structure in sex estimation. Through examination of a large sample of thyroid cartilages, significant differences in pattern of ossification between the sexes were demonstrated. Presented here are sex-specific sequences of ossification and accuracy rates for this new method of sex estimation.

This study was conducted in two parts using macerated thyroid cartilages. To observe and establish expected sequences of ossification for males and females, 390 ossified thyroid cartilages from individuals of known sex and age were examined. A subsample of 48 ossified thyroid cartilages was then employed in independent, blind tests of sex estimation.

A significant difference in the pattern of ossification was observed between males and females, specifically in the relationship between the ossification of the superior horns (cornua) and the anterior superior margin (laryngeal prominence). Ossification begins along the lateral inferior margins of the thyroid cartilage in both sexes. Ossification then extends to the superior horns and along the anterior inferior margin in females. In males, ossification extends along the anterior inferior and anterior superior margins and the laminae prior to any development of the superior horns. Thus, the superior horns appear to be the last portion of the cartilage to ossify in males, occurring after the complete ossification of the laryngeal prominence at the anterior superior margin, and at times appear not to ossify at all. In contrast, the superior horns appear early in the ossification process in females, prior to the ossification of the laryngeal prominence, which is rarely observed as ossified in females. In the subsample of 48 ossified thyroid cartilages, 17 of 28 males (60%) exhibited some degree of ossification of the superior horns (cornua) and of these, 17 of 17 (100%) also exhibited ossification of the anterior superior margin (laryngeal prominence). Twenty of 20 females (100%) exhibited ossification of the superior horns and of these, 0 of 20 (0%) exhibit ossification of the anterior superior margin.

Using these criteria, a series of independent blind tests were performed to evaluate the degree of inter-analyst correspondence and the accuracy of sex determination. Under macroscopic observation, sex was accurately estimated with a 96% two-way correspondence and 97% accuracy. These results demonstrate that the sex-specific patterns of ossification observed in this research may be employed as supplementary support in estimates of sex in individuals.

Recent immunohistochemical research demonstrates sex-specific differences in cartilage mineralization of the laryngeal structures.[9-11] These studies suggest that apoptosis and HADH-TR positive chondrocytes play a role in the ossification of cartilage in males and females, and that the chondrocytes “stay alive” longer in females than males.[11] This research also proposes that hormonal changes in men and women contribute to the ossification processes.[9] Still, these studies remain tied to the concept of age as the driving factor behind ossification, hindering interpretations of the process and the establishment of any clear sex-specific ossification sequences.

The patterns of ossification observed here appear to affirm the hypothesis that multiple cartilage types are responsible for ossification of the components of the thyroid cartilage, and those elements respond in a sex-specific manner during ossification. Further, this research demonstrates a very high accuracy rate for sex estimation using the ossified thyroid cartilage. Finally, the results of this study have implications for the forensic pathology community as well, as understanding the female pattern of superior horn ossification may alter standard autopsy procedures to better capture potential injury in strangulation cases.

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Thyroid Cartilage, Ossification Patterns, Sex Estimation

A4 A Craniometric Recapitulation of Genetic Estimates of Ancestry for Individuals of Hispanic Identity: Temporal, Geographic, and Identification Trends

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After attending this presentation, attendees will better understand the importance that population dynamics plays in forensic anthropological casework, especially when considering individuals of Hispanic identity and Latin American geographic origin.

This presentation will impact the forensic science community by illustrating how craniometrically derived estimates of admixture recapitulate the results previously reported using forensic genetic markers, offering new evidence in support of a case identification bias.

Using genotypic data for United States-Mexico border-crossing fatalities, Hughes et al. reported greater indigenous ancestry in individuals who are currently unidentified and have been recovered in more recent years — what they called an "identification bias" in the forensic case analysis of individuals of Hispanic identity and Mexican origin.[1,2] These findings are consistent with recent demographic trends among living migrants and forensic casework statistics on undocumented border-crosser deaths. As such, they have significant implications for both casework logistics on the United States-Mexico border and the larger study of Hispanic population dynamics. It is necessary, therefore, to validate these genotypic trends using other sources of biological information relevant to the forensic context. Given the importance of craniofacial morphology to the estimation of ancestry and the historical use of quantitative cranial traits as reasonable proxies for neutral genetic markers, craniometrics were used to recapitulate these prior results.

The hypothesis that the dynamic temporal, geographic, and identification trends in United States-Mexico border casework revealed with genetic ancestry information can be equally accessed using craniometrically derived admixture estimates was tested. Hispanic cases were selected from the Forensic Anthropology Data Bank (FDB) such that craniometrics, birth year, geographic, and Identification (ID) status information were available. Proportions of admixture were sourced from the population structure analyses of Algee-Hewitt.[3]

To test for temporal trends, correlation coefficients, ρ, are calculated between birth year and the estimated admixture proportions. A significant correlation ($p < 0.05$) is found between birth year and the Native American (ρ=0.32) and European estimates (ρ=-0.31, p=0.0053). These analyses were repeated, after partitioning the sample into two groups: cases with documented place of birth and cases with recovery location only, serving as proxies for identified and unidentified cases, respectively. Significant correlations are obtained between birth year and the Native American estimates for the location born subset (ρ=0.31). For the recovery location subset, significant, and stronger, correlations are produced between birth year and both the Native American (ρ=0.50) and European (ρ=-0.49) estimates. When case year replaced birth year, the associations remain consistent and these two measures are positively related (ρ=0.60).

To assess identification bias, the location born and location recovered cases are compared for their level of indigeneity and identification status. Native American ancestry appears higher for the recovery location sample (mean 50%, median 58%) than for the sample with known birthplace (46%, 39%). When grouped by ID status codes, the location born cohort contains many more cases with confirmed identifications (89%) than the recovery location cohort (63%). A cross-classification using identification status and location categories is significant (R^2=0.12, df=2, X^2=19.04, $Prob > X^2$= <0.0001). Inverse correlations between identification status and both case year (ρ=-0.33) and Native American proportions (ρ=-0.12) are produced for the location found subset. Identification status and case year (ρ=0.12) are positively related and Native American proportions (ρ=-0.12) are negatively related for the location born subset. Over time, confidence in identification decreases for the location found and increases for the location born data. Most importantly, for both analyses, case identification success decreases as indigenous ancestry increases. These findings are supported by regression analysis of the Native American proportions for the location found subset. The full model (R^2=0.28, df=3, F = 3.56, $Prob > F$ = <0.0274) and effects tests for birth year

($F = 8.13$, $Prob > F = <0.0082$) and ID status-by-birth year ($F = 5.95$, $Prob > F = <0.0216$) are all significant, and lack of fit is not significant ($F = 0.52$, $Prob > F = <0.8622$) at $\alpha=0.05$.

These results attest to an inverse relationship between the amount of European and Indigenous ancestry and suggest an increased case representation of peoples with more Indigenous ancestry in more recent years. These findings support an identification bias among the Hispanic-labeled cases in the FDB. Individuals with higher proportions of Native American membership have less frequent birth location information on record and are more often assigned low identity status scores. They most often represent individuals not yet positively identified by other means, such as DNA profile, fingerprint, or antemortem record matching.

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Hispanic Ancestry, Identification Bias, Geo-Temporal Trends

A5 Population Identifiability From Forensic Genetic Markers: Ancestry Variation in Latin America

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After attending this presentation, attendees will better understand how the ancestry content of forensic genetic markers can contribute to the study of Latin American variation and how it can assist forensic anthropologists in choosing population-specific methods when inferring other biological profile parameters.

This presentation will impact the forensic science community by demonstrating that forensic Short Tandem Repeats (STRs), such as the Combined DNA Index System (CODIS) markers, are valuable resources for population structure analyses at micro-regional levels, specifically in Latin America. This presentation also quantifies and confirms the agreement between ancestry/admixture patterns produced from a small panel of forensic genetic markers and a large gold-standard dataset.

Ancestry information content in panels of forensic STRs, including the CODIS loci, has been most recently addressed by Algee-Hewitt et al., who demonstrated that forensic STR markers with high individual identifiability carry a non-trivial amount of information on ancestry.[1] These findings support the legacy of population research in anthropological genetics that uses forensic genetic markers in the study of population history and the analysis of contemporary variation. The observed link between individual and population identifiability also has implications for forensic anthropology. These markers have both research and practical importance: the individual identification profiles often represent the only source of genetic information for understudied populations and they provide another source of biological information on admixture and ancestry that is of concern for, broadly, casework logistics and, specifically, individual skeletal case analysis. While small sets of forensic STR loci have been used to reveal latent structure, estimate ancestry, or generate proportions of admixture, the appropriateness of these applications using these markers has not been thoroughly studied. The goal, here, is to determine the compatibility of ancestry/admixture estimates generated from forensic and non-forensic STR markers and to articulate the value of forensic loci for different levels of population identifiability in forensic anthropology.

Genetic data representing Latin America variation are used to evaluate the utility of forensic microsatellite markers for fine-grained population research. This region is of special forensic relevance because of the humanitarian crisis at the United States-Mexico border and the challenges that the identification of undocumented migrant fatalities pose for forensic anthropologists. Moreover, Latin America is composed of highly admixed populations with varied patterns of ancestry. Genotypes were sourced from the Wang et al. dataset, which includes 240 admixed individuals from 13 Latin American populations genotyped for 678 microsatellite markers.[2] Within this "Full" dataset, 9 of the traditional 15 forensic loci were identified, and tetranucleotide STR markers that recapitulate key properties of the CODIS loci. Drawing on this sample of nine CODIS and the identified CODIS-like markers, ten unique "Test" datasets, which contain 15 STRs each, were generated to reflect the traditional size of a forensic panel. Parallel STRUCTURE analyses are run for all datasets. K=2 solution, including Native American and European parental populations, is the preferred model.

As the purpose of this analysis is to determine if forensic STR panels display structure patterns and confer ancestry information in amounts statistically similar to traditionally large, microsatellite datasets not used in forensic identity testing, the Full dataset was designated as the non-forensic, gold standard. A consensus solution is obtained from the STRUCTURE results for each of the ten Test datasets (15 loci). It is compared against the results generated using the Full dataset (678 loci). The similarity statistic computed between these two sets of admixture-cluster results is high (0.86). Cross-classification using the hard (indigenous) cluster assignments from STRUCTURE is X^2 significant (R^2=0.55) and classification error is remarkably low (4.24%). Significant and strong positive correlations exist between the indigenous component estimates from the Test and Full datasets when partitioned by subpopulation ($0.66 \leq p \leq 0.96$, $p < 0.001$).

Results of two-sided paired *t*-tests for mean indigenous cluster assignments between the Test and Full datasets are significant for 3 of the 13 Latin American samples. The average of the mean differences in indigenous cluster

assignments is 6%. Kolmogorov-Smirnov testing identifies a significant distributional difference for only one sample. The cumulative analyses indicate that the CODIS/CODIS-like markers tend to overestimate the amount of indigenous ancestry, when K=2; however, this overestimation is small, systematic, and corrected when the STRUCTURE analysis includes African parental, K=3. Analysis of Variance (ANOVA) reveals significant subpopulation mean differences for the Test (R^2=0.81) and Full (R^2=0.92) datasets. Post-hoc comparisons find fewer among-population, microregional differences for the Test dataset, indicating that forensic markers provide a lower-resolution, more conservative picture of Latin American variation.

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Forensic Genetics, Ancestry, Latin America

A6 Vulture Scavenging of Pig Remains at Varying Grave Depths

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After attending this presentation, attendees will better understand how vultures (*Coragyps atratus* and *Cathartes aura*) respond to and modify graves of different depths containing buried carrion. Attendees will also gain insight into the behavior of these vultures regarding digging and human disturbance at a decompositional facility.

This presentation will impact the forensic science community by providing results from a controlled experiment in an area with very little previous research. This presentation will augment research in forensic taphonomy by adding to the body of knowledge in terms of how vultures respond to carrion that is buried in a shallow grave and how they respond to human disturbance. This presentation will suggest that researchers' daily presence has so modified vulture behavior that it is now altering vulture studies at the Forensic Anthropology Research Facility (FARF) at Texas State University-San Marcos, indicating there may be a "shelf life" for outdoor decompositional facilities when used for vulture scavenging research.

Forensic anthropologists occasionally encounter human remains in burial scenarios such as shallow graves (i.e., depth of ≤1 meter) that show signs of vultures having disturbed the scene.[1-4] The purpose of this study was to examine how vultures detect graves, disturb the area, remove, disarticulate and skeletonize remains, and finally abandon the different graves in comparison to a surface deposition. Accumulated Degree Days (ADD), humidity, and wind speed were used to assess different environmental and climatic factors that may have affected the timing of vulture activity.

From November 2012 to January 2013, four pig carcasses (*Sus scrofa*) were buried at varying shallow grave depths at FARF. A fifth pig served as a control on the surface. Modification of the graves and surface deposit was recorded through the use of five motion-sensing cameras and daily on-site observation. The research intended to examine how vultures (*Coragyps atratus* and *Cathartes aura*) respond to and modify shallow graves of varying depths.

Vultures in this study did not locate or unearth the carcasses in the burials and this may be explained by vulture seasonality and migration, feeding behavior regarding digging and the specific vulture species, and most importantly, the presence of human disturbance. Season may have an effect on the migration patterns of turkey vultures, especially because the study was conducted in the early winter in a location at the edge of the summer turkey vulture range. The lack of turkey vulture involvement may have created an intervening variable in which black vultures would not and/or could not access the carcasses. Although vultures usually return to scavenging opportunities shortly after being displaced by humans (~20 minutes), consistent human presence at FARF may have influenced vulture behavior and is likely another limitation to the study. Vultures may have moved on to more consistent and secluded sources of food (known and reliable locations of carrion with minimal disturbance, human or otherwise).

This presentation highlights the first attempt at understanding vulture response to buried remains in an outdoor decompositional facility. It is suggested that future research should be conducted in a location that is not frequented by humans and/or during a season when turkey vultures are more prevalent. More information is needed regarding vulture seasonality, feeding habits, and the digging abilities of turkey vultures. Finally, it is suggested that future vulture research at FARF should be conducted in a location that is secluded and as close as possible to conditions found in nature.

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Forensic Anthropology, Taphonomy, Vulture Scavenging

A7 3D Image Technology in Forensic Anthropology: Assessing the Validity of Biological Profiles Derived From Computed Tomography (CT) 3D Images of the Skeleton

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After attending this presentation, attendees will understand the benefits and limitations the use of 3D technology poses on the creation of biological profiles in forensic anthropology. Attendees will also be informed as to how these issues can be addressed in current and future research.

This presentation will impact the forensic science community by exploring research results that contribute to the growing literature of virtual anthropology, the use of which could theoretically enable forensic anthropologists to derive skeletal collections from living populations and by creating population-specific standards as well as digitizing entire osteological collections, which would enable research to be conducted without the limitations that travel and accommodation costs impose on researchers.

This project explores the reliability of building a biological profile for an unknown individual based on 3D images of the individual's skeleton. 3D imaging technology has been widely researched for medical and engineering applications, and it is increasingly being used as a tool for anthropological inquiry. While the question of whether a biological profile can be derived from 3D images of a skeleton with the same accuracy as achieved when using dry bones has been explored, bigger sample sizes, a standardized scanning protocol, and more inter-observer error data are needed before 3D methods can become widely and confidently used in forensic anthropology.

3D images of CT scans were obtained from 130 innominate bones from Boston University School of Medicine's skeletal collection. For each bone, both 3D images and original bones were assessed using the Phenice and Suchey-Brooks methods. Statistical analysis was used to determine the agreement between 3D image assessment versus traditional assessment. A pool of six individuals with varying experience in the field of forensic anthropology (at the beginner, intermediate, and expert levels) scored a subsample (n=20) and the data obtained were used to explore inter-observer error. The results of the inter-observer portion of this study exhibited mixed results. While a high agreement was found for age and sex estimation for specimens scored by an expert, the inter-observer study shows that observers found it difficult to apply standard methods to 3D images. Higher levels of experience did not result in higher agreement between observers, contrary to what was expected. These results were also contrary to those obtained for the main portion of the study, in which a good correlation was found between scores obtained for dry bones versus those obtained for their 3D counterparts (n=130). Thus, a need for observer training in 3D visualization before applying anthropological methods to 3D bones is suggested. Future research should explore inter-observer error using a larger sample size in order to test the hypothesis that training in 3D visualization will result in a higher agreement between scores. The need for the development of a standard scanning protocol focusing on the optimization of 3D image resolution is highlighted.

Applications for this research include the possibility of digitizing skeletal collections in order to expand their use and for deriving skeletal collections from living populations and creating population-specific standards. Further research for the development of a standard scanning and processing protocol is needed before 3D methods in forensic anthropology are considered as reliable tools for generating biological profiles.

Virtual Anthropology, 3D Technology, Biological Profile

A8 The Accuracy and Reliability of the Klales et al. Non-Metric Pelvic Sexing Method

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After attending this presentation, attendees will better understand the accuracy and inter-observer and intra-observer error rates for the Klales et al. method of sex estimation utilizing three non-metric traits of the pelvis.[1]

This presentation will impact the forensic science community by contributing an external validation study on the accuracy and reliability of using pelvic non-metric traits to estimate sex for unknown individuals.

When attempting to estimate sex from a set of unknown skeletal remains, the pelvis is considered to be the most reliable element. In 1969, Phenice created a now widely used method that evaluates three nonmetric traits of the pelvis: the ventral arc, subpubic concavity, and medial aspect of the ischiopubic ramus.[1] In 2012, Klales et al. created an ordinal scoring method for the Phenice traits in an attempt to provide robust statistical analysis, posterior probabilities, and error rates, as required by *Daubert*.[2] As non-metric pelvic traits are commonly used in estimating sex in forensic cases, it is imperative that this method be externally validated for both accuracy and reliability.

The Klales et al. method was used to score three nonmetric pelvic traits in United States White and United States Black individuals from the Hamann-Todd Collection (N=279).[2] Two different observers scored each of the innominates to evaluate overall accuracy rates using the ordinal scores and logistic regression equation provided in the 2012 publication. The entire sample was also utilized to examine inter-observer error rates, while intra-observer error was evaluated on a subsample of 50 innominates that were rescored by each observer. Additionally, after rescoring the traits on this subsample, each observer also blindly provided an overall "gestalt" evaluation of sex based on the traits, other morphological characteristics, size, and personal experience.

Observers A and B attained similar accuracy rates of 73.1% and 72.4% with the Klales et al. regression formula, with each observer's results exhibiting a large sex bias. Females were correctly identified most of the time by both observers (95.7% and 97.1%, respectively), while males were correctly identified with accuracy approximately equal to chance (50.7% and 47.9%, respectively). Fifty-three individuals were incorrectly sexed by the Klales regression formula by both observers, including 51 males and only 2 females. In contrast to the regression equation, both observers achieved high accuracy based on their gestalt estimation of the intra-observer error subsample (98.0% and 90.2%, respectively).

Pairwise comparisons completed using Cohen's weighted kappa were used to evaluate observer error. Both the inter-observer and Observer A's intra-observer comparison exhibited moderate agreement for the ventral arc and subpubic concavity ($kappa$=0.539 and 0.549, respectively) and substantial agreement for the medial ischiopubic ramus ($kappa$=0.651 and 0.669, respectively). Observer B's intra-observer comparison revealed moderate agreement for the ventral arc (0.572) and substantial agreement for the subpubic concavity and the medial ischiopubic ramus (0.634 and 0.645, respectively) (all $p < 0.001$). The intra-class coefficient correlation tests found similar patterns, with agreements ranging from 0.693-0. 832 (all $p < 0.001$).

Results demonstrate that each of the three non-metric pelvic traits can be scored consistently both between and within observers, with a low incidence of systematic observer bias; however, the high accuracy rates originally reported by Klales et al. were not reached. These results, combined with the high sex bias in accuracy, reveal problems with the method that will likely impact the method's use in forensic contexts when sex estimation of an unknown individual is considered to be the most important aspect of the biological profile. Additional research should be directed toward utilizing less subjective techniques to better quantify the traits and creating a more representative set of descriptions and visual guides for scoring.

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A9 Organic Staining on Bone

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After attending this presentation, attendees will understand the taphonomic process of organic staining on bone deposited in aqueous environments, with different organic materials introduced for extended intervals.

This presentation will impact the forensic science community by displaying taphonomic data of color staining, which will aid in investigations when questions arise concerning the environment in which remains were deposited and what type/species of plant material have made contact with the bones.

Organic staining results largely from tannins leaching from plant materials, including wood and leaves, and therefore can be seen on bone deposited in wooden coffin environments or on terrestrial surfaces. This study hypothesized that the degree of staining observed on skeletal elements would increase as the length of exposure to the organic matter increased and that different plant materials would leave different degrees or colorations of staining.

The skeletal elements consisted of 150 commercially available pig (*Sus scrofa*) femora that had the epiphyses removed and were completely defleshed. The total sample was divided into three groups with differing conditions and/or types of organic material introduced. Some were buried in a marshy environment within wooden boxes constructed of ten wood types commonly utilized in coffin construction throughout American history: chestnut (*Castanea*), walnut (*Juglans*), cherry (*Prunus*), soft maple (*Acer*), mahogany (*Swietenia*), yellow pine (*Pinus*), poplar (*Populus*), cedar (*Cedrus*), oak (*Quercus*), and spruce (*Picea*). Additional femora were deposited in plastic containers lined with the same wood types as above and filled with tap water. An additional five control bones were deposited in a container with tap water. Five additional bones were placed in a container with commercial tannic acid. The final group of femora was deposited on the ground surface surrounded by four types of dead vegetation: evergreen pine needles, northern red oak leaves (*Quercus rubra*), sugar maple leaves (*Acer saccharum*), and acorns.

The bones were removed once a month from their experimental environments and left overnight to dry to allow for the color to be recorded. The level of staining that manifested on the osseous material was recorded qualitatively using a Munsell Color Chart and a 40-watt daylight light bulb. The color staining was recorded after two months upon initiation of the study and every following month until the study's completion. After the color staining was recorded, the bones were returned to their experimental environments, and this process was repeated throughout the study.

In all of the experimental environments, staining was present after two months of contact with the organic materials and the color darkened or expanded across the bone surface with each data collection. Both groups exposed to the wood types displayed staining across the entire bone surface with a mixture of colors expressed. Over time, a few colors became dominant while minor colors were only expressed along the margins of the bone or as small patches along the shaft. As the buried boxes began to break down, which is commonly observed in coffin burials, soil was able to infiltrate the boxes and contact the bones. This resulted in multiple shades of brown (10YR 4/3; 10YR 3/3) to be present in the staining across bones in multiple wood types. The bones in the plastic containers with wood exhibited a larger variation in color staining, likely due to a higher concentration of tannins restricted to a smaller area around the bones. The staining ranged from red (10R 5/4) for bones with the mahogany to brown (7.5YR 5/3) for bones with the cedar to even dark gray (10YR 4/1) or black (10YR 2/1) on bones with the walnut. The bones in plant matter differed from the other two groups in that the organic staining was sporadic, often with large areas of very pale brown (10YR 7/3) or yellowish brown (10YR 6/4) coloration and with smaller patches of darker brown shades (10YR 4/3; 10YR 5/2).

The results from this study indicate that staining can manifest on bone within a relatively short time frame once skeletonization occurs and a variety of colorations or degrees of staining can manifest based on the plant material. This research will aid in the determination of which types of organic material have contacted osseous remains and the potential environments in which they were deposited.

A10 Postmortem Environment and DNA Quality: Investigating the Effect of Seasonal Taphonomy on Skeletal DNA Quality

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After attending this presentation, attendees will appreciate the impact taphonomy has on both skeletal morphology and DNA, which will assist attendees in making informed decisions regarding victim identification during routine molecular analyses.

This presentation will impact the forensic science community, specifically within the field of victim identification, by providing an insight into the survivability of skeletal DNA within harsh taphonomic environments, thus increasing the likelihood of obtaining positive identifications of descendants.

The identification of unknown decedents occurs through the collaboration of multiple forensic disciplines. When skeletal remains are discovered, identification is often the responsibility of forensic anthropologists. The analyses they perform are primarily used to ascertain estimations of age, sex, stature, ethnicity, and trauma, which collectively are known as a biological profile. The biological profile is used to narrow the pool of potential matches. Issues arise when there is a lack of antemortem information available to cross-reference with the biological profile and when taphonomy alters the physical morphology of bone such that routine anthropological assessment is no longer accurate. In these situations, the use of molecular biological practices, such as skeletal DNA extraction and amplification, is sought to aid in the identification process.

Investigating the effect that seasonal weather fluctuations, particularly wet/dry cycles as well as freeze/thaw cycles, have on the structural integrity of bone and therefore the effect this may have on the sequencability of skeletal DNA forms the basis of the current investigation. The goals of the present study were threefold: (1) to ascertain the effect that wet/dry and freeze/thaw cycles have on the sequencability of skeletal DNA; (2) to determine the effect skeletal fragmentation has on the ability to extract sequenceable DNA; and, (3) to determine whether nucleic or mitochondrial DNA is a more appropriate target for skeletal DNA amplification.

A total of 16 porcine long bones (femurs) were used as substitutes for human skeletal remains. Half of the bones were left intact and half were medially fragmented. Two taphonomic environments were simulated during the investigation: (1) wet/dry cycling, to mimic seasonal rainfall, was achieved by submerging long bones in one liter of water for two weeks, then allowing them to dry within a fume hood for a further two weeks; and (2) freeze/thaw cycling, to mimic seasonal temperature variations, was achieved by housing the bones at -20°C for two weeks followed by a two-week thaw period (20°C) under a fume hood. Two-week cycles were continued for a total of five months with skeletal DNA extraction occurring monthly. Amplification was achieved via Polymerase Chain Reaction (PCR) and Sanger sequencing using two mitochondrial DNA primers and one nucleic primer that targeted pig growth hormone. DNA sequencability was judged based on quantity (nanodrop values) and quality as measure by trace (Phred) scores following sequencing.

The results of the investigation saw dramatic morphological changes occur within both environments; bones subjected to wet/dry cycling lost structural integrity with periosteal flaking and cracking as well as disarticulation of the epiphysis. Despite the physical alterations, the results indicate that sequenceable DNA could be extracted from both groups with varying levels of success. The lowest quantity and quality of DNA as measured by low nanodrop values and trace scores occurred for bones within the wet/dry group after three months of cycling; however, readable sequences were still available for these bones indicating that over a short duration, despite relatively harsh taphonomy, skeletal DNA survivability is possible.

The most significant factor for the study was shown to be the choice of primer, with significant preferential preservation of mitochondrial DNA over nucleic DNA shown for all treatment groups.

The results of this investigation indicate that sequenceable mitochondrial DNA can be extracted from skeletal remains exposed to harsh environmental conditions after relatively short periods of time. The outcome of the present study impacts the field of victim identification for scenarios in which skeletal DNA extraction would otherwise have

been considered redundant.

Skeletal DNA, Seasonal Taphonomy, Forensic Anthropology

A11 A Transition Analysis of Pediatric Fracture Repair Stages

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The goal of this presentation is to discuss the utility of transition analysis in the estimation of the age of pediatric healing fractures using radiographic images.

This presentation will impact the forensic science community by providing the ages of transition between stages of fracture repair from a large known pediatric sample, as measured by the date of injury to the date of radiographic imaging. This may have significant ramifications for investigations of child abuse and the interpretation of cases involving healing skeletal fractures.

Multiple fractures in various stages of healing are considered highly indicative evidence of physical child abuse.[1] The estimation of time since fracture, while complicated by factors such as age and anatomical location, has the potential to assist in the identification of physical abuse. In both forensic and clinical settings, a radiographic skeletal survey is generally performed to assess for occult and healing fractures, and this imaging is often the basis upon which an assessment of fracture healing is performed. Yet, despite a handful of studies examining pediatric fracture healing using radiographic images, the timing of fracture healing has yet to be fully understood.[2]

The goal of this project is to present the ages of transition between fracture repair stages for the interpretation and analysis of pediatric healing fractures. This study used data collected from a series of radiographs of lower limb and forearm bone fractures from 116 individuals within the age group most at risk for child physical abuse, individuals aged 0 to 5 years. Some individuals exhibited multiple fractures for a total of 185 forearm fractures and 107 lower leg fractures examined. These data were collected and previously examined by Malone.[3] This project reinvestigates this data with a different statistical approach: using transition analysis to determine mean ages and ranges of transition between fracture repair stages.

The original analyst (Malone) scored all radiographs within the following six-phase system: Stage 1 – No healing; Stage 2 – Granulation; Stage 3 – Callus; Stage 4 – Bridging; Stage 5 – Clinical Union; and, Stage 6 – Completion.[3] Using Analysis of Variance (ANVOA) tests, Malone found statistically significant differences in rates of healing between lower leg and forearm bones, with forearm bones healing faster, as well as differences in age, with individuals aged one year or less spending less time in Stage 1 than individuals aged two years or older.[3]

The present reanalysis uses these same data; however, the goal was to generate ages of transition between different fracture healing stages. To extend transition analysis for ordinal traits, a restricted cumulative probit regression was used. All analyses used the VGAM package in R.

Results found that for all transitions except one (Stage 4 to Stage 5), the mean age of forearm fractures was ahead of the mean age of the corresponding lower leg fractures. Further, when examining individuals aged one year or less versus individuals aged two years or more, there was no trend in mean-age transition timing, with neither group consistently ahead of or behind the other.

There was a great deal of overlap in transitions, especially between the first three stages. This overlap indicates that the present six-stage system does not provide a great deal of discriminating power between the current morphological stage descriptions. Revising stage descriptions or generating new evaluative criteria for pediatric fracture healing has the potential to increase accuracy of fracture age estimates. For example, distinguishing between early features of fracture healing, such as subperiosteal new bone formation versus callus formation, has been suggested to improve interpretation of time since injury.[1] In addition, the effect of variables that influence rate of healing, such as age and anatomical location, should be further explored and considered in future work. Overall, transition analysis provides a more in-depth examination of pediatric fracture healing, allowing for broader interpretation when analyzing pediatric fractures.

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Fracture, Healing, Child Abuse

A12 Musculoskeletal Stress Markers as Evidence of Manual Labor: The Allumiere Cemetery Case

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After attending this presentation, attendees will better understand the importance of musculoskeletal stress markers on human bones.

This presentation will impact the forensic science community by providing an additional tool to enhance the biological profile and osteobiography of unknown human skeletal remains.

Entheses are muscle and tendon attachment sites on bones that can vary morphologically in relation to muscle activity. The analysis of these marks on skeletal remains can help to identify physical activity carried out by a subject during life. An enthesis is an interface between hard and soft tissues where high biomechanical stress is concentrated. The result of this stress could be represented by acute or overuse injuries that may develop into an enthesopathy.[1] Marks on skeletal tissue could be also influenced by different factors, such as intense muscle activity, postural habits, and medical or nutritional conditions.[2]

For this study, an Italian historical skeletal collection housed at the Department of Biology of the University of Rome Tor Vergata was examined. The skeletal remains belong to a cemetery in Allumiere (Rome, Italy), an area historically related to alum exploitation. A preliminary anthropological analysis was performed in order to assess sex, age at death, stature, and general osteobiographic traits. The sex ratio value (45M:7F) supports the theory that the cemetery area was mainly used by miners.

The analysis of entheses was performed according to the protocol proposed by Mariotti and colleagues; the 23 entheses proposed in that protocol have been integrated with those proposed by Borgognini Tarli and Reale.[3-5]

The analysis focuses on three parameters: robusticity, osteophytic formations and osteolytic enthesopathies. Robusticity represents the normal bone marks at a muscle, tendon or ligament attachment site, and it is nearly always recognizable, with the exception of extensive osteolytic processes affecting the entire osseous area.[3] The analysis of the degree of development is conducted following a scaling system: degree 1 (weak-moderate enthesal expression), degree 2 (strong development), and degree 3 (very strong development). Nevertheless, subcategories (1a, 1b, 1c) were provided for degree 1, corresponding to "very slight," "low," and "medium" development. Even for the other two parameters, a scaling system was used from 0 (absence) to 3 (marked) osteophytic or osteolytic process.

High-level biomechanical stress was detected analyzing single bone entheses. The results were then clustered into functional groups (shoulder, elbow, forearm, hand, hip, knee, foot) and all entheses of the entire upper and lower limb were considered in order to reconstruct the biomechanics and body movements.

The mechanical stress experienced by a surface is proportional to the force experienced in each unit area of that surface.[6] It is undeniable that the relationship between muscle activity and enthesal morphology is neither as simple nor as obvious, but morphological variation in the attachment site does exist and likely reflects some aspects of *in vivo* stimuli. Skeletal entheses are thoroughly connected with the attaching muscles so, through them, it is possible to investigate the *in vivo* activity of those muscles.[7]

The availability of data regarding the different phases of alum production and the related labor categories opened the possibility of associating every individual to a specific work task correlated to alum extraction and exploitation making the presented case a "collector's item."[8] It was possible to assign different task groups. For instance, a young adult male showed a marked development of pectoralis major in addition to osteolytic lesions corresponding to costoclavicular ligament insertion on clavicle; this biomechanical stress that involved both the upper limb and the shoulder girdle could be related to the extraction in the cave through iron picks.

The goal of this pilot study is to demonstrate the relationship between musculoskeletal stress markers on human bones and activity patterns through a multidisciplinary approach that involves different points of view, such as

anthropological, biomechanical and historical.

In addition, this research demonstrates the potential application in forensic contexts in which anthropologists have to investigate compromised unknown human remains (decomposed, mummified, mutilated, burned, and dismembered). The possibility of identifying musculoskeletal and occupation stress markers and moreover to reconstruct *in vivo* biomechanical stimuli related to daily tasks could become an important improvement to biological profiling opening the possibility of reconstructing muscle body movements and hypothesizing activity patterns, in order to increase the chance of sorting candidates for identification.

Presented here is a pilot study that, even if limited by the small sample size, demonstrates the potential of an empirical experiment and provides an important additional tool to forensic investigations.

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Entheses, Osteobiography, Activity Patterns

A13 A Statistical Method for Reassociating Human Tali and Calcanei From a Commingled Context

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After attending this presentation, attendees will understand the utility of using metric methods for sorting commingled human remains.

This presentation will impact the forensic science community by presenting a statistically valid means of sorting commingled remains when traditional non-metric methods need to be supplemented.

Compact human bones are highly resistant to taphonomic change and are usually found intact in the field.[1] The two largest tarsal bones, talus and calcaneus, are frequently recovered intact and in high abundance. In a commingled context, assessing that a talus and a calcaneus correspond to the same individual could become the primary step for accurately sorting human remains. In this framework, the present study seeks to develop a more accurate method for sorting an individual's talus and calcaneus by using measurements of these bones' joint surfaces.

For this purpose, the lengths and widths of the trochlea, posterior calcaneal articular surface, and posterior talar articular surface were measured in 197 individuals (105 males, 92 females) of the Athens Collection.[2,3] This skeletal collection consists of individuals of known sex, age, occupation, and cause of death. All specimens examined lived in the second half of the 20th century in Athens, Greece. Their biological age ranged between 22 and 99 years. The degree of significant correlation among measurements was calculated using the Pearson's correlation coefficient. Simple and multiple regression analyses were performed for the development of functions for reassociating an individual's talus with its corresponding calcaneus.

Simple and multiple linear regression analyses produced a total of 12 equations (six for each side) as the best statistical models for predicting measurements of the calcaneus using measurements of the talus. The standard error of the estimate ranged between 1.03mm and 2.02mm. Pearson's correlation analyses demonstrated statistically significant strong positive correlations among measurements (0.69-0.93, $p<0.05$). The coefficient of determination (r^2) scored overall higher in multiple regression analyses (0.72-0.87) compared to simple regression analyses (0.48-0.86).

In conclusion, the regression equations developed in this study are found to be suitable for sorting commingled human tali and calcanei. Future development of similar methods for other joints of the human skeleton would be beneficial for the anthropological analysis of commingled remains.

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Commingled Remains, Talus, Calcaneus

A14 Long-Term Observer Error, Observer Experience, and the Value of Trait Standardization in Macromorphoscopic Trait Analysis

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After attending this presentation, attendees will understand the implications of observer experience and trait standardization on intra-observer error in the analysis of macromorphoscopic trait scores commonly used in the forensic estimation of ancestry. Attendees will also gain insight into the impact of this type of error in the estimation of ancestry.

This presentation will impact the forensic science community by addressing the role of experience, standardization, and validation in long-term observer error studies and the impact of these factors on the estimation of ancestry using macromorphoscopic trait scores.

As one part of a much larger investigation into the macromorphoscopic traits used in the estimation of ancestry from skeletal remains, a 14-year (2002 to 2016) intra-observer error study was conducted. Motivated by the development of a large macromorphoscopic database, which could potentially utilize data collected in 2002, quantification of the impact on observations caused by observer error, technological improvements in macromorphoscopic trait collection, and observer experience was necessary. To maximize comparisons between the two samples, only ten macromorphoscopic traits were assessed: (1) anterior nasal spine; (2) inferior nasal aperture; (3) interorbital breadth; (4) malar tubercle; (5) nasal aperture width; (6) nasal bone contour; (7) nasal overgrowth; (8) postbregmatic depression; (9) posterior zygomatic tubercle; and, (10) zygomaticomaxillary suture.

Paired data were collected from 185 American Black (n=127) and White (n=58) individuals from the Robert J. Terry Collection. The 2002 sample was collected on paper forms using then-standard texts, references, and line drawings. The 2016 sample was collected using Macromorphoscopic Traits v.1.61 (MMS), a data collection program designed specifically for macromorphoscopic trait analysis. Following data collection, the 2002 and 2016 samples were combined into a single data table for analysis. A traditional (unweighted) Cohen's Kappa is used to quantify disagreement between two assessments, but does not control for the degree of disagreement. Therefore, when the ratings are ordered ($1 < 2 < 3$), as in macromorphoscopic trait expressions, a quadratic weighted Cohen's Kappa is better suited to assess intra-observer error.

There was good agreement between the two observation periods for seven of the ten traits. The frequency of agreement ranged from 74.05% (postbregmatic depression) to 28.11% (zygomaticomaxillary suture). The three underperforming traits are: (1) malar tubercle (confidence interval $\kappa_w = 0.029 – 0.046$; levels = 4); (2) zygomaticomaxillary suture (CI $\kappa_w = 0.031 – 0.093$; levels = 3); and, (3) posterior zygomatic tubercle (CI $\kappa_w = 0.029 – 0.046$; levels = 4). Discrepancy between observations can be attributed to the following explanations. First, these three traits are not commonly used in forensic ancestry estimations and are therefore less familiar. Second, difficulty distinguishing between the various character state manifestations due to poorly worded definitions or inadequate capture of trait variants may occur. Finally, the experience level of the observer is potentially the most interesting factor influencing low observer agreement. Intermediate values for all ten traits demonstrated a lower proportion of agreement between observation periods; the 2016 sample demonstrated a higher proportion of intermediate scores, possibly indicating extreme trait values are less likely as the experience of the observer increases. As a final point, classification accuracies from a canonical analysis of the principal coordinates using all ten traits and a reduced model using the seven traits with good observer agreement were calculated and assessed for statistically significant differences between the 2002 and 2016 samples. Despite the noted error, all classification accuracies were promising and similarly distributed. The 2002 sample correctly classified 92.2% (seven variables) and 93.5% (ten variables) of the total sample. Similarly, the 2016 sample correctly classified 91.1% (seven variables) and 88.2% (ten variables)/

The results of this research suggest a moderate level of observer error should be expected as an analyst becomes more familiar with a methodology. As new technologies supplant older approaches, there is potential to reduce observer differences. Although observer error was significant for three of the ten traits documented in this study,

their influence on classification accuracies was not demonstrably significant.

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Ancestry, Macromorphoscopic Trait Analys, Inter-Observer Error

A15 The Impact of Cognitive Bias in Skull Sexing

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WITHDRAWN

A16 Collagen Structural Changes and Decomposition in Burnt Bone and Their Significance for Forensic Anthropology — New Insights Via Amino Acid Racemization

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After attending this presentation, attendees will better understand the changes bone collagen undergoes when subjected to heating and the implications these changes have on subsequent forensic analyses.

This presentation will impact the forensic science community by shedding new light on the thermal stability of bone collagen, allowing for an improved prediction of the success rate of collagen extraction for subsequent analyses, such as dating or isotope analyses.

All amino acids found in proteins exist as one of two possible stereoisomers, known as L-amino acids and D-amino acids. With the exception of glycine, all amino acids are L-amino acids, which have the ability to change into the D-form over time. The process of reaching an equilibrium between the L and the D form is called racemization. Amino Acid Racemization (AAR) is a time- and temperature-dependent process that has found application in the dating of materials, paleothermometry, and age-at-death estimation. Currently, there is no clear consensus in the literature regarding the thermal stability of collagen, an issue on which this research sheds more light.

Sheep (*Ovis aries*) ribs were cut into 4cm-long pieces and burnt at temperatures between 100°C and 1,000°C in 50°C increments for 45 minutes. All samples were weighed pre- and post-burning. Four demineralization samples were suspended in 0.5 M HCl, which was exchanged every two days. After ten days, the HCl was removed and replaced by distilled water until a solution of pH 3 was obtained. Samples were heated at 70°C for 48 hours and subsequently filtered. The extracted collagen was frozen at -20°C and 250µL of solution from each sample were placed in a sterile glass vial, adding 100µL 7 M HCl per sample. The vials were flushed with nitrogen, heated at 110°C for 18 hours, and subsequently dried under vacuum in a centrifugal evaporator. Samples were rehydrated for analysis. The sample's amino acid composition was analyzed by reverse-phase High-Performance Liquid Chromatography (HPLC) using fluorescent detection. Then 2µL of sample was injected and mixed with 2.2µL derivitizing reagent. The amino acids were separated on a C18 HyperSil™ BDS column (5mm * 250mm) at 25°C using a gradient elution of three solvents: sodium acetate buffer, methanol, and acetonitrile. The fluorescence detector uses a xenon-arc flash lamp at a frequency of 55Hz with a 280nm cut-off filter, an excitation wavelength of 230nm, and emission wavelength of 445nm.

The D and L isomers of 13 amino acids could be analyzed, namely serine, L-threonine, L-histidine, glycine, L-arginine, alanine, tyrosine, valine, phenylalanine, leucine, and isoleucine, as well as aspartic acid and glutamic acid. The amino acid concentration rapidly decreases from 250°C onward, being below reliable detection levels from 400°C. Up to temperatures of 250°C, only aspartic acid racemizes, reaching a D/L ratio of 0.3 at 250°C. From 300°C onward, the other amino acids commence racemization. From 400°C onward, the total amino acid concentration is too low to accurately depict D/L ratios. The composition of amino acids was dominated by collagen up to 400°C.

The findings illustrate the thermal degradation of bone collagen. Up to 250°C, virtually no amino acid racemization takes place, with the exception of aspartic acid, which is one of the only amino acids which can racemize while still internally bound. Collagen, when heated, begins to locally unravel its triple helix, releasing the collagen-stabilizing H-bonded water, which leads to a gradual collapse of the triple helical structure at approximately 150°C. At temperatures between 250°C -300°C, a sudden drop in total amino acid concentration as well as the commencing of racemization of all other amino acids can be observed, indicating a catastrophic breakdown of collagen. The now-free amino acids continue racemization until their complete combustion from approximately 400°C onward.

Successful collagen extraction is the basis for a multitude of forensically relevant analyses, such as stable isotope analysis, radio carbon dating, or genetic profiling. Being able to accurately determine the point at which collagen denaturizes and amino acids are lost is therefore of paramount importance for forensic analyses.

Burnt Bone, Collagen, Amino Acid Racemization

A17 Trace Element Analysis of Dental Enamel for the Geographic Attribution of Unidentified Remains

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After attending this presentation, attendees will understand the potential of utilizing trace elements in both surface and subsurface enamel to add pertinent information to biological profiles of unidentified individuals.

This presentation will impact the forensic science community by adding to existing geographic attribution methodologies to assist in the identification of unidentified remains. Ultimately, aspects of this methodology could be implemented in a forensic setting to benefit the Undocumented Border Crosser (UBC) crisis at the United States-Mexico border.

The remains of hundreds of UBCs must be examined every year and identification efforts are frequently hindered by the rapid early decomposition due to the extreme desert environment. Therefore, calcified tissues that preserve well (i.e., dental enamel) may hold the key to improving identification methods. The static, sub-surface enamel of permanent dentition remains unchanged during life after its formation *in utero* and for a brief post-natal period and thus can reflect an individual's birthplace, whereas the dynamic nature of the exchange of ions at the enamel surface can reflect the recent geographic residence of the individual.[1-3] Previous studies have shown that trace elements in dental enamel and bone, namely strontium isotopes, can be influenced by diet and geographic locality in humans.[4,5] Two drawbacks of these studies are addressed by this project: (1) lack of evaluation of other trace elements which are inevitably present; and, (2) utilization of bulk dental enamel alone.[1] By looking at both surface and subsurface (bulk) enamel separately, rather than solely bulk enamel, greater discrimination may be obtained (i.e., birthplace and recent residence geographic locality), which will aid in identification efforts.

This study examined ca. 60 teeth from 33 individuals obtained from the Virginia Commonwealth University (VCU) Dental Clinic with Institutional Review Board (IRB) consent. These individuals currently reside in Virginia, but originated from a variety of countries, including El Salvador, Mexico, Guatemala, China, and Somalia. Surface enamel was etched directly using a solution of trace metal-free nitric acid and glycerin, and bulk enamel was dissolved in trace metal-free nitric acid. Samples were analyzed for the following trace elements via Inductively Coupled Plasma/Mass Spectrometry (ICP/MS): ^{7}Li; ^{24,25,26}Mg; ^{27}Al; ^{49}Ti; ^{58,60}Ni; ^{63,65}Cu; ^{64,66,68}Zn; ^{69,71}Ga; ^{75}As; ^{86,87,88}Sr; ^{137,138}Ba; and ^{204,206,207,208}Pb. Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) were performed to assess multivariate relationships among samples and determine which trace elements drive compositional differences among the samples and the locality groups.

Based on results from the surface enamel, a correlation-matrix PCA plot was generated using ^{55}Mn, ^{63,65}Cu, ^{137,138}Ba, and ^{206,207,208}Pb isotopes as these isotopes yielded the best clustering of individuals originating from the same locality. Standardized function coefficients demonstrated that all isotopes used in the statistical analysis (i.e., ^{55}Mn, ^{63,65}Cu, ^{137,138}Ba, and ^{206,207,208}Pb) had a relatively equal influence on the separation of the individuals' teeth chemical compositions (the range of function coefficients was from 0.339 to 0.363). Variables, such as enamel erosion and age, are likely to influence the persistence of certain trace metals and require further investigation to determine their effects.

In conclusion, Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) analysis of surface and deeper enamel is a promising method for determining the birthplace and recent residence of individuals, providing essential information for identification purposes.

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Dental Enamel, Trace Elements, Geographic Attribution

A18 Traditional Morphometrics of the Juvenile Skull: An Analysis of Ontogenetic Growth Patterns

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After attending this presentation, attendees will understand a novel method of juvenile age estimation involving linear measurements of the cranium.

This presentation will impact the forensic science community by providing a novel method of juvenile age estimation.

This study assesses patterns of ontogenetic growth and, by association, morphological variation in juvenile crania from a Western Australian population. Current methods of juvenile age estimation largely relate to quantifying dental development and skeletal growth; the latter occurs in a predictable and known pattern and is not as readily affected as other skeletal elements by nutritional and other environmental disruptions during growth and development. The current study develops age prediction models using linear measurements of the juvenile cranium and explores novel methods of age estimation based on ontogenetic growth. The specific goals are: (1) determine the most accurate single predictor polynomial model for age; (2) determine a model of age estimation that is derived from all linear terms of predictors; (3) determine a model that includes linear, quadratic, and cubic terms of all predictors; and, (4) create age prediction models based on linear measurements.

The study sample comprised 174 cranial Multi-Slice Computed Tomography (MSCT) scans of individuals ranging in age from birth to 18.76 years (98 males and 76 females). The MSCT scans were acquired from the Western Australia Department of Health Picture Archiving and Communication Systems (PACS) database. The MSCT scans are visualized using OsiriX® and 52 homologous landmarks are acquired in each cranial scan. MorphDB and morphologika are then used to calculate 27 linear measurements between landmarks.

Prior to all data collection and analyses, the level of accuracy and precision was quantified to ensure data are repeatable and reliable. Measurement error was quantified using Technical Error of Measurement (TEM), relative Technical Error of Measurement (rTEM), and coefficient of Reliability (R). The linear measurements are analyzed with Statistical Package for Social Science (SPSS) and Excel®. Asymmetry was evaluated through correlations between bilateral measurements; a paired sample *t*-test was performed to explore the statistical significance of any bilateral variation. The root mean squared error of prediction (the calculated difference between the actual and predicted age) was used to determine the most accurate age prediction model for each measurement. Multivariate models were also considered to determine whether using more predictor variables had beneficial effects on being able to predict the response.

Age prediction models were developed based on interlandmark distances. Individual and pooled sex models were produced, with prediction accuracy ranging from ±1.3 to ±2.7 years. The single most accurate predictor of age in the pooled sex sample was palatine height (±2.3 years). Age prediction models were formulated using single and multiple linear and polynomial regression analyses; accuracy rates were deemed acceptable relative to extant methods of juvenile age estimation. This study has developed novel methods of age estimation in juvenile crania and also provides a strong foundation for further research.

Juvenile Age Estimation, Craniometric Analysis, Virtual Anthropology

A19 A Preliminary Investigation Into the Effects of Previous Freezing on Human Decomposition

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After attending this presentation, attendees will understand the effects of prior freezing on human decomposition and whether frozen human corpses display the suite of decompositional traits previously identified in frozen pig proxies.[1]

This presentation will impact the forensic science community by providing preliminary answers to the questions of how previous freezing impacts the rate and pattern of human decomposition and whether there are visible external features that may identify previously frozen human corpses during decomposition, as were identified in pig proxies.

Freezing is an important forensic taphonomic variable as subjects may be stored in the frozen condition prior to use in taphonomic studies. Additionally, perpetrators may freeze victims in attempts to delude investigators by complicating Postmortem Interval (PMI) estimation. Freezing may impact decomposition by decreasing the viability of enteric bacteria responsible for driving putrefaction and research has shown that previous freezing significantly decreases the rate of decomposition in pigs.[1-3] In line with recent calls to validate results of forensic taphonomic studies using animal models with follow-up research utilizing human subjects, this study investigated human frozen decomposition.[4,5] It was hypothesized that the decrease in viable bacteria after freezing would result in a slower rate of decomposition in frozen human subjects, and human and pig subjects would show similar externally visible effects of previous freezing.

All subjects in this study were deposited at the Complex for Forensic Anthropology Research (CFAR) at Southern Illinois University. To understand how previous freezing alters the progression of "normal" human decomposition, two comparisons were made. A previously frozen human subject deposited on September 18, 2015, the fall season in southern Illinois, was compared to a never-frozen human control deposited on the same date and to never-frozen human subjects deposited in the fall of 2012-2015 (n=5). Additionally, the pattern of human frozen decomposition was compared to previously reported results from a similar study using pig proxies to identify whether the markers of decomposition observed in frozen pigs are identifiable in frozen humans as well.

One adult human subject was frozen at -18°C for 172 days. Fresh subjects were deposited immediately upon arrival at CFAR. Human donors arrived at CFAR within eight days of death and were kept in morgue refrigeration prior to deposition. All subjects were placed nude, directly on ground surface, within chain-link enclosures to minimize scavenging. Total Body Score (TBS), abdominal circumference, digital images, and written observations concerning insect activity, scavenging, and weather were collected daily.[6] To quantify decomposition rate, Kelvin Accumulated Degree Days (KADD) were used to measure the thermal energy required for each subject to reach several TBS thresholds: early decomposition (TBS≥6.0); halfway through early decomposition (TBS≥12.5); advanced decomposition (TBS≥19.0); halfway through advanced decomposition (TBS≥23.0); and skeletonization (TBS≥27.0). Bloat was quantified by analyzing the percent difference in abdominal circumference for each subject.

The previously frozen human subject decomposed faster than its paired never-frozen human control during both early and advanced decomposition. Percent difference in abdominal circumference was 105% for the frozen subject compared to 123% for the control. Bloat in the control subject was more rapid, substantial, and sustained. In addition to minimal bloat, the previously frozen subject displayed several differences in decomposition pattern, including grayer, pasty overall color, an absence of green discoloration, earlier onset of bone exposure, and a faster deflation of tissues compared to the control subject. With the exception of earlier onset of bone exposure, differences in decomposition pattern were consistent with previous frozen pig decomposition research. Compared to never-frozen human subjects deposited in the fall at CFAR, the previously frozen subject fell within the range of thermal energy necessary to achieve early and advanced decomposition, but at TBS≥23.0 and TBS≥27.0, the frozen subject decomposed more rapidly.

In this preliminary investigation, prior freezing was found to increase the rate and alter the pattern of human

decomposition. Additionally, the rate of frozen human decomposition did not progress as expected based on previous research on frozen pig decomposition, but expected effects on visible external features were observed. Further research is ongoing.

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Forensic Taphonomy, Decomposition, Freezing

A20 The Influence of Stature on Sex Estimation

Haley E. Horbaly, BS, University of Tennessee, Knoxville, 250 S Stadium Hall, Knoxville, TN 37996*

After attending this presentation, attendees will better understand whether body size influences the expression of traits used in forensic sex estimation.

This presentation will impact the forensic science community by illustrating the importance of understanding human variation when looking at skeletal traits in forensic anthropology.

Sex estimation of the adult skeleton is a key part of the biological profile in forensic anthropology due to its utility in feasibly eliminating half of the missing persons pool and enhancing the accuracy of other aspects of the biological profile, such as stature and ancestry; however, sexual dimorphism in the human skeleton is relatively small, and sex estimation can be further complicated by aspects of the individual's life or genetic background. The degree of sexual dimorphism is known to vary between populations, and while global patterns have been well-studied, the underlying causes of this variation are still unclear. Previous studies have established a secular pattern of increasing sexual dimorphism with increasing stature, a reduced accuracy of sex estimation methods on short-bodied populations, and have shown that body size affects morphological trait expression used for age estimation.[1-3] These findings give support to the present study, which seeks to establish whether body size also influences expression of traits useful in forensic sex estimation. The hypotheses for this study are: (1) variation in expression of sexually dimorphic traits covary with stature; and, (2) taller individuals are more accurately sexed due to a greater expression of dimorphic traits.

Sex estimation has been known to achieve 95% accuracy when a complete set of skeletal remains is present, though forensic contexts often produce incomplete remains.[4] In an effort to account for this potential limitation, this study employs three widely-used non-metric sex estimation methods, as metric methods depend on significant skeletal preservation. The three methods selected for this study (Walker, Klales et al., and Rogers) rely on the cranium, pelvis, and humerus, respectively.[5-7] Should differences in sexual trait expression in individuals of varying stature be observed, the use of three different sex estimation methods will illustrate whether this pattern is observable in multiple regions of the skeleton. This research was conducted at the William M. Bass Donated Skeletal Collection curated at the University of Tennessee, Knoxville, one of the most comprehensive modern skeletal collections of known demography. The derived sample of 127 individuals (63 males and 64 females) was controlled for ancestry and age by consisting strictly of White adult males and females. Stature is used as a proxy for body size and is treated as a continuous variable. Body mass is not considered since skeletal growth (height) is more tightly canalized than storage of fat.[8]

Preliminary statistical analyses were conducted. Overall, the methods show a higher misclassification rate than has been previously reported for reference collections (Walker: 24% misclassified; Klales: 17% misclassified; Rogers: 22% misclassified). Logistic regressions were used to test whether stature can explain the probability of individuals being assigned male or female for each method. Regression results reveal that stature is significantly correlated (p <0.001 for all cases) with all the sex estimation methods used in this study; however, this is expected due to the fact that there is sexual dimorphism in human stature and that forensic sex estimation methods are explicitly designed to detect other sex-related differences. Therefore, to remove the impact of sex on stature variation, new binary regressions were conducted for each method within sex groups to determine if the misclassified individuals can be explained by body size. The results indicate that within each sex, incorrect sex estimations were not related to individual stature (Walker: p = .080 for males/p = .250 for females; Klales: p = .058 for males/no females were misclassified using this method; Rogers: p = .447 for males/p = .808 for females). In conclusion, the misclassifications are not the result of stature, and future studies will pursue other sources of biological variation, such as donor weight or Body Mass Index (BMI), to test the hypothesis that body-size dimorphism is associated with sexual dimorphism in the skeleton.

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Sexual Dimorphism, Stature, Non-Metric Method

A21 The Potential Use of Five Cranial Traits for Sex Assessment in Forensic Cases

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After attending this presentation, attendees will better understand five sexual dimorphic non-metric cranial traits and their potential in forensic cases.

This presentation will impact the forensic science community by illustrating the usefulness of the foramen magnum shape, the Zygomatic Arch Ending (ZAE) with respect to the external auditory canal, the sigmoid notch, the mandibular ramus flexure, and the Gonial Angle (GO) area muscle attachment for sex determination.

Sex assessment is an essential step in human identification, in both legal cases and archeological context. The highest accuracy for sex determination is achieved when the complete skeleton is available, although there are situations such as cremated, dismembered, and otherwise taphonomically altered skeletal remains in which the use of a complete skeleton is not possible. The goal of this study is to evaluate the usefulness of five non-metric cranial traits that are considered resilient in sex assessment and their potential application in forensic cases.

Previous described non-metric cranial traits (foramen magnum shape, ZAE in respect to external auditory canal, sigmoid notch, mandibular ramus flexure and GO area muscle attachment) were analyzed in 100 skulls, 45 females and 55 males, from 34 to 92 years of age. All individuals were White adults from the W.M. Bass Donated Skeletal Collection at the University of Tennessee. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 15. Comparisons between groups were conducted using X2. Discriminant function analysis model was performed to develop specific formulas for sex determination.

Foramen magnum, sigmoid notch, and mandibular ramus exhibited no significant differences between males and females. In contrast, ZAE and GO morphology revealed strong significant differences between these two groups. Based on these two parameters, the function obtained by discriminant analysis, Sex = -1.992 + (ZAE*2.123) + (GO*0.631) with a zero result pointing to males and one result pointing to females, correctly classified 79.8% of the original cases. In fact, taking into account all five cranial traits on the discriminant function analysis, this demonstrated the usefulness of ZAE and GO for sex determination, arriving at the same formula and correctly classifying 79.8% individuals according to their sex.

This study evaluated the usefulness of five different cranial traits and their potential value in forensic cases and indicated that ZAE and GO were the best indicators for sex assessment. These two anatomical regions correspond to two highly resistant skeletal structures, allowing forensic anthropologists to use them for this purpose alone or in combination with other methodologies, when the preservation and taphonomic factors affected skeletal material, although traits that are sexually dimorphic in one population may be much less so in another. Therefore, further studies in other populations are necessary to extend the diagnostic value of these sexually dimorphic traits.

Cranial Traits, Sex Assesment, Non-Metric Traits

A22 Forensic Proteomics: A New Perspective for Age of Death and Postmortem Interval (PMI) Estimation in Porcine Bone Within the Burial Context

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After attending this presentation, attendees will better understand the new potential applicability of proteomics to the world of forensic sciences. This presentation will introduce attendees to the basics of proteomic analyses and to the study of post-translational protein modifications related to the decay process. New biomolecular markers occurring both *in vivo* and postmortem may shine a light on the progress of tissue aging and on the estimation of the PMI from skeletonized remains.

This presentation will impact the forensic science community by illustrating the potential of a new proteomic method to address important forensic questions such as the age of death and the PMI estimation from a new, unexplored perspective. Some protein post-translational modifications, and in particular protein oxidation, deamidation, and racemization, have already been suggested as "molecular clocks" for protein turnover, development, and aging, both *in vivo* and postmortem; however, none of these modifications have been used until now on bones for forensic purposes.[1-3] This presentation will describe their new potential applications in this field to improve understanding of the biological and taphonomic decay of bone.

One of the most debated themes in forensic anthropology is the estimation of PMI as well as the approximation of the age of death from skeletal remains.[4-6] Many analytical and morphological methods have been published so far to address these goals, but the inherent limits regarding the accuracy of the current methods warrant the application of possible alternatives, including relatively new techniques, such as proteomics, to forensics. This study sought to apply proteomic methods to pig skeletal remains to look for new biomarkers that can help in estimating the animal's age of death as well as its PMI.

The first element of the study compares porcine skeletal remains from different aged animals to verify any proteomic difference between the various specimens, as well as to compare different skeletal elements within the same animal to evaluate any intra-skeletal difference. Bones from five different juvenile pigs were sampled, and their proteome was analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) techniques. The proteomes of these buried bones with one fresh bone purchased from a local butcher were also compared to evaluate the impact that the burial environment can have on proteome recovery. For the second portion of the study, an experimental burial environment in the field was created, in which four piglets of similar biological age were buried and the bones were recovered after different time intervals. The goal of this second stage was to focus on proteome decay postmortem, in order to look for biomarkers to correlate PMI with particular peptide modifications.

The results reveal intriguing differences within the proteome of bones from different aged piglets, paving the way for the estimation of the age using proteomics. Surprising differences within the same piece of bone tested in different areas across the length of the bone were also noted. While the midshaft gave the least variance, biological replicates collected at the ends of long bones (especially the proximal end) exhibited significant differences in terms of their proteome composition. Differences were also present within different bones from the same individual, underlining the intra-skeletal variability of the proteome. Furthermore, important differences between the buried bone and the fresh bone were also observed, confirming that the burial environment can affect the proteome, as these lost a notable amount of proteins due to leaching. Hence, results are proving the applicability of these types of studies to forensic remains, and that they are promising approaches for future studies on age estimation. This presentation will conclude with the comparison of these results to those obtained from the burial experiments to evaluate applicability to PMI estimation.

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Postmortem Interval, Proteomics, Aging

A23 Making Up for Missing Pieces: Scanning Electron Microscopy With Energy-Dispersive X-Ray Spectroscopy (SEM/EDX) Gunshot Residue (GSR) Analysis of the Human Cranial Bone

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After attending this presentation, attendees will understand the utility of nanotechnology for detecting GSR in bone. A forensic case will be presented to illustrate how the combined use of SEM and X-ray elemental microanalysis (in the form of EDX) demonstrates the presence of signature inorganic compounds found in GSR.

This presentation will impact the forensic science community by demonstrating the application of a rarely used technique that practitioners can employ to distinguish gunshot trauma from other forms of skeletal trauma when diagnostic fracture patterns or wound signatures are absent.

Cranial gunshot wounds are easily detected even in the absence of soft tissue by examining bony traumatic defects for characteristic attributes (e.g., internal or external beveling); however, these features may be absent in partial or fragmentary remains. In these instances, the forensic anthropologist cannot reliably distinguish between fractures caused by gunshot versus blunt force trauma.

Research by Berryman et al. found that SEM/EDX analysis was able to detect trace amounts of lead, barium, and antimony — components of bullet primer — in experimentally shot fleshed pig bones.[1] GSR is a combination of primer and gunpowder particles (burnt and unburnt) derived from the bullet cartridge. Ignited primer and gunpowder exit as a gaseous plume from open surfaces of the gun. Primer mixtures often comprise three inorganic compounds: (1) lead styphnate (initiator); (2) antimony sulfide (fuel); and, (3) barium nitrate (oxidizer). Sulfur, charcoal, and potassium nitrate are common organic compounds in GSR derived from the gaseous plume. SEM reveals GSR morphology, while EDX yields quantitative elemental analysis of the inorganic compounds (primer).

Partially skeletal remains were found in a shallow hole and scattered in the surrounding wooded area by animal activity. Fragments of women's clothing were also recovered. At pathological examination, the remains had areas of residual intact skin, which were devoid of injury. Residual internal organs were severely decomposed. The skull was fragmented and incomplete due to trauma.

The anthropological analysis revealed a nearly complete skeleton and partial skull. The occipital bone was absent, and the right parietal, left and right temporal, and vomer were partially absent. Four linear fractures consistent with peri-mortem trauma were present on the right parietal, and a small linear fracture was visible on the right hard palate. The etiology of these fractures could not be ascertained with scientific certainty, but the pathologist and anthropologist strongly suspected gunshot trauma.

In order to determine if the cranial fractures were caused by gunshot trauma, four cranial fragments and a control specimen of a thoracic vertebral spinous process were analyzed with SEM/EDX for elemental components of GSR. The cranial fragments were cut from the lateral and posterior aspects of the right parietal, the posterior portion of the left parietal, and the left temporal. Fragments were sputter coated prior to analyses of the inorganic residue components. Trace amounts of barium, antimony, and lead were detected on the cranial fragments, but not on the control specimen, indicating the likelihood of GSR in the skull.

A missing person fitting the biological profile provided by the anthropologist was found, and identity was confirmed via dental comparison. The cause of death was certified as disruptive head trauma and, given the circumstances of the case, the manner of death was certified as homicide. The investigation of the case is ongoing.

Forensic pathologists and anthropologists frequently work together to ensure a thorough analysis of skeletal trauma, but some cases present circumstances that require additional experts. Nanotechnology such as SEM/EDX provides a useful tool for detecting GSR in cases in which fracture patterns are atypical or remains are too fragmentary to conduct macro-morphological analyses with scientific certainty. This technique can also provide an additional line of evidentiary support, even in cases in which gunshot trauma is easily recognizable.

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Gunshot Residue, Skeletal Trauma, EDX

A24 Testing the Performance of a New Age-Estimation Method on an Asian Sample

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After attending this presentation, attendees will be well versed in pubic symphyseal morphologies commonly used in age-estimation techniques.

This presentation will impact the forensic science community by providing the results of a validation study of a new component-based method that uses developmental and degenerative traits of the pubic symphysis to estimate age in individuals less than 40 years of age.

Age estimation is a crucial yet challenging task that forensic anthropologists face during the development of a biological profile. Previous methods used to analyze the pubic symphysis for indicators of age involved a higher probability of misclassification due to the overlapping physical features and broad age ranges. Additionally, previous methods have primarily used samples from American White individuals, often from samples that date to the 20th or even 19th centuries.[1-3] Validation studies of statistical models built using American White populations have shown reduced accuracy when tested on populations outside of the reference sample.[4] Practitioners at the national and international level work with skeletal samples from a large number of ancestral categories and often find that established methods for estimating the biological profile perform differently on different geographic and temporal populations. Therefore, it is necessary to assess the performance of methods on populations outside the reference sample used in initial model building. Forensic anthropologists ultimately require a methodology that is applicable to a range of ethnicities for assessing age and the first step in reaching this lofty goal is to ascertain how a method performs on samples that were not used to construct the method.

To examine the accuracy and precision of the Dudzik and Langley method, this study tested the performance on modern Asian samples collected for this study.[5] The original method was produced using forensically relevant modern American samples of known age, sex, and ancestry from the Maricopa County Forensic Science Center in Phoenix, AZ, as well as donated individuals from the William M. Bass Forensic and Donated Collections at the University of Tennessee, Knoxville. Specifically, this method assessed the accuracy of distinct morphological features of the pubic symphysis in young adults that are typically observed in ages less than forty. Five indicators were used in the decision tree to estimate the age category of each individual. The five features included the pubic tubercle epiphysis, symphyseal billowing, presence of the ossific nodule at the superior apex, presence of the dorsal plateau, and presence of the ventral rampart.

The Asian samples included Chinese individuals from Kunming Medical University (n=85), Japanese from Jikei University and Chiba University (n=50), and Thai individuals from Khon Kaen University and Chiang Mai University (n=50). Testing the method on the Chinese sample produced 72% accuracy, which is lower than the reported results using American White and Black individuals, which delivered correct percentages not lower than 83%. Accuracy rates for the pooled Asian samples were not as high as the percentages identified in the American samples reported previously and were generally less than 80%.

This study reveals the results of an age estimation method that uses a decision tree approach for component-based traits of the pubic symphysis. This analysis confirms the need for population-specific methodologies for not only age estimation but all aspects of the biological profile. Further research involving samples that span larger coverage of world populations is necessary for forensic anthropologists that practice in the ever-globalizing population of the United States. Method validation using larger sample sizes will help to reveal what aspects of the decision tree method produces the highest percentages of accuracy and identify which age categories experience the most variation across different ancestral groups. Future work in this arena will allow for modifications of existing age estimation techniques to better serve forensic anthropologists who work closely with a varied number of ancestral groups.

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Age Estimation, Pubic Symphysis, Validation

A25 Evaluating Differential Nuclear DNA Yield Rates Among Human Bone Tissue Types: A Synchrotron Radiation Micro-Computed Tomography (SR micro-CT) Approach

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After attending this presentation, attendees will understand whether differences in bone microstructure may be used to explain differential nuclear DNA yield among bone tissue types.

This presentation will impact the forensic science community by improving current understanding of the relationship between nuclear DNA yield and osteocyte lacunar abundance, thus informing bone-sample selection for nuclear DNA analysis in a forensic context.

Molecular human identification has conventionally focused on DNA sampling from dense, weight-bearing cortical bone tissue from femora or tibiae. A comparison of skeletal elements from three contemporary individuals demonstrated that elements with high quantities of cancellous bone yielded nuclear DNA at the highest rates, suggesting that preferentially sampling cortical bone is suboptimal.[1] Despite these findings, the reason for the differential DNA yields between cortical and cancellous bone tissues remains unknown. Evidence from bone microarchitecture may help explain this variation and enrich the understanding of bone microstructural features.

The primary goal of this research is to determine whether the 3D examination of osteocytes and the quantification of their associated cellular spaces (lacunae) can be used to explain differential nuclear DNA yield among cortical and cancellous bone tissue types. Osteocytes and other bone cells are recognized as housing DNA in bone tissue, thus examining the density of their lacunae may explain why nuclear DNA yield rates differ among bone tissue types.

Methods included visualizing and quantifying osteocyte lacunae using SR micro-CT at the Canadian Light Source synchrotron facility in Saskatchewan, Canada. Forty-three bones were selected for SR micro-CT imaging from the 55 elements per skeleton sampled for DNA by Mundorff and Davoren. Representatives from each skeletal element type were chosen and bones from the left side only were sampled. Regions Of Interest (ROIs) from cortical and cancellous bone tissues (n=129) were comparatively analyzed.

Osteocyte lacunae were separated from the high-density bone using global thresholding and segmentation. Despeckling was conducted to remove noise (structures <10μm^3). Features >2,000μm^3 were assumed to be canals and remaining structures were designated as lacunae. Standard nomenclatures for lacunar indices were applied for the analysis of 3D lacunar parameters within the ROIs. The variables measured included: Total ROI Volume (TV), total Canal Volume within ROI (Ca.V), average Canal Diameter (Ca.Dm), total Number of Lacunae (N.Lc), and average Lacunar Volume (Lc.V). To determine lacunar density per mm^3 (N.Lc/BV), Bone Volume (BV) was calculated as TV minus Ca.V (TV-Ca.V). BV for cancellous bone ROIs was calculated as TV minus the volume of the marrow spaces.

Statistical analyses tested the primary hypothesis that the abundance and density of bone's cellular spaces vary between cortical and cancellous bone tissue types. Bones identified by Mundorff and Davoren to yield more DNA per mass of sample were directly correlated against the number of lacunae and osteocyte lacunar density data.

Results demonstrated that osteocyte lacunar abundance and density varied between cortical and cancellous bone tissue types, with cortical bone ROIs containing a higher lacunar abundance and density than cancellous bone. The osteocyte lacunar density values are independent of nuclear DNA yield, suggesting an alternative explanation for the higher nuclear DNA yields from predominantly cancellous bones.

At the time of this writing, this work represents the first examination of inter-element variation in osteocyte lacunar properties from cortical and cancellous bone tissues in various human skeletal elements. The use of SR micro-CT allowed for a scale of analysis that revealed a high range of variation in lacunar abundance in both tissue types and soft tissue remnants within marrow spaces. As such, it is hypothesized that soft tissue remnants, only observed via SR micro-CT, within the medullary cavities of primarily cancellous skeletal elements are driving the

high nuclear DNA yields.

The results have implications that improve current understandings of the relationship between nuclear DNA yield and osteocyte lacunar abundance, and normal variation of osteocyte lacunar parameters. Results of this work also have broader applications as they offer promise for the development of a refined method for identifying the bone tissue type most likely to yield nuclear DNA. The procurement of small, primarily cancellous bones with associated soft tissues within marrow spaces should be preferentially sampled and no longer dismissed as potential DNA sources in favor of cortical bone tissue.

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3D Imaging, Nuclear DNA, Osteocyte Lacunae

A26 The Effect of Embalming on Discoloration of Burnt Bone

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After attending this presentation, attendees will understand how embalmed bone changes color during burning in comparison to fresh bone.

This presentation will impact the forensic science community by enhancing knowledge of how bone reacts to burning.

Although there has been previous research regarding burnt bone, it has focused on repeating and confirming color changes from previous studies, while the effect of embalming on bone is largely unknown. This study is the first to examine the interaction between embalming and exposure to heat.

This project observes the color changes associated with burned bone after embalming. It was hypothesized that embalmed bone will show no differences from fresh bone after burning as the embalming process focuses on soft tissue and only affects hard tissue after being embalmed for several months. It was also hypothesized that bones soaked individually in formalin react differently to heat exposure due to formalin solutions reportedly leaching out inorganic material from bone.

Forty femora from domestic pigs (*Sus scrofa domestica*) were obtained from a local butcher. Half were burned without alteration and half were immersed in a 10% formalin solution for a week prior to burning. Ten whole pigs were embalmed using the same process used to embalmed deceased humans in the United Kingdom, after which femora were removed for burning.

Four different temperatures were chosen based on their use as milestones in the color change of bone during heating: beginnings of color change at 200°C; carbonization at 350°C; beginnings of calcination at 500°C; and complete calcination at 700°C.

A Munsell soil color book was used to analyze the color of the bone after burning. Each code in the Munsell soil color book refers to the hue, value, and chroma of a particular color, and each code also has a visual description that goes along with it. After initial observation, although there are differences in the assigned color code, there are multiple codes that all refer to the same color; therefore, it appears that there is no significant difference between the visual descriptions of the final discolorations of the fresh, immersed, and embalmed bones. Bones heated to 200°C result in a yellow-brown or brown color, bones heated to 350°C result in black or very dark gray, bones heated to 500°C result in variations of gray or bluish gray, and bones heated to 700°C result in white coloration. Both bone from embalmed pigs and bone immersed individually in formalin exhibit post-burning coloration that is consistent with that of fresh bone. When looking at the Munsell color codes, it can be seen that both the initial color and the final discoloration of the immersed bones were more homogenized. While the multiplications of fresh and embalmed bones revealed several different color codes for a particular temperature, the immersed bones only displayed two at most.

Embalming, Burnt Bone, Color Change

A27 Modeling Thermal Alterations on Burned Human Remains

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After attending this presentation, attendees will better understand the variation in thermal alterations on human remains and the variables that can contribute to these heat-related changes.

This presentation will impact the forensic science community by highlighting the limitations of current models and the need to create a new model that can be more broadly applicable to the forensic community.

Since its original publication in 1996, forensic anthropologists have used the Crow-Glassman Scale (CGS) to characterize heat-related damage to human remains.[1] The CGS was developed from a mass casualty fire in Waco, TX, where the remains exhibited advanced stages of heat-related damage. As a result, this scale progresses from blistering to fragmentation in only five stages, thus, limiting its utility. The CGS does not include descriptions of times or temperatures, nor does it quantify surface area or percentage of body affected. This scale is also not typically used by medical examiners, who instead use calculations of total burned surface area and the rule of nines to describe the extent of burn injuries. As such, there are inconsistencies in the reported descriptions of burned bodies from medical examiners from those provided by forensic anthropologists.[2-5] This research seeks to develop a method more applicable to and consistent with remains encountered in fires, thus bridging the work between the various forensic sciences.

This study involves observational experiments of the burning of 40 donated human cadavers between 2014 and 2016. 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 vehicle fires, structure fires, outdoor contexts, and in confined spaces. All physical alterations to both soft and skeletal tissues were documented with digital photography and thermocouples. The visual assessment of the burned bodies was guided by the existing CGS and supplemented with additional time and temperature data. Skin splits, subcutaneous fat exposure, muscle exposure, and presence or absence of soft tissue color banding were among the soft tissue variables recorded for each individual. Skeletal color banding, charring, calcination, fracturing, and fragmentation were among the skeletal changes recorded.

The amount of thermal damage to remains is correlated with maximum temperatures and exposure times in each fire environment. Individuals placed in the front seat of vehicles exhibited limited soft tissue loss and bone exposure, consistent with exposure to high temperatures (CGS score of 3). Individuals placed in trunks exhibited calcination and fragmentation, consistent with prolonged exposure to high temperatures (CGS score of 5). The individuals in outdoor fires exhibited calcination, fragmentation, and heat-related fracturing, consistent with prolonged exposure to high temperatures (CGS score of 5). Remains in structure fires exhibited partial soft tissue loss and soft tissue color banding, which is consistent with exposure to intermediate temperatures (CGS score of 2). Remains from confined space fires exhibited charring, muscle exposure, and limited presence of soft tissue, consistent with intermediate temperatures (CGS score of 3).

The results of this study demonstrate notable and patterned differences in physical alterations, making it possible to model heat-related damage. The presence of soft tissue, muscle exposure, and skin splits on some remains illustrates the need for refining the CGS to include these variables. As data collection progresses, a more robust model to describe heat-related damage in addition to temperature data will be created that can be more broadly applicable to remains encountered in fatal fires.

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Burned Human Remains, Crow-Glassman Scale, Forensic Anthropology

A28 Scavengers at Real and Taxidermied Carrion

Lauren R. Pharr, PhD, 5709 Lyons View Pike, Apt 2208, Knoxville, TN 37919*

After attending this presentation, attendees will be aware of misconceptions regarding when and how scavengers locate carrion.

This presentation will impact the forensic science community by illustrating that forensically important animal scavengers appear early in the postmortem interval, regardless of whether the food is actually decomposing or simply appears to be decomposing.

Acceleration of decay by animal scavengers has been attributed to turkey vultures, which use smell to locate decay, and black vultures, which rely on sight. Once vultures hone in on a carrion source, they land and rapidly remove soft tissues from the body. Once skeletonization has been reached, terrestrial scavengers will then scatter and damage the bones.

Although researchers are aware that turkey vultures can locate food through smell, the ability of black vultures to use the sense of smell to locate food is debatable. New knowledge on vultures' ability to locate food via visual versus olfactory indicators will contribute to the understanding of both vulture scavenging behavior and the postmortem roles vultures have in forensic contexts involving buried or concealed remains.

To test the role of sight versus olfaction in vultures' ability to detect carrion, a taxidermied juvenile pig was placed seven times at three different site types where vultures had previously scavenged real juvenile pigs in the fresh stage of decay. The three site types included the Texas State Forensic Anthropology Research Facility (FARF), a cattle pasture located more than 1km from FARF, and rotating pig placement sites that changed locations with each trial. The Rotate Sites were located at least 7km from the other two site types.

On September 9, 2012, the project began by placing a juvenile pig in the fresh stage of decay at each of the three site types. This process was repeated every two weeks eight more times to determine if the site type affects vulture scavenging. Three real pigs were placed at the three sites during each of Trials 1-4. After the fourth placement of the decaying pigs, vultures had scavenged 11 of the 12.

Beginning on November 4, 2012 (Trial 5), both real pigs and the taxidermied pig were placed, and the taxidermied pig was placed at sites where the real pigs had been scavenged by vultures. As more real pigs were placed and scavenged by vultures, additional sites to place the taxidermied pig became available. During Trials 5-9, the taxidermied pig was placed biweekly at five of the Rotate Sites that had involved vulture scavenging of the real pigs. Following the conclusion of the ninth trial, the taxidermied pig was placed a sixth and seventh time at the cattle pasture and FARF sites, respectively. All pigs were monitored with a motion-activated game camera.

Results reveal that animal scavengers arrive early in the postmortem interval, regardless of whether the food source is dead or appears to be dead. Scavengers at the taxidermied pig included turkey vultures, a red-tailed hawk, a crow, coyotes, dogs, foxes, opossums, raccoons, and a bobcat. Other animal visitors included deer, cows, and rabbits. Surprisingly absent was the black vulture, a scavenger said to rely on sight. This is surprising because 121 black vultures were present at the real carrion placed during the nine trials.

In addition to advancing the discussion on how scavengers detect a carrion source, this research contributes to the discussion of the trauma scavengers inflict. During this study, scavengers removed an ear and the tail of the taxidermied pig and left linear scratch marks on the abdomen. This trauma is consistent with scavengers going for easily removable and accessible parts of the body as seen on real carrion.

The unexpected presence of forensically important scavengers at the taxidermied pig, a pig unable to decompose and unaffected by accumulated degrees, will promote a discussion on the need to include avian and terrestrial scavengers in future taphonomic studies.

Scavenging, Taphonomy, Taxidermy

A29 The Effect of Partial Wrapping of Buried Rabbit Cadavers on the Decomposition Rate: A Comparative Taphonomic Study

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WITHDRAWN

A30 Robbed Burial Sites: A Comparative Investigative Approach Using Geographic Information Software (GIS) to Locate Secondary Burial Sites

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After attending this presentation, attendees will: (1) better understand the phenomenon of "robbed" mass graves in armed conflict contexts; (2) become familiar with examples from Spain, Bosnia, and Cyprus; and, (3) more thoroughly understand the motives behind the clandestine removal of bodies from graves, which can be studied to help determine the location of the secondary depositions. Attendees will also appreciate the negative consequences of this grave robbing for forensic professionals, who are called on to exhume residual remains from primary sites and reconstruct the sequence of events concerning the victims, and for the families of the missing, who are offered partial skeletal remains (possibly at different time intervals) for reburial.

This presentation will impact the forensic science community by demonstrating a new investigative approach which uses GIS to assist in the discovery of secondary deposition sites. This study builds on previous research, analyzing patterns of primary grave distribution, and examines whether a more specific analysis of similar patterns may assist in identifying secondary grave locations.

This study reviews a set of 130 graves in Cyprus, of which five were subject to the clandestine removal of bodies, and 403 graves in Bosnia, of which ten sites were robbed. Robbed bodies from sites in Bosnia were redeposited in 39 secondary graves, whereas secondary burial locations in Cyprus are unknown. Several examples of robbed graves from the Spanish Civil War, which relate to different motives and actors (i.e., by families of the victims or by the postwar government), are also used to assist in the analysis, albeit in a qualitative rather than quantitative way due to the limited number of confirmed sites and positive identifications from secondary sites.

This study examines the possible elements that set the robbed graves apart from others that were left intact. Observations from all three contexts indicate that bodies from robbed graves were of both civilians and military personnel; however, factors such as victim demographics (sex, age) and whether the victims were battle casualties, executed prisoners of war, or mass killings of civilians, have been identified as important factors influencing the decisions of those responsible for the robbing.

In order to assist in the search for secondary graves in Cyprus, GIS was used to analyze primary and secondary grave locations in Bosnia for spatial clustering and distances relative to settlements and territorial borders. The analyses indicate a 24% increase in distance from settlements between primary (mean of 1,049 meters) and secondary (mean of 1,303 meters) graves. The mean distance of secondary graves from their robbed primary graves is 14.85km with a standard deviation of 9.21km. Of particular interest is that the bodies robbed from primary graves closest to international borders and territory controlled by the military associated with the victim group were relocated farther than other robbed bodies. Consistent with previous studies, there is significant clustering of secondary graves. These results all indicate a coordinated effort to hide victim bodies in response to the threat of international investigations in Bosnia, which subsequently firmly established military responsibility for grave robbing.

This study hypothesized that, similar to Bosnia, high-level political developments — at an international scale — combined with considerations regarding the victim count and demographics, spurred the large-scale, organized, and costly operations of the clandestine removal of human remains from mass graves in Cyprus. Qualitative evidence and observations from Spain indicate that *internal* (rather than international) high- and low-level decisions also motivated grave robbing, but the spatial patterns between primary and secondary graves in Spain contrast with the case of Bosnia.

Through the analysis of data collected from all three contexts, this study is able to construct site location models to assist in the search for identifying secondary deposition sites, particularly in Cyprus, but with implications for other contexts as well. Such mechanisms offer the forensic community useful guidelines and insights to help manage expectations of the families of the missing. This will also help to work toward a more complete return of victim remains to families, honoring their fundamental right to know the fate of their loved ones.

A31 A Comparison of Skin Color Change in Terrestrial and Aquatic Decomposition and Its Potential Value as an Indicator of Postmortem Interval (PMI)

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After attending this presentation, attendees will better understand how skin color changes during decomposition in terrestrial and aquatic (submerged) environments and how the use of reflectance spectrometry can allow objective quantification of skin color as a function of time.

This presentation will impact the forensic science community by providing results from three controlled porcine experiments in an area of very little previous research. This presentation will add to existing research in the field of forensic taphonomy by broadening understanding of porcine decomposition in terrestrial and aquatic conditions and by enabling a better appreciation of these processes in human decomposition. This research presents a novel in-field reference guide for estimating: (1) the PMI or Postmortem Submergence Interval (PMSI) of an unknown individual; and, (2) the original skin color of the unknown individual and, as such, adds to the existing armory of techniques for the identification of severely decomposed human remains.

Bodies found in aquatic environments show markedly different decomposition to those found in terrestrial situations.[1] One of the most obvious, visually arresting differences is in skin color. The processes of decomposition can change the original skin color of an individual dramatically, particularly in cases with long PMIs.[2] As skin color can be used to make a preliminary assessment of the ancestry of an individual, any skin color changes as a result of decomposition can cause misidentification of unknown remains, as was the case in the aftermath of the 2004 Asian tsunami.[3] In addition, skin color changes can cause confusion regarding the stage of decomposition reached, complicating PMI estimation.[4]

This presentation combines the results from three separate porcine decomposition experiments, conducted between 2013 and 2016 in outdoor decomposition facilities at Cranfield University and the University of Huddersfield in the United Kingdom. As such, it represents a substantial accumulation of knowledge on the visible alterations to skin color as a result of decomposition in terrestrial and aquatic environments and marks the first time such experiments have been attempted in a United Kingdom climate.

In the first experiment, the cadavers of two domestic pig (*Sus scrofa*) were left on the surface to decompose for 49 days, while skin color (represented by L*a*b* scores) was recorded across the torso and back using a hand-held Konica Minolta reflectance spectrophotometer. The second experiment utilized four domestic pig cadavers, two surface deposited and two submerged in tanks of tap water for a total of 56 days; skin color measurements were taken across the bodies using a hand-held HunterLab MiniScan EZ 4500L spectrophotometer. The third experiment focused on the color change of eight excised swatches of porcine skin, submerged in canal water (freshwater) at 5°C and 18°C for a total of 35 days.

In the data from both terrestrial and aquatic environments, preliminary statistical tests found a significant correlation ($p < 0.01$) between PMI and skin color, measured using L*a*b* scores. In the surface-deposited pigs, the skin visibly darkened as PMI increased, the correlation between PMI and L* reaching a peak at day 45 ($R^2 = 0.83$). In the submerged pigs and skin swatches, a significant correlation ($p < 0.05$) was found between PMSI and skin color, particularly in the a* scores, which represent hues on the red-green spectrum. The nature of the water in which the submersion occurs was found to influence the skin color change as well. The skin of the pigs submerged in tap water turned a peachy-pink color, whereas those in canal water lightened visibly and gained an obvious greenish hue.

The linear relationship and statistically significant correlation between L*a*b* scores and PMI and PMSI suggest that it may be possible to use quantifiable skin color change as a method for estimating PMI or post-submersion interval, particularly in originally light-skinned individuals.

A preliminary reference tool produced from combined L*a*b* scores for in-field use in time-sensitive forensic cases is presented here. The color swatches can be used: (1) to give an early indication of PMI or PMSI; and, (2) even to allow back extrapolation to estimate the original shade of an individual's skin color. This has important implications for the identification of unknown human cadavers found in terrestrial, surface depositions and those found submerged in freshwater aquatic environments.

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Decomposition, Skin Color, Postmortem Interval

A32 Estimating the Postmortem Interval (PMI): A Metabolomics/Lipidomics Approach

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The goal of this presentation is to explain the utility of Glycerophospholipids (GPLs) unique to the membranes and organelles of human skeletal muscle tissue cells in estimating Time Since Death (TSD).

This presentation will impact the forensic science community by demonstrating the use of targeted metabolomics and lipidomics assay platforms to provide more accurate and robust biomarkers of the PMI.

During the initial stages of decomposition (within 72 hours), body temperature is used to determine PMI. Prior to the onset of putrefaction, the potassium content of the vitreous humor is also a useful estimator. After this point, visual inspection of the body for color changes, the onset of bloat, and the stage of insect activity can be used to predict TSD; however, longer PMIs are more difficult to estimate with precision using visual inspection, especially if scavengers have consumed some of the soft tissues. Consequently, these estimates are often open-ended and limited in forensic utility (e.g., a minimum of number weeks or months). Biochemical analyses have shown promise, but the actualistic studies were conducted on small samples and lack extensive validation data. Furthermore, the analytical capabilities of mass spectrometers have increased exponentially since these studies were conducted.

This study utilizes high-resolution mass spectrometry analytical platforms to examine biomarkers of time-dependent postmortem tissue degradation. Twenty bodies were placed in protective cages at the University of Tennessee's Anthropological Research Facility. Daily samples (20mg-40mg) were collected from vastus lateralis muscle, four to six inches proximal to the patella and lateral to the femur using muscle tissue biopsy needles. If vastus lateralis muscle was not available, samples were obtained from other thigh muscles or from the leg. Temperature and humidity data were collected daily as well. Samples were stored in a -80°C freezer until transported to Lincoln Memorial University-DeBusk College of Osteopathic Medicine for mass spectrometer analyses. The muscle samples were placed into solution of 50ul standard, 1ml of deionized water and 1ml of methanol. The solution was sonicated with polytron, mixed with 1ml tert-butyl ether 99%, shaken for 30 minutes, and centrifuged for 15 minutes. A 1ml organic layer was extracted, vacufuged for four hours, and 150 ul ammonium acetate was added to sample for mass spectrometry.

The results obtained from the analytical platforms for sterol sulfates, short chain fatty acids, Very-Long-Chain Fatty Acids (VLCFA), Ethanolamine Plasmalogens (PlsEtn), Choline Plasmalogens (PlsCh), and phosphatidyl glycerophospholipids were analyzed with multiple linear regression analysis with stepwise variable selection using Accumulated Degree Days (ADD) as the dependent variable. An r-squared value of 0.81 indicates that these variables account for variation in temperature-related postmortem changes in a corpse. The equations are being tested on a separate validation sample of known-PMI tissues to provide the forensic community with validated equations to derive accurate postmortem interval estimates based on biomarkers of long PMIs.

This research was supported by the National Institute of Justice.

Time Since Death, Postmortem Interval, Mass Spectrometry

A33 The Growth and Development of Secondary Sex Characteristics in the Human Skull

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After attending this presentation, attendees will better understand the growth and development of sexual dimorphism in the human skull, including which sexually dimorphic craniofacial traits reach adult expression before skeletal maturity, and the implication that these traits may be useful for sex estimation from adolescent skeletal remains.

This presentation will impact the forensic science community by addressing the common assumption that sex cannot (and should not) be assessed from immature remains and by suggesting avenues for further research to improve sex estimation methods in adolescent populations.

Although high accuracy rates have been achieved for sex estimation from adult skeletal remains, estimating sex in subadults remains a vexing issue facing forensic anthropologists. Many previous studies have focused on measuring sexually dimorphic skeletal features that are commonly used to estimate sex in adults, such as pelvic morphology and cranial traits, and have applied these methods to juvenile humans. As these are sexually dimorphic features whose expression is either absent or incomplete until adolescence, these methods have enjoyed mixed success when applied to immature skeletal remains; however, the growth and development of these sexually dimorphic secondary sex characteristics have not been systematically studied. The timing of their sexually dimorphic expression therefore remains unknown, and practitioners frequently and perhaps erroneously assume that these traits are not fully developed until adulthood. To assess the value of these traits for diagnosing sex among subadults, their expression must be measured during the adolescent period when growth and development of the secondary sex characteristics occurs.

This study was performed on a longitudinal sample of lateral radiographs from the Denver Growth Study, which includes males and females of European ancestry (n=5 males; n=5 females; n=96 total scans). To assess the growth of secondary sex characteristics in the human skull, four linear distances were measured that approximate the areas commonly scored in non-metric sex assessment: the nuchal crest, mastoid process, glabella, and mental eminence. These four measurements were taken of each individual at multiple ages; the youngest age cohort is defined as prior to the eruption of the second permanent molars, which is correspondingly before the onset of sex-based differential growth of cranial secondary sex characteristics. Measurements were taken on every subsequent scan for each individual until the measurement in question reached full adult expression (i.e., maximum size). Skeletal maturity was defined as the age of alveolar eruption of the maxillary third molar, with all earlier radiographs being characterized as immature. The age of skeletal maturity was then compared to the age of adult expression for each measurement. One-tailed, paired Wilcoxon rank-sum tests were performed to compare the pooled-sex sample's age at full expression of each trait to the age at skeletal maturity. The age of adult expression of the mental eminence was significantly younger than the sample's age of skeletal maturity (p<0.001), while the age at adult expression of the nuchal crest was significantly older than the age of skeletal maturity (p=0.02). The mastoid process and glabella ages at adult attainment did not differ significantly from the sample's age of skeletal maturity (p=0.27; p=0.34, respectively).

The results indicate that the mental eminence matures in size relatively early in adolescence and suggest that this feature is suitable for further analysis and the development of sex estimation criteria in adolescent skeletal remains — effectively pushing back the age at which sex can be assessed from crania. These results also support the use of the mastoid process and glabellar regions for sex estimation at (and after) M^3 eruption, but indicate that caution should be exercised when scoring the nuchal crest in young adults, as this trait does not reach full expression until after skeletal maturity. None of the traits included in this study are routinely employed for sex estimation in subadults. Yet their utility in this context can be assessed through the use of a longitudinal database of radiographs that began 85 years ago at the Denver Growth Study. The novel insights into adolescent growth and development of sexually dimorphic traits provided by this study demonstrate how even evolving forensic methods rely upon past research efforts.

Sex Estimation, Growth and Development, Adolescence

A34 Sex Estimation: A Novel Protein-Based Sex Assignment Technique Using Human Tooth Enamel and Mass Spectrometry

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After attending this presentation, attendees will better understand a powerful new methodology in which proteinaceous sample sources such as tooth enamel can be used as an alternative to DNA for the forensic analysis of humans. This presentation will inform attendees that compromised samples lacking DNA can still be used to determine information about the identity of a subject. Specifically, sex estimation can be achieved by proteomic analysis of human tooth enamel proteins.

This presentation will impact the forensic science community by offering an alternative science-based forensic technique using proteins as a biological sample source to achieve human sex estimation from human tooth enamel.

Human sex-estimation provides a fundamental metric essential for human forensic and bioarchaeological practice. Compromised samples that are influenced by fire, explosions, extreme environments, and long periods of time often do not meet the quality standards necessary for success of current sexing techniques. These techniques rely on anatomical sexual dimorphisms, which are particularly problematic in subadult skeletons' DNA-based analysis of sex chromosomes. Protein is chemically more stable than DNA and has an increased resistance to deterioration in the environment. Tooth enamel is the hardest tissue in the human body and is an ideal sample source in this context due to its preservation in harsh conditions. Probing the sex-linked protein amelogenin that is found in tooth enamel offers a novel way to sex compromised or subadult skeletons that lack DNA.

Using Mass Spectrometry (MS) -based proteomic techniques, the sex-linked isoforms of amelogenin can be detected directly by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) peptide sequencing. Approximately 20mg-30mg blocks of enamel were resected from each tooth. Protein extractions were performed by a milling, acid-etching, disulfide reduction and alkylation steps. Peptides were generated by proteolysis in the presence of the surfactant with trypsin. The resulting peptides were separated by high-pressure LC and analyzed by a Thermo™ Q-Exactive™ plus hybrid orbitrap/linear ion trap MS. Mass spectra were then matched to peptide sequences and a subset of peptides containing amino acid sequences peptides unique to the X- and Y-isoforms of amelogenin was generated. In this study, five male, five female, and one archaeological sample from a male and a female were tested for amelogenin X/Y isoform peptides in their tooth enamel. After the initial detection of unique isoform peptides was achieved, specific mass-to-charge ratios of interest were chosen for additional data acquisition targeted to collect MS/MS data correspondingly. Synthetic peptides were purchased for validation of mass-to-charge ratios and retention times of the peptides identified within experimental data. Optimization of protein extraction from tooth material was achieved by testing different extraction conditions (different chelating agents and acid-etching time/temperatures) on a homogenized standard sample composed of eight different teeth. Dentin and cementum were also excised from teeth for amelogenin isoform detection.

X-isoform peptides were detected within every sample of the enamel dataset and peptides unique to the Y-isoform were consistently detected within all male samples of this group, yielding a positive predictive value of male and female sex-estimation of 100% in this cohort. These results reveal the potential of this technology for sexing of a given individual using a proteomic approach. By exploiting the durability of enamel in combination with the stability of protein, this technique provides a new pathway for analysis of compromised human remains in both forensic and bioarchaeological settings. Expansion of this research will likely establish that proteomic analysis of the isoforms of amelogenin in tooth enamel can be used for sex assignment when other established forensic and bioarchaeological techniques are ineffectual.

This work performed under the auspices of the United States Department of Energy by Lawrence Livermore National Laboratory under contract.

Sex Estimation, Proteomics, Tooth Enamel

A35 Sex Estimation Using Relative Pubis Length

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After attending this presentation, attendees will: (1) better understand how to estimate sex using relative pubis length; (2) be informed regarding a method that provides statistical analysis to support previous literature describing the pubis as longer in females than in males; and, (3) realize the importance of utilizing a replicable pubis length measurement.

This presentation will impact the forensic science community by providing a method of sex estimation using the pubis that is not reliant on morphological features. This research adds to the already existing methods of sex estimation and provides a method developed on a modern sample.

Sex estimation is an integral part of the biological profile. Other aspects of the biological profile require the estimation of sex before their evaluation can be considered. Both age at death and stature use sex-specific regression formulas. Estimations of sex are most accurate after the individual has reached adulthood, due to the emergence of secondary sex characteristics.[5] The pubis bone is continually used in sex estimation based upon its identification as the best indicator of sex due to childbirth; however, there are discrepancies on how to measure the pubis.[1]

In this presentation, an index to determine relative pubis length is proposed, using "the nearest border of the acetabulum to the superior point of the pubic symphysis" for measurement of pubis length and the "distance from the most superior point on the iliac crest to the most inferior point on the ischial tuberosity" for measurement of os coxa height.[2,3] Relative pubis length is proposed as a sex estimation method in order to provide metric assistance to the assessment that the pubis is more "stretched" in females.[4] Four hundred eighty-nine individuals of known age at death, sex, and ancestry from the W.M. Bass Collection at the University of Tennessee were measured to determine relative pubis length. Two hundred seventeen females and 272 males make up the 489 individuals measured from the W.M. Bass Collection. Relative pubis length is an index, created by dividing pubis length by os coxae height. This information was then applied in an Analysis of Variance (ANOVA) to determine the statistical significance of relative pubis length and sex estimation. In preliminary results, there is a significant relationship between sex and relative pubis length (p=2.2x10^{-16}) and in a two-way ANOVA, the only significant relationship found was between sex and relative pubis length; all other factors were not significant (ancestry and age at death). Additional measurements were taken using the Hamann-Todd Collection to assess the effect of secular trends on relative pubis length. Five hundred nineteen individuals were measured from the Hamann-Todd Collection; this data is currently undergoing evaluation.

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Relative Pubis Length, Sex Estimation, Pelvis

A36 The Accuracy of Nutrient Foramen Versus Midshaft Measurements of the Tibia for Sex Determination

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After attending this presentation, attendees will understand the utility of nutrient foramen and midshaft measurements of the tibia for sexing by discriminant function analyses.

This presentation will impact the forensic science community by providing usable discriminant function analyses for sex determination using the tibia.

The postcranial skeleton has been used for sex determination of an individual for more than a century. These methods have been developed and re-assessed to find the most accurate methods. In the tibia, discriminant function analysis has been employed, including measurements of diameter taken at the nutrient foramen. The location of the nutrient foramen varies from person to person and can be located in different areas of the bone from the right to left tibia in the same individual. This inter- and intra-individual variation has led some standards to adopt midshaft measurements in place of, or in addition to, the existing nutrient foramen located measurements.

To understand the implications this variation may have on the accuracy in sex determination, comparative consideration of midshaft measurements in place of nutrient foramen level measurements were tested with the following questions: Is intra-person variation in the location of the nutrient foramen great enough to cause significant mismatching of tibias left applied to left, left applied to right? Is there a significant advantage to using measurements collected at the midshaft in addition to, or in place of, measurements collected at the level of the nutrient foramen? It is hypothesized that there will be no significant difference in correct sex classification between the two measurements.

A sample of 400 individuals were measured from the Robert Terry Anatomical Skeletal Collection. Measurements of the tibia were taken via standard osteometric protocols. Because of discrepancies in the standards, medial-lateral diameter at the nutrient foramen was collected both in a 90° rotation from the anterior posterior measurement and from the interosseous crest.

Data were randomly divided into a testing set and a training set, each consisting of 200 individuals. Discriminant function analyses were run in the statistical programming environment R, using only left measurements or left and right measurements from the training set. Initial tests of ancestry differences determined no significant differences, and thus ancestry groups were pooled. The resulting discriminant functions were applied to classify individuals from the testing set using left and right measurements combined. Three variables, maximum length and proximal and distal epiphyseal breadth, were tested.

Results indicate breadth measurements of the proximal and distal epiphyses were consistently good predictors with high loading values higher in all combinations. Of the medial lateral measurements of the diaphysis, the measurement taken from the crest to a point directly opposite was a better predictor of sex than measurements taken at a 90° rotation from the anterior posterior measurement (90% versus 91.5%). Of the measurements taken at the midshaft, minimum diameter and circumference were both considered good predictors (89% correct). In the combined analysis medial-lateral diameter at the nutrient foramen, minimum at the midshaft and circumference at the midshaft were the best predictors (89% correct). There is no significant difference in accuracy between a discriminant function created with only lefts and applied only to lefts and the same function applied to lefts and rights (always a <1% difference in accuracy).

This investigation identifies that there is no significant advantage of sex determination based on measurements taken at the nutrient foramen compared to those taken at the midshaft. In cases involving fragmented remains, measurements taken at the level of the nutrient foramen would, of course, have more utility. It would be most valuable to collect both midshaft- and nutrient foramen-based measurements.

Tibia, Discriminant Function Analysis, Sexing

A37 Skeletal DNA Preservation and Bone-Associated Microbes: The Implications for DNA Sampling Strategies in Forensic Identification

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After attending this presentation, attendees will better understand microbe-mediated skeletal DNA degradation.

The results of this study provide novel insights into the diverse fungal and bacterial communities capable of colonizing and possibly influencing bone degradation. This presentation will impact the forensic science community by contributing to attendees' current understanding of microbe-mediated skeletal degradation and by informing attendees of current skeletal DNA sampling strategies for forensic identification.

Skeletal DNA degradation occurs at differential rates during human decomposition, and which elements are more susceptible to mechanisms of degradation is currently debated. Some research points to long bones as better reservoirs of high-quality DNA, while others point to small cancellous bones.[1-7] Mundorff and Davoren ranked skeletal elements from three individuals based on their overall DNA yield and ability to ascertain a full forensic profile.[6] This was the first study of its kind that looked at both intra-individual and inter-individual variation in human skeletal DNA yield. That study found that small cancellous bones, on average, yielded greater amounts of better-quality DNA than their larger, denser cortical bone counterparts, a pattern that held true over time in postmortem intervals of up to 21 years.

Yet, the mechanisms behind skeletal degradation and preservation remain largely unknown. Microbial colonization is likely a critical component, since bacteria and fungi readily degrade organic material, including DNA. Therefore, it was hypothesized that microbial intrusion and community composition will be related to human skeletal DNA preservation. While there have been several published reports on microbes associated with soft tissue decomposition, there is little information on the quantity and community composition of microbiota in human bone. The present study seeks to improve understanding of bone-associated microbes and skeletal DNA degradation by looking at bacterial DNA yields and microbial community composition across multiple bone types within and between three skeletons following natural (surface) decomposition.

Human skeletal DNA extracts previously generated and analyzed for Mundorff and Davoren were used to directly compare bone-microbial colonization with DNA quality and quantity.[6] Specifically, extract from 49 bones, from each of the three individuals, were characterized for microbial intrusion using Illumina® MiSeq®. Both 16S recombinant DNA (rRNA) and 18S rRNA gene sequences were obtained for characterization of bacterial and fungal communities co-extracted with human skeletal DNA. Additionally, total bacterial DNA was quantified using quantitative PCR (qPCR), targeting the 16S rRNA gene.

Preliminary results reveal major contributions from the phyla Proteobacteria (20-35%), Actinobacteria (2-11%), Bacteroidetes (2-11%), Firmicutes (1-10%), with additional contributions from Verrucomicrobia, Planctomyetes, Chlamydiae, and Deinococus-Thermus; however, the proportion of these phyla throughout the skeleton do not appear to be uniform and also show varying influences from rarer taxa. Of the fungal communities sequenced, Ascomycota (40-60%) and Chytridiomycota (15-40%) were highly representative, with lesser contributions by Basidiomycota.

Preliminary qPCR results are highly variable within and between individuals; mean 16S rRNA gene abundances range from 4.10×10^8 gene abundances per gram of bone powder in teeth to 1.58×10^{10} gene abundances per gram of bone powder in 1st proximal foot phalanges. The bones with the least amount of bacterial DNA were teeth, femora, humeri, ulnae, the 12th ribs, and occipital bones, respectively. Conversely, the highest quantities of bacterial DNA were found in the 1st proximal foot phalanges, 3rd metacarpals, 1st proximal hand phalanges, capitates, and sterna. When considering human DNA yield, the highest human DNA to bacterial DNA ratios were found in teeth, 3rd metatarsals, humeri, mandibles, femora, and the tibiae. Bones with a lower ratio of cortical to trabecular bone appear to be more readily colonized by microorganisms than those with a higher ratio of cortical to trabecular bone.

This is the first study to systematically examine microbial intrusion and microbial community composition throughout the human skeleton.

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Skeletal DNA, Necrobiome, Skeletal Degradation

A38 A Morphometric Analysis of the Neurocranium in an Adult South African Sample

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After attending this presentation, attendees will better understand the human variation among modern Black, White, and mixed-ancestry South Africans and will understand the statistical framework used to describe similarities and differences among these groups.

This presentation will impact the forensic science community by not only contributing to the knowledge of human variation in modern South Africans, but also by revealing a more accurate method of estimating ancestry and sex from full and partial crania in different ancestral populations occupying the same geographical space.

Many questions in anthropology relate to variation in shape of anatomical structures. Geometric morphometrics allow evaluation of shape variation by "removing" the effect of size and has been applied to many fields within anthropology, primarily utilizing the cranium. The high crime rate in South Africa makes it important to develop methods to gain information such as sex and/or ancestry from skeletal material that is likely to be recovered in a forensic context. This study examined morphological variation in the crania of a South African sample in relation to the associated demographic information and assessed the accuracy with which such information could be estimated.

The purpose of this study was to use cranial morphometrics to evaluate ancestral variation and sexual dimorphism among White, Black, and mixed-ancestry groups as a means to explain current variation and more accurately identify unidentified remains.

A total of 774 crania of Black (160F, 123M), White (90F, 130M), and mixed ancestry (104F, 167M) were used from the Universities of Cape Town, Stellenbosch (Kirsten), Pretoria, and Witwatersrand (Raymond A. Dart) skeletal collections with ages ranging between 20 to 100 years at death. A total of 11 to 12 landmarks for each cranial element was digitized to generate various linear measures, angles and subtenses, and 14 landmarks for the whole cranium. Discriminant function analysis was employed and South African groups were tested against themselves to test classification accuracies. All accuracies were cross-validated. Multivariate analyses were used to assess differences between sex, ancestry, and sex-ancestry groups.

The analyses demonstrate that males have relatively and absolutely larger crania than females. Females tended to have a slightly larger medio-lateral dimension of the cranium, but an antero-posteriorly longer occipital region compared to males. This resulted in a more steeply sloped forehead but less steeply sloped occipital region in females. This revealed that the frontal bone had the best results for classifying each of the sexes with between 75.5%-83.7% accuracies

The most accurate identifiers for ancestry in Black individuals were found on the frontal bone (88%). This was similar for the mixed-ancestry individuals (83.7%); however, for White individuals, the whole cranium was best for ancestry identification (90.2%).

These results illustrate that morphometric analysis of the frontal bone alone could be used to estimate certain demographic parameters, which may be used in constructing a biological profile for forensic purposes. The accuracy of such analysis is similar to or exceeds that of traditional analyses.

Ancestral Variation, Sexual Dimorphism, Geometric Morphometrics

A39 Additional Information for Identification Purposes Via the Study of Cam-Type Deformity of the Hip

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The goal of this presentation is to highlight the importance of a morphological variation, such as the cam-type deformity, as a useful tool for personal identification.

This presentation will impact the forensic science community by taking into account the cam-type deformity to gain important information in identifying unknown human remains by providing information about life style and possible pathologies.

The cam-type deformity of the hip is a morphological alteration of the femoral head neck junction. Such manifestation is considered part of the Femoral-Acetabular Impingement Syndrome (FAI). This disease develops consequently to hip flexion because of an abnormal contact between the acetabular cavity and femoral head neck junction. This causes the deformity and the loss of the round shape of the head of the femur, due to several and repeated traumas toward the upper labrum of the acetabular cup and the adjacent chondral structures, resulting in a deformity of the acetabulum of the pelvis. The cam-type deformity's main feature is the formation of extra bone in the upper segment of conjunction between the body and the head of the femur.

The presence of this clinical condition can either be painless or cause pain and functional limitation of the hip, due to the deformities of the acetabular labrum and articular cartilage. As a consequence of the FAI, the cam-type deformity can be linked to hip dysplasia, previous fracture of the pelvis and femur, childhood hip disorder, and septic arthritis. Scientific literature states that significant morphological variations of the femoral head-neck junction, due to physical activities and/or occupational stress, can be a possible manifestation. Sports such as soccer, hockey, and horseback riding may be the cause of this deformity of the head and body of the femur. In these cases, the peculiar stance of the subject, as in hockey for example, causes a morphological response of the bone structure of the proximal epiphysis of the femur. This feature is also typical in sports or in jobs in which the subject remains standing for long periods of time. In these cases, the deformity is caused by the normal and involuntary favoring of one of the legs.

By the anthropological examination of the bones of a subject, both of the femur and of the whole skeleton, it is possible to detect this type of modification of the physiological structure. This study compares two subjects, both affected by cam-type deformity due to different pathological and occupational conditions. Bones belonging to these individuals have been macroscopically examined with the goal of finding features leading to pathology or occupational stress. Particular physical activities or pathological conditions linked to cam-type deformity, such as limping, can provide additional and useful information for the reconstruction of identity in cases of recovered unidentified corpses.

International literature is not lacking information about the cam-type deformity, yet this feature has not yet been taken into account for identification purposes.

Cam-Type Deformity, Identification, Forensic Anthropology

A40 A Cranial-Postcranial Approach to Metric Ancestry Estimation Among Modern South Africans

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After attending this presentation, attendees will better understand the potential of employing a combined cranial-postcranial approach to estimate ancestry in modern South Africans. The combined approach will be compared to previous cranial and postcranial studies to highlight the improvement in accuracy over previous assessment methods.

This presentation will impact the forensic science community by contributing to knowledge on craniometric and postcraniometric variation observed among modern Black, Colored, and White South Africans and the potential of a combined methodology for estimating ancestry.

The cranium is recognized as the most reliable indicator of ancestry and is the preferred bone for assessing ancestry in South African anthropological casework. Recently a postcraniometric approach to ancestry on a South African sample yielded comparable results to accuracies achieved by previous craniometric studies, demonstrating the potential use of postcrania in ancestry estimation.[1] Despite high accuracies obtained with both cranial and postcranial data, large amounts of overlap between the three major South African groups limits the predictive accuracy of the elements in providing a definitive ancestry estimate.[2] A more holistic method assessing both cranial and postcranial measurements simultaneously would include the most discriminatory variables of the entire skeleton, resulting in better group separation than when crania and postcrania are evaluated separately.[1] The current research seeks to explore a combined cranial-postcranial approach to metric ancestry estimation in a modern South African sample.

A total of 38 standard measurements were taken from the cranium and ten postcranial bones. The sample consisted of 360 modern South African individuals (120 Black, 120 White, 120 Colored) from the Pretoria Bone and Kirsten Collections housed at the University of Pretoria and the University of Stellenbosch, respectively. Group differences were explored with Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test. Multivariate classification models were assessed using Linear Discriminant Analysis (LDA). Classification accuracies achieved for a variety of multivariate models were compared to previous cranial and postcranial studies to evaluate the potential of the holistic approach.

The results demonstrated variable patterns of group overlap. Consistent with previous studies, Black and Colored South Africans displayed overlap for the majority of the variables; however, 11 variables inclusive of all lengths of the distal limb segments, breadth measurements of the orbit and nasal aperture, interorbital breadth, mastoid height, and the femoral transverse subtrochanteric diameter were found to be significantly different between the two groups ($p<0.05$). White South Africans displayed larger measurement means for all postcranial variables, except the lengths of distal limb segments, whereas Black South Africans had the largest means. Cranially, White South Africans also displayed the largest measurement means for all variables, except inter- and bi-orbital, nasal and minimum frontal breadths, for which the measurements were the smallest of all three groups. For both crania and postcrania, Colored South Africans had either the smallest or intermediate measurement means. A series of multivariate subsets were created to present numerous different measurement combinations and achieved accuracies that ranged from 85% to 91% (using LDA), with only one to two misclassifications for White South Africans, creating almost complete separation of the group from Black and Colored South Africans. Overall, White South Africans had the highest correct classification rates, followed by Black and Colored South Africans, respectively.

Substantial heterogeneity among the three South African groups decreases the positive predictive power of the cranial and postcranial methods, making them of limited reliability in a forensic setting. The current research demonstrates that when used in combination, cranial and postcranial measurements have the potential to outperform the current standard methods for estimating ancestry in South Africa.[2] Combining the skeletal elements accounts for more between-group variation and decreases the amount of overlap observed among Black, Colored, and White

South Africans, ultimately yielding improved accuracies. Using a computer program, such as FORDISC® 3.1, that combines or selects the most discriminatory variables on a case-specific basis, may be the best approach to ancestry estimation.

Reference(s):

1. Liebenberg L., L'Abbé E.N., Stull K.E. Population differences in the postcrania of modern South Africans and the implications for ancestry estimation. *Forensic Sci Int.* 2015;257:522–9.
2. L'Abbé E.N., Kenyhercz M.W., Stull K.E., Ousley S.D. Craniometric Assessment of Modern 20th-Century Black, White, and "Colored" South Africans. *Proceedings of the American Academy of Forensic Sciences*, 65th Annual Scientific Meeting, Washington, DC. 2013. p. 444.

Classification Models, LDA, Human Variation

A41 The Decomposition of Child-Sized Remains in Different Depositions

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After attending this presentation, attendees will be better acquainted with the specific environmental factors that influence seasonal decomposition.

This presentation will impact the forensic science community by presenting the significant environmental factors that affect decomposition during each season.

Thirty-seven *Sus scrofa* (16 juvenile and 21 fetal) remains were obtained fresh from the North Carolina State University (NCSU) swine farm in the summer, fall, winter, and spring months. The traditional calendar for the start of each season was used as the initial day of placement from summer 2013 to spring 2015. Juvenile pigs were used as a proxy for human children up to 9 years of age (35-50 pounds) and fetal pigs were used as a proxy for human neonatal remains (4-6 pounds). Two juvenile pigs were placed on the surface each season, one fetal pig was placed in a plastic bag, and the other fetal pig was wrapped in a baby blanket. From spring 2014 to spring 2015 (5 seasons), a third fetal pig was placed on the surface as a control. All remains were enclosed in cages to prevent scavenging.

Total Body Score (TBS) was used to record decompositional observations. Accumulated Degree Days (ADD) were calculated from daily maximum temperature with data obtained from the State Climate Office of North Carolina Lake Wheeler Road Field Lab weather station located one-half mile from the open-air site. In addition to daily temperature, daily precipitation, relative humidity, fly activity, soil temperature, and soil moisture were collected.

A mixed random coefficients model, which is useful for analyzing repeated measures, was used to examine the relationship between the dependent (ADD) and independent variables (TBS, daily temperature, daily precipitation, soil temperature, soil moisture, and deposition). All statistical analyses were performed using JMP® Pro 12.1. For the fall season, TBS, deposition, fly activity, and relative humidity were not significant effects in the fetal remains (*deposition* (bag, blanket) DFNum = 2, DFDen = 116, F ratio = 0.107, Prob >F = 0.956; *relative humidity* DFNum = 1, DFDen = 116, F ratio = 57.469, Prob > F = 0.470; *fly activity* DFNum = 1, DFDen = 116, F ratio = 0.086, Prob >F = 0.770; *TBS* DFNum = 1, DFDen = 116, F ratio = 1.99, Prob >F = 0.160;); however, daily precipitation, soil moisture, and soil temperature were significant at the <.0001 level. The juvenile remains followed the same pattern in the fall as the fetal pigs with the only significant effects being daily precipitation, soil moisture, and temperature. For the spring, all variables were significant (0.01-0.0001 level) with the exception of the fly activity for the fetal remains (p-value = 0.119). The juvenile remains displayed a different pattern with TBS, soil moisture and temperature as significant factors in the spring. Summer yielded a different pattern with only two significant effects for fetal remains (*soil temperature* DFNum =1, DFDen = 104, F ratio = 7.27, Prob >F = <.0082; *soil moisture* DFNum =1, DFDen = 104, F ratio = 19.69, Prob >F = <.0001) and only TBS (p-value = 0.05) for the juveniles. Winter also showed a different pattern with TBS, deposition, fly activity, and soil moisture having significant effects (*TBS* DFNum =1, DFDen = 140, F ratio = 90.20, Prob >F = <.0001; *Deposition* DFNum =2, DFDen = 140, F ratio = 4.77, Prob >F = <.0099; *fly activity* DFNum = 1, DFDen = 140, F Ratio = 9.77, Prob>F = 0.002; *soil moisture* DFNum =1, DFDen = 201, F ratio = 70.87, Prob >F = <.0001). For the juvenile remains, only TBS and soil temperature were significant effects in winter (*TBS* DFNum =1, DFDen = 54, F ratio = 42.03, Prob >F = <.0001; *soil temperature* DFNum =1, DFDen = 54, F ratio = 17.50, Prob >F = <.0001).

Results demonstrate that size has a significant impact on the pattern of decomposition and further support the importance of seasonal and geographic-specific indices for estimating the postmortem interval. This project was supported by a National Institute of Justice grant.

PMI, Decomposition, Seasonal

A42 Changing Impact Angles: The Mechanics Involved in Blunt Force Cranial Trauma and Their Importance in Investigating Curb-Stomping Cases

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After attending this presentation, attendees will better understand how fracture types can differ as a result of blunt force trauma produced by differing angle strikes.

This presentation will impact the forensic science community by providing an experimental model that aids in the understanding of fracture mechanics produced when force is applied to the cranium from differing angles.

The majority of studies on the infliction of blunt force trauma to the cranium assess only the initial impact site. This method can therefore result in a loss of essential data relating to the circumstances in which the injury was sustained. The goal of this study was to create an experimental model that would provide a more realistic picture of the damage sustained during a violent attack to aid investigators.

Twenty adult pig heads (*Sus scrofa domesticus*) were placed on a solid base, resting on the mandible. The base could be angled so that the impact angle to the skull could be altered for each strike. Using a drop hammer rig, modified with a replica hammer head (modeled after a 16oz claw hammer), each pig head was struck once over the frontal bone from a height of one meter. A total of five angles were assessed in this preliminary study (0°, 9°, 18°, 27°, and 36°), with each angle tested a minimum of three times. It was not possible to strike the frontal region of the pig head at any angle greater than 36°. To monitor the acceleration, timing, and force of each strike, a piezoelectric accelerometer was attached to the drop hammer, with data recorded at a rate of 10,000 scans per second. Following maceration, the fractures present were compared with previously published images and descriptions, with measurements taken of the width, length, and depth of each depression fracture.[1]

It was noted that a number of mandibles had also fractured when struck using a more direct angle (0°, 9°, and 18°). To establish that this was a result of the impact study, a further set of pig heads were radiographed prior to the strikes. A further radiograph following the impact confirmed that the mandibular fractures had caused a transference of the force through the cranium when struck from above.

A total of 22 fractures were observed between the cranium and mandible. Depression fractures (n=10) demonstrated a decrease in size as the angle increased and radiating fractures (n=4) were present on angles from 18°. Mandibular fractures (n=8) were only present up to 18° in this study, with the severity ranging from complete break to partial fractures as the angle increased. It was also noted that the angle of the fracture on the mandible differs as the angles increase.

Presented here is a pilot study that exhibits the need to further investigate the issues surrounding violent assaults using blunt force trauma, such as bludgeoning with a hammer; however, an unexpected finding was the secondary trauma inflicted to the mandible as a result of resting on the solid base plate, which mimicked the scenario faced by curb-stomping victims. Although the traditional "biting the curb" posture is not exhibited in this experiment, it provides information on how the transference of force can travel through the skull and exhibit in fractures elsewhere.

There are increasing numbers of reports in the media of violent crimes involving blunt force trauma taking place that utilize everyday household objects.[2] It has also been highlighted in studies that blunt force trauma to the head is one of the most effective methods of murder, but that the weapons most commonly involved are hands and feet, also referred to as human strength.[3]

This study is limited by the small sample size, but has provided information that could direct further research into violent assaults using blunt force trauma. It would be beneficial to repeat the study using a larger sample size, bone substitutes to more directly simulate the cranial biomechanics of a human skull, and by modifying the drop hammer to investigate how increasing the surface area impact will affect the results.

Reference(s):

1. Wedel V.L., Galloway A. Broken bones: anthropological analysis of blunt force trauma. Charles C Thomas Publisher, 2013.
2. Verzeletti A., Bin P., De Ferrari F. Homicide by blunt trauma in Brescia County (Northern Italy) between 1982 and 2012. *Am J Forensic Med Pathol.* 2014; 35(1):62-67.
3. Mohanty M.K., Kumar T.S.M., Mohanram A., Palimar V. Victims of homicidal deaths – An analysis of variables. *J Clin Forensic Med.* 2005; 12(6):302-304.

Blunt Force Trauma, Cranial Fracture, Secondary Injuries

A43 Comparing the Decomposition of Partially Suspended (Semi-Recumbent) Pigs With Fully Suspended Hanging Pigs and Fully Recumbent Pigs in Direct Contact With the Ground

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After attending this presentation, attendees will better understand the major differences in the decomposition patterns and rates between fully suspended hanging pigs, semi-recumbent pigs, and pigs decomposing on the ground.

This presentation will impact the forensic science community by adding to current knowledge concerning decomposition in hanging bodies and the appropriate decomposition scoring scales to use, which, when combined with Accumulated Degree Days (ADD), will allow for the calculation of time since death.

Establishing the Postmortem Interval (PMI) is an essential part of any death investigation. By using decomposition scoring and ADD, the PMI can be estimated. The findings of a decomposition study conducted under controlled conditions at the Taphonomic Research in Anthropology: Centre for Experimental Study (TRACES), University of Central Lancashire, United Kingdom, will be presented. Thirty freshly killed pigs (*Sus scrofa*) of the same age were used as human analogues. Twenty pigs were hung by the neck using nylon rope attached to hooks hung from A-frames built from scaffolding poles. Ten pigs were hung, fully suspended, with their hind feet approximately 100cm off the ground, with the remaining ten hung partially suspended, so that the flanks and hind legs were in direct contact with the ground. The animals were spaced 60cm-90cm apart. To protect the pigs from vertebrate and avian scavengers, each of the A-frames was surrounded with chicken wire up to a height of 60cm above ground and bird netting was stretched over the whole frame. A further ten control pigs were placed on the ground under scavenger-proof cages.

The pigs were observed and the patterns of decomposition recorded and photographed for head and neck, torso, and limbs at approximately 50 ADD intervals until 1,078 ADD. Ambient temperature was recorded by dataloggers every six hours. Total Body Scores were assigned to the control pigs (TBS_{surf}) at each visit using the Megyesi et al. decomposition scoring scale, adjusted to score from zero for fresh bodies.[1] Decomposition scores for the hanging pigs (TBS_{hang}) were obtained using the Lynch-Aird et al. hanging scale.[2] Initially, the semi-recumbent pigs were scored using both of these scales to assess the whole body until it became clear the upper and lower portions of the animals were displaying different decomposition patterns. The upper sections of the semi-recumbent pigs followed the same decomposition pattern as the hanging pigs and were scored using the hanging scale, covering the head, upper limbs, and upper torso, while the lower sections followed the same pattern as the control pigs and were scored using the surface scoring scale, covering the lower limbs and lower torso (the head was not scored), to give Partial Body Score Torso plus Limbs ($PBST_{surf} + PBSL_{surf}$).

The hanging and semi-recumbent upper bodies displayed the same levels of TBS response to ADD, with no statistically significant difference between the two groups (p=0.53, $F_{2,197}$=1,402). The TBS versus ADD responses for the lower sections of the semi-recumbent bodies were compared with the corresponding partial body scores for the controls; there was no statistically significant difference between these two groups either (p=0.8, $F_{2, 197}$=1,157).

Reference(s):

1. Megyesi M.S., Nawrocki S.P., Haskell N.H. (2005) Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains. *J Forensic Sci*. 50(3) pp.618-26.
2. Lynch-Aird J., Moffatt C., Simmons T. (2015) Decomposition Rate and Pattern in Hanging Pigs. *J Forensic Sci*. 60(5) pp.1155-1163.

Taphonomy, Hanging, Decomposition

A44 The Effects of Cranial and Pelvic Asymmetry on Accurate Sex Classification

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After attending this presentation, attendees will better understand how asymmetry impacts non-metric sex estimation methods.

This presentation will impact the forensic science community by examining the potential and actual impacts of the presence, frequency, and type of asymmetries on the classification accuracies of two methods based on cranial and pelvic markers: the original Walker and the Klales et al. methods.[1,2] This presentation will also provide recommendations for the application of these methods in asymmetrical individuals.

Sex estimation is one of the most important aspects of the biological profile, as a reliable estimation of this parameter allows for the application of more accurate sex-specific methods for the estimation of age, ancestry, and stature. Sex estimation still relies to a large extent on non-metric evaluation of the pelvis and skull, as these areas are both strongly dimorphic and geometrically complex, with subtle morphological traits and differences that are hard to capture metrically. By convention, forensic anthropological protocols typically suggest using the left side when estimating sex from bilateral traits; however, if asymmetry is common, this criterion could introduce fundamental biases and compromise accuracies. For example, if the population displays directional asymmetry, by preferentially selecting the left side, a systematic decrease in the classification accuracy for either females or males would occur, depending on whether that side is the one favored or disfavored by the directional trend. Even if the asymmetry does not consistently favor one of the sides, more asymmetrical individuals could be reflecting systemic effects, such as increased developmental stresses, affecting the accuracy of not only one, but both sides of the individual. In this case, the presence of asymmetry should be factored when reporting the corresponding sex estimates, as asymmetrical individuals would be expected to render lower accuracies than reported for the overall method.

An experienced observer blindly collected ordinal score data for the two bilateral cranial traits in the Walker method (Mastoid (M) and Supra-Orbital (SO) margin) as well as for the three bilateral pelvic traits in the Klales et al. method (Ventral Arc (VA), Subpubic Contour (SPC), and Medial Aspect (MA) of the ischio-pubic ramus). Data were also collected for the three unilateral Walker traits in order to test the classification accuracy of the method equations. The left and right sides were scored from a sample of 1,310 individuals (523 females, 787 males) from the Bass, Terry, and Hamann-Todd donated skeletal collections, as well as from the Texas State Operation ID collection.

Asymmetry was observed in 55.6% of individuals in at least one of the two cranial traits and in 59.8% of individuals in at least one of the three pelvic traits. With a frequency of 40.4%, the M was significantly more asymmetrical than the remaining traits ($p < 0.001$), well above SO and VA (31.0% in both cases; $p = 0.734$), while two of the Klales et al. traits, MA and SPC, were the least asymmetrical (27.2%; $p = 0.427$); although the difference between the two pairs SO-VA and MA-SPC ($p = 0.018$) is not significant after Bonferroni/Holms correction.

Even though asymmetries are common, the intra-class correlation coefficient revealed high levels of agreement between the left and right trait scores of individuals (M 0.849, SO 0.859, VA 0.952, SPC 0.984, and MA 0.932) with the vast majority of asymmetries consisting of only one score point difference. Directional asymmetries were detected, with males being right dominant for all traits ($p < 0.05$), while females only displayed directional asymmetries at the 0.05 α-level for the cranial variables, also exhibiting right dominance for the two traits.

As expected from the presence of directional asymmetries, selecting always the left side resulted in some cases in increased accuracies for females, at the expense of lower ones for males. Although, also as expected from the high intra-class correlations, the effect sizes of these between-sex discrepancies in accuracy were basically negligible in most cases (with the notable exception of Walker's equation 6). These results recommend reporting both the presence of asymmetries and the estimates for both sides when asymmetries are present.

Reference(s):

1. Walker P.L. Sexing skulls using discriminant function analysis of visually assessed traits. *Am J Phys Anthropol.* 2008;136:39-50.
2. Klales A.R., Ousley S.D., Vollner J.M. A revised method of sexing the human innominate using Phenice's nonmetric traits and statistical methods. *Am J Phys Anthropol.* 2012;149:104-114.

Asymmetry, Sex Classification, Cranium and Pelvis

A45 Experimentally Derived Protocols for Generating 3D Cranial Surface Scans for Forensic Anthropological Applications

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After attending this presentation, attendees will be familiar with the utility and limitations of cranial 3D surface scans in forensic anthropological contexts, including the need for further development of standard operating procedures.

This presentation will impact the forensic science community by providing an experimentally derived protocol for the use of the NextEngine® Desktop Scanner in scanning cranial elements for forensic anthropological contexts.

The prevalence of 3D laser technologies employed in contemporary forensic anthropological research introduces the need for assessments of accuracy and systemic standardization of these techniques. The NextEngine® Desktop 3D Scanner is the most widely implemented 3D scanning technology within the discipline due to its user-friendly capabilities, relatively inexpensive cost, and resolution; however, at this time there are no published or implemented standard operating procedures for the collection and utilization of 3D scans for forensic purposes. This can have sizable consequences in forensic examinations, as the application of various settings chosen by the user ultimately affects the accuracy of the scan and 3D model. The purpose of this study was to systematically evaluate the effect of various scanning settings and determine the optimal settings for scanning the cranium. The cranium was selected due to its significance and frequented application in forensic anthropological estimations of the parameters of the biological profile, including age, sex, and ancestry.

Using the NextEngine® Desktop 3D Scanner, cranial scan trials were conducted using 8 divisions, 12 divisions, and 16 divisions for each of the following Points Per Inch (PPI) settings: Quick3, SD1, SD3, HD1, and HD3. In total, 15 scans were conducted. All scan post-processing was performed in the associated ScanStudio™ HDPro software. Following completion, the scans were coded to facilitate an unbiased evaluation and an observer was asked to perform a quality assessment by comparing the scans to the actual specimen and ranking a number of features on a scale of 1 to 10, 1 being terrible and 10 being a perfect representation of those features. Comparative analyses of average scan quality rankings, time requirements, and memory demands in relation to implemented scan settings were performed in order to determine those settings that produce optimal scans for forensic anthropological contexts.

The results of the comparative analyses indicate that the optimal settings for scanning the cranium include 12 divisions at the HD1 PPI setting. Time and memory demands for scan procurement proved to correspond highly, and ultimately determined that the HD3 PPI setting was inoperative, as the memory demands were too high for the scan to be reopened after obtained, even when using a high-end graphics computer. The representative accuracy of trial scans was assessed with regard to unique features, taphonomic qualities, and traits associated with evaluations of the biological profile. In none of the scans, regardless of employed settings, did the observer feel confident in assessing cranial suture closure by means of the Meindl and Lovejoy 1985 age estimation method; however, the observer felt comfortable applying non-metric sex and ancestry estimation methods to the cranial scans.[1] Using the optimal 12 divisions, HD1 settings, the observer felt the scan exhibited a suitable representation of surface coloration and suture representation (score 5). In addition, the observer felt able to discern cranial landmarks (score 5.3), muscle markings (score 5.7), nasal aperture shape (score 6), cranial foramina (score 7), and distinct skeletal markings (score 8).

To further evaluate the accuracy of scans produced using the proposed settings, 24 measurements taken on the cranium were compared to those taken virtually on the 3D scan. The average difference in measurements was 0.62mm (*SD* = 0.51mm), equating to an average percent difference of 1.17%. The highest deviation was found in measurement of the orbital height (1.7mm), which resulted in a 4.5% difference; however, the subjectivity in the definition of this measurement may have factored into this discrepancy. The next highest error was obtained with orbital breadth (2.9%). All other measurements had error rates less than 1.8%.

Once established, a standard operating procedure for the use of the NextEngine Desktop® 3D Scanner would

allow further documentation of known error rates in compliance with the *Daubert* ruling and the 2009 National Academy of Sciences (NAS) Report and render them operative practices within forensic anthropological contexts.[2,3]

Reference(s):

1. Meindl R.S., Lovejoy C.O. Ectocranial suture closure: a revised method for the determinations of skeletal age at death based on the lateral-anterior sutures. *Am J Phys Anthropol.* 1985:68(1):57-66.
2. *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.
3. National Academy of Sciences. *Strengthening Forensic Sciences in the United States: A Path Forward.* Washington, DC: The National Academies Press, 2009.

3D Laser Scanning, NextEngine Desktop 3D Scanner, Cranial Scans

A46 Testing Computational Age Estimation Methods Using Laser Scans of the Adult Pubic Symphysis on Modern Hispanic Populations

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After attending this presentation, attendees will have a better understanding of the applicability and effectiveness of three newly published computational methods that focus on 3D laser scans of the pubic symphysis to estimate age at death for individuals of Hispanic identity.[1-3]

This presentation will impact the forensic science community by demonstrating how age at death can be estimated in a more accurate, precise, and objective manner by utilizing fully computational methods and 3D scans of the pubic symphysis on populations of Mexican and Puerto Rican descent. This presentation will also provide recommendations and best practice applications of these standards for forensic casework.

Age-at-death estimation techniques have received considerable attention within the anthropological community, especially among forensic anthropologists, as knowing age can narrow the list of potential missing persons in a forensic investigation. Even though the estimation of age is a crucial parameter of a biological profile, it is also one of the most challenging to attain as it greatly depends on the practitioner's ability to associate age changes with a set of population-specific criteria, usually represented by a series of pre-defined age phases. Along with this issue is the lack of population-specific standards for underrepresented populations in the United States, as the most established of the age-at-death techniques are based on individuals of European and, to a lesser degree, African ancestries from late 19th- and mid-20th-century anatomical collections. In order to address the lack of population-specific methods in age-at-death estimation for contemporary Hispanic casework, this study sourced data from individuals of Mexican and Puerto Rican origin with the goal of testing the newly published framework for age-at-death estimation of Slice and Algee-Hewitt and Stoyanova et al.[1-3]

Slice and Algee-Hewitt and Stoyanova et al. have recently developed objective, fully computational, and statistically robust techniques that offer several advantages over the conventional bone-to-phase matching methods.[1-3] These new techniques utilize coordinates obtained from 3D scans of the pubic symphysis. These data are subjected to numerical shape algorithms and multivariate regression analysis to produce age-at-death estimates. The goals of this study are: (1) to expand these new standards so they can be utilized in populations of Mexican and Puerto Rican origin; and, (2) to produce more accurate and precise estimates of age at death for these populations with reduced error and subjectivity than currently possible using the traditional macroscopic assessment methods.

Data for this study consists of laser scans for both sides of the pubic symphysis from skeletal collections with known age at death housed at the Universidad Nacional Autónoma de Mexico, Institute of Forensic Science in Puerto Rico, and Pima County Office of the Medical Examiner's in Arizona. For each pubic symphysis, 3D scans were created using the NextEngine® 3D Desktop Scanner, 2020i. The resulting scans were aligned and fused in order to generate a multidimensional model of the pubic symphysis. The 3D coordinates representing the symphyseal surface were extracted from each scan and subjected to the Slice Algee-Hewitt (SAH) -Score method, the thin plate splines/bending energy-based method, and the ventral curvature method.[1-3] Multivariate regression models were used to combine the resulting measures and obtain the final age estimate for each individual.

Preliminary results of match-paired *t*-tests find no significant differences between known age at death and inferred age when utilizing a combination of all three methods and when both left and right sides were pooled, such that $0.03 \leq p \leq 0.71$. Furthermore, when left and right sides of the pubic symphysis were tested separately, non-significant results are produced: SAH+curvature for left, p=0.28; BE+curvature for left, p=0.80; BE+curvature for right, p=0.47. Bonferroni corrections were imposed on α=0.05 for multiple comparisons. Pearson correlations between true and inferred ages for all methods and side configurations appeared consistently high (r >0.91), indicating good agreement among values. Results do suggest a tendency, though not significant, to overestimate

known age notably with the SAH+curvature method, generating the greatest mean difference, 9.3 years, for the right side. This study has confirmed that objective and reliable age-at-death estimation can be obtained for populations of Mexican and Puerto Rican descent by the use of computational methods and 3D laser scans of the pubic symphysis.

Reference(s):

1. Slice D.E., Algee-Hewitt B.F. Modeling Bone Surface Morphology: A Fully Quantitative Method for Age-at-Death Estimation Using the Pubic Symphysis. *Journal of Forensic Sciences.* 2015;60(4):835-43.
2. Stoyanova D., Algee-Hewitt B.F., Slice D.E. An enhanced computational method for age-at-death estimation based on the pubic symphysis using 3D laser scans and thin plate splines. *American Journal Of Physical Anthropology.* 2015;158(3):431-40.
3. Stoyanova D., Algee-Hewitt B.F., Kim J., Slice D.E. A Fully Computational Framework for Age-at-Death Estimation from the Adult Skeleton: Surface and Outline Analysis of Three-Dimensional Laser Scans of the Pubic Symphysis. *Journal of Forensic Sciences.* 2016, in review.

Age Estimation, 3D Scans, Hispanic Populations

A47 Recording Total Skeletal Completeness: Introducing a New Approach

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After attending this presentation, attendees will better understand a new standardized and objective method for recording skeletal completeness.

This presentation will impact the forensic science community by providing an improved method for recording total skeletal completeness in osteological analyses of adult individuals. This presentation will also illustrate the value, and provide an example of one possible application, of Computed Tomography (CT) volume-rendering techniques for forensic anthropological research.

Recording the preservation (condition and completeness) of human skeletal remains is the foundation of osteological analyses for forensic and archaeological skeletal material. Guidelines for the recording of skeletal preservation typically include a statement on the total skeletal completeness; that is, how much of the total skeleton is present for analysis. This component of skeletal preservation recording is essential as the quantity of the skeleton preserved determines the extent to which it is possible to develop a biological profile and provide an interpretation about skeletal trauma; however, current osteological methods for recording the total skeletal completeness are non-standardized and subjective. In order to provide practitioners with an objective and standardized means to accurately quantify the total skeletal completeness preserved in an adult skeleton, percentage values for each skeletal element have been established and can be applied to the skeletal remains of any adult individual.

Percentage values for each skeletal element were calculated by establishing the proportion of the volume of each bone relative to the total volume of a complete skeleton. The postmortem CT scan of a young adult male (undertaken with a 128-row helical CT, the SOMATOM® Definition Flash, as part of the Victorian Institue of Forensic Medicine's routine autopsy process) was used. Volume measurements for each skeletal bone and the complete skeleton were generated using Philips IntelliSpace Portal, V7, CT visualization software. The proportion of each skeletal element relative to the complete skeleton, and their subsequent conversion into percentage values, was calculated using basic descriptive statistics.

Percentage values ranged from 0.01% (select hand and foot bones) to 10.83% (cranium). A variety of mediums (visual, written, and an application designed for mobile operating system devices) are provided to ensure the user-friendly calculating of percentages.

Results of this study will provide an accurate and objective means of calculating total skeletal completeness; a component of skeletal preservation recording that is important in medicolegal contexts (to guide recovery operations and provide information about the deceased to their family) and in archaeological contexts (influence the outcomes of research).

Total Skeletal Completeness, Percentages, Volume Rendering

A48 A Study of Modern Commingled Human Remains From a Korean War Recovery Site — Gum Riverside, Junla Province

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After attending this presentation, attendees will better understand two of the most commonly used methods in pair-matching and segregation of commingled remains and their effectiveness when applied to Korean samples.

This presentation will impact the forensic science community by providing results that compare and validate two methods — visual pair-matching and osteometric sorting — as well as demonstrating that the osteometric sorting method can be reliably used as a basic guideline in segregating commingled remains in situations such as mass disasters or archeological sites.

In a situation in which commingled remains are recovered from mass disasters or mass burial, segregating the remains is one of the most fundamental and crucial processes.[1]

The purpose of this study is to analyze the effectiveness of the two widely used methods in pair-matching and segregation of individual remains — visual analysis and osteometric sorting. In this study, samples were collected from the commingled remains recovered from a Korean War recovery site.

Of the 1,969 samples recovered from Gum Riverside in Junla Province, four skeletal elements (femur, tibia, innominate, and humerus) were chosen and the two pair-matching methods were separately applied. Using the visual analysis method, the samples were evaluated by looking at the similarities in bone morphology and taphonomy, and the osteometric sorting, a quantitative method, was used to find possible matches through a statistical evaluation of size similarities between homologs.[2-4] The matched homologs were then compared to DNA analysis of the samples in order to confirm the validity of the methods.

As a result, the concordance rate of the samples pair-matched using the visual pair-matching method to the DNA analysis ranged between 58% and 94%. Specifically, 94% (29/30) of the pair-matched samples were in concordance with the DNA testing result for femurs, 88% for tibias (14/16), 100% for innominates (5/5), and 58% for humeri (7/12).

Using the osteometric sorting method, the pair-matching concordance rate ranged from 83% to 97%; 97% for femurs (29/30), 94% for tibias (16/17), 100% for innominates (5/5), and 83% for humeri (10/12).

The result showed a relatively high concordance rate (94%~100%) for both methods when applied to the femur and tibia; however, the rate was comparatively low (58%~83%) when applied to the humerus. The result indicates that both pair-matching methods are reliable when applied to the femur, tibia and innominate, but not for the humerus; however, the low rate of successful pair-matching of the humerus could be due to the small sample size as well as the samples being highly fragmented, which limited the analysis using the visual and osteometric methods.

Although it is difficult to be conclusive due to the small sample size, the result for the innominate was noteworthy as it exhibited a 100% concordance rate despite its highly fragmented condition. It is hypothesized that distinctive features of the samples, such as sciatic notch and auricular surface, contributed to the result.

Despite a recent study suggesting that the osteometric pair-matching method is unreliable, the conclusion reached through the results from this study is that the quantitative method is adequately utilizable in segregating commingled remains in situations such as mass burial or archeological sites.[5] Also, this study demonstrated that one can expect a positive result even for highly fragmented samples, especially if the samples have distinct features that can be visually evaluated and distinguished. Thus, in sum, the osteometric sorting method has its value in pair-matching commingled remains and would be even more so through some adjustments and when used in combination with the visual pair-matching method.[6]

In this study, the equation of regression using the size of bones and the *t*-score for the comparison of adjoining bones at joints could not calculated because the sample size of the Korean remains collected from Gum Riverside

was not significant enough to apply the statistical method. Through further studies, the calculation of the equation of regression that is suitable for the Korean data by accumulating Korean samples is expected, along with the establishment of a pair-matching method that can be used in combination with the visual analysis method to effectively analyze and segregate commingled remains in various situations.

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Pair-Matching, Comminged Remains, Osteometric Sorting

A49 The New Method of Implementing 3D Scanners and X-Rays on Commingled Remains Recovered From a Korean War Recovery Site

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After attending this presentation, attendees will better understand how to approach the new method of implementing 3D scanners and X-rays in the calculation of Minimum Number of Individuals (MNI) for commingled remains.

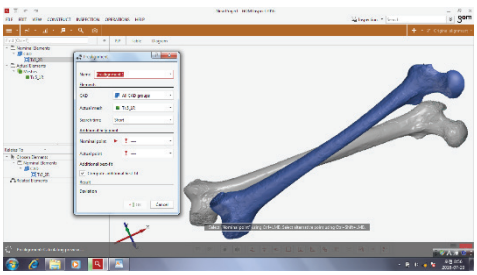
This presentation will impact the forensic science community by providing information on the challenges faced when calculating MNI for commingled remains in mass burial cases.

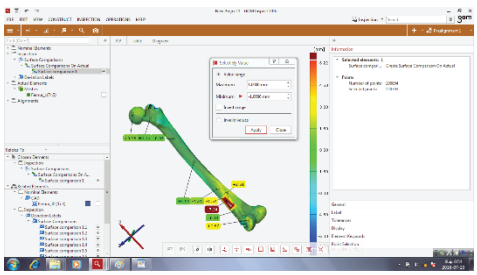
The pair-matching method has been a useful means of identifying each individual from commingled remains. Until this point, physical and metrical (osteometric sorting) analyses were commonly used for pair matching; however, both methods face certain limitations. As physical analysis is subjective in nature, results may differ depending on anthropologists' personal experiences. With metrical analysis, there may be difficulties in applying this method when the bones are either impaired or damaged.

This study will review the effectiveness of employing 3D scanners and X-ray images as part of pair matching to minimize the limitations faced by the more traditional methods. In this study, samples were collected by the Ministry of National Defense (MND) Agency for KIA Recovery and Identification (MAKRI) as a part of their mission to recover Korean War remains in Gum riverside in Junla province, Republic of Korea. The MNI calculation for the physical and metrical analyses of commingled remains recovered was 41 individuals; however, 49 individuals were identified through DNA analysis. The eight individuals who could not be matched using the traditional pair-matching methods were 3D scanned and X-rayed.

Once 3D scanning was completed, an analytic program was used to produce mirror images of the bone elements and compared: (1) to the same bone element of different individuals; (2) to the left and right bone elements of different individuals; and, (3) to the left and right bone elements of the same individual for the degree of superimposition, then deviation was calculated.

To compare the same bone element of different individuals through bone marrow cavity of extent and width, thickness of compact bone, and bone curvature and angle, the bone elements were X-rayed in anterior to posterior and lateral directions.





Through 3D scanning and X-ray analysis, five out of the eight individuals were pair matched, which could not be accomplished using only the physical and metrical analyses. Based on these results, it is possible to infer that X-ray and 3D scanners, when used in combination with the traditional methods such as physical or metrical analysis methods, are effective enough to enhance the accuracy from 84% to 94%. The mirroring technique of the 3D analysis method is especially effective in inferring or confirming the degree of superimposition on damaged or impaired bones, which cannot be seen by physical or metrical analysis methods. X-ray provides valuable information that cannot be gained by superficial examination as it allows for a comprehensive evaluation of internal structures of bone elements. Thus, using 3D scanners and X-ray with pair matching is highly recommended; however, it is necessary to collect additional samples in further studies to truly understand its effectiveness. In addition, the possibility of implementing Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) in pair matching

commingled remains should also be considered.

This presentation will discuss the effectiveness of the use of 3D scanners and X-ray in pair matching commingled remains.

Commingled Remains, 3D, Mass Burial

A50 A Grading System to Assess the Sex and Parity Status for the Preauricular Sulcus: A Step Forward

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After attending this presentation, attendees will better understand the sexual dimorphic variability of the preauricular sulcus in the human adult os coxae.

This presentation will impact the forensic science community by providing additional tests on a new grading system for the preauricular sulcus (p.s.) as a sexually dimorphic trait.

During the 68th AAFS Annual Scientific in Las Vegas, a new grading system for the p.s. to assess sex and parity status was presented. This scoring system provides grades from zero (no p.s. present) to four (large, well-defined p.s.) and was developed on two British Medieval populations. Even though the samples used were archaeological, the collections have been previously seriated (aged and sexed) by using multiple established methods and compared through inter-observer error with the estimations produced by other forensic anthropologists.

In the present research, the proposed scoring system was tested on a modern documented British collection. The samples (*n*=353) are from the St Bride's Church and curated through the Museum of London. For the entire collection, the sex (170 females; 183 males) and age at death of each individual was known. The parity status of 35 females was established by archive research and coffin plates data.

The results confirmed the findings of the previous study, exhibiting a significant difference in the occurrence rates of p.s. in males and females. A p.s. is present in 98% of females and not present in only 2%, while for males, p.s. is only present in 51.4% of cases and is absent in 48.6% of the samples. When the sulcus is detected in males, the grade is very low: 73% have grade one and 27% show grade two. No grade three or four sulcus have been found in males, supporting the hypothesis that the aspect and modifications related to these grades are reliable sexual dimorphic traits. The results also demonstrated that parity status had a significant correlation with the grades of the p.s. and an association with the highest grades (three and four) in the scale provided.

As an additional step forward in the analysis, the effect of pelvic shape on the p.s. morphology was observed. A selection of 68 pairs of coxae that was possible to anatomically articulate with the sacrum was made from the same documented collection. The diameters that describe both the greater and lesser pelvis as well as the measurements that describe the sacrum shape have been recorded with sliding callipers and analysed.

The results reveal that there is a significant correlation between some of the measurements and the morphology of the sulcus. In particular, the transverse diameter, the bispinous diameter in the pelvis, and the maximum sacral width seems to affect the development of a well-defined preauricular sulcus. An interesting finding indicates the direct correlation in females between the increasing of the transverse diameter and the increasing of the p.s. grade. This suggests the needs of future investigations on the link between the p.s. and the process of giving birth.

Considering that pelvic morphometric characteristics are related to both sex and parturition, this study suggests the validity of the new system proposed to describe the p.s. as a tool for the objective description of a trait that is not only a sex-indicator but also a possible parity marker.

With the new test performed on the preauricular sulcus grading system, this study demonstrates the reliability of this instrument as a useful tool for establishing a biological profile of unidentified skeletal remains.

Forensic Anthropology, Preauricular Sulcus, Sexual Dimorphism

A51 Biological Indicators of Aging and Their Application in Individualized Biological Anthropology

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After attending this presentation, attendees will better understand, and gain insight into, the application of biological indicators in age estimation of adult persons on skeletal remains and will become familiar with the limitations of anthropological age estimation techniques that could be expanded with a novel approach that uses biological indicators such as mineral bone density and mitochondrial DNA (mtDNA) for narrowing ranges for age estimation.

This presentation will impact the forensic science community by presenting a novel methodology of personalized biological anthropology that enables narrowing the intervals for age estimation on skeletal remains of adults.

Besides chronological age, biological indicators of aging such as telomeres or GlycanAge Index are often employed to enlighten the aging dynamics. Additionally, an important aging indicator is mineral bone density. The results of this research will be used for the development of this novel approach — personalized biological anthropology, which will use both morphological age indicators on skeletal and mitochondrial haplogroup data in order to more precisely estimate age. In the case of the positive correlation of mtDNA haplogroups and other aging indicators, the biological anthropologist could, after estimating age with traditional methods, use the results of the mtDNA analysis to additionally narrow down the estimated age range. One of the main aggravating factors of the reconstruction of person's biological profile is the fact that anthropologists estimate the age in a five-year range (often even wider) using morphological skeletal indicators. Thus, the main goal of this study is to investigate the correlation of mitochondrial genetic markers with mineral bone density and biological aging indicators.

This research was conducted on a sample that consisted of 969 adults from the island of Korčula, Croatia, as a part of the 10,001 Dalmatians project. Data used in research were basic data (such as age and sex) as well as data about behavioral risk factors previously known to be associated with mineral bone density, which were collected by questionnaire. Mineral bone density was measured on calcanei using a densitometry method with a mobile Dual-Energy-X-ray Absorptiometry (DEXA) Calcsan device. Measurements were conducted on standard bone sites to estimate the density of cortical and trabecular bone. Three additional data acquisition methods were employed: genotpisation, telomere length measuring, and the analysis of N-glycans. Genotipisation was conducted on a venous blood sample. After an isolation using a commercial QIAGEN® kit, the samples were genotyped using the Illumina® CNV370 chip. Telomere length was measured on amplified DNA by Polymerase Chain Reaction (PCR) and the relative ratio of telomere repeats to a single-copy gene was compared with a reference sample. The glycans from plasma were measured using Hydrophilic Interaction Chromatography (HILIC). Glycan analysis was based on solubility measured in glucose units and obtained data were compared to the reference values in Glyco-Base (available on: http://glycobase.nibrt.ie) due to structure determination. Glycans were separated by the number of sialic acid molecules using weak anion-exchange high-pressure liquid chromatography. The analysis was performed using a Prozyme GlycoSep™ C 75mm x 7.5mm column. Linear regression was employed to simultaneously examine the effect of multiple variables and their interaction in the same analytical model.

The results of the mitochondrial haplogroup analysis revealed a total of 120 different haplotypes that were afterward classified into seven haplogroups to facilitate computation. Association of mtDNA haplogroups and target outcomes was not found for the majority of variables, except for the mineral bone density that showed a statistically significant association. Results revealed that persons with haplogroup H have significantly higher mineral bone density values compared to the average, while people with haplogroup HV have significantly lower bone density. Overall, it is shown that there is statistically significant association between mtDNA haplogroups and mineral bone density (additionally controlled for the known factors that influence the mineral bone density — lifestyle, calcium intake, physical activity and sun exposure). On the other hand, a significant association of mtDNA with telomere shortening, N-glycan profile, and the association of the Single Nucleotide Polymorphisms (SNPs) with the named

markers of aging was not found.

Mineral bone density, considered as a valuable method for estimation of age at death on skeletal remains, stands out as a valuable indicator of the biological age of an individual, but also indicates the great potential for the application of personalized biological anthropology. The importance of mineral bone density and mtDNA analysis is even greater because, unlike glycans and telomere analysis, those methods can also be applied to dry bone.

Personalized Anthropology, mtDNA, Mineral Bone Density

A52 The Use of the Palate for Ancestry and Sex Estimation

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After attending this presentation, attendees will better understand the use of the palate in the estimation of ancestry and sex for adult individuals.

This presentation will impact the forensic science community by providing insight into a method for ancestry and sex estimation in adult individuals using metrics of the palate. In addition, this presentation demonstrates the need for further research into the use of palate shape and depth for sex and ancestry estimation.

The estimation of ancestry remains the most difficult parameter of the biological profile and, due to the changing demographic structure within the United States, ancestry estimation is becoming increasingly difficult. Until recently, utilization of the palate for ancestry estimation focused on non-metric traits of palate shape and the dentition itself. In 2015, Maier and colleagues developed three landmarks to measure palate depth and found significant ancestry and sex differences.[1] The goal of the present research is to metrically examine size and shape differences between four groups using Maier et al.'s palate landmarks: (1) Black Males (BM); Black Females (BF); (2) White Males (WM); and, (4) White Females (WF).

A total of 519 dentulous adult individuals of known ancestry, sex, and age were sampled from the Hamann-Todd Osteological Collection housed at the Cleveland Museum of Natural History: 259 Blacks (130 females and 129 males) and 260 Whites (130 females and 130 males). Using a digitizer, one new coordinate was collected, the midpoint between the Central Incisors (CI), as well as three additional coordinates in accordance with Maier et al.: (1) the most posterior aspect of the Incisive Foramen (IF); (2) the Intersection of the median and transverse palatine Sutures (IS); and, (3) the most posterior aspect of the Palate/Posterior (PP) nasal spine.[1] Interlandmark Distances (ILDs) were calculated and subjected to jackknifed Linear Discriminant Function Analysis (LDFA) to explore size differences and classification accuracy between ancestry/sex groups. Next, Geometric Morphometric Analyses (GMA) were conducted to explore shape differences. Classification accuracy was again assessed using LDFA and the Procrustes Coordinates (PCoords) generated from the GMA, as well as the Principal Components (PCs) from the principal component analysis.

Using LDFA of the ILDs, correct classification among the four groups was 50.5%. With the exception of BF-WF (p=0.0106) and BF-WM (p=0.1528), the Mahalanobis Distance (D^2) between the groups was significant at the p<0.01 level. Correct classification between the two ancestral groups (pooled sexes) was 62.8% and between the sexes (pooled ancestry) was 72.3%. Blacks were significantly larger (p<0.05) than Whites for three ILDs except IF-PP and CI-IF, which were not significantly different between ancestry groups, and IS-PP, which was larger in Whites. Males were significantly larger (p<0.05) than females for all ILDs except CI-IF and IS-PP, which were not significantly different between the sexes. Classification between the four groups using LDFA of the PCoords was 52.79%, slightly higher than, but similar to, the size analysis. LDFA classification between the two ancestral groups was 72.5% (pooled sexes) and between the sexes was 82.3% (pooled ancestry). T he first six PCs explained 99.9% of the variance. Using LDFA and these six PCs produced 52.0% classification accuracy between the four ancestry/sex groups. Sex classification was 69.0% and ancestry classification was 62.2% using PCs one to six.

The results of this study show statistically significant differences among the palate of the four reference groups. Classification accuracy for the four groups was highest with the shape analyses; however, the results from the size analysis were comparable. Classification by sex and ancestry independently was highest using LDFA of the Procrustes coordinates. The results from this study are similar to the Maier et al. study examining palate depth in which significant differences existed between Whites and Blacks and between males and females. The combination of size and shape analyses from this preliminary study with the measurement of palate depth, as conducted by Maier et al., has the potential to produce even higher classification accuracies.

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A53 Subaerial Bone Weathering in North Africa: An Experimental Study in Algiers, Algeria

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After attending this presentation, attendees will understand the ways in which subaerial weathering research can enhance estimates of the Postmortem Interval (PMI) in forensic anthropological contexts, particularly in the region of Algiers, Algeria.

This presentation will impact the forensic science community by providing new subaerial weathering data from the Mediterranean region of North Africa, specifically in the region of Algiers, Algeria.

Taphonomy is the study of all of the processes that occur after death until the recovery of an organism and is critical to the reconstruction of forensic anthropological contexts. The study of taphonomic effects on bones, particularly those related to subaerial weathering, sometimes allows for the estimation of the PMI in medicolegal contexts. Scholars have demonstrated that numerous variables, including solar radiation, heat, moisture, and freeze/thaw cycles, affect human skeletal remains over time.[1] Moreover, researchers have developed six Weathering Stages (WS) to describe the degree and extent of osseous changes linked to subaerial weathering.[1] While several studies representing diverse geographic contexts have been undertaken to document subaerial weathering, there have been no studies related to this topic in Algeria, despite the high frequency of forensic casework recovered from outdoor surface contexts there, particularly in urban zones located along the Mediterranean coastline. This study presents WS data generated from an actualistic study conducted in the Algerian capital of Algiers, located in the country's northernmost region along the Mediterranean coast. Algiers is characterized by a mild climate with moderate seasonality (Köppen climate classification (Csa). The mean annual temperature is approximately 18.3°C (65°F) and there is approximately 600mm (24 in) of precipitation annually.

The experiment consisted of depositing fresh, defleshed and fleshed ovine (*Ovis aries)* and bovine (*Bos taurus*) bones on the surface of the grounds of the Institut National de Criminalistique et de Criminologie de la Gendarmerie Nationale Algérienne (INCC/GN) (the National Institute of Criminalistics and Criminology of the National Gendarmerie) in Algiers. All skeletal elements were deposited in June 2014 and observed over a period of two years. Corresponding WS stages were recorded visually and documented with photography.

Regarding the defleshed skeletal elements, WS 0-2 were observed during the two-year observation period. Bones remained in WS 0 for nearly a year, with WS 1 reached after 11 months. WS 2 was reached after 23 months. These results are similar to other regions of the world where WS 1 and WS 2 have been observed 1-2 years after death. For example, on the African continent, scholars observed WS 1 two years after death in Zaire.[2] WS 2 has been observed beginning after two years in Kenya.[3]

Though these results provide some preliminary data on subaerial weathering in Algiers, this research is ongoing. There are plans to continue this study to better understand when and if WS 3-5 appear. Moreover, this study will hopefully be expanded to the southern region of Algeria to document differences in subaerial weathering caused by exposure to the Sahara Desert. Ultimately, this study will contribute to refining PMI estimates for Algerian forensic anthropological contexts.

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Taphonomy, Subaerial Weathering, Algeria

A54 Metric Pair-Matching of Calcanei in Commingled Remains Cases: A Case Study From South Africa

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The goal of this presentation is to highlight the use of osteometric sorting in commingled remains cases. After attending this presentation, attendees will understand the benefits of using metric analysis to assist in pair-matching elements before applying visual assessment.

This presentation will impact the forensic science community by expanding methods for resolving commingled remains cases, ensuring that the individuals are more complete and reducing the potential for mismatched elements. By utilizing osteometric sorting, the number of elements requiring visual pair-matching decreases; this is beneficial for improving time and costs for analyses in the field.[1]

Sorting commingled remains in order to re-associate skeletal elements of the individual has been investigated using both morphological and metric methods. Recent research has been conducted utilizing metric assessment to remove any subjectivity and increase the accuracy and repeatability. Pair matching a left element to its corresponding right element has traditionally been based on visual assessment and relies largely on the experience of the observer.[1-3] Pair matching using quantitative and statistical methods is known as osteometric sorting; this method relies on the ability to "formally characterize normal size and shape relationships" among skeletal elements.[1,2] Osteometric sorting of skeletal remains is possible for a number of long bones (e.g., the femur and humerus) and some smaller bones (e.g., metacarpals). Previous methods utilized for predicting element pairs has reduced the number of potential pairs requiring visual assessment.[1-3]

Research involving the pair matching of tarsal bones is scarce. This study tested the ability to pair match the calcaneus, similarly to Thomas' and colleagues' method, based on metric analysis and utilization of a statistic (M).3 The objectives were: (1) to investigate bilateral asymmetry of the left and right calcanei in the South African colored population for pairing unmatched human remains; (2) to investigate the degree of asymmetry between left and right calcanei within each individual; and, (3) to use the M-statistic to assess applicability for pair matching left and right calcanei.

In this experiment, left and right calcanei from 70 males and 70 females were measured (*N*=280) from the South African colored population group. Two additional re-samplings of the calcanei from 5 males and 5 females (*N*=20) were measured for inter- and intra-observer error analyses. Six measurements of the left and right calcaneus were assessed and the M statistics were calculated for each pair of measurements. The maximum value of M, and 90th and 95th percentiles of M for each variable, were tabulated for use in assessing possible pairs. When attempting to pair match calcanei, the value of M for the possible pair is compared with the tabulated values; if the value of M is greater than the 90th or 95th percentile of M, then the null hypothesis can be rejected. The values of M for males and females exhibited no statistically significant difference between the sexes, therefore pooled M statistics can be utilized to pair match. A test application was performed in which measurements of a left calcaneus were compared to a sample of 140 right calcanei (including the homolog). Values of M were compared to the 90th percentile of M, 95th percentile of M, and the maximum value of M; this resulted in a reduction in the number of potential pairs requiring visual pair matching up to 90%, 93%, and 74%, respectively. Further analyses will compare this data to other population groups and the use of pooled values of M will be explored. These results may have the potential to assist in the re-association of individuals from commingled remains cases, no matter the population group nor the level of admixture of the sample.

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Forensic Anthropology, Osteometric Sorting, Commingled Remains

A55 A Validation Study of the Mandibular Canine Index Method of Sexual Assesment Using Two American Populations

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After attending this presentation, attendees will be informed concerning a dental sexing method developed from a South Indian population and its application to American populations. Attendees will understand that further investigation is needed in the application of this method.

This presentation will impact the forensic science community by acknowledging the idea that not all sexing techniques are useful for all populations, yet specific techniques are still applied too broadly. This presentation will present a validation study of a sexing technique on an American population in which the technique has not previously been implemented.

The analysis of biological sex is important to the forensic community during any investigation of an unknown decedent. Due to the fact that human remains and especially skeletal remains may be incomplete, it is important to create, and also test, sex assessment methods on as many skeletal elements as possible in case the more traditional methods cannot be used.

In 1989, Rao and colleagues studied canine measurements in South Indian male and female populations.[1] Using the data they gathered, the Mandibular Canine Index (MCI) was created: the mesio-distal length of the mandibular canine crown, which was then divided by the width of the mandibular canine arch length (that is, the distance between the left and right canine cusp tips measured at the midline). Males were found to have a significantly higher mandibular canine index than females.[1] Since the Rao article was published; more studies have been conducted in the South Asian populations. Some of these studies have come to the same conclusion as Rao and colleagues, while others have found no significance between males and females using this method.[2] Issues have been found with the mandibular canine index technique, including the fact that teeth that are not in correct occlusion can affect the results.[3] Further research on populations from different regions may clarify the benefits and issues of this method.

The purpose of this current study was to test the effectiveness of the mandibular canine index for sex discrimination in modern North American White and Black populations. Twenty-one individuals from the C.A. Pound Human Identification Laboratory archived collection were measured in addition to 24 individuals from the Wichita State University donated collection. The measured sample consists of 23 females and 22 males. The two ancestry groups were pooled due to the lack of significant differences between the two groups (p value of MCI = .64 for females and .34 for males). The individuals were chosen based on having a known identity and having full dentition that was in correct occlusion. Individuals with dental pathologies or missing teeth were excluded. The maximum mesio-distal lengths of both mandibular canines and the canine arch width were measured using sliding calipers. The MCI was calculated using the left canine. A two sample t-test was completed to determine if there were any significant differences between the male and female canine measurements.

On average, females had significantly smaller canine mesio-distal lengths than males (p = .04 for right and .002 for left). Female right canines averaged 6.68mm compared to the male canines that averaged 7.05mm. Left canines were slightly larger than right canines. Female left canines were 6.7mm while the men's were 7.18mm. Males also had large canine arch widths, with an average of 29.62mm to the females 27.32mm (p=.01)

The mandibular canine index showed no significant differences between males and females. Using either the right or left canine in the MCI ratio leads to females having a slightly larger mean of .25 compared to the males .24 (p=.48 for right and p=.83 for left.)

Due to the small sample size, only tentative conclusions can be made. From the above results it appears that the Mandibular Canine Index may not be an adequate tool for sex analysis. The mesio- distal lengths and the canine arch widths may be better tools. Further testing is needed.

Reference(s):

1. Rao, Nageshkumar G., Nirmala N. Rao, M. Lakshman Pai, M. Shashidhar Kotian. 1989. Mandibular canine index—a clue for establishing sex identity. *Forensic Science International.* 42(3): 249-254.

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Sexual Dimorphism, Mandibular Canine Index, Anthropology

A56 Identity by the Numbers: Cancerous Lesions and Likelihood Ratios

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After attending this presentation, attendees will better understand how the frequency of skeletal cancerous lesions can contribute to a correct identification using a likelihood ratio framework drawn from Bayesian theory.

This presentation will impact the forensic science community by further demonstrating the utility of likelihood ratios when identifying an unknown individual. Additionally, the likelihood ratio generated in this study can be used multiplicatively with likelihood ratios derived from other parameters of the biological profile, thereby increasing the probability of a correct identification.[1]

The use of Bayesian theory has been gaining popularity within the forensic anthropology community for its ability to model the way in which decisions are made based upon varying levels of confidence. Through the evaluation of evidence in court, participants unknowingly utilize Bayesian reasoning when determining the weight of evidence while considering the legal proceedings and their personal biases.[1] As prior probabilities representing attitudes and effects of evidence on the jury's decision cannot be determined realistically, it is impossible to create posterior probabilities representing the weight of anthropological evidence. Therefore, this study instead focuses on the generation of a likelihood ratio representing the strength of macroscopic skeletal cancer when making an identification from a closed population of known antemortem records.

This project consists of skeletal analysis of a sample of adult individuals from the Bass Donated Skeletal Collection (BDSC) who self-reported as having cancer at the time of death or having had a previous cancer diagnosis (n=337). A preliminary randomized sample of these individuals (n=75) was initially analyzed for the presence/absence of macroscopic lesions and is the focus of this study. The BDSC was then used to represent the "population at large" (total n=1,432) from which the likelihood ratio numerator was drawn, this equation having been modeled using Steadman et al.'s likelihood ratio for skeletal pathology.[1]

Data collection involved physical examination of all skeletal elements for signs of primary bone cancer or secondary metastases. Of the 75 individuals in the preliminary sample, 16 exhibited macroscopic cancerous lesions (21.3%). Assuming this frequency remains constant to the subset of all individuals who reported having or having had cancer, 72 out of the 337 would be expected to show visible lesions in the skeleton. Thus, the expected likelihood ratio of visible cancerous lesions from the BDSC is 1,432/72, or 19.9. These results indicate that if a possible identification of an individual within a closed population matches the BDSC demographic, has a medical history indicative of cancer, and exhibits visible cancerous lesions, then it is 19.9 times more likely that they have been correctly identified than not. This preliminary likelihood ratio only represents a single category of pathology and is intended for combination via the product rule with other independent likelihood ratios representing additional parameters of the biological profile.

Although this study is limited by scope and composition of the BDSC (primarily middle-aged to elderly individuals), this presentation provides a further proof of concept for the generation of likelihood ratios representing pathological conditions and how they can be used to influence the quantitative probability of making a correct identification, even if DNA evidence is not available.

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Forensic Anthropology, Identification, Cancer

A57 The Application and Accuracy of 3D Surface-Scanned Postcranial Bones

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The goal of this presentation is to provide attendees with an understanding of the complexity of 3D surface scanning of human remains, the principles of osteological scanning methods, the necessary considerations for successful scanning, and the current applications and limitations of a mid-range surface scanner.

This presentation will impact the forensic science community by highlighting current research assumptions and areas of necessary testing and by addressing the difficulties of osteology replication via surface scanning using the NextEngine®, a popular and economical tabletop scanner.

3D scanning provides forensic anthropologists with permanent records of human remains that can be shared globally and analyzed in innovative ways on 3D platforms with 3D tools. To utilize this growing technology in a scientifically meaningful way, set standards need to be implemented.

The objective of this research is to determine whether standardized surface scanning methods can accurately record skeletal characteristics for use in display, teaching, or research applications. Five categories of skeletal traits were selected: (1) gross morphology; (2) rugosity; (3) non-metric variation; (4) pathology; and, (5) trauma. These aspects were selected because of their effect on analyzing age, sex, and health. Parameters set to evaluate the scanning success for this study required 100% similarity in all categories to be used in research, 75% similarity to be used in teaching laboratories, and a 50% similarity to be used in display. The postcranial bones selected for this sample were from the ancient Maya trading site Moho Cay, Belize (AD 600-800), which was situated at the mouth of the Belize River. Although well-preserved, the human remains excavated from Moho Cay are fragile due to cyclic wet and dry seasons.

3D surface scanning the remains created a durable record of the Moho Cay Maya. Bones from nine adult males were selected for this study, including: (1) the sternal end of an unnumbered left rib; (2) a left ulna; (3) a lumbar vertebra; (4) a left clavicle; (5) a right scapula; (6-7) a left and right radius; and, (8-9) a left and right humerus. The selection included large, medium, and small sizes and complex and simple geometric shapes. All of the selected bones could be evaluated for rugosity, pathology, and trauma.

Scanning was carried out in the Louisiana State University (LSU) Digital Imaging and Visualization in Archaeology (DIVA) Lab. A trial scan was performed first, which began at the lowest division (lowest number of individual laser scans) and then increased in number of divisions until the complete geometry of the trial bone was captured. The method was then applied to all nine postcranial bones.

The bones and the 3D digital scans were separately evaluated according to the five categories of gross morphology, rugosity, non-metric variation, pathology, and trauma. Categorically, rugosity was the only trait with a 100% similarity. Gross morphology, non-metric variation, and trauma showed mid-range success but did not rate above 90% similarity. Pathology was the lowest categorical similarity, rating at only 11%. Using the parameters of similarity set up for this study, all of the 3D scanned bones could be used in display, approximately 50% of the bones could be used in teaching, and none of the digitized bones were precise enough to be used in research. Suggestions for improving precision in 3D surface scanning of skeletal material are discussed, including increasing the number of individual laser scans (rotations), increasing the point density for scanning, and adding macro-scans of bone features.

3D Scanning, Osteology, Digital

A58 An Exploration of the Variation in the Sternal Rib Ends of Infants

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After attending this presentation, attendees will understand the gross morphological variation of the developing human rib, in particular at the Costochondral Junction (CCJ) of infants up to one year of age.

This presentation will impact the forensic science community by providing insight into sternal rib morphological variation for infants between 0 and 12 months of age in order to improve the accuracy of fracture identification on the costal surface of the CCJ using macroscopic analysis.

While multiple studies have looked at fracture patterns in infants, little research has been conducted focusing on the topography of the anterior rib at the CCJ. Remote fractures at this location are difficult to identify due to the lack of data on morphological variation during development in normal infant ribs. This study examines the gross morphology of infant rib costal surface at the CCJ in order to establish what features are normal, likely the result of human development, and what may result from other factors, such as remote trauma and possibly pathological changes.

To address this goal, this study reviewed a total of 73 sternal rib ends from 39 individual cases ranging from the ages 0 to 12 months old at the Harris County Institute of Forensic Sciences (HCIFS). All cases but one received Cardiopulmonary Resuscitation (CPR). Case data on age and whether or not remote trauma was observed elsewhere on the skeleton was collected from the Infant Injury Database housed at the HCIFS. Traits on the costal face observed included pits, crevices, and bony spicules. Pits were defined as areas on the bone surface where there were holes or depressions with distinct, relatively sharp edges. Crevices were defined as areas on the costal surface where the bone folds over or into itself. Bony spicules were defined as outgrowths of bone on the costal face that were texturally distinct from the rest of the surface that disrupted the topography and protruded at sharp angles. All characteristics were scored according to presence or absence. Additionally, the number of pits present on the costal surface was tallied.

Pits were found on 57.5% of the ribs. The majority of ribs that had pits had more than one pit present on the surface (43.8% of the total cases). Most pits were found on ribs with ages of 0 to 3 months (76.2%). The correlation between age and the presence or absence of pits approached significance (X^2=16.479, *d.f.*=10, p=0.087); however, there was no correlation between presence of pits and the presence of remote trauma on the skeleton (X^2=0.190, *d.f.*=1, p=0.663). Crevices were found on only 37.0% of ribs. The presence of crevices was not correlated with age (X^2=14.174, *d.f.*=10, p=0.165). Crevice presence also did not correlate with the presence of remote trauma on the skeleton (X^2=0.220, *d.f.*=1, p=0.639). Finally, bone spicules were observed in 28.8% of the cases. The presence of bone spicules was also not correlated with age (X^2=12.157, *d.f.*=10, p=0.275) or with remote trauma on the skeleton (X^2=0.271, *d.f.*=1, p=0.602).

The results suggest that the presence of pits, crevices, and bone spicules are likely part of normal human developmental variation for this age group. The large amount of gross morphological variation observed on the costal face may limit the ability to diagnose subtle remote fractures or fracture healing on the costal surface of the CCJ macroscopically. This study therefore recommends the use of caution in diagnosing subtle remote fractures at the costal surface of the CCJ using macroscopic analysis based on these variants in topography when lacking other definitive indicators of trauma.

Infant Osteology, Rib Morphology, Trauma Analysis

A59 Reciprocating Saws as Tools of Dismemberment

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After attending this presentation, attendees will be familiar with class characteristics of sharp force trauma caused by reciprocating saws, specifically those characteristics most affected by variations in blade type. The results of qualitative analysis of sharp force trauma inflicted by a reciprocating saw with interchangeable blades will be presented.

This presentation will impact the forensic science community by demonstrating the effects of reciprocating saws, which may be used in cases of dismemberment. This presentation will add to previous research by broadening the understanding of a different type of mechanical saw and its effects on bone.

The analysis of sharp force trauma characteristics to bone is undertaken to determine the class of implement used to inflict the trauma. Specific features of kerfs vary based upon saw blade properties, including the morphology of the blade teeth, how the saw is powered, and how it is wielded in reference to the bone. The present study focuses on the characteristics created by reciprocating saws, which previously have not been investigated in detail. The hypothesis tested is that reciprocating saws have unique class characteristics that can be used to differentiate them from other classes of saws, and that unique blade configurations used on reciprocating saws can be differentiated from each other.

Six saw blades were used in the study: five types of reciprocating saw blade with varying numbers of teeth-per-inch and one hacksaw blade for comparison with a common hand-powered saw. Partially fleshed hind and forelimbs of white-tailed deer (*Odocoileus virginianus*) were utilized as a proxy for human remains. A minimum of ten complete kerfs and two false-start kerfs were made using each saw blade on the remains. In addition, three partially fleshed cervical vertebrae segments of white-tailed deer were sawn a variable number of times by the reciprocating blades of the lowest and highest teeth-per-inch, as well as the hacksaw blade. Following sawing, the remains were further defleshed using scalpels, macerated, dried, and then placed in a dermestid colony to remove any residual soft tissue.

The remains were examined macroscopically and microscopically for a suite of class characteristics as denoted in Symes and Symes et al.[1,2] Features examined include: kerf floor shape, minimum kerf width, blade drift in kerf, cut surface drift, exit chipping, cut consistency, tooth imprint, entry flare, kerf flare, bone islands, entrance shaving, pull-out striae, energy transfer, harmonics, and striation regularity/visibility. The features were described qualitatively and/or scored as present/absent, with the exception of minimum kerf width, which was measured using digital calipers.

Qualitative analysis identified several key characteristics that varied based upon blade type and power source: size and location of exit chipping, cut surface drift, striation regularity/visibility, and energy transfer. Kerfs created by the hacksaw blade were readily differentiated from those made by the reciprocating saw blades. Hacksaw kerfs had minimal exit chipping and little cut surface drift, demonstrating a consistent blade path. The kerf walls were deeply etched, with readily visible striae that varied in regularity throughout the sawing event. The kerfs did not show any evidence of energy transfer (i.e., polishing of the cut surface).

The reciprocating saw kerfs varied based upon blade type but overall displayed lesser blade control and less visible kerf wall features. Kerfs showed relatively larger exit chipping and across a greater degree of the kerf edge. Chipping varied in size based on the Teeth Per Inch (TPI) of the blade but was found both externally and within the medullary cavity of the element. Interior exit chipping was not observed in the hacksawed bones. The reciprocating saw kerfs also displayed cut surface drift, as the path of the blade was not consistent. The striae were irregular and decreased in visibility with lower TPI blades. The kerf walls were highly polished, demonstrating extensive energy transfer.

In conclusion, while the degree of expression of these characteristics varied based upon blade, reciprocating blades can be confidently differentiated from hand saws. Furthermore, this research provides data regarding varied kerf characteristics for different reciprocating blades.

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2. Symes S.A, Chapman E.N., Rainwater C.W., Cabo L.L., Myster S.M.T. 2010. *Knife and Saw Toolmark Analysis in Bone: A Manual Designed for the Examination of Criminal Mutilation and Dismemberment.* Report, National Institute of Justice, Grant 2005-I-J-CX-K016.

Sharp Force, Trauma, Reciprocating Saws

A60 Assessing the Accuracy of Historical Associations in a Commingled Assemblage

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After attending this presentation, attendees will understand the complexities associated with massively commingled historical assemblages of human remains.

This presentation will impact the forensic science community by identifying the extent of commingling that resulted from the application of legacy data and methods and how the Defense POW/MIA Accounting Agency (DPAA) laboratory is developing new strategies for resolving this issue.

Commingled assemblages present specific challenges for identification. In order for the medicolegal community to render individual identifications, remains must be segregated from the assemblage by medicolegal authorities into discrete units, representative of a single missing person. The complexity in achieving individual identifications from commingled settings is driven by the number of fatalities, the nature of the incident, and myriad postmortem processes that affect the provenience of recovered remains.

The commingled remains of the U.S.S. *Oklahoma* are believed to represent 388 individuals. These remains were recovered from the day following the attack on Pearl Harbor to May 10, 1944. After initial burial in two cemeteries on the island of Oahu, HI, the remains were disinterred in 1947 and attempts at individual identifications were made. Two years later, without successful individual identification for the majority of the remains, the American Graves Registration Service (AGRS) decided to consolidate the remains for a single group burial, and further commingled the remains by placing "like" elements together in caskets. When the group burial recommendation was denied by the United States Army Quartermaster Corps, the parent agency with oversight of the AGRS, an attempt was made to segregate the remains into individuals using articulation, morphology, age, color, texture, and/or proportion. Failing to segregate the remains adequately for identification, they were all reburied as unknowns, but this time individually divided into "bundles" of the most parsimonious associations.

Based on historical documentation, it is assumed that placement of skeletal elements in a single bundle was based on the belief that these elements represented a single individual. To test the accuracy of historical pair matches and associations, and to better understand the extent and patterning of commingling, a sample of 25 "paired" humeri and 28 "paired" tibiae were examined; followed by an assessment of 47 historical associations of upper (humerus) to lower (tibia) long bones. Only those complete or nearly complete humeri and tibiae that were placed together in a bundle and for which there is currently a mitochondrial DNA (mtDNA) sequence available were considered in this research. Mt DNA sequences were used to confirm or refute a potential pair match.

Of the 25 historically paired humeri in the test sample, 11 (44%) pairs were correctly determined and 13 (56%) pairs were incorrectly matched. For the 28 pairs of tibiae, 13 (46%) were correctly paired, and 15 (54%) were incorrectly paired. Comparing the association of humeri and tibiae within a single bundle, 3 (6%) were correctly associated to the same individual based on mtDNA sequence, while 44 (94%) were incorrectly associated. The total number of humeri and tibiae inventoried from segregated bundles are: left humeri, n=278; right humeri, n=282; left tibiae, n=296; and right tibiae n=304.

Testing the legacy assumptions provides insight to the extent of the problem to be addressed by the DPAA Laboratory today. If initial associations proved correct more often than chance, then more weight would be placed on the legacy association than not. As a result of this initial assessment, the bundles of most parsimonious associations made in 1949 appear to be better than chance alone, but there is enough error to warrant subsequent comparisons across the entire assemblage. Furthermore, it should be noted that correct associations of nearly 50% for pair matching, given the number of possible pairwise comparisons to be made with around 300 elements from each side, may represent a best-case scenario for pair matching in a historical sense.

These data indicate legacy pair matches and associations based on historical methods must be reexamined, and they highlight the need for incorporating osteometric and DNA data into the segregation process, particularly in large-scale commingling cases such as the U.S.S. *Oklahoma*. Further research is being conducted to incorporate

all available data types that will not only permit sorting and data-filtering capabilities, but will also permit front-end applications for applying algorithms to execute the complex computations required to cluster 10,000 skeletal elements into nearly 400 individuals.

Commingling, Historical Pair Matches, U.S.S. Oklahoma

A61 An Age-Based Approach to Establishing Minimum Number of Individuals (MNI) in Commingled Juvenile Skeletal Material

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After attending this presentation, attendees will be informed of a step-by-step approach to constructing an age-based MNI from durable age-informative elements. Attendees will understand the situations in which the application of the method is warranted, as well as any limitations inherent in this approach.

This presentation will impact the forensic science community by providing an additional tool for assessing MNI in commingled assemblages, particularly in situations such as mass graves or mass disasters in which there may be an unknown number of young individuals of varying ages and states of skeletal preservation and recovery.

The goal of this presentation is to describe a systematic, replicable, and defensible approach to computing an age-informed MNI from commingled assemblages containing a large proportion of juvenile remains. This presentation will end with two case studies that demonstrate how this approach can provide a more accurate picture of burial demographics.

Incorporating age information into MNI is a common strategy for reducing the downward bias inherent to this estimator.[1] Such an approach is especially helpful with juvenile remains, as age estimation is more precise for younger individuals, but elements are more fragile and poor preservation may inhibit other approaches. The method presented here was developed during the analysis of two large archaeological burial features from the Lower Illinois River Valley, Helton 20-36 and Carter 2-15; however, this method is structured to be applicable and replicable in diverse situations, including modern mass graves or fatalities.

The goals of this method are replicability, utility in analyzing assemblages with poor preservation or recovery, flexible age categorization, and reduction of the downward bias of the traditional max(L, R) method of MNI calculation in which the MNI is computed as the largest number of a single element from the same side of the body.[2] The resulting procedure differs from many other age-based MNI approaches in that it focuses primarily on the dentition and uses flexible rather than fixed age categories. This approach could theoretically be useful for practitioners in legal settings as it produces a well-documented and supported MNI that can be presented in a way easily understood by laypeople.

The age-based MNI is calculated using the following procedure: (1) an age- and sex-informed MNI is calculated for all adult elements; (2) age at death is estimated for all maxilla and mandible fragments using associated teeth; (3) maxillae and mandibles are paired based on age matches; (4) age is estimated for each unassociated (loose) tooth; (5) unassociated teeth are placed with paired maxillae and mandibles based on age and absence of that tooth, tracking the age of each set of dentition as teeth are added; (6) any remaining teeth are grouped into as few units as possible based on age; and, (7) additional elements are used (e.g., the petrous pyramid) to ascertain any missing age categories, such as infant or fetal, as well as to provide support for the age ranges constructed from dentition.

For Helton 20-36, the max(L, R) MNI was 16 and the age-based MNI was 20. In Carter 2-15, the max(L, R) MNI was 10 and the age-based MNI was 13. Constructing an age-informed MNI made it possible to more accurately assess both the size and demography of the features by including age categories that would have otherwise been overlooked. In a forensic scenario, this would mean establishing the presence of more individuals whose remains could then be individuated through other means, such as DNA testing.

Reference(s):

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Commingling, Juvenile, Minimum Number of Individuals

A62 Accuracy in Osteometric Reassociation: Comparing Geometric Morphometric Landmark Data and Linear Measurements

Kyle A. McCormick, PhD, 1561 Wilhelmina Rise, Honolulu, HI 96816*

The goal of this presentation is to inform attendees regarding accuracy rates for resolving small-scale, closed-population commingled assemblages using either geometric morphometric landmark data or linear measurements.

This presentation will impact the forensic science community by comparing the performance of two forms of bone size/shape quantification in resolving commingling.

Commingled assemblages present a common situation in osteological analysis in which discrete sets of remains are not readily apparent, thereby hindering biological profile construction and the identification process. Of the methods available for resolving commingling, osteometric sorting is reliable and relatively objective.[1] Traditional osteometric sorting methodology is a decision-making, error-mitigation approach, in which possible matches are eliminated if the accompanying *p*-value exceeds an analyst-defined threshold.[1] Elements are reassociated if all other possibilities are eliminated and the assumption of a closed-population is met; however, recent research has demonstrated that commingled remains can be accurately reassociated in small-scale assemblages using geometric morphometric landmark data in a predictive framework.[2]

The primary goals of the current study are twofold: (1) examine the accuracy (as assessed through correct classification rates) of a predictive framework for reassociation; and, (2) compare two forms of data for quantifying long bone morphology — geometric morphometric landmark data and linear measurements.

To accomplish these goals, landmark data from 208 individuals and linear measurements from 435 individuals were analyzed from the William M. Bass donated skeletal collection. Raw landmark data were fit using generalized Procrustes analysis to extract log-centroid size and Procrustes coordinates. Procrustes coordinates were subjected to partial least squares analysis to extract relevant components. Ten individuals were randomly removed from the total sample, acting as a small-scale, closed-population commingled assemblage. One element was chosen from the commingled assemblage as the independent variable, with the ten possible matching elements representing the dependent variable. Using the remaining total sample, Bayesian regression via Hamiltonian Markov chain Monte Carlo was used to estimate a range of possible dependent variable values. These values were smoothed into a probability density function using kernel density estimation and the ten possible matches were evaluated against this distribution to calculate predictive probabilities. The element with the highest predictive probability was considered the best match. This process was repeated 1,000 times for femur antimere comparisons for both linear measurements and landmark data.

Matches were correctly classified for 78.2% and 93.2% of the commingled assemblages, using landmark data and linear measurements, respectively. These results suggest that bones can be accurately reassociated using the predictive framework, without the need to eliminate all possible matches. Linear measurements performed markedly better than landmark data for resolving commingling, demonstrating that linear measurements contain ample information for predicting correct matches. Furthermore, the ease of data acquisition and analysis of linear measurements in comparison to landmark data make the former a better choice for resolving commingling.

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2. McCormick K.A. 2016. Osteometric Reassociation Through Quantifying Long Bone Size and Shape and Prediction Using Bayesian Regression Via Hamiltonian Moarkov Chain Monte Carlo (MCMC). *Proceedings of the American Academy of Forensic Sciences*, 68th Annual Scientific Meeting, Las Vegas,NV. 2016.

Commingling, Osteometrics, Bayesian Modeling

A63 Performance Assessment for Osteometric Pair-Matching

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After attending this presentation, attendees will understand how to measure the performance of a forensic test method and will understand what to expect from osteometric pair-matching.

This presentation will impact the forensic science community by providing attendees with error rates that can be used to understand results in the laboratory.

Byrd and Legarde provided osteometric methods for testing the association of paired bones when resolving a commingled assemblage.[1] The overall approach utilized a significance test with a null hypothesis which states that two bones are of a size to have originated from the same person. The reasoning is counterfactual in nature, as in: If one sampled left and right femora of individuals within a random sample of the relevant population and applied this test to each individual, the test would rarely reject the association. This approach does not provide a means to prove that two bones must have originated from the same person. To do that, one must employ a second line of counterfactual reasoning: If one sampled left and right femora of individuals within a random sample of the relevant population and applied the test to bones from different individuals, one would rarely accept the null hypothesis. The first line of reasoning is a straightforward significance test whose efficacy in future applications can be easily evaluated. The second line of reasoning is not straightforward — its veracity will be determined by the circumstances in which the method is applied. Specifically, the diversity of sizes among the commingled individuals will drive how rare it will be to accept the null hypothesis.

Byrd and Adams recommended using $p<0.10$ as a standard for rejecting an association of two bones.[2] This standard was intended to balance power against risk in applications. It is not intended to be a draconian cutoff, and analysts are encouraged to use the attained *p*-value to evaluate the association. As a rubric for evaluating performance, one can view a null hypothesis rejection ($P<0.10$) as a "positive" result and acceptance as a "negative." The False Positive Rate (FPR) is the rate at which associations that actually come from the same individual are rejected. When applying the Byrd-Legarde paired whole bone models (six bones) to DPAA reference data, the FPR ranges from 0.05 to 0.20, with a mean FPR of 0.10. Acknowledging that with $p<0.10$ as a standard, one can expect to attain variation in the FPR, these results largely meet expectations. One bone, the ulna, has an FPR (0.20) that is significantly higher than expected (one-sided binomial test $p=0.00083$). Vickers et al. applied the Byrd-Legarde models to the Forensic Databank (FDB) and attained very similar results (mean FPR=0.10) to include higher error with the ulna.[3] LeGarde applied a larger variety of models (12 models from three bones) to the Bass Collection and found the FPR to range from 0.07 to 0.13, with a mean FPR of 0.10.[4] She also applied the Byrd models to the collection at Chiba, Japan, and attained an FPR range 0.04 to 0.15, with a mean FPR of 0.09.

One cannot directly address the second counterfactual given above because it is difficult to project what variation in body sizes will hold in future forensic cases; however, one can use the size distribution of the reference sample as a proxy. A sample (N=1,000) of the right and left long bone measurements in the reference sample were randomly paired for comparison and any instances of the same individual removed from the analysis. This yielded a large number of random pairings of bones from different persons. These comparisons provided a basis for assessing false negatives along with other possible outcomes. With these random comparisons combined with the above metrics, the false discovery rate (Q_{actual}), sensitivity, specificity, positive predictive values, negative predictive values, and efficiency were estimated. The results from the six whole bone models (DPAA reference data) are in simple terms: Q_{actual} 0.01-0.03; sensitivity 0.67-0.84; specificity 0.80-0.95; positive predictive value 0.97-0.99; negative predictive value 0.17-0.52; and efficiency 0.69-0.86. The meaningfulness of these results will be determined by how representative the size distribution of the reference data is. Under strong assumptions about the size distributions of future cases, it is possible to project the future performance of these models.

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1. Byrd J.E., LeGarde C. Osteometric Sorting. In: Adams B.J., Byrd J.E., editors. *Commingled Human Remains: Methods in Recovery, Analysis, and Identification.* Amsterdam: Academic Press, 2014:167-192.

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4. LeGarde C. Asymmetry of the humerus: The influence of handedness on the deltoid tuberosity and possible implications for osteometric sorting. (Thesis). Missoula (MT): The University of Montana, 2012.

Performance, Osteometric, Statistics

A64 Mentoring by Example: Lessons in Cremation Analysis From the Forensic Case Files of Walter H. Birkby, PhD

Thomas Michael Fink, MA, BioArch LLC, 2959 N 68th Place, #115, Scottsdale, AZ 85251-6872*

After attending this presentation, attendees will appreciate the skill and knowledge of Dr. Birkby in the field of cremation analysis and the example he set for his students.

This presentation will impact the forensic science community and the field of forensic cremation analysis by demonstrating that: (1) heavily fragmented cremains can still be identified, especially when examined by a highly skilled analyst such as Dr. Birkby; and, (2) when plying their skills in police cases, forensic anthropologists may have to contend with politically charged, high-profiles cases.

Attendees of this presentation will understand that the foundations of forensic anthropology lay, as with Dr. Walter H. Birkby, in "mentoring by example." Students learned by observation — not only the methods for studying human remains but the subsequent presentation of the results. This reliance on training by example, rather than on textbooks, epitomizes the formational stages of forensic anthropology.

Birkby often cautioned his students about making overreaching inferences from osteological data, saying, "Don't say anything in writing that you couldn't defend on the stand." Practical lessons in human osteology were often emphasized through similar, often humorous, sayings known as "Birkby-isms." These phrases captured the essence of the principles behind lessons taught by example.

While the presenter worked and studied with Dr. Birkby from 1975 to 1980, he learned from Birkby's emerging interest in the analysis of cremated and calcined bone. Birkby became an acknowledged expert in the study of human cremains and it was through him the presenter acquired a major interest in this mode of burial.

When it came to cremation analysis, Birkby said: "You get out of cremations what you put into them." His meticulous approach to cremains is well exemplified in one of his early forensic cases and represents a unique and important study. Unfortunately, it went unpublished.

The case dates to the late 1960s when a male member of the Black Panther Organization in the San Francisco Bay area was reportedly shot at a rural training facility. As the ground was rocky, the perpetrator(s) could not bury the body and, instead, burned the remains for several hours on a pile of redwood branches, repositioning the body parts to facilitate cremation. Once the fire had consumed the corpse, the calcined skeleton was broken up and the fragments were scattered on the side of a hill. The bony fragments remained *in situ* until 1971 when an informer, who may have assisted in the disposal, told local authorities what had happened and where the individual's remains could be located. A search of the scene revealed a number of personal items along with bone fragments.

The California authorities requested Birkby's help in examining the cremains and, in the summer of 1971, the calcined bone was sent to him for study. As the authorities knew the probable identity of the deceased, they also sent antemortem radiographs of his head and chest. After working for several weeks identifying and reconstructing fragments Birkby was able to identify several points of similarity between bony features in the cremains and those in the radiographs. The similarities included age and sex, mandibular and frontal sinus morphology, and a lambdoidal ossicle.; however, and most importantly, was the remnant of a healed surgical wound in the reconstructed fragments of the right temporal bone that matched a defect present in the decedent's radiographs. Birkby was able to extract from these burned and scattered bones the critical elements needed to cinch the case. Positive identification was later officially established by California authorities. The likely perpetrator was, himself, killed in 1972.

This case had significant impact on the direction of forensic anthropology. First, it proved that burning a body did not prevent identification when faced with someone of Birkby's skill and dedication. Second, this case showed that experts, like forensic anthropologists, often navigate dangerous waters around high-profile cases.

Similarly, Birkby's work on the Black Panther case also had an impact on the bioarchaeological study of cremains. The very same effort and attention he afforded forensic cases was given to prehistoric cremation burials. His work in the field of bioarchaeology likewise served as an example of what can be accomplished with fragmentary calcined bone.

The "Birkby-isms" hold true no matter what the year or what the nature of the case. By not over-reaching and

by devoting the time and effort, Birkby was able to advance a difficult and politically charged case long before most of the anthropological studies on cremation were conducted. These examples are those that guided his students and "mentoring by example" lies at the heart of forensic training.

Birkby, Mentorship, Cremains

A65 Walter H. Birkby's Influence on the Science of Mummy Studies

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After attending this presentation, attendees will gain an appreciation of Walter Birkby's influence on mummy studies. His own unpublished work, and fostering research on mummified remains by others, resulted in methods applied today in forensic studies.

This presentation will impact the forensic science community by increasing attendee awareness of the heretofore unknown contributions of Walter Birkby to forensic science.

For many decades, the Arizona State Museum at the University of Arizona curated one of the largest mummy collections in the United States. Walter Birkby was the curator of these collections. Several prominent archaeological sites were represented by the mummies. These included Ventana Cave, the excavations of which established the first chronology of extensive time depth and represented Archaic, Hohokam, and Tohono O'odham cultures. Mummies from Vandal Cave and Painted Cave represented Ancestral Pueblo peoples.[1] The mummies from Ventana Cave were reburied in 1992, representing the first major mummy collection reburied without full study.

Birkby's curatorial role was complicated by defleshing of mummies that occurred in the 1950s, long before his curatorship began. Birkby curated the resulting skeletal remains. He had the foresight to curate coprolites that were recovered when some Ventana Cave mummies were defleshed. The excavator of Ventana Cave, Emil Haury, mention two unpublished mummy studies.[2] It is noteworthy that these studies were relatively sophisticated approaches to mummy studies. Radiology was employed in an inclusive search for pathology related to valley fever (coccidioidomycosis). Also, the mummies were tested for ABO antigens. In this pioneering study, nine mummies were positive for type 0 and one for AB.

Birkby was perhaps the first paleopathologist to conduct studies of head lice (*Pediculus humanus capitis*) from mummies.[1] His work inspired the later paleoepidemiological approach to lice in large mummy collections.[3] This approach has expanded and now louse epidemiological studies are being based on analyses of large samples of mummies, especially in the Andes.

Significant for the forensic investigation of mummies, Birkby provided Ventana Cave coprolites for analysis.[4] The analyses of these laid the quantification method framework now used in the forensic analysis of mummy intestinal remains.[5] Analysis of parasites, pollen, starch, and other microscopic remains provided information relevant to diet and season of death. Macroscopic remains provided additional remains of diet. For the Ventana Cave prehistoric mummies, two dietary episodes were discerned. One was composed of mesquite and another of saguaro cactus seeds and pollen. The cactus reliance was especially interesting. The evidence conclusively demonstrated that buds of saguaro or organ pipe cactus were eaten. The use of buds from these cacti had never before been noted. These methods of analysis have since been applied to mummies of forensic relevance. These showed the location of death and last meals for a Nebraska homicide victim and of a starvation diet for a Korean War POW.[5]

The contribution of Birkby's direct research on mummies and his sponsorship of intestinal analysis laid a foundation for two important developments. Louse analysis of mummies has become a focus of paleoepidemiology. Importantly, the methods of coprolite analysis have been applied in the forensic setting, both in domestic homicide contexts and in military investigations.[5]

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2. Haury E.W. *The Stratigraphy and Archaeology of Ventana Cave*. Tucson, AZ: University of Arizona Press, 1950.
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4. Reinhard K.J., Hevly R.H. Dietary and parasitological analysis of mummy 5, Ventana Cave, Arizona. *Kiva.* 1991; 56:314-325.
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Walter H. Birkby, PhD, Mummies, Botany

A66 Birkby, Bones, and Bodies: The Making of a Mentor

Madeleine J. Hinkes, PhD, San Diego Mesa College, Office of Institutional Effectiveness, 7250 Mesa College Drive, San Diego, CA 92111*

After attending this presentation, attendees will better appreciate the special mentoring role that Dr. Walter Birkby played in the lives of many forensic anthropologists in practice today.

This presentation will impact the forensic science community by reminding mentors of the key role they play in shaping their students and by urging students to learn as much as they can from their mentors.

Most people think of Walt Birkby as an exceptional forensic anthropologist, but his early career focused on archaeology and osteological analysis of the recovered human skeletal remains. Arriving at the University of Kansas in the late 1950s, by 1963 Walt had earned a BA and an MA under Bill Bass. During that time, he excavated sites in the Great Plains, worked his way up from crew member to crew chief to research assistant, and was Bill's Teaching Assistant (TA) for human osteology classes. Walt published on general skeletal identification, with forays into analysis of dentition, the suborbital fossa, non-metric traits, and craniometric assessment of race and sex.

Walt arrived at the University of Arizona (UA) in 1963 and completed his PhD in 1973. He rose from Instructor to Physical Anthropologist to Curator of Physical Anthropology at the Arizona State Museum. Just as Walt arrived in Tucson, AZ, the UA archaeological field school began work at Grasshopper Pueblo, a 500-room, 14th-century Mogollon pueblo located on the modern White Mountain Apache Indian reservation. Excavations continued there for 30 years and uncovered 672 burials (700 individuals). These remains were turned over to Walt at the Arizona State Museum for preservation, analysis, and curation.

That collection brought me to the UA in 1977 after I too had earned an MA at the University of Kansas. Because Walt curated that collection, I would be working with him. I became his first female graduate student, and for the next six years, my home was Birkby's Body Shoppe on the fourth floor of the Anthropology Building. My dissertation research focused on skeletal evidence of stress in subadults (a population of approximately 400) at Grasshopper. Walt helped me hone my osteological skills and taught me how to take and develop X-rays, since he was certificated in medical X-ray technology. I bought my first Single-Lens Reflex (SLR) camera, and he taught me how to take photos and the importance of documenting my work. He encouraged me to follow him into forensic anthropology, and I attended my first AAFS meeting in 1978.

During my time as his graduate student, Walt instilled many lessons in me and my colleagues, which have continued to influence our work today. He was always accessible and unselfish with his time. He taught us by example to take our work, but not ourselves, seriously; to take the time to do the job well (i.e., if forensic anthropology is the application of science to the law, you need to get the science right). Observing both his ease with law enforcement officers and his role as a forensic team member taught us how to conduct ourselves, too. His most important lesson: always take time for lunch.

Probably the best evidence of his influence on me begins with a 1980 incident in which 27 Salvadoran refugees fleeing a right-wing military regime decided to head north through the southwestern Arizona desert in July. After their smuggler abandoned them, only 14 were rescued. The other 13 were found dead — at that time, the deadliest incident involving migrants at the United States-Mexico border. Our team worked the case over the next few days. Just five-and-a-half years later, I was at Dover Air Force Base faced with a mass disaster of 248 fatalities from the crash of an airplane bringing the 101st Airborne Division back from the Persian Gulf. I was the only forensic anthropologist on the team with military pathologists and dentists, but I knew what to do and how to do it, thanks to Walt. Every one of the victims was identified.

Walter H. Birkby, PhD, Human Skeletal Remains, Forensic Anthropology

A67 The Birkby Paradox — Forensic Anthropology Participation and Gender

Alison Galloway, PhD, University of California, Chancellor's Office, Santa Cruz, CA 95064*

After attending this presentation, attendees will better understand the critical nature of support for diversity by gender in training programs in forensic anthropology and the current status of such diversity.

This presentation will impact the forensic science community by considering the current situation and discussing what levels of support are needed for the future in training and developing forensic anthropologists.

Walter H. Birkby was a superb osteologist and an excellent teacher, was dedicated to forensic anthropology, and possessed a great instinct for legal context. He and his program produced many students, both male and female; however, there was also a negative side. His laboratory featured pictures of women no longer considered acceptable and, from the perspective of his female students, his relationship with male and female students differed. He favored his male students in support, but he also gave rein to the women in his program, allowing us to move forward. His laboratory trained a significant number of female graduate students.

Women used a variety of strategies to survive and succeed: (1) alliances and support networks; (2) finding funding outside of Walt's laboratory and outside employment both factored into completion; (3) taking on the challenges; and, (4) focusing on the issues.

From the 1970s through 1990s, the Birkby laboratory epitomized the transitions in the discipline. The remainder of this presentation reviews the changes in participation by gender in forensic anthropology. Material is drawn from publicly available sites. Unfortunately, a binary separation into two genders had to be used, ignoring gender fluidity and those who may be gender queer.

Forensic science and forensic anthropology developed slowly, with the American Academy of Forensic Sciences being founded in 1948. Largely male for many decades, women were present and active, however, it was not until 1979 that a female served as president.

The Anthropology section has seen a steady growth of women. Membership lists as of 2016 show women at the Student Affiliate and Trainee Affiliate levels outnumber men by a 4:1 margin. At the Associate Member level, women outnumber men by almost 3:1. The margins are more balanced at Member level (3:2) but only reach equivalency at the level of Fellow.

At the Member and Fellow ranks, the numbers of men and women with doctorates are approximately the same; at the Associate Member level, the numbers are notably different with more women having a Master's degree (46.8%) than men (16.1%). There are suggestions that a number of women have found productive careers in forensic laboratories and coroners' offices with a Master's degree and have remained at that level for years.

The American Board of Forensic Anthropology has seen a similar transformation in the numbers of women. Begun by men in 1977, the Board gradually added a few females. Over the next two decades, the percentage of women gradually rose from 20% to more than 30%. Currently, the number of active male and female diplomates is approximately equal, having seen a noticeable upswing in the past few years.

Forensic anthropology has paralleled the trend of increasing the numbers of women in the forensic sciences. Universities report that forensic sciences program enrollments are 74%-90% female. Houck noted that forensic sciences constitute a marked departure from the gender gap in most of the Science, Technology, Engineering, and Math (STEM) fields.[1] There are many suggestions as to why this has occurred, including strong role models in popular media and a commitment to public service.

What is left of the "glass ceiling" is in leadership and in the pipeline to it. Women are common in state crime laboratories at the bench and leadership levels, they are rarely directors of the larger federal laboratories.

The increase in women in forensic sciences, and in forensic anthropology in particular, began in the mid-1980s, at the time that Walter Birkby and many of his generation of professors were at the height of their programmatic careers. Despite the often noticeable discrepancies in <u>how</u> they supported female students, they encouraged their entry into the field and provided opportunities for these women to gain field experience.

Reference(s):

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Career Development, Gender Diversity, Birkby

A68 The Recordkeeping of Walter H. Birkby, PhD

Bruce E. Anderson, PhD, PCOME, Forensic Science Center, 2825 E District Street, Tucson, AZ 85714*

After attending this presentation, attendees will better understand the advantages of keeping a parallel set of records derived from forensic anthropology examinations and the associated medicolegal death investigations.

This presentation will impact the forensic science community by illustrating the potential for augmenting current medicolegal case investigation data and for performing research into old and cold cases that can lead to case resolution.

The late Walter H. Birkby performed his first forensic anthropology examination in 1965 and continued from that date to keep a set of medicolegal death investigation records parallel to that of Law Enforcement Agencies (LEA) and Medical Examiners/Coroners (ME/C) for each of the more than 3500 forensic anthropology consultations that he performed over his illustrious career of over forty years. These records, arranged within a separate folder for each individual consultation, were a combination of original forensic anthropological notes, forms, sketches, chain-of-custody documents, photographs, radiographs, reports, and photocopies of law enforcement and ME/C reports. Many copies of court interviews and depositions, as well as a plethora of newspaper clippings from selected cases, are also included in this collection.

The vast majority of these records survive to date and are housed at the Pima County Office of the Medical Examiner (PCOME), where many of these are consulted on a regular basis by PCOME forensic anthropologists still attempting to identify unknown individuals or assisting LEA or family requests on resolved cases. The value of these records cannot be overstated for those cases in which governmental retention schedules have served to drastically reduce, or even destroy, the entire original governmental case file. More than 150 such files of currently unidentified individuals, including Dr. Birkby's first case from 1965, are the sole source of surviving archival records. Several identifications have been made over the past decade utilizing these records, with the existence of the original anthropological data and copies of ME/C data proving essential in establishing everything from chain-of-custody to explaining an identification to the family of the decedent. Because Dr. Birkby consulted in every county in Arizona and several counties in California over the course of his career, other forensic anthropologists in other jurisdictions have made inquiries to the PCOME regarding a few old cases with the hope that Dr. Birkby's records might clarify or establish that a suspected old case actually existed.

Examples of the diversity of the information contained within these 3,500 records will be highlighted and their importance to unresolved cases will be emphasized.

Forensic Anthropology, Case Reports, ME/C Case Records

A69 The Skeletal Trauma Casework of Walter H. Birkby, PhD: Setting a Standard for Future Generations

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After attending this presentation, attendees will understand the impressive scope of the skeletal trauma casework performed by Dr. Walter Birkby during his long and distinguished career.

This presentation will impact the forensic science community by discussing the influence Dr. Birkby's peri-mortem skeletal trauma casework had on his students, as well as on future generations of forensic anthropologists.

The distinguished career of the late Walter H. Birkby spanned more than 40 years and encompassed more than 2,300 forensic anthropology cases. From the years 1965 to 2008, Dr. Birkby consulted for medical examiner offices and law enforcement agencies across Arizona and the west. Approximately 15% of his total cases involved the analysis of peri-mortem skeletal trauma. Interestingly, his very first forensic case, on November 2, 1965, included a description of sharp force defects to the skeleton.

Of Dr. Birkby's 310 peri-mortem skeletal trauma cases, 169 cases were gunshot wounds (55%), 94 were blunt force trauma (30%), and 47 were sharp force trauma (15%). From 1965 to 1979, his number of trauma cases was modest, totaling approximately 40. Beginning in the 1980s, there was a sharp increase in trauma consultations, and this trend continued through the 1990s. Over the two-decade period from 1980 to 1999, Dr. Birkby worked 88% (272) of his total trauma cases. These ranged from the analyses of homicides and suicides to skeletonized undocumented border crossers to the re-evaluation of cause of death from exhumed remains. Indeed, the scope of casework performed by Dr. Birkby proves difficult to summarize as it encompasses so many aspects of forensic anthropology.

Despite not publishing extensively on skeletal trauma, his legacy involving trauma analysis is a powerful one, both through the large number of trauma cases he worked and through his keen analytical skills, careful descriptions paired with meticulous diagrammatic and high-quality photographic documentation, and the succinct, streamlined case report writing style he developed. Most importantly, at the foundation of his legacy is the impact he had on his graduate students; one that is squarely centered on his daily interactions with those who worked at his side on many of his cases.

In the days before the Scientific Working Group for Forensic Anthropology (SWGANTH), Dr. Birkby trained his students with his own best-practice guidelines on trauma analysis. Many students were given the opportunity to write first drafts and co-author reports with him, following successful assistance with trauma casework. His intensive case volume allowed for training in the recognition of the mechanisms of trauma, as well as distinguishing antemortem and peri-mortem trauma from the postmortem damage generated by the denizens of the Sonoran Desert. Above all, though, he trained his students to be conservative analysts, to front-load analyses with careful, clearly stated descriptions, and to follow up with interpretation only when appropriate.

Many of Dr. Birkby's students are now board-certified forensic anthropologists who have their own graduate students, direct their own laboratories, consult on trauma cases, and publish on skeletal trauma, including Alison Galloway who wrote *Broken Bones: Anthropological Analysis of Blunt Force Trauma*, the benchmark of blunt force trauma. [1]

Most certainly the presenting author's case experience and training under Dr. Birkby was the impetus for the ongoing interdisciplinary research initiative focused on pediatric and adult cranial and long bone fracture initiation and propagation at Michigan State University. In this collaborative research between forensic anthropologists and biomechanical engineers, a science of skeletal trauma is being built based on controlled biomechanical experiments that are performed in order to test hypotheses and establish relationships between fracture characteristics and variables related to the injury scenario. This application of the scientific method to skeletal trauma analyses follows in the footsteps of Dr. Birkby's objective of understanding the processes behind observations made during skeletal analyses. This, along with the involvement of graduate students in both casework and research, continues the

legacy set forth by Dr. Birkby to engage and train the next generation of young scholars interested in pursuing new directions in trauma interpretation and forensic anthropology.

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Trauma Analysis, Walter H. Birkby, PhD, Forensic Anthropology

A70 Human Bone Taphonomy and the Use of Consolidants: An Example From Cyprus

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After attending this presentation, attendees will better understand human bone taphonomy and the use of consolidants; Walter H. Birkby utilized bone consolidants both in forensic anthropology casework to preserve fire-damaged teeth and in bioarchaeology to prepare human skeletal remains for a museum display in Cyprus. This presentation will look historically at the use of consolidants commonly used for human bone and also note more recent work examining the analytical effects of these materials that may affect choices made in the field today.

This presentation will impact the forensic science community by examining the taphonomy of human bone and by making known Walter H. Birkby's contributions to conservation sciences.

Walter H. Birkby, in addition to devoting his career to teaching and practicing forensic anthropology, was a bioarchaeologist, often working on archaeological field projects during the summer months. His skills in the analysis of skeletal disease and trauma, as previously noted by Dr. Fenton in this session, were invaluable, both in the context of casework in forensic anthropology and in applications to archaeology. Walt's archaeological work in Arizona is noted in Dr. Hinkes's presentation on the human skeletal material from Grasshopper Pueblo and in Dr. Reinhard's presentation regarding Walt's research in mummy studies. In historical archaeology, Walt spent a field season excavating in the Rocky Mountains, where he and a team excavated and analyzed the victims of the first convicted cannibal in United States history, Alferd Packer.

In international endeavors, Walt consulted on at least one forensic case in Mexico, and in classical antiquity, Walt worked at both of the University of Arizona excavations, directed by David Soren, at the Roman sites of Lugnano in Italy, and at Kourion in Cyprus. As with casework in forensic anthropology, Walt took his students with him into the field, and Kourion was no exception. It was in Cyprus where victims of the AD 365 earthquake were recovered and prepared for a museum display.

Animal bone is highly mineralized and it is generally recovered from archaeological contexts in Cyprus in better condition than human bone. Unfortunately, human bone on the island often tends to be demineralized, with the organic component of the bone having been highly degraded through taphonomic processes. Human bone prefers a constant environment, such as in Egypt, for example, where it is hot and dry, or in the Russian tundra where the climate remains relatively cold. Both conditions tend to produce more well- preserved human bone.

The situation in Cyprus, though, is not one of stability, but rather one of flux, due to the constantly changing environmental conditions, alternating between hot and dry summers and cool and wet winters. Furthermore, bone prefers a neutral soil pH, and Cypriot soils tend to be either highly alkaline or highly acidic. To exacerbate the situation, Cypriots in the past, as they do today, often reused tombs and practiced secondary burial, thus commingling human skeletal remains and causing further damage to the bone in the process.

Walt worked in conjunction with archaeological conservators at Kourion, in both the consolidation of human skeletons and in block-lifting them as was required among two different contexts at the site by the Department of Antiquities of Cyprus. Walt understood the value of using consolidants in the field, both in forensic anthropology casework for the preservation of fire-damaged teeth and in archaeology for preserving human skeletal remains, in particular, for those destined for museum display.

This presentation will provide a historical view of the use of consolidants commonly used for human bone and also note more recent work examining the analytical effects of these materials that may affect choices made in the field today.

Human Bone Taphonomy, Bone Consolidants, Walter H. Birkby, PhD

A71 Standing on the Shoulders of Giants: The Evolving Legacy of Walter H. Birkby, PhD

Laura C. Fulginiti, PhD, Forensic Science Center, 701 W Jefferson, Phoenix, AZ 85007*

After attending the presentation, attendees will better understand the importance of strong mentoring and reflecting backward as they progress forward.

This presentation will impact the forensic science community by elucidating the evolving role of forensic anthropology in medicolegal death investigations and the importance of relying on and providing strong mentoring.

Everyone stands on the shoulders of those who came before them: parents, grandparents, iconic and admired predecessors. Mentors wittingly and unwittingly shape the individuals they impact and, in doing so, drive the evolution of the professional discipline related to their mentorship. Forensic anthropology has evolved in just such a manner; mentors influencing students and students becoming mentors, all the while putting their particular stamp on the discipline.

Walt was a student of William Bass and his initial loves were skeletal biology and archaeology. As he moved into his own career at the University of Arizona, he was slowly drawn into the medicolegal community. He forged strong ties with the local medical examiner and personnel from local and regional law enforcement. Students gravitated toward him as knowledge of forensic science trickled out into the collegiate community. Eventually Walt became one of the founding members of the American Board of Forensic Anthropology (ABFA) and the Physical Anthropology (now Anthropology) section of the American Academy of Forensic Sciences (AAFS). He served as an officer for both entities and encouraged his students to be active participants in both.

Over time, Walt's interests shifted from skeletal biology to assessing traumas in bone. He became a recognized expert both in Arizona and nationally, and was known for his down-to-earth sensibility. As Walt honed his skill, Forensic Anthropology also began to expand nationally and internationally. Developing the biological profile for unidentified skeletons as a side line to an academic career morphed into full-time employment in medical examiners' offices and active engagement in human rights movements globally. Anthropologists have always been able to adapt their skill set as necessary and forensic anthropologists were no exception. The career path burgeoned and during the 1980s and early 1990s, Walt's student population exploded to more than 20. Many of those individuals left and turned their careers to different aspects of anthropology; indeed, many of them are presenting in this symposium.

Forensic anthropology has evolved from the weird old aunt that is at every family reunion to a respected mainstream science. Walt's students have emerged from the shadows to become leaders in the field, positioned around the country and even the world as engaged scientists who care passionately about their work and their contributions to the discipline. Those contributions include mentoring the next generation who, in turn, will put their own mark on forensic anthropology.

The 2009 National Academy of Sciences Report has changed forever the way forensic sciences will be viewed in the United States. Where before academicians like Walt could dabble in the medicolegal world, practitioners will now be required to earn a PhD in Anthropology and board certification will become the norm. Standards that have never before been applied universally will be developed by the National Institute of Standards and Technology (NIST) Scientific Area Committee (SAC) and forensic anthropologists will have to adhere to a more uniform code. Medical examiner and coroner offices will move toward accreditation and the anthropologists and odontologists will move with them. The forensic anthropologist will become part of a standard death investigation. Many of these changes are already being implemented internationally. The discipline is growing up and good mentorship will create the practitioners who will thrive in this new world. This presentation will discuss the changing roles of forensic anthropology domestically and internationally; using Walt as a focus to reflect back will project out to the future of the science in the next 20 years.

Walter H. Birkby, PhD, Legacy, Forensic Anthropology

A72 A New Application for Estimating Ancestry Based on Dental Morphology

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After attending this presentation, attendees will better understand how dental morphological traits vary across the globe and how this variation can be used to estimate the ancestry of individuals in a forensic context.

This presentation will impact the forensic science community by providing an additional method for ancestry estimation that can be adopted by forensic anthropologists for use in the development of the biological profile.

A statistical application using R was developed to estimate the ancestry of unknown individuals using tooth crown and root morphology.[1] A global reference sample of 21 dental traits was created from the Christy G. Turner II database consisting of approximately 30,000 individuals. These individuals were subdivided into seven geographic regions (American Arctic and Northeast Asia; Australo-Melanesia and Micronesia; East Asia; American Indian; Southeast Asia and Polynesia; Sub-Saharan Africa; and Western Eurasia) for ancestry estimation. To classify an unknown individual, dental traits are scored according to the Arizona State University Dental Anthropology System and entered into the application as present, absent, or unobservable, according to predetermined breakpoints.[2] The statistical program first derives a Nei's distance matrix, followed by a hierarchical clustering tree using an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm with complete linkage. Posterior probabilities are then assigned for the unknown set of remains to each of the seven biogeographic groups using a naïve Bayes classifier algorithm.

A web-based application of this program, called rASUDAS, was used to test the accuracy of the method. In an analysis of a test set of 150 individuals of known ancestry from all parts of the world, the application correctly assigned individuals to their geographic group between 57% and 92% of the time. When limited to the broader ancestry groups of European, African, and Asian, correct assignment increased to between 82% and 92%.

The rASUDAS program is extremely flexible; it is able to accommodate missing data and allows the user to choose which biogeographic groups to include in the model; however, in its current form, the reference sample for this program is based predominantly on individuals from archaeological sites with considerable temporal depth. More work is needed to understand the effects of secular change on dental morphology before the method can be broadly adopted in a medicolegal context. The ultimate goal is to grow the reference sample to include modern individuals to enhance the utility of the program.

This study identifies broad patterns in crown and root traits as related to ancestry. The efficacy of the method outlined here highlights the utility of these data as part of the biological profile. As dental traits are directed by different developmental processes than those traditionally analyzed for ancestry estimation (i.e., cranial metrics, cranial morphoscopics, and postcranial morphology), the inclusion of dental morphological data can provide a more robust view of population histories and improve the estimation of ancestry within forensic anthropology.

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Dental Morphology, Ancestry Estimation, rASUDAS

A73 Molar Crenulation Trait Definition and Population Variation

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After attending this presentation, attendees will better understand how molar crenulations vary in modern human populations and how this variation can be used to estimate the ancestry of individuals in a forensic context.

This presentation will impact the forensic science community by providing a standardized scoring system for molar crenulations that can be adopted by forensic anthropologists for use in the development of the biological profile. Additionally, this presentation outlines frequency data on molar crenulations in various modern human samples — data that has been lacking within forensic anthropology.

The condition of crenulated (i.e., wrinkled) molars has been described extensively in the paleontological and anthropological literature. Within forensic anthropology, molar crenulations have often been identified as being encountered at higher frequencies among samples of individuals of African ancestry.[1,2] Despite the recognized importance of this trait to differentiate contemporary samples, very little data exist that document the distribution of molar crenulations. Part of the difficulty with implementing research programs investigating molar crenulations may stem from the fact that they are not well defined. In fact, they do not form a part of the standard suite of dental morphological characteristics that are described in the Arizona State University Dental Anthropology system.[3] In this work, a clear and operational definition and scoring system for the trait is provided, as well as inter- and intra-observer error rates. Frequencies for modern groups are documented and the use of the trait for differentiating between these populations is explored.

Molar crenulations are defined here as curved fissures and ridges that surround the primary ridges (i.e., cristids) of each main molar cusp. These crenulations are found on the occlusal surface of all three molars of both the mandible and maxilla. The scoring system is: 0 = no crenulation; 1 = crenulations are shallow and do not involve all cusps; and, 2 = crenulations are deep and involve all major cusps. Data were collected on several modern skeletal collections (n=420). These samples include individuals from Japan (n=98), Hispanic migrants (n=54), American Whites (n=111), American Blacks (n=17), and casts of South African Bantu-speaking peoples (n=140). To test inter- and intra-observer error, data were again collected on the same set of individuals (n=50).

Inter- and intra-observer error rates were assessed using Cohen's Kappa. Rater agreement was moderate to high (<0.41) for all teeth, indicating the reliability of this scoring system.[4] The frequency of molar crenulations was low among the Japanese, Hispanic, and American White samples. When the trait was observed, it was typically on the third molar and was a score of 1. Crenulation frequencies increased with the American Black sample and greatly increased with the South African sample. In these samples, the trait was present on all molars and scores of 2 were much more frequent. Univariate statistics (Chi-square) indicated a difference in crenulation presence between groups for all molars when raw scores were used. When data were dichotomized and the breakpoint set at one, this same pattern was found. When the breakpoint was set at two, all teeth show group differences except for the lower first molar.

Based on this research, it is argued that molar crenulations become part of a suite of traits that can be used to estimate ancestry. It is further advocated that crenulations (as well as other dental morphological traits) be included in statistical frameworks to improve accuracy rates of ancestry estimation as part of the biological profile.

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Dental Morphology, Ancestry Estimation, Molar Crenulation

A74 Observer Error and Its Impact on Ancestry Estimation Using Dental Morphology

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After attending this presentation, attendees will understand how observer error impacts data collection on dental morphology and methods that use dental morphological data to estimate ancestry.

This presentation will impact the forensic science community by evaluating the utility of dental morphology in the estimation of ancestry as well as providing a comprehensive study on inter- and intra-observer rates for dental morphological data.

Traditionally, only a small set of dental morphological variants have been used as part of the "trait list" in the biological profile to estimate ancestry. As dental morphology has gained greater visibility within forensic anthropology, several methods have been developed to estimate ancestry that employ a range of traits; however, many forensic anthropologists have received limited training on the 36 dental traits that make up the Arizona State University Dental Anthropology system.[1-4] This study serves to explore how error can affect estimations of ancestry.

Data were collected on the same set of nine dental casts by ten different participants. The dental casts form part of the Economides Orthodontic collection housed at the University of New Mexico. Of these nine casts, three had an identified ancestry as European-American, three were Hispanic, and three were African American. Dental morphological data collected for each cast included 19 traits on multiple teeth. Volunteers were provided with extensive trait descriptions, dental cast exemplars, and scoring sheets. Participants supplied data on level of training in dental morphology, total time taken, as well as their highest degree and area of study. Data were collected on the same set of casts twice. Data from each observer for each cast were then evaluated for ancestry using the method proposed by Edgar.[2]

Intra-observer error on 636 data points using raw scores (not dichotomized) provided a Kappa statistic of 0.452, indicating moderate rater agreement. Inter-observer error varies by trait and observer experience. When data were input into the method of Edgar, results varied widely.[2] Correct ancestry assignment per individual ranged from two to seven (out of nine total), with an average of 3.5 correct. Total time spent on data collection ranged from 0.5 to 8 hours, with an average of 2.9 hours. There was a strong bias toward an ancestry assignment of Hispanic (74% of the results, 60/81). Two participants classified every single individual as Hispanic, and two more classified all but one individual as Hispanic. These researchers indicated moderate experience with dental morphology and took the least amount of time to collect the data (average 1.95 hours). Individuals who indicated low or high experience with dental morphology took more time to collect data (average 3.85 hours) and had higher rates of correct ancestry assignment (average 4.75 correct).

It is likely that the bias toward an estimate of Hispanic ancestry is related to improper recordation of the dental variation in these sets of casts. The Edgar method classifies individuals as Hispanic if any shoveling is present at a grade of one or higher, and/or the metacone/hypocone have a score of five.[2] Care must be taken to ensure that these traits are in fact present at these scores. Additionally, methods that employ breakpoints at the high and low ends of the variant scores appear to be more susceptible to observer error.

The results of this study indicate that ancestry estimation methods based on dental morphology have utility in forensic anthropology; however, they are only effective when the practitioner has proper training and care is taken in scoring traits.

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Observer Error, Dental Morphology, Ancestry Estimation

A75 The Utility of Stable Isotope Ratios of Tap Water and Human Teeth as Predictors of Region of Origin in Central and Southern Mexico

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The goals of this presentation are to explore the relationship: (1) between oxygen and hydrogen isotopes in tap water sources available to Mexican populations from traditionally high immigrant sending areas; and, (2) between these water samples and tooth samples of known origin.

This presentation will impact the forensic science community by presenting data on the relationship between Mexican tap water and human tooth enamel and by examining the utility of this relationship to act as a predictor of region of origin.

The use of oxygen and hydrogen isotopes in human drinking water, hair, bone, fingernails, and teeth has demonstrated the ability to track movement and identify the region of origin for modern populations.[1] In this study, the ability of human tooth enamel to predict region of origin in modern Mexican populations by estimating drinking water from tooth enamel is tested.

The water samples consist of 224 samples of tap water collected from 13 contiguous states in Central and Southern Mexico. Hydrogen and oxygen water isotopes were measured for tap water samples for all locations using laser absorption spectroscopy at the University of Utah Stable Isotope Ratio Facility for Environmental Research (SIRFER) laboratory. Sixty-four human tooth samples were collected from four states (Yucatan, Michoacán, Oaxaca, and Mexico) and analyzed for ^{18}O and ^{13}C isotopes at the University of California Santa Cruz (UCSC) Keck isotope facility. All carbonate analysis was conducted for the tooth samples using a Thermo™ Finnegan Gas Bench II connected to a Thermo™ Delta Plus XL continuous flow mass spectrometer. Replicates of NBS-19 resulted in a reproducibility of 0.1‰ and 0.2‰ for $\delta^{18}O$ and $\delta^{13}C$. The results are reported here using delta notation and the Vienna Standard Mean Ocean Water (VSMOW) and Vienna Peedee Belemnite (VPDB) scale. Drinking water values from tooth enamel were estimated using the following equations by Iacumin et al. and Daux et al.[2-3]

All reported data has been calibrated to the VSMOW-Standard Light Antarctic Precipitation (SLAP) scale. Tap water values spanned a range from -12.7‰ to +0.4‰ and -91.7‰ to -4.2 ‰ for $\partial^{18}O$ and $\partial^{2}H$, respectively. The most ^{2}H and ^{18}O depleted tap water samples were distributed over the inland high-altitude regions of Puebla, Morelos, and Oaxaca. The local Meteoric Water Line (LMWL) for tap waters was $\partial^{2}H=7.78 * \partial^{18}O+7.0$, $r^2=0.96$. The slope of the LMWL for tap waters is similar to that of the Global Meteoric Water Line (GMWL), which has a slope of eight.

Oxygen values for tooth enamel ranged from 23.1‰ to 28.1‰. Estimated drinking water values ranged from -4.8‰ to -11.3‰, with the most depleted drinking water estimates coming from inland Michoacán and the most enriched drinking water estimates coming from Coastal Oaxaca. A one-way Analysis of Variance (ANOVA) was conducted to determine if the means of estimated drinking water values and measured drinking water values were different for four regions in Mexico. Means for measured and estimated drinking water were statistically significantly different between regions $F(7,104)=7.817$, ($p<0.05$). Tukey Kramer post hoc analysis revealed that estimated drinking water values from each of the four regions were not statistically significantly different from actual measured drinking water values from the regions; however, the test also revealed a substantial amount of overlap between estimated drinking water values and actual drinking water values between regions. In each case, estimated drinking water values were not significantly different from at least two alternate locations in addition to the region of origin. For example, estimated drinking water values for Mexico State were not significantly different from Michoacán actual drinking water values ($p=0.167$), nor Yucatan actual drinking water values ($p=0.162$).

Although clear patterns exist among measured water samples from different regions, there is also a significant amount of overlap. In order for patterns in measured water samples to provide useful information on provenance, products such as IsoMap or ArcGIS must be used to identify regional differences and identify areas of overlap based on estimated drinking water values.

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Isotopes, Water, Teeth

A76 Cranial Non-Metric Variability and Ancestry Assessment in Modern Japanese and Thai Individuals

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After attending this presentation, attendees will better understand how cranial non-metric traits can be used within a statistical framework to identify Japanese and Thai individuals. Additionally, attendees will be more informed concerning several issues associated with using non-metric traits in forensic ancestry assessment.

This presentation will impact the forensic science community by identifying the non-metric traits that are the most useful in identifying Japanese and Thai individuals. This presentation will also provide forensic anthropologists with statistical models to differentiate between Japanese and Thai individuals using non-metric traits.

Effective biological profiles in forensic anthropology and bioarchaeology depend on the development, validation, and refinement of population-specific methods; however, most biological profile methods were developed in North America on individuals of African and European descent, and it is unlikely that such methods accurately determine biological profiles for Asian individuals. Further, Native Americans have served as biological proxies for Asians due to their distantly shared genetic history, resulting in the largely untested assumption that Native Americans and Asians share a unique suite of cranial traits that can be used to ascertain ancestry. The continued reliance on methods developed from non-Asian populations is especially problematic in forensic contexts in which Asian remains are likely to be recovered, including mass disaster contexts and large western United States cities where significant populations of East and Southeast Asians reside.

This study explores non-metric cranial trait variability in 1,397 Japanese and Thai individuals, 17 to 96 years of age. The Japanese sample is composed of 209 individuals from the late 19th to early 20th centuries (Chiba University) and 572 individuals from the modern era (Jikei University). The Thai sample is composed of 616 individuals from the modern era (Khon Kaen and Chiang Mai Universities). Trait frequencies and chi square analyses for 37 traditional and novel cranial and mandibular traits used in ancestry assessment were calculated to determine if the Japanese and Thai differ from each other and from Native Americans in trait expressions. Subsequently, binary logistic regression equations and Chi Square Automatic Interaction Detection (CHAID) decision trees were calculated to identify the traits that best differentiate Japanese and Thai individuals. Additional non-parametric statistical tests examined the affects of sex, age at death, inter-trait correlations, intra-observer error, and time period on the non-metric traits.

The results indicate that 32 (87%) of cranial and mandibular traits used in ancestry assessment significantly differ in frequency between the Japanese and Thai. Additionally, logistic regression equations and CHAID decision trees correctly classified 61%-91% of individuals, with the logistic regression equations performing the best and all classifications favoring the Japanese individuals. The traits that performed the best at differentiating the two groups include the inferior nasal aperture, nasal aperture shape, prognathism, occlusion, maxillary incisor orientation, gonial muscle attachment ridging, mandibular tori, and chin projection. Further, the Japanese and Thai differ from Native Americans in the frequency distributions for most non-metric traits; however, non-metric traits are influenced by several factors, including sex, age at death, inter-trait correlations, intra-observer error, and time period, thereby complicating their use in ancestry assessment.

This study demonstrates that the Japanese, Thai, and Native Americans are not skeletally homogenous, as they exhibit differences in the expression of cranial trait frequencies due to unique population histories. Thus, non-metric traits can be used within a statistical framework to identify Japanese and Thai individuals, despite the factors that influence the expression of non-metric traits. Moreover, the findings of this research underscore the importance of developing population-specific biological profile methods for diverse and understudied Asian populations.

This research was funded by the National Science Foundation, the Japanese Society for the Promotion of Science, and the National Institute of Justice.

Ancestry Assessment, Non-Metric Traits, Asia

A77 Population Classification Using Discriminant Function Analysis of Combined Mandibular Osteometrics and Non-Metric Traits

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The goal of this presentation is to evaluate various combinations of mandibular non-metric traits and osteometrics for their efficacy in classifying individuals based on ancestry.

This presentation will impact the forensic science community by demonstrating that classification rates using the mandible for ancestry assessment can be used accurately and reliably.

It is widely known that the cranium is the most diagnostic area of the skeleton for ancestry assessment; however, the cranium is often damaged or distorted by trauma or taphonomic processes, making accurate morphological and metric assessments difficult or even impossible. Thus, other skeletal elements must be evaluated for their usefulness in assigning ancestry to unknown skeletal remains. Widely available tools such as FORDISC® 3.0 allow one to make an assessment based on mandibular osteometrics, however, in practice, inclusion of mandibular variables tends to limit population sample sizes. When used in conjunction with other standard cranial measurements, their inclusion may confound some discriminant function model interpretations. Researchers have recently turned to analyzing non-metric traits of the mandible, yet these traits are rarely used in combination with the full suite of morphological characteristics utilized for ancestry assessment.

In the current study, non-metric traits previously found useful for population-based classifications (inversion of the ramus, eversion at the gonial angle, chin shape, shape of the mandibular border, and presence/absence of a mandibular torus) were combined with several standard mandibular osteometrics (mandible length, gonial width, bicondylar width, mandibular angle, and minimum ramus breadth) and tested for classification accuracy using Linear Discriminant Function (LDF) and canonical variates analysis. Scoring of these mandibular variables are different than those previously published by Berg.[1] A total of 963 individuals of European (205 male, 207 female), African (292 male, 195 female), and Asian (40 male, 24 female) ancestry, spanning three continents, were analyzed using the custom database feature in FORDISC® 3.0. These samples include individuals of European and African ancestry from South Africa as well as the United States, while the Asian sample derives from Thailand. Using the ten combined traits, this study seeks to discern whether correct classifications can be obtained and refined for various logical iterations of "lumping" and "splitting" the populations based on shared geographic ancestry and sex (e.g., pooled males and females of combined African, European, and Asian groups).

It is recognized that combining ordinal and continuous data violates some assumptions of LDF analysis; thus, the various iterations and variables are analyzed critically. Correct classification rates, positive predictive values, specificity, sensitivity, and efficiency are used as tests of efficacy. In a three-way test of European, African, and Asian males and females combined (n = 547), the model provides an overall cross-validated Correct Classification Rate (CCR) of 76.2% using ten variables. Two-way tests between African and European (n = 485), African and Asian (n =345), and Asian and European (n = 264), using the same ten variables, yield CCRs of 81.0%, 89.3%, and 85.2%, respectively. Results indicate that the classification rates based on these combinations are on par with Berg's discriminant functions for "forensically interesting groups," which have accuracies ranging from 58.1%-91.4%.[1] This further demonstrates the validity of "mandible only" ancestry determination methods as an accurate and reliable technique for ancestry assessment.

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Ancestry, Mandible, Discriminant Function Analysis

A78 Forensic Age Estimation by Medial Clavicle Epiphysis Ossification Using Computed Tomography (CT)

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After attending this presentation, attendees will better understand the principles of Forensic Age Estimation (FAE), its importance for law enforcement, how to increase the accuracy of FAE, the use of CT to enhance the precision of clavicular maturity, and CT's practical application.

This presentation will impact the forensic science community by providing knowledge about the role of maturation stages of the clavicle in FAE. In addition, this presentation will highlight the role of CT scan s in increasing the accuracy of FAE.

Age estimation is of paramount importance in assisting law enforcement.[1] In many countries, the relevant age limits in criminal laws for the existence of criminal responsibility are between 14 and 21 years. Maturation of sexual characteristics, dental and skeletal, have been used to determine age.[2] By 18 years of age, most developmental sites have completed their growth, except for the medial epiphysis of clavicles. Therefore, in some countries, radiography or CT scan of the clavicles is used to estimate age in persons presumed to be older than 16, 18, and 21 years.[2] For example, as per the Association of German Forensic Age Diagnostics (AGFAD) guidelines, X-ray examination of the clavicle to confirm the chronological age of 21 is mandatory.[3] Correlations between clavicle maturation and age have long been studied in dry bones and at autopsy.[4] Recent studies have revealed the utility of imaging tools and, among these, CT has particular advantages.[5]

A retrospective analysis of 556 CT scans of the neck or chest of patients aged between 10 and 30 years was performed. Medical records were reviewed for date of birth and date of performing the CT. All axial and coronal images of 1mm slice thickness were used in the evaluation. The clavicular maturation stages were scored separately by two radiologists without knowledge of the patient's age. The five stages of maturation described by Schmeling et al. and sub-stages of stage 2 and 3 by Kellinghaus were followed.[4,5]

The results of gender-based comparisons revealed statistically significant differences in mean age at the maturation stages of 1, 3b, and 5. The maturation at stage 1 and stage 3b occurred earlier in females by 16 and 17 months, respectively. In contrast, maturation at stage 5 occurred 12 months earlier in males compared to female counterparts. No statistically significant differences were noted in other maturation stages (2a, 2b, 2c, 3a, 3c, and 4). Maturation stage 3a was first presented at 16 years of age for both sexes. Maturation stage 3b was first presented at age 18 in females and age 16 in males. Maturation stage 3c was first presented at 21 years of age for both sexes.

In conclusion, the CT scan is a useful visualization tool for estimating chronological age. The Kellinghaus sub-stage classification criteria improved accuracy of age estimation, particularly in stage 3. Results suggest using stage 3a of maturation to represent ages >16 years, stage 3b of maturation to represent ages >18 years, and stage 3c to indicate ages >21 years in FAE.

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Forensic Age Estimation, Clavicle Ossification, Computed Tomography

A79 The Role of Third Molar Impaction on Dental Development and Age Estimation in Males

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After attending this presentation, attendees will understand how third molar impaction influences dental development and estimates of age as part of the biological profile.

This presentation will impact the forensic science community by evaluating the utility of impacted third molars in the age estimation of males.

Numerous dental development charts exist to aid in overall age estimation from the dentition.[1-3] These charts incorporate the third molar; however, this tooth is often treated separately to establish independent methods of age estimation. While there is substantial variation in third molar development, it is still a useful measure of age in late adolescence, when other teeth are fully developed. Third molar development may also prove effective in conjunction with other age indicators in determining if an individual has attained the age of majority, which could have legal ramifications.

While a clear relationship between chronological age and third molar development has been established, the effect of impaction on crown and root development is not well understood. The vast majority of impacted teeth are third molars, and a worldwide survey found that approximately 24.4% of the population has impacted third molars.[4] While prophylactic extraction of the third molars is exceedingly common in the United States, there is growing debate over the necessity of this procedure, and the presence of third molars may become more common in forensic casework. The goal of this study is to investigate the effect of third molar impaction on development and its concomitant impact on age estimation methods.

Data were collected on radiographs of individuals who were analyzed in the Central Identification Laboratory within the Defense POW/MIA Accounting Agency. Due to the unique mission of this agency, the vast majority of individuals recovered are males; therefore, this study is only comprised of males (n=90). Radiographs of skeletonized individuals were taken by forensic odontologists. These radiographs were then scored for dental development according to the stages defined by Demirjian et al.[5] Two cohorts were created from this sample, individuals with impacted third molars (n=38) and those without impacted third molars (n=52). Age was assigned based on the appropriate ancestry-based method.[6-7] Differences between identified age and predicted dental age in each group were assessed to determine if molar impaction affected root development and age estimations.

In the cohort with third molars that were not impacted, age was correctly assigned in 90.1% of third molars. In the cohort with third molar impaction, age was correctly assigned for 66% of impacted teeth and 61.5% of non-impacted teeth. In every case of incorrect age assignment, the individual was estimated to be younger than his identified age.

Based on this research, individuals with at least one impacted third molar tend to have underdeveloped third molars, even in those that are not impacted. Therefore, care should be taken in age estimates of individuals who display any impacted third molars; however, more work is needed to explore the effect of impacted molars and development among females and other ancestry groups.

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Molar Impaction, Age Estimation, Dental Development

A80 The Challenges of Comparative Radiography in the Developing Skeleton

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After attending this presentation, attendees will recognize the ability to positively identify juvenile individuals by means of comparative radiography using radiographs taken years apart.

This presentation will impact the forensic science community by identifying comparative radiography as a method that can be used in the scientific identification of subadults. Specifically, strategies can be implemented to approximate postmortem radiographs by adapting radiographic techniques to accommodate changing growth.

Positive or scientific identifications are a common practice in medical examiner's offices when decedents are not visually identifiable. In Michigan, law also requires definitive identification when a death is the result of an incident involving two or more individuals of approximately the same age, sex, ancestry, height, weight, hair color, and eye color. Scientific identifications can be achieved using DNA, fingerprints, and dental and medical radiography. While each technique has its advantages and disadvantages, they each require the availability of antemortem and postmortem data for comparison. While these are commonly used for adult decedents, there are few reports or validation studies of appropriate identification methods for juveniles. Juveniles present a unique challenge in that they are growing and developing rapidly and may not have antemortem data, such as fingerprints, for comparison. Depending on their age, dental records may or may not be available. The first challenge in these cases is determining if there are radiographic features that retain morphological stability in the growing and maturing juvenile skeleton. An additional difficulty is the change of the gross morphology and orientation of skeletal elements with development requiring the adaptation of standardized techniques to approximate antemortem radiographs. Six cases in which antemortem radiographs from infancy or childhood were used in the scientific identifications of juveniles or young adults are presented.

One case concerned a motor vehicle accident involving two sisters who had the same skin, hair, and eye color and were approximately the same size since one was four years old and the other was five years old. One of the girls died as a result of the accident. The other girl survived but was unresponsive in the hospital. Footprints from birth had not been documented for the presumed decedent but were available for the decedent's sister. Unfortunately, there was not enough detail in these prints to confirm an identification. Antemortem chest radiographs of the decedent at eight months old were obtained and were able to be used for positive identification. Another case involved the discovery of subadult remains requiring identification. A postmortem vertebral radiograph of the 7-year-old decedent was compared to an antemortem chest/abdomen radiograph of the presumed decedent at three years of age to establish aid in identification. A multiple fatality house fire required the identification of two juveniles, a 12-year-old female and a 10-year-old male. The female was identified from a hand radiograph taken 4.5 years prior and the male was identified from chest and abdomen radiographs from between 2.5 and 3.5 years prior. The final cases resulted from a motor vehicle accident in which three young adults, two males and one female, died. One male had fingerprints on record taken 1.5 years prior, from which he was positively identified. The other male had dental records and dental radiographs from 8.5 years prior, when he was 11 years old. The 20-year-old female had an antemortem chest radiograph taken when she was 12 years of age. Using fingerprint comparison and comparative dental and medical radiography, each of these decedents was positively identified.

Comparative radiographic identification cases present unique challenges that require an understanding of radiographic techniques and positioning to approximate the antemortem radiograph. Juveniles can be especially difficult to positively identify due to growth changes and morphological changes to the skeleton. Although there are gross developmental and morphological changes to skeletal elements, these cases demonstrate that specific skeletal radiographic traits are maintained for long periods and can be used to make positive identification in the postmortem setting.

Identification, Radiography, Juvenile

A81 Examining the Effect of Region Of Interest (ROI) Size on the Ability to Accurately Estimate Age at Death From Osteon Population Density (OPD)

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After attending this presentation, attendees will understand the current issues concerning histological age estimation and the effect that ROI size has on the relationship between OPD and age-at-death estimation.

This presentation will impact the forensic science community by demonstrating that the amount of bone sampled/analyzed in histological age assessment can have a profound impact on the ability to accurately estimate age at death. This will inform future histological research, resulting in higher quality forensic research and practice.

As a complement to macroscopic aging methods, or when necessary macroscopic elements are damaged/absent, age can be estimated through histological examination of remodeling events in cortical bone. To date, the femoral midshaft has been the most commonly employed site for histological studies; however, a consensus is lacking on how much bone to analyze when quantifying remodeling, as existing methods employ ROIs that differ in size, number, and location.

Recently, histological research at the femoral midshaft has proliferated due to the revival of the Ericksen femur collection as an active resource. The current study is a meta-analysis of three recent studies that provide a unique opportunity to assess the effect of ROI size on the relationship between OPD and age. All three studies analyzed here (the Ingvoldstad, Crowder/Dominguez, and Gocha methods) primarily utilized the Ericksen collection, examined the anterior region of the femur, and quantified remodeling according to the same standard histological definitions.[1-3]

The Ingvoldstad method examined 200 individuals (97 males, 103 females), aged 30-97 years (average=71 years), all from the Ericksen collection; a fixed ROI size of 3.00mm^2 was used to quantify remodeling at eight anatomical and biomechanical locations around the femoral cortex, though only anterior data are analyzed here. The Crowder/Dominguez method examined 320 individuals (170 males, 150 females), aged 15-97 (average=66 years), 87% of whom were from the Ericksen collection. This method used a topographic sampling strategy, separating a 5mm-wide section of the anterior femur into ten columns and reading every other frame using a Merz reticule; this resulted in an average ROI size of 18.30mm^2, with an average of 9.52mm^2 of bone analyzed. The Gocha method examined only 30 individuals (15 male, 15 female), aged 21-97 years (average=59 years), 83% of whom were from the Ericksen collection. This method examined remodeling over the entirety of the femoral midshaft, though only anterior octant and quadrant data are analyzed here; average octant ROI size was 41.82mm^2, average quadrant ROI size was 89.93mm^2.

Statistical analyses were performed in SPSS 23. Kolmogorov-Smirnov tests demonstrated OPD values for all methods to be normally distributed (all p-values >0.070). The relationship between OPD and age was assessed through Pearson's correlation coefficients, as well as the adjusted R^2 value of linear regression predictive models; all of these statistical measures were statistically significant (all p-values <0.043). The person who collected the majority of the data for the Crowder/Dominguez method also performed inter-observer error measures for the Ingvoldstad and Gocha studies, neither of which demonstrated significant differences between observers.

The correlation coefficient for the Ingvoldstad method was R=0.143, and the adjusted R^2=0.016, indicating OPD explained only 1.6% of the variation in age at death. The correlation coefficient for the Crowder/Dominguez method was R=0.681, and the adjusted R^2=0.462, indicating OPD explained 46.2% of the variation in age-at-death. The correlation coefficient for the Gocha Octant method was R=0.907, and the adjusted R^2=0.817, indicating OPD explained 81.7% of the variation in in age at death. For the Gocha Quadrant method, the correlation coefficient was R=0.918 and the adjusted R^2=0.838, indicating OPD explained 83.8% of the variation in age at death.

Results indicate that ROI size has a significant effect on the ability to predict age at death from histological remodeling. Examination of small, isolated ROIs is not recommended, as such an approach is more susceptible to random variation in variable distribution and can negatively affect interpretation. Instead, future studies should examine larger ROIs to maximize histological remodeling's ability to predict age at death.

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Age Estimation, Skeletal Histology, Forensic Anthropology

A82 Age Estimation Using the Development of the Foot and Ankle

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After attending this presentation, attendees will understand the changes that have occurred in the development of the foot and ankle in subadults since the 1930s as well as a new subadult aging technique utilizing radiographs of the foot and ankle. By using the presence or absence of ossification centers in the subadult foot and ankle along with new statistical methods, a new means of subadult age estimation will be discussed.

This presentation will impact the forensic science community by providing an improved subadult aging technique of the foot and ankle that can be used in forensic anthropology and pathology. Additionally, by using only radiographs, this study presents a less intrusive age estimation method for subadults.

Age estimation in subadults can assist with narrowing missing person searches substantially. The developmental timing of multiple areas of the skeleton, such as the wrist, knee, and elbow, have been researched thoroughly in recent years in relation to subadult age estimation; however, multiple areas of the skeleton should be used for age estimation when available.[1] The goal of this study is to first test for differences in age of appearance of ossification centers in the foot and ankle between historical and modern cohorts, and, if differences are noted, to develop a model using these modern data for subadult age estimation.

A total of 1,520 radiographs (871 males, 649 females) of the right foot and ankle from White individuals aged from birth to 13 years were analyzed from the Pediatric Radiology Interactive Atlas (PATRICIA) and funded by a National Institute of Justice grant. Eleven ossification centers were chosen to be scored based on their previously published predictive value for age.[2] Each ossification center was scored as either present, absent, or not able to be scored. Data from each ossification center were analyzed using logistic regression, which removes assumptions of a normal distribution and linearity. Most importantly, a logistic regression model produces probabilities for ossification center appearances at any age. There were two goals for using logistic regression to analyze these data. The first was to test for differences in each ossification center between the modern and historical samples; the 5th and 95th probabilities produced from the logistic regressions were used for one-sided confidence intervals for age. The second goal was to test for sexual dimorphism in ossification center appearance. The regression tree method was applied to these data in order to estimate age using explicit intervals based on the scores of multiple epiphyses.[3] Inter-observer and intra-observer studies were completed on 39 individuals to test the validity and reliability of the method. All statistical analyses were completed using R software.[4]

Results of the inter-observer and intra-observer studies demonstrate that this scoring method is both reliable and valid for 9 out of the 11 ossification centers, with the ossification centers of the ankle, tarsals, and metatarsals being more reliable and valid than ossification centers of the phalanges. Two ossification centers located on the phalanges were valid for estimating age but were very often difficult to score. For this reason, these two ossification centers had a limited amount of observations because they were rarely visible enough to score in the radiographs.

Seven out of the 11 ossification centers appear earlier in the modern males than they did in the historical males. In the modern females, 9 of 11 ossification centers developed later than the historical females. Sexual dimorphism was seen in 9 of the 11 ossification centers, with 8 of the 11 ossification centers appearing statistically significantly earlier in females. Noting these observed changes in the modern sample, this study explored two different methods for age estimation, the first being age estimates based on one-sided 5^{th} or 95^{th} probabilities of the ossification center scores for age, and the second being the application of regression trees. The results of this study prove that an updated age estimation method for the foot and ankle is necessary to use on modern populations, and that the application of regression trees in this type of study provides a valuable new age estimation technique.

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Age Estimation, Subadult, Radiograph

A83 The Influence of a Pathological State on Fetal Biometric Age Estimation

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The goal of this presentation is to highlight the influence of fetal growth-affecting diseases on the estimation of fetal biometric age calculated from conventional anthropometric tools. This presentation reveals the potential mistakes inherent in the estimation of fetal age in cases in which judicial consequences are crucial. This study challenges the anthropometric tools traditionally used for age estimation in healthy fetuses and suggests more relevant markers of fetal growth in pathologic conditions. With brain maturation being the most relevant marker of fetal maturation, skull base bony structures could be a useful witness of fetal growth.

This presentation will impact the forensic science community by providing diagnostic tools useful in all fetal diseases characterized by a short femur length.

Pars basilaris growth is not influenced by the diseases characterized by a short femur length; thus, the decoupling between pars basilaris growth and that of long bones is a diagnostic element for these diseases. Thirty-seven Computed Tomography (CT) fetal scans, *in utero* or postmortem, conducted after observing a short femur length were compared to a control population of 97 CT postmortem fetal scans. Twin pregnancies were excluded. The study population was divided into two groups: (1) a "skeletal anomalies" group involving fetuses suspected of, or that were carriers of, a constitutional bone disease or a chromosomal abnormality; and, (2) an "isolated short femur length" group involving fetuses without suspicion of syndromic context. A reconstruction of the long bones (humerus, radius, ulna, femur, and tibia) and the pars basilaris of each fetus allowed the calculation of the maximum long bones diaphyseal lengths and the dimensions of the pars basilaris (maximum width, maximum length, and sagittal length). For each fetus, the ratio of diaphyseal lengths to Femoral Length (FL) as well as that of FL to pars basilaris dimensions were calculated. Between study and control populations, a significant difference in the humeral, radial, femoral, and tibial lengths was observed ($p<0.05$), whereas no difference between the pars basilaris dimensions was found. Age estimation calculated from the FL underestimated the true age of the population. The FL/Maximum Width Basilaris ratio was the most relevant diagnostic tool of the diseases characterized by a short femur length, and the Humeral Length/FL ratio was useful for the differential diagnosis between intra-uterine growth retardation and constitutional bone disease.

A study associating both pars basilaris and long bones growth estimation allows: (1) reducing the risk of gestational age underestimation and, thus, its legal consequences; and, (2) making a diagnosis of disease affecting fetal growth, which is a challenge in anthropological practices.

Fetal Age, Short Femur Length, Anthropology

A84 Exploring the Gap Between Anthropological and Clinical Literature on Pediatric Fracture Healing

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After attending this presentation, attendees will understand the differences within and between anthropological and clinical literature regarding the interpretation of the time since injury of pediatric healing fractures.

This presentation will impact the forensic science community by serving as a resource for understanding the variability that exists within current literature and how this impacts the applicability of certain fracture healing timelines for use in forensic contexts. Further, inconsistencies across the literature and between fields call into question the appropriateness of published timelines for use in legal contexts.

Several methods to assess time since injury using radiographic signs of healing have been proposed specifically for identification of child physical abuse. The goal of this project is to present major gaps in these methods, compare clinical and anthropological literature, and define issues that warrant future research. Through a review of scientific literature, 18 published timelines of fracture healing were identified and compared. Of these, more than half were based solely on previous literature or clinical experience. In fact, little scientific data exist to support the understanding of the rate of pediatric fracture healing and only 8 of the 18 timelines examined were based on primary research.

The most significant discrepancy across the literature is a failure to take into account intrinsic variables that may influence fracture repair, including age, anatomical location, specific bone location, and within-bone fracture location. Of the nine timelines of fracture healing with an associated age range, the majority pool various age groups of different years together with little to no consideration given to the effect of age on fracture repair. Three timelines pool adults and teens with children. Given the definite differences between mature and immature skeletons, timelines derived from such samples should be treated with extreme caution. Even pooling smaller age ranges is questionable; age ranges of 0-5 years, while more appropriate to pool together than adults and children, still include pre-ambulatory and ambulatory children whose skeletons are distinct from one another structurally and morphologically.

Further, different bone functions likely influence the healing process between broad anatomical locations (upper vs. lower extremities). Half of the published timelines examined did not take into account anatomical location of the healing fracture, though it has been preliminarily suggested to influence fracture repair rate. Malone et al. found that upper extremity fractures heal more rapidly than lower extremity fractures, yet more than half (55%) of time-since-injury methods either do not cite the specific skeletal elements from which their research was derived or they combine skeletal elements of various anatomical regions (28%).[1] Further, there is significant overrepresentation of long bones (98% of the timelines examined), with 33% represented by radius fractures; however, fracture locations used in time-since-injury studies, such as the radius, do not necessarily overlap with fracture locations that are considered specific for abuse. Finally, published timelines do not take into account within-bone location and its influence on fracture repair, particularly in the early stages of healing. This is despite the fact that there are several clinical methods to assess radiographic union for specific within-bone locations.

Finally, stages, features, and timelines of fracture healing are inconsistent both within and between fields. There is a general lack of published timelines including early evidence of fracture healing, especially within anthropological literature. These early characteristics are of particular importance to identify occult healing fractures seen in child physical abuse. In anthropological literature, there is a tendency to use a stage system, while clinical literature tends to examine traits of specific radiographic characteristics of healing. Not only are the radiographic features of fracture healing inconsistent, the timelines presented in the literature vary significantly between one another, even for the same features.

In conclusion, time since injury of pediatric healing fractures is of great importance to the forensic field as evidence of child physical abuse; however, inconsistencies across the literature and between fields call into question the appropriateness of published timelines for use in legal contexts. This work identifies the gaps in current knowledge surrounding pediatric fracture healing. Further research is needed to determine the influence of certain

variables on fracture healing and to work toward incorporation of those variables into time-since-injury estimation methods and timelines.

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Child Abuse, Fracture, Healing

A85 Skeletal Sun Bleaching and Weathering Patterns in Central Florida: An Approach for Estimating Time Since Death (TSD)

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After attending this presentation, attendees will better understand the progression of skeletal sun bleaching and weathering over time. This presentation will focus on patterns of bone sun bleaching and weathering in order to fill a gap in the literature regarding the time frame in which bones become bleached and weathered in the Central Florida environment during the Postmortem Interval (PMI).

This presentation will impact the forensic science community by discussing the timing and extent of skeletal sun bleaching and weathering. Factors influencing sun bleaching and weathering that will be discussed include the effect of microenvironments (full sun versus shade), length of the PMI, and bone type.

After soft tissue decomposition, taphonomic modifications to the remaining skeletal elements can provide valuable information to investigators regarding TSD. It is therefore important to investigate the timing of sun bleaching and weathering patterns observed during the PMI in order to understand how these taphonomic modifications can contribute to TSD estimations. Investigation into the timing and progression of sun bleaching and weathering was undertaken over six months in Central Florida. Four pig (*Sus scrofa*) carcasses were placed in two microenvironments: two in open, full sun and two in shade. After skeletonization occurred, ten bones from each pig carcass location were assessed weekly for sun bleaching and weathering changes and included cranial elements, mandibles, scapulae, vertebrae, ribs, os coxae, and long bones. The total sample was reduced to 37 bones due to animal scavenging. Bone sun bleaching was assessed once a week using the Munsell soil color charts, with the lightest coloration of the exposed bone scored.[1] A bone was considered to be sun bleached when the lightened color corresponded to the WHITE PAGE, and the extent of the sun bleaching was estimated as <25%, 25% to 50%, >50% to 75%, and >75% to 100%. Additionally, bone weathering was evaluated using the Behrensmeyer stages by scoring weather-related changes on a scale from 0 to 5.[2]

Analysis of sun-bleached skeletal elements demonstrated that microenvironment had the greatest influence for the presence or absence of sun bleaching. Overall, bones in full sun locations exhibited a greater rate of sun bleaching than bones in full shade locations. By month four of the investigation, skeletal elements in full sun locations exhibited approximately 25% more sun bleaching on the exposed bone surface than elements in full shade locations. Bones in full sun locations exhibited approximately 25% to 50% bleaching on the exposed bone surface by day 100 and approximately 50% to 75% bleaching on the exposed bone surface between day 100 and 135. Additionally, bones in full sun and partial sun locations exhibited initial signs of bleaching around day 22 and day 36 of the investigation, respectively; however, by day 135, bones in full shade still exhibited minimal signs of bleaching (<25% of the exposed bone surface).

Skeletal element types displaying sun bleaching earliest in the PMI included ribs and scapulae, whereas os coxae appeared to exhibit bleaching latest in the PMI out of all bone types studied. While the majority of the skeletal elements that were analyzed contained no soft tissue remnants, a few elements that were studied contained minimal desiccated and adhered soft tissue, including articular cartilage, which may have partially shielded the surface of the bone from sun bleaching. It is important to note that the most challenging issue with scoring sun bleaching was the periodic presence of moist bones from rainfall that affected bone surface coloration, as compared to dry bones. Overall, minimal weathering was noted on the remains after five months. While bones in full shade generally exhibited Stage 0, bones in partial shade and full sun exhibited Stage 1. In addition, one mandible and one scapula in full sun exhibited the beginning progression of Stage 2 on less than 25% of either bone. In conclusion, the combined utilization of sun bleaching and weathering as a taphonomic model that is regionally specific may aid standardization of future studies by increasing accuracy in estimating TSD.

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Taphonomy, Sun Bleaching, Bone Weathering

A86 Optimization of a Protocol for Visualizing Vascular and Cellular Pore Networks in Human Bone Using Multiphoton Confocal Microscopy

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After attending this presentation, attendees will appreciate the potential of multiphoton confocal microscopy for 3D visualization of human bone loss at the histological level.

This presentation will impact the forensic science community by optimizing a protocol for 3D imaging of vascular and cellular spaces in bone tissue. Future forensic applications for imaging these pore structures include: (1) identifying bone tissue sites at high risk for spontaneous fracture; (2) distinguishing between different bone types at the tissue level based on pore geometry, which appears highly sensitive to mechanical loading environment; and, (3) demonstrating the advantages of multiphoton confocal microscopy for histomorphological analysis in general.

Throughout life, bone is remodeled to replace old tissue, repair microscopic damage, or resorb bone in response to decreasing mechanical strain. Osteoclasts tunnel into the old bone, and osteoblasts fill this resorption space with new bone, leaving a central Haversian canal to transmit a blood vessel. This "vascular porosity" increases with age as osteoblasts decline in their capacity for bone formation, while osteoclasts increasingly resorb bone in response to declining physical activity and muscle strength. Vascular pores are stress concentrators for microdamage, which can initiate and propagate into a spontaneous fracture within the highly interconnected pore network. Bone tissue also has a separate network of "cellular porosity," composed of the lacunae that house osteocyte cells and their connecting canaliculi. Porosity shows promise for distinguishing between bone regions and types based on mechanical loading environment. Regions of lower mechanical strain within a given bone have higher vascular porosity. Loaded bones have been found to have larger lacunar volumes and more branched canaliculi compared to the unloaded bones.

3D pore geometry is poorly characterized. Forensic scientists interested in associating pore geometry with different bone types (e.g., normal/osteoporotic, young/old, human/non-human, loaded/unloaded) need an effective protocol for imaging these pore structures. Ciani et al. stained rat tibiae with Fluorescein Isothiocyanate Isomer I (FITC) and imaged cellular pore networks with single-photon confocal laser scanning microscopy.[1]

The suitability of this protocol was tested for imaging both vascular and cellular pore networks in human bone. Multiphoton confocal microscopy was used as it can penetrate far deeper into a sample with infrared light. Ten cross sections, approximately 500µm to 600µm in thickness, were cut from the midshaft of a fresh cadaveric human rib. Each cross section was placed in a 15mL volume of freshly prepared 4% formaldehyde and fixed for 24 hours at room temperature under gentle rotation. Cross sections were dehydrated in ascending grade ethanol (75%, 95%, and 100% for five minutes each). FITC was diluted in 100% ethanol at concentrations of 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.2%, and 0.1%. Each 10mL solution was gently rotated for one hour, then filtered. Each cross section was placed in a different concentration of solution for four hours under gentle rotation. Each cross section was then rinsed in 15mL of 100% ethanol under gentle rotation for 30 minutes, then air dried. Small regions were imaged using an Olympus® FV1000 MPE Multiphoton Laser Scanning Confocal microscope with N.A. 1.05, 25x water immersion objective, 800nm laser excitation wavelength, resolution 1,024 x 1,024 pixels, step size 0.63µm, and 2x Kalman averaging. 3D vascular and cellular pore networks were analyzed for volume, orientation, and connectivity in the image analysis software FIJI.

Results were best for a concentration of 1% FITC, as resolution was lost as most features became indistinguishable at a depth of approximately 185µm into the sample. Vascular and cellular pores were clearest to a depth of approximately 100µm. Pores displayed fairly uniform brightness within a given depth. At concentrations of FITC <1%, the resolvable depth decreased, and pore structures became more variable in brightness within a given depth.

These results demonstrate that the method from Ciani et al. is suitable for 3D imaging of both vascular and cellular pore networks in human bone tissue. Multiphoton confocal microscopy facilitates deep penetrance into the sample to allow quantification of pore volume, orientation, and connectivity.

Reference(s):

1. Ciani C., Doty S.B., Fritton S.P. 2009. An effective histological staining process to visualize bone interstitial fluid space using confocal microscopy. *Bone.* 44: 1015 – 1017.

Skeletal Histology, Confocal Microscopy, 3D Imaging

A87 Reconstructing Personhood: A Historical Perspective on Fragmentary Remains, DNA Technology, and Family Acceptance of Positive Identifications of United States War Dead

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After attending this presentation, attendees will better understand the historical evolution of identifications based on fragmentary remains as reconstituting the personhood of the decedent from the perspective of forensic practitioners and next of kin.

This presentation will impact the forensic science community by expanding the discussion regarding the identification of increasingly fragmentary remains consequent to improvements in scientific methodologies, including how those identifications may or may not reconstitute the "person" to the next of kin. A renewed awareness of the different meanings of "identification," "remains," and "person" to scientists and family members will positively impact attendees' abilities to practice a contextualized and empathetic form of forensic science.

The identification of remains from past United States conflicts is meant to provide answers for family members and contribute to a semblance of closure, despite the significant time depth often associated with these losses. The dead themselves — their bodies, their memories, their deaths — have agency. In some cases, a minute quantity of physical remains, accompanied by a report consisting of sequences of nucleotide bases and statistical probabilities, is willingly recognized as proof that a loved one was located, recovered, and identified. At first glance, this acceptance of very fragmentary remains appears to be a modern artifact of the genomic era; however, this presentation argues that the trend of accepting smaller and smaller portions of the body as representative of the whole "person" has deeper historical roots. Specifically, public acceptance for the identification of less-than-whole bodies appears to have begun during the Civil War, the first United States conflict in which families faced the realities of decomposition and fragmentation on a massive scale. Over time, the developing rhetoric around the recovery and repatriation of service members has increasingly made the possibility of receiving partial remains, or none at all, acceptable to the public.

Trends were analyzed among identification year, duration of identification process, completeness of remains, and use of mitochondrial DNA (mtDNA) analysis in a sample of remains identified at the Defense POW/MIA Accounting Agency-Central Identification Laboratory (DPAA-CIL).

Data were collected from the DPAA-CIL on a subset of identifications made from 1996-2015 and accessioned as early as 1982 (n=888). A subset of the cases accessioned after 1995 (n=621) was also analyzed (1995, the year in which the Defense Science Board Task Force released their *Report on The Use of DNA Technology for Identification of Ancient Remains*, represented a benchmark for DNA-led identifications of United States war losses). Positive identifications from cemetery disinterments were not considered, as these disproportionately targeted more complete cases for exhumation. Similarly, to counteract possible biasing effects, group identifications and additional portions from previously identified individuals were not considered.

The majority of cases in the sample were less than 50% complete. Results indicated that the completeness of remains had a significant relationship to the amount of time taken between initial accession and identification in both datasets (p<0.001 for both). The use of mtDNA as an identification method also had a significant relationship with completeness of remains (p<0.001) and the year the identification was made (p<0.001). The overall trend in the data is that more fragmentary remains take longer to identify, and those identifications tend to use mtDNA methodology.

As methods for sequencing degraded DNA improve (e.g., next generation sequencing), fragmentary remains, once unidentifiable, may become the basis for positive identifications and repatriations. The last entire year considered here (2014) includes the highest numbers of identified cases consisting of remains <50% complete. Yet, anecdotal evidence suggests that it is an existing historical foundation that facilitates the acceptance of incomplete remains identified through modern DNA-based methodologies. In 2015, for example, family members of a service member implied hesitance in accepting a recent positive identification, instead finding closure in witness testimony:

as *The Charlotte Observer* reported, "(They) still weren't convinced (of the DNA-based identification) until finally they heard what happened that day from the helicopter's co-pilot …."[1]

Loss, acceptance, and closure are part of a process that is individual; however, previous research across disciplines reveals some commonalities in this process with regard to war losses. As advocates for the dead, forensic anthropologists would do well to acknowledge the perspectives of the next of kin when making and presenting positive identifications.

Reference(s):

1. Perlmutt D. Home again: After 45 years, family greets return of fallen soldier. *The Charlotte Observer*. 2015 April 8 (http://www.charlotteobserver.com/news/local/article17918333.html).

Fragmentary Remains, Positive Identification, Mitochondrial DNA

A88 Changing the Mentorship Paradigm: Survey Data and Interpretations From Forensic Anthropology Practitioners

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After attending this presentation, attendees will understand current perceptions of the roles of mentors and protégés within the field of forensic anthropology.

This presentation will impact the forensic science community by informing future mentor-protégé interactions and guiding forensic scientists to focus on the positive influence that mentorship can have — not only on protégés' careers, but also on the personal development of the mentor and the overall well-being of the field.

The concept of mentorship originated in ancient Greek mythology and referred to a relationship between a knowledgeable person (mentor) and a less-experienced person (protégé). Today, mentorship can be conceptualized as the informal transmission of knowledge over a sustained period of time and in a domain in which the mentor and protégé have unequal knowledge. Traditionally, mentors are seen as older, wiser advisors who provide counsel to younger, less-experienced individuals in their professional or social sphere. Certainly, the role of this high-level mentor has been paramount in developing the field of forensic anthropology; however, anyone with greater knowledge in a specific domain who exerts a positive influence on another individual's professional or social development can be considered a mentor, regardless of age or experience. This presentation contends that peer-to-peer interactions (e.g., leading by example, sharing diverse work experiences, and dispensing advice) also qualify as important mentorship behaviors.

This research explores how the roles of mentors and protégés are perceived throughout the diverse academic and applied contexts of the field of forensic anthropology. All users of the American Academy of Forensic Sciences (AAFS) Anthropology Section listserv received a link to an anonymous, Institutional Review Board (IRB) -approved Qualtrics.com survey. Instructions encouraged participants to share the survey link with other practitioners of varying skill and experience levels, including students and other non-members. Consenting participants answered 23 multiple-choice and 12 open-answer questions regarding their demographic information, opinions about mentorship, and experiences as both mentors and protégés.

A total of 96 professional forensic anthropologists and anthropology graduate students participated in the survey. The majority of respondents specialized in biological anthropology, had completed a PhD, and worked in the academic or medical examiner setting. Most had been in the field for fewer than 21 years and were less than 45 years of age. The majority self-identified as being of female gender and European ancestry.

Respondents were nearly unanimous regarding the importance of mentorship in forensic anthropology, and many felt that being mentored directly contributed to their career success. Nearly all respondents had benefitted from the influence of multiple mentors, and many had also mentored multiple protégés. As expected, most respondents reported being mentored by a thesis/dissertation advisor; however, many respondents also reported peers as an important category of mentor (68%) and peers emerged as the most commonly reported category of protégé (75%). More respondents had received mentorship in career-related areas than they had in social interactions and social issues. Likewise, when asked in which areas they desired to provide and receive additional mentorship, more respondents selected these latter, social categories. Still, differences between distributions for mentorship provided/received and additional desired mentorship provided/received were not statistically significant (α=0.05; Wilcoxon rank-sum test; R). Further, the most commonly selected category for additional desired mentorship (both received and provided) was "none," implying overall satisfaction with the mentorship experience.

This study indicates that the traditional mentorship paradigm is already shifting. In forensic anthropology, the mentorship paradigm does not solely consist of vertical-level interactions, but often includes horizontally oriented interactions. If the future reflects the past, then forensic anthropologists must honor the long-valued (and still valuable) role of the traditional mentor, while emphasizing the non-traditional mentorship behaviors that can

enhance the careers and lives of both trainees and experienced practitioners.

Career, Professionalism, Mentor-Protégé Interactions

A89 Rethinking Adult Skeletal Age Estimation: An Expanded Approach to Transition Analysis Using Binary Traits

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After attending this presentation, attendees will understand that potentially useful variation exists in areas of the adult skeleton that are not traditionally used for age estimation.

This presentation will impact the forensic science community by demonstrating that age can be estimated when standard features, such as the sternal ribs, pubic symphyses, and auricular surfaces, are unavailable for analysis.

For more than 100 years, the development of age estimation methods for adult skeletons has been heavily weighted toward portions of the skeleton that experience numerous changes throughout life. Despite repeated testing and revisions of techniques based on the cranium, pelvis, and ribs, problems remain. The most notable of these is ubiquitous age estimation bias that worsens after approximately age 50.

This work, with collected data, is a proof of concept that low-information, binary features can be used to produce age estimates throughout adulthood. Five rounds of preliminary data collection, analysis, and trait revision at the W.M. Bass Donated Skeletal Collection were used to investigate more than 250 trait variants. In each iteration of data collection, traits with strong age-related patterns were retained, while those that provided little to no age information were revised for one or more rounds of analysis before being eliminated if useful age-related patterns failed to emerge. Fifty-three skeletal features were ultimately selected and investigated in a larger sample of individuals from the W.M. Bass Donated Skeletal Collection, the Maxwell Museum Documented Skeletal Collection, the University of Iowa-Stanford Collection, and the J.C.B. Grant Collection. These samples were intentionally chosen to represent as heterogeneous a group as possible within a single, broadly defined ancestry category (European/White). This is because in many forensic situations the most appropriate reference sample for an individual case is impossible to identify. Thus, a conservative, widely applicable method is desirable.

The combined sample of 1,010 individuals (677 males and 333 females) from the four skeletal collections was used to select a suite of features that collectively show age-related change throughout adulthood. These data were then used as a reference sample to estimate age for 350 modern and historic individuals from the Athens, Greece, collection and the St. Bride's crypt collection in London. Estimates generated using several dozen binary features are compared to those produced using the existing Transition Analysis procedure and other commonly used age-estimation methods for the pubic symphysis and auricular surface.[1] The Greek sample demonstrates how the selected traits perform on well-preserved modern remains. Individuals from St. Bride's crypt illustrate how the traits perform on individuals from a population with significantly different diet, activity level, and disease exposure than the reference sample. Because this sample is also less well-preserved than the Athens collection or the reference sample, it is a test of how well the procedure performs under less than optimal conditions, similar to those encountered in forensic settings when skeletal elements are often missing or damaged. The produced maximum likelihood estimates of age collectively exhibit less bias than estimates from other methods, even though no data from the cranial sutures, rib ends, pubic symphyses, or auricular surfaces were used.

A wider array of features similar to those used here is currently under investigation by a National Institute of Justice (NIJ) -funded research team using a diverse sample of modern populations from four continents. A reference manual and computer software are also under development as part of the larger project.

This research is supported by a National Science Foundation (NSF) Doctoral Dissertation Research Improvement Grant (DDRIG) award.

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A90 Examining the Effectiveness of Mastoid Process Measurements in Estimating Sex

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After attending this presentation, attendees will understand the applicability of using mastoid process measurements to estimate sex of the fragmentary cranium.

This presentation will impact the forensic science community by examining the use of the "mastoid length" measurement, as described by Buikstra and Ubelaker, in determining sex of a fragmentary cranium using the mastoid process.[1] This presentation will also evaluate the inter-observer variability of the mastoid length measurement due to its wide use by forensic anthropologists.

Due to sexually dimorphic morphology, the skull is considered to be very useful when determining the sex of skeletal remains as part of the biological profile. The mastoid process is a feature typically cited as a good indicator of sex based on differences in length and volume between males and females. A system of scoring the mastoid process, and other cranial features, on a 1-to-5 scale has been developed to aid in sex estimation of cranial remains[1]. In addition, in recent years, attempts have been made to analyze the mastoid triangle region for sexual dimorphism with varying results; however, no attempt has been made to solely use the mastoid length measurement described by Buikstra and Ubelaker, which is likely the most common mastoid process measurement.[1] Nevertheless, there is a general consensus among forensic anthropologists that this measurement is difficult to take and inconsistent between analysts.

This research examines the usefulness of mastoid length and mastoid triangle area as an indicator of sex using a Southeast Asian sample. A total of 122 crania (47 female, 75 male) from the skeletal collection at Khon Kaen University in northeast Thailand were used in this study. Each mastoid process was scored on a 1-to-5 scale following Buikstra and Ubelaker, referred to as estimated sex, and the mastoid length measurement was taken.[1] Measurement of the mastoid triangle was conducted to obtain distances between porion, asterion, and mastoidale. The semi-perimeter of the triangle was computed and used to calculate the triangle's area using Heron's formula. Twenty crania were measured by a second researcher to test the level of inter-observer error.

Correlations between variables were measured using Pearson's correlation coefficient. As expected, significant correlations (p <0.05) were observed for both males and females between estimated sex and mastoid length, estimated sex and area, and mastoid length and area. A significant correlation was observed between estimated sex and age in females, the only significant correlation between age and other variables in females. No significant correlation was identified in males between age and remaining variables.

Discriminant function analysis was performed using several different combinations of variables to test whether mastoid length alone can be useful in determining sex. When all six measurements were used to discriminate between sexes, leave-one-out cross-validation reached an accuracy of approximately 80%, the highest level of accuracy reached of any analysis. When only mastoid length was used to discriminate between sexes, cross-validation accuracy was approximately 77.6%. This suggests that when presented with a fragmentary temporal bone, a single mastoid length measurement can be used with significant accuracy to estimate sex.

Inter-observer error was examined for estimated sex and mastoid length. Cohen's kappa was used to measure the level of agreement between observers. A Cohen's kappa value of 0.100 (p = 0.222) suggests a low level of agreement between observers. The percentage of difference between 40 mastoid length measurements (both left and right sides) was calculated with an overall average of 4% difference. Interestingly, in 18 of the 26 cases in which estimated sex differed between observers, the observer that scored the mastoid process highest also had the larger mastoid length measurement. Given that estimated sex was assessed prior to taking the mastoid length measurement, this suggests the estimated sex score may bias the researcher's measurement of mastoid length.

The results of this analysis are promising for the applicability of using the mastoid length measurement as a method for estimating sex of fragmentary crania; however, further testing on non-Asian samples should be conducted to corroborate these results.

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1. Buikstra J.E., Ubelaker D.H. 1994. Standards for Data Collection from Human Skeletal Remains. *Arkansas Archeological Survey Research Series No. 44.* Fayetteville, AR.

Mastoid Process, Sex Estimation, Discriminant Function Analysis

A91 The Effect of Body Composition on Outdoor Human Decomposition

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After attending this presentation, attendees will understand the phases of human decomposition and how they are affected by body composition. The underlying goals are to improve the determination of Postmortem Interval (PMI), provide information useful to the positive identification of remains, and to spark further research into the decomposition variables that show correlation to body composition.

This presentation will impact the forensic science community by providing results of a study regarding how body composition affects the association between human decomposition milestones/phases and Accumulated Degree Days (ADD). This presentation will add to the general understanding of PMI estimation, human decomposition, and one of its key variables, body composition.

This research examined the differences in the rate of decomposition in humans with reference to their Body Mass Index/body composition. For instance, Simmons and colleagues found no effect of body mass on the rate of decomposition, while Sutherland et al.'s study found that small pigs decomposed almost three times as rapidly as large carcasses and Meadows and colleagues study found that obese individuals "quickly loose body mass due to the liquefaction of the body fats," which was supported by Zhou and Byard's case studies.[1, 3-5]

For this study, it was predicted that the body composition will affect the length of various phases of decomposition. Furthermore, it was predicted that more or less ADD will be required to reach specific decomposition milestones depending on body mass. Additionally, body mass may have an effect on how long (ADD) it takes the body to transition from one phase to the next.

A modified version of Megyesi et al.'s total body scoring system was used to examine the influence of body composition on human decomposition using 35 study subjects at the Texas State University's Forensic Anthropology Research Facility (FARF).[2] A total of 32 phases/time periods were established for three anatomical regions. The phases and time periods of decomposition were examined daily until full mummification occurred and ADD were calculated. These results were further statistically analyzed using a Student's Slope *t*-test.

The milestones of decomposition were examined for each individual following Megyesi and colleagues' method every day until full mummification occurred.[2] The dates from the beginning and the end phases and when milestones were reached was recorded and ADD were calculated to measure the thermal units that were required for the subject to reach certain decomposition landmarks (discoloration, bloat, and mummification) and for the bodies to complete certain stages of decomposition (Fresh, Discoloration, Loss of Tissue, Maggot Activity, Bloating and Caving In of Abdominal Cavity, Purge, And Mummification).

The results exhibited a strong statistically significant correlation between ADD and BMI for 7 of the 32 phases and time periods. The Head's Loss of Tissue phase and the Limbs' Placement until Start of Mummification time period showed strong statistically significant correlations (R^2 = 0.70051 and R^2 = 0.77258, respectively). The strongest and most prominent correlations were seen in the Trunk:Purge (R^2 = 0.77396), Placement until End of Purge (R^2 = 0.73464), Caving In (R^2 = 0.77991), Placement until End of Caving In (R^2 = 0.6888), and Mummification (R^2 = 0.71958). The statistical analyses of how phases and time periods correlate to each other presented that the slopes of the Trunk Mummification and Trunk Placement until Mummification and Trunk Purge and Placement until Purge End phases and time periods do not reveal a significant difference and are therefore comparable.

Overall, the study demonstrated that body composition is a factor in human decomposition but is not always a statistically significant one. Therefore, the results should be further examined in order to establish exactly how the correlation works and how the correlation can be used to improve PMI estimation.

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2. Megyesi M.S., Nawrocki S.P., Haskell N.H. 2005. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci.* 50(3), pp.618-26.

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Body Composition, Human Decomposition, Forensic Anthropology

A92 The Accuracy of Estimating Ancestry in Undocumented Migrants Along the South Texas Border Using Dental Morphological Traits: A Comparison to Craniometrics

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After attending this presentation, attendees will understand the advantages and disadvantages of using dental morphological traits and craniometrics to estimate the ancestry of Hispanic individuals.

This presentation will impact the forensic science community by testing the validity of using dental morphology to estimate ancestry in Hispanic individuals and by comparing dental morphology to more traditional craniometric results.

Ancestry estimation is an essential factor of the biological profile, but accurately estimating ancestry in Hispanic individuals is difficult.[1] While it is customary to use craniometrics to quantitatively estimate ancestry of unknown individuals, the use of dental morphological traits for ancestry estimation is becoming more common. In this presentation, the accuracy of discriminant function equations using dental morphological traits established by Edgar and traditional craniometrics to differentiate between Hispanic and non-Hispanic individuals were compared.[2] The goal is to determine if these two accepted methods of ancestry estimation can accurately classify undocumented migrants discovered along the South Texas border.

The sample consists of ten individuals (nine male, one female) discovered along the South Texas border who are thought to be Hispanic based on anthropological analyses and cultural profile. A total of 13 dental traits were observed and scored on both antimeres, when present, using the Arizona State University Dental Anthropology System (ASUDAS) and the expression count method.[3] The expression count method uses the more complex or higher score of the antimeres to represent the scored trait for that individual.[4] Only permanent maxillary and mandibular teeth were observed and scored. Those teeth that exhibited wear, breakage, caries, modification, or calculus were observed to the extent possible. A discriminant function equation established in Edgar was used to differentiate between Hispanic and non-Hispanic. The rate of accurate Hispanic classification was then compared to the rate of accurate craniometric classification.

Standard craniometric landmarks were taken with a Microscribe® G2 3D digitizer and recorded using 3Skull. Twenty-four inter-landmark distances were then imported into FORDISC® 3.0 in order to estimate ancestry. FORDISC® is a program that uses discriminant function analysis to classify individuals into ancestral groups in reference to data from the Forensic Data Bank (FDB).[1] Each individual was compared to four ancestral groups in FORDISC®: White, Black, Hispanic, and Guatemalan. These four groups were chosen to be consistent with Edgar sample groups of Spanish-speaking regions that include South America, Cuba, Mexico, and Puerto Rico.[2]

Results from a sample of N=10 revealed that the two methods do not give similar ancestry estimations. According to the dental results, one out of ten individuals classified as Hispanic, while the craniometric results indicated that five out of ten individuals classified as Hispanic (one of which was Guatemalan). Further, of the ten individuals, ancestry estimations for only three individuals matched between both methods. A goodness of fit was used on the results and showed that there is a statistical significance between the two methods at the 95% confidence level; however, these results may be due to the small sample size of N=10.

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Ancestry Estimation, Dental Morphology, Craniometrics

A93 What Stature and Weight Do We Actually Estimate?

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After attending this presentation, attendees will understand the type of body size (reported versus cadaver size) to which estimates by commonly used regression equations are most closely related.

This presentation will impact the forensic science community by investigating and quantifying the relationship between estimated body size and reported/cadaver size in contemporary Americans. This information will assist forensic investigators and researchers in selecting body size estimation methods appropriate for their casework and research.

In forensic anthropology, the primary purpose of body size estimation is identification of unknown skeletal remains. Thus, the emphasis is generally placed on estimating victims' body size *at the time of death*. Various estimation methods have been devised based on different types of body size (e.g., living stature/weight, cadaver-adjusted living stature, reported stature/weight), and they are frequently believed to be estimating living or reported size; however, how accurate or biased the results they produce are in terms of the reported and cadaver size, which are likely to represent one's body size at the time of death, have not been systematically tested. In this study, the estimates from the commonly used methods are compared to reported and cadaver size to see if they actually represent living stature/weight or other types of size.

Forty-three American White skeletons (23 males and 20 females) in the W.M. Bass Donated Skeletal Collection, University of Tennessee, were used. Statures of the donations were estimated using the anatomical method (Raxter et al., equation 1), Ousley (femur equation), and Trotter and Gleser (femur equation).[1-3] Weights were also estimated using the morphometric method (Ruff et al.), McHenry, and Grine et al.[4-7]. Then, the bias between estimates and reported/cadaver size (Σ(cadaver or reported size – estimated size/n)) was calculated.

Stature estimates tended to be closer to cadaver stature than reported stature. The only exception was the Ousley's female equation that yielded less-biased estimates for reported stature (bias of -0.24cm and -1.13cm for reported and cadaver stature, respectively).[2] As far as cadaver stature is concerned, the anatomical method and Ousleys equation produced least-biased results for females and males, respectively (bias of -0.38cm and -0.29cm, respectively).[2] Estimates closest to reported stature were obtained by Ousley's equations for both sexes (bias of -0.24cm and 1.93cm for females and males, respectively).[2] Trotter and Gleser tended to underestimate cadaver stature (bias of 3.12cm and 2.01cm for males and females, respectively).[3] Considering that cadaver stature is reportedly 2.5cm larger than living stature, Trotter and Gleser appear to estimate living stature with least bias.[3] All the weight equations underestimated both cadaver and reported weight. Like the stature equations, smaller bias was produced when cadaver weight was estimated compared to reported weight. The least-biased cadaver weights were obtained from the morphometric method and Ruff et al. for males and females, respectively (bias of 5.26kg and 4.33kg, respectively).[5] When estimating reported weight, Ruff et al.'s equations performed best for both sexes, but the bias reached up to 14.74kg and 6.78kg for males and females, respectively.[5] McHenry's equation turned out to produce most biased results among the weight equations.[6]

Stature and weight equations produce estimates of differential degree of bias depending on the type of body size (reported versus cadaver). Once forensic investigators and researchers decide the type of body size based on their purpose, the most appropriate equations should be chosen based on the degree of bias associated with the size type.

Reference(s):

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4. Ruff C., Niskanen M., Junno J.A., Jamison P. Body mass prediction from stature and bi-iliac breadth in two high latitude populations, with application to earlier higher latitude humans. *J Hum Evol.* 2005:48(4):381-92.

5. Ruff C., Scott W.W., Liu A.Y. Articular and diaphyseal remodeling of the proximal femur with changes in body mass in adults. *Am J Phys Anthropol.* 1991:86(3):397-413.
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Body Size Estimates, Reported Size, Cadaver Size

A94 A Retrospective Analysis of FORDISC® Performance at the C.A. Pound Human Identification Laboratory (CAPHIL)

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After attending this presentation, attendees will better understand performance discrepancies between FORDISC® 2.0 and FORDISC® 3.1, as well as some causes for these differences.[1,2]

This presentation will impact the forensic science community by highlighting facets of ancestry assessment, particularly in metric analyses, that continue to be problematic for the identification of human remains.

The goal of this presentation is to evaluate the performance of FORDISC® and its use in analyses at the University of Florida CAPHIL.

Since its establishment in 1972, the CAPHIL has utilized cranial Discriminant Function Analyses (DFA) to help ascribe ancestral affiliations to skeletonized human remains, beginning with the DFA methods outlined by Giles and Elliot.[3] Despite the 1993 release of FORDISC® 1.0, the CAPHIL did not regularly employ this program until after the release of FORDISC® 2.0,[1,4] This study uses FORDISC® 3.1 to provide a retrospective analysis of metric ancestry assessment at the CAPHIL.[2]

Four CAPHIL analysts accessed cranial metric data from 60 individuals originally processed in FORDISC® 2.0 by the CAPHIL between 1997 and 2002.[1] All included individuals were positively or strongly tentatively identified by Medical Examiner's Offices (MEOs). For each individual, all measurements included in the original FORDISC® 2.0 assessment were entered into FORDISC® 3.1 and compared against all groups in the FORDISC® 3.1.309 Forensic Data Bank (FDB) using a forward mean percentage stepwise analysis.[1,2] The results of the FORDISC® 2.0 and FORDISC® 3.1 ancestry analyses were then compared to each other and to the individual's MEO-issued identification.[1,2] Results from each FORDISC® version were considered "correctly classified" if in agreement with the MEO identification.

FORDISC® 3.1 analyses agreed with the original FORDISC® 2.0 analyses for 40 cases (66.7%)[1,2] FORDISC® 2.0 did not provide a classification matching the MEO-provided demographics in ten cases (16.7%).[1] FORDISC® 3.1 did not provide a classification matching the MEO-provided demographics in 17 cases (28.3%); in four of these cases, FORDISC® 3.1 did not classify the individual into any group.[2] Importantly, for six cases (10%) the reference group selected by FORDISC® 3.1 was not included in the original FORDISC® 2.0 analysis, even though the reference group was available in the FDB at the time.[1,2] In five of these cases, the FORDISC® 2.0 result matched the MEO-issued identification; in the sixth case, a reference population representative of the decedent was not available in either FORDISC® version.[1] FORDISC® 3.1 results matched the MEO-provided data in none of these six cases.[2] In two additional cases, FORDISC® 3.1 selected a reference group that was not available in FORDISC® 2.0; in both cases the FORDISC® 3.1 classification was incorrect and the FORDISC® 2.0 classification correct.[1,2]

In this study, FORDISC® 3.1 showed no improvement over FORDISC® 2.0 in providing correct (i.e., matching MEO-issued identification) classifications of the Florida forensic sample.[1,2] These results may be influenced by discrepancies between the DFA options used by past CAPHIL analysts and those used in this study, including differences which may be chosen (e.g., by self-limiting reference groups based on *a priori* assumptions) or imposed (i.e., program updates between the two FORDISC® versions resulted in the addition of new reference groups and statistical functions). Nonetheless, the results prompt several interesting questions that will be discussed. Should forensic anthropologists expect updated versions of FORDISC® to increase discriminating power, or should researchers instead expect them to provide more realistic (and most likely lower) statistical probabilities? Is there a balance between increasing the amount of human variation captured by the program and maintaining practical classificatory efficacy? Further, as sample sizes increase and more comparative groups based on ethnic categories (e.g., Hispanic) are added, how confident are researchers that FORDISC® reference samples, though based on "known" identities, meaningfully reflect ancestry (or even self-identification, in cases where "known" ancestry is ascribed by the MEO)?

With the imminent release of FORDISC® 4.0, this comparative study provides important insight into DFA and its use in forensic anthropology. Understanding how updates to past FORDISC® versions and historical variation in analysts' use of FORDISC® have affected results through time may strengthen DFA implementation and interpretation as the field advances. In this manner, these reflections on the past can guide the continuing evolution of best practices in forensic anthropology into the future.

Reference(s):

1. Jantz R.L., Ousley S.D. FORDISC®2.0: Computerized forensic discriminant functions. The University of Tennessee, Knoxville, 1996.
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3. Giles E., Elliot O. Sex determination by discriminant function analysis of crania. *Am J Phys Anthropol.* 1963; 53-68.
4. Jantz R.L., Ousley S.D. FORDISC®1.0: Computerized forensic discriminant functions. The University of Tennessee, Knoxville, 1993.

FORDISC®, Ancestry, Identification

A95 The Undergraduate Forensic Internship Experience: A Path to Employment for Anthropology Students

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After attending this presentation, attendees will understand the impact of forensic internships on employment opportunities for anthropology students.

This presentation will impact the forensic science community by identifying opportunities to enhance career development for undergraduate anthropology students.

The popularity of forensic-themed television shows focusing on aspects of forensic science (*Bones, CSI, Forensic Files,* etc.) has led to a concomitant increase in university enrollments in forensic-related fields, including forensic anthropology. Many of these undergraduate students lack the means or desire to pursue advanced degrees immediately upon matriculation, but employment opportunities in forensic-related fields for the graduate with an Anthropology BA are limited in Florida due to Florida Department of Law Enforcement (FDLE) regulations requiring a Bachelor of Science degree (in any field) prior to employment in all entry-level forensic science positons. Since 2006, the Department of Anthropology at the University of West Florida (UWF), in conjunction with the District One Medical Examiner's Office (MEO) in Pensacola, has offered forensic anthropology students the opportunity to intern at the MEO for academic credit, in the hopes of improving their employment prospects after graduation.

The forensic internship as developed at UWF is designed to allow students to clarify their career goals, to integrate theoretical concepts and knowledge obtained in the classroom with real-world employment situations, to gain practical on-the-job experience in a professional setting, and to gain work-related references and networking opportunities, which will enhance their marketability as professionals. Over the course of their internships, students have the opportunity to observe and assist with autopsies, attend and help document death scenes, assist in the field recovery of surface or buried skeletons, process/macerate decomposed bodies, familiarize themselves with Florida statutes governing the medical examiners system and the disposition of human remains, as well as to learn basic office management skills.

Over the past ten years, more than 30 anthropology students have participated in the internship program. The purpose of this presentation is to track the career trajectories of these students, and to evaluate the impact of the internships on the career prospects of undergraduate students in forensic anthropology. Of those interns whose post-graduate careers could be tracked, approximately 10% ultimately decided against a pursuing a career in the forensics field, 15% found employment immediately after graduation in an MEO, either locally or in another state, 27% were accepted into anthropology graduate programs, and 17% were accepted into forensic science graduate programs. The remaining students found employment in fields other than forensics or anthropology.

Internship, Medical Examiner's Office, Career Development

A96 The Detection of Genetic Information in the Enamel Proteome

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After attending this presentation, attendees will better understand alternative scientific and statistical approaches for calculating measures of human identity from teeth.

This presentation will impact the forensic science community by informing attendees that genetically variant peptides from tooth enamel can potentially be used to infer the status of Single Nucleotide Polymorphism (SNP) loci in a subject's genome. The past few decades have seen the impact of nuclear and mitochondrial DNA typing on forensic science. The method discussed in this presentation introduces a third identifying method based on protein typing.

Human identification frequently depends on the ability to extract genetic information from human remains. In most cases, DNA typing is sufficient to statistically link an individual with biological material. In the event that DNA is absent or degraded, additional identification methods are required. Given the increased need for laboratory-based quantitative forensic methodologies, genetic variation in protein is an attractive alternative to develop novel identification technologies.

Enamel is the most robust human tissue and persists for long periods in the environment. The calcium apatite crystals encase and protect endogenous protein that contains genetic information in the form of single amino acid polymorphisms, the result of non-synonymous SNPs. This type of genetic variation is distributed across many populations and can be used to develop measures of human identification.

Blocks of enamel (20mg) were obtained from four subjects and were milled and dissolved in HCl for 1h at 56°C in the presence of reductant. The resulting supernatant was pH neutralized, carboxymethylated, and digested with trypsin protease in the presence of mass spectrometry-compatible surfactant. The resulting peptide mixtures were applied to a Thermo™ Q Exactive™ plus hybrid Orbitrap/linear ion trap mass spectrometry instrument. The resulting proteomic datasets were analyzed by using the "Global Proteome Machine" peptide spectra matching algorithm (www.thegpm.org). The peptide datasets were screened for previously characterized and genetically validated variant peptides. Six genetically variant peptides were observed in the enamel proteome across the four subjects and originated from five proteins, including COL1A2, one of the two major forms of collagen in teeth and bones.

This data indicates that genetically variant peptides in enamel are a potential and orthogonal means to develop statistical measures of human identification in the event of compromised, degraded, or absent DNA. This will have particular relevance for skeletal remains in which DNA is either degraded or absent. In the context of mixed skeletal assemblages, profiles of genetically variant peptides may also provide information about biodistance between different skeletal components.

This work was performed under the auspices of the United States Department of Energy by Lawrence Livermore National Laboratory under contract.

Enamel, Genetically Variant Peptide, Protein Typing

A97 Accumulated Decomposition Score (ADS): An Alternative Method to Total Body Score (TBS) for Quantifying Gross Morphological Changes Associated With Decomposition

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After attending this presentation, attendees will be aware of the foundation of a novel standardized method for quantifying human decomposition for the purpose of estimating the Postmortem Interval (PMI) based on gross morphological changes. Attendees will also have a better understanding of the pattern of decomposition in Central Texas as it relates to Accumulated Degree Days (ADD).

This presentation will impact the forensic science community by resolving issues anthropologists face when using the current TBS method to estimate PMI.[1]

Megyesi and colleagues presented a method for quantifying gross morphological changes during decomposition known as the TBS, as well as a regression equation for calculating the ADD since death based on the TBS; however, subsequent research has shown that the progression of descriptive traits used to calculate the TBS may not be universal.[1,2] Therefore, a new method of quantifying gross morphological changes associated with decomposition is needed.

In this presentation, the preliminary results of a new method of quantifying gross changes in the body during decomposition that resolves many of the issues associated with the progressive stages of the TBS are introduced. The proposed method, ADS, utilizes component scoring of traits, which allows for quantification of variability within and between regions. The ADS is calculated based on the appearance and progression of traits, including discoloration, skin slippage, marbling, bloat, purge, liquefaction, desiccation, mummification, and skeletonization. Although a sequence of decomposition is implied in this method, the ADS includes flexibility for adding the traits as they appear. For example, Megyesi and colleagues describes marbling and skin slippage in the trunk as occurring simultaneously; however, this is often not the case.[1] Additionally, because discoloration can be variable during decomposition, the ADS is designed not to emphasize coloration details, but to simply observe the initial appearance of this trait. Like the TBS, the ADS segments the body into regions for scoring; however, unlike the TBS, the ADS requires scoring the upper and lower limbs separately since they differentially decompose. Thus, the ADS observes the head and neck, torso, upper limbs, and lower limbs as four separate regions.

Four individuals from the Texas State University Willed Body Donation Program were scored throughout active decomposition using the ADS method. Observations were made both in the field and based on photographs taken throughout the decomposition process. ADD for each observation (n=138) was calculated using the average of the minimum and maximum temperature for the entire day. The ADS was then regressed against ADD, and the regression formula was tested using ten additional static observations at different stages of decomposition. Preliminary results indicate that the ADS correlates exponentially with ADD ($y= 25.574e^{0.0926x}$, R^2=0.84689), explaining a significant proportion of the variation observed in the rate of decomposition in Central Texas. This pilot study suggests the ADS method is a good predictor of PMI based on ADD.

This research introduces a new standardized method of collecting decomposition data that can be used to develop regionally specific regression formulas in order to more accurately estimate PMI. This will lead to a greater understanding of the PMI and the process of decomposition in forensic anthropology, may assist in condensing missing persons reports, and aid in generating a timeline for law enforcement investigation.

Reference(s):

1. Megyesi M.S., Nawrocki S.P., Haskell H. 2005. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci.* 50(3):1-9.
2. Suckling J.K., Spradley M.K., Godde K. 2016. A longitudinal study on human outdoor decomposition in Central Texas. *J Forensic Sci.* 61(1):19-25.

A98 To Group or Not to Group?

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After attending this presentation, attendees will understand whether or not populations should be grouped in osteometric analyses.

This presentation will impact the forensic science community by describing which measurements exhibit statistically significant differences when comparing White, Black, and Asian (Japanese) groups and the implications for osteometric analyses, including osteometric sorting.

Osteometric sorting is a statistical tool utilized in cases of commingling to sort remains based on size. The reference dataset currently used for osteometric sorting uses multiple population groups and both sexes. This has primarily occurred to increase the sample size, but as the dataset increases, the need to pool groups may be unnecessary. In addition, differences in size between populations may decrease the performance of the method. The purpose of this study is to determine whether differences between groups exist and whether particular measurements should be utilized with a grouped sample.

Three populations were utilized in this analysis: American/European Whites, American Blacks, and Asian (Japanese). A total of 152 skeletal measurements (74 left, 74 right, and 4 midline), which includes standard and non-standard measurements (77 and 75, respectively), were compared via a two-tailed Student's *t*-test to determine whether a significant difference in group means exists ($p \leq 0.05$). White vs Black, White vs. Asian, and Black vs. Asian were compared with sexes pooled and separated by males and females, as well as males and females compared within each group, for a total of 12 comparisons per measurement. Because osteometric sorting utilizes the difference between left and right pairs (the "D" value), this value was calculated for each measurement and the same comparisons via *t*-tests were performed ($p \leq 0.05$). The mean, minimum, and maximum were also calculated for each measurement and D-value for every group.

The majority of the skeletal measurements were significantly different between the groups. When comparing Whites and Blacks, the number of statistically significant different measurements ranged from 33.8% to 38.8%, while the number of significant measurements ranged from 69.2% to 90.1% when comparing Whites and Asians. When comparing Blacks and Asians, there were between 63.8% and 75.0% significant.

The number of statistically significant tests was much lower when comparing the D-value between groups. When comparing Whites and Blacks, the number of statistically significant differences in D-values across elements ranged from 8.6% to 14.9%, while the number of significant D-value tests ranged from 2.9% to 17.6% when comparing Whites and Asians. When comparing Blacks and Asians, there were between 9.9% and 20.3% significant. The female group comparisons had the fewest number of significant results for all tests, which indicates a smaller difference in size between the females in these populations.

Overall, results of this study suggest that, although the majority of skeletal measurements are significantly different between groups, the difference between left and right elements when compared across groups is not significantly different. This suggests that the majority of measurements can be used with populations grouped in the reference dataset for osteometric sorting. Measurements with significant D-value tests for the majority of the between-group comparisons may need to be avoided in osteometric analyses when samples are grouped. These include the maximum length of the radius and ulna, the physiological length of the ulna, and the maximum distal epiphyseal breadth of the tibia.

Ancestry, Osteometry, Commingled Remains

A99 Disturbed Soil: Unexplored Variables and the Postmortem Interval (PMI) in Southwest Florida

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After attending this presentation, attendees will understand how subsurface variables, such as soil moisture, temperature, and pH value, are influenced by the act of disturbing soil in the absence of human decomposition in Southwest Florida. The environment of Fort Myers, FL, will be delineated in an effort to determine how the simple act of agitating soil influences these ecological variables upon which well-established PMI estimation methods rely.

This presentation will impact the forensic science community by increasing the accuracy of PMI estimations.

Time-Since-Death (TSD) and Time-Since-Burial (TSB) estimation methods have grown from research conducted within the University of Tennessee–Knoxville's Forensic Anthropology Center. As a result of decades of research at this facility, Dr. Arpad Vass helped to establish two standardized equations to estimate the PMI associated with human body decomposition.[1] Of specific interest to this study was Dr. Vass' equation to estimate TSD. Key variables used in this equation are soil moisture and soil temperature. Similarly, other prominent PMI estimation methods, such as Megyesi's Accumulated Degree Days (ADD) system, also rely upon variables, such as average daily temperature and atmospheric humidity.[2] It is well established in the literature that these variables are proximal influences upon decomposition rates as: (1) warm temperatures increase in the rate of decomposition (per Van't Hoff's Law); and, (2) an increase in soil moisture slows body decay rate; however, this current study proposes that while the relationship of these variables to decomposition rates is well described, previous research has neglected to consider how the act of disturbing soil strata layers when a clandestine grave is dug without the addition of a body may influence these variables.[1]

Twice a year, as part of Florida Gulf Coast University's (FGCU's) forensic anthropology graduate and undergraduate course, five mock clandestine burials containing plastic skeletal specimens are uncovered from a pine flatwood ecosystem. The specimens are buried at a depth of 40 to 60 centimeters within an area of approximately 250 square meters.

From June 19 through July 1, 2016, a Science, Technology, Engineering, and Math (STEM) Student Research Opportunity (SRO) was conducted in conjunction with the Whitaker Center for STEM Education. This training included instruction in forensic anthropological field methods which allowed the SRO students to assist the FGCU Human Identification and Trauma Analysis (HITA) graduate students and faculty in large-scale data collection. Soil temperature, moisture, and pH readings were taken at surface level (0-3cm), the level of the skeletal specimen, and the grave bottom using Vernier probes. These same readings were also taken from the control test pits.

The statistical analysis revealed no significant differences in soil temperature (R = -0.053, t = 1.98, p = 0.08), skeleton level (R = 0.30, t = -0.039, p = 0.7), grave bottom (R = -0.36, t = -2.87, p = 0.34), or moisture (R = 0.22, t = 0.42, p = 0.68; pH (R = -0.53, t = 1.98, p = 0.08) between the undisturbed soil of the test pit and the previously disturbed soil of the mock graves. These findings therefore indicate that the agitation of soil does not significantly influence the soil temperature, moisture, or pH of a clandestine grave as compared to a control test pit. Caution must be used in the application of these findings because exogenous variables, such as insect activity and local floral species, may have an unaccounted-for impact upon these soil readings.

Accurate estimation of the PMI and TSB is imperative in forensic anthropology casework. A truly universal PMI formula would be an invaluable tool for forensic anthropologists. For this to be a future possibility, a complete understanding of the variables that influence decomposition rate is paramount; additionally, the calibration of environmental impact on those variables by using a control test pit in each experiment should be implemented as a standard.

Reference(s):

1. Vass A.A. The elusive universal post-mortem interval formula. *Forensic Sci Int.* 2011: 209(1-3): 34–40.

2. Megyesi M.S., Nawrocki S.P., Haskell N.H. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci.* 2005: 50(3): 619–626.

Anthropology, PMI, Burials

A100 Mapping Surface Scatter of Scavenged Human Remains Using Drone Aerial Photography

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After attending this presentation, attendees will better appreciate the suitability of mapping the spatial distribution of major elements in outdoor surface remains using images collected by an Unmanned Aerial Vehicle (UAV).

This presentation will impact the forensic science community by demonstrating the accuracy, benefits, and drawbacks of aerial photography via UAV for mapping major skeletal elements present in outdoor crime scenes without disturbing the scene.

High-resolution aerial images collected from UAVs are more commonly being used by law enforcement to map crime scenes and to search for clandestine human remains.[1] Small UAVs can be rapidly deployed and photograph a scene in a fraction of the time required when using traditional methods. Computer software can stitch the multiple photographs to render a 3D map of the scene that can be viewed and measured from multiple positions; however, in outdoor scenes containing skeletonized human remains, scavengers often distribute the small bones over a relatively large area, making them difficult to identify in aerial photographs.

The purpose of this pilot study was to compare maps of outdoor human remains sites based on UAV-collected photographs to traditional baseline maps. Two scattered remains sites at the Forensic Anthropology Research Facility at Texas State University were used in the study. Both sites were more than two years old, in an open grassy area, and the bones had been scattered over an approximately 50-meter area by scavengers.

Prior to deployment of the UAV, each scatter was mapped utilizing traditional baseline mapping techniques used in archaeology. A 2'x2' black/white aerial target was then placed near the scattered remains for reference during the UAV flight. In addition, the baseline points were marked with pink flags to provide easier visibility in the photographs. A multi-rotor UAV was then deployed and a series of aerial photographs were taken at approximately 40 feet above ground surface for each site. Two images were also imported into the open-source software, ImageJ. The baseline was drawn and the scale was set using known distances. The measure tool was then used to measure the distance from the baseline to the bone that could be observed. Additionally, an ellipse was fitted around the areas of the scatters to measure the total distribution of the scatters.

Baseline measurements using traditional techniques were compared to baseline measurements acquired from the photographs. An analysis of the two methods exhibits an average percent error of less than 2% (1.5%) for major skeletal elements. The comparison of maps demonstrates that the major skeletal elements can be accurately mapped using aerial photographs collected from an UAV equipped with a high-resolution camera; however, mapping smaller elements is difficult. Though small elements can be seen in the photographs, they are not discernible enough to accurately measure. The advantage of the aerial photograph maps is that they can be rapidly produced without disturbing the scene, and the stitched map can be viewed three-dimensionally from any position.

As drones equipped with high-resolution cameras become more readily available, they have the potential to be used to develop accurate 3D maps of outdoor crime scenes. While aerial photographs collected from UAVs cannot replace the more detailed mapping necessary in forensic anthropological cases, they do provide a valuable additional tool. Furthermore, initial mapping can be conducted prior to any disturbance of the scene.

Reference(s):

1. Katz E., Halámek J. (2016). *Forensic science: A multidisciplinary approach*. Weinheim, Germany: Wiley-VCH.

Surface Scatter, Drone Mapping, Spatial Distribution

A101 Assigning Region of Origin in the Southeastern United States Using Stable Oxygen Isotopes of Modern Human Enamel

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After attending this presentation, attendees will understand the importance of developing regional stable oxygen isotope drinking water comparisons. Attendees will be informed of the application of assignment models to determine accurate predictions of region of origin in forensic investigations using isotopic signatures.

This presentation will impact the forensic science community by demonstrating the advantages of applying human enamel from individuals with known residential histories to tap water isoscapes for accurately predicting the decedent's region of origin in forensic investigations.

Isotope analysis has become increasingly popular for narrowing the region of origin for unidentified human remains in forensic investigations. Stable oxygen isotopes ($\delta^{18}O$) in enamel reflect early life residential history and stable hydrogen isotopes ($\delta^{2}H$) in hair keratin records later geographic movement, months before death. Skeletal preservation in the southeastern United States is typically poor due to the humid subtropical climate, sometimes limiting the isotope analysis package to $\delta^{18}O$ of enamel.

Distinct spatial $\delta^{18}O$ distributions in tap water assist in narrowing the decedent's region of origin in unidentified persons cases. Human oxygen isotope measurements are compared to the spatial tap water distributions using baseline tap water isoscapes. Furthermore, understanding the relationships of enamel and water isotopes improves the assignment prediction models in forensic investigations. The goals of this research were to investigate the relationship between enamel and tap water oxygen isotopes in the southeast and use the relationship to improve region of origin predictions for modern human enamel.

Enamel (n=11) samples obtained by the United States Air Force Academy (USAFA) from individuals whose third molars were extracted between 2004 and 2005 were processed.[1] Residential history information recorded for the participants included birth city and state, and residential history including the year and geographic information of relocations. The USAFA sample measurements ($\delta^{18}O$ carbonate) reported on the Vienna Peedee Belemnite (VPDB) scale were converted to $\delta^{18}O$ phosphate (Vienna Standard Mean Ocean Water (VSMOW)).[2,3] Tap water data were extracted from previously reported oxygen tap water values for the United States.[4,5] Converted AFA data, (i.e., $\delta^{18}O_{ep}$) from individuals with early southeastern United States residential origins were regressed against tap water isotope $\delta^{18}O_{tw}$ values.

To determine if the relationship would improve predictions for human enamel in the southeast, an assignment model based on likelihood predictions was used to estimate region of origin. Likelihood assignment methods based on probability are more accurate and may incorporate prior probability using Bayes' rule to directly estimate origin based on previous data. A likelihood assignment model was developed using Geographic Information Software (GIS) and the USAFA sample (AFA134) from Southaven, MS, was assigned to the calibrated isoscape.

The relationship between the southeastern individuals and the tap water was $\delta^{18}O_{ep} = 0.704 * \delta^{18}O_{tw} + 20.07$ ($R^2 = 0.89$) and was calibrated into the isoscape assignment model. The $\delta^{18}O_{ep}$ for the state of Mississippi ranged from 16.22‰ to 16.94‰, compared to AFA134 whose oxygen isotope value was 15.846‰. Sample AFA134 was an ideal participant for this study, being a local resident of Southaven, MS, from birth until the age of 20. The likelihood model predicted the region of origin for AFA134 from a geographical band spanning from southern Washington County to central Alcorn County on the northern Tennessee boundary. The Southaven area had a slightly lower predicted outcome than the 90% probability band just south of the actual residential origin of AFA134.

Since enamel reflects the $\delta^{18}O$ sources of body water, the human signature is enriched compared to the $\delta^{18}O$ of the tap water. Therefore, the tap water isoscape was calibrated to make it comparable when assigning region of origin for modern human dentition in the southeastern United States. Individuals sampled from Florida were omitted from the study due to disproportionate relationships with tap water. The relationship between tap water

and human enamel from this study ($\delta^{18}O_{tw}$ = 1.2704 * $\delta^{18}O_{ep}$ - 26.01) is similar to the relationship Daux et al. ($\delta^{18}O_{tw}$ = 1.54 * $\delta^{18}O_{ep}$ - 33.72) demonstrated for human $\delta^{18}O$ values.[6] The differences between the two conversion equations may relate to source water and sample availability, suggesting that specific human-to-drinking-water $\delta^{18}O$ conversions should be applied dependent upon the geographic region of the research study. Variability is expected as many factors complicate regional $\delta^{18}O$ values, especially in modern humans and globalization.

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Isotope, Oxygen, Assignment Model

A102 The Differences in the Rate of Decomposition Between Frozen and Non-Frozen Human Remains

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After attending this presentation, attendees will have a greater understanding of the differences in the decomposition rate between previously frozen and never-frozen human remains that have been allowed to decompose in an outdoor setting.

This presentation will impact the forensic science community by adding to the research being conducted involving decomposition of human remains and how freezing impacts the rate of decomposition.

There is a need to understand the effect of freezing on decomposition rates. In some cases, research facilities need to store human remains before they begin documenting decomposition in an outdoor setting. There are also instances when remains from a homicide victim may have frozen due to low external temperatures before decomposing or the remains are stored in a freezer prior to relocation.

Freezing slows bacterial growth and therefore greatly reduces the rate of decomposition while the body is in a frozen state; however, there have been mixed results in studies examining the effect of freezing on post-thaw decomposition rates. An experiment conducted examining the effect of freezing on muscle tissue found no significant difference between frozen and unfrozen samples.[1] A more recent study using domestic pigs (*Sus scrofa)* found that previously frozen subjects and non-frozen subjects differ in rates of decomposition, with decomposition rates being slower in previously frozen pigs.[2] There have been no studies that have used whole body human donations in an outdoor setting to examine differences in decomposition between previously frozen and never-frozen subjects.

In this experiment, a total of ten human remains were placed in an outdoor setting at the Forensic Anthropology Research Facility in San Marcos, TX. All of the remains were placed on the ground surface, with half of them having been previously frozen. Each subject was placed unclothed in a supine position under a wire cage to prevent scavenging. The frozen and unfrozen remains were pair matched for season of placement and body size. A Total Body Score (TBS) was calculated for each subject at approximately 100, 300, and 500 Accumulated Degree Days (ADD).[3]

Paired samples were compared using a scale of TBS <10, TBS 10-20, and TBS >20. There were no statistically significant differences in TBS between previously frozen and never-frozen remains at any ADD period; however, after 100 ADD, frozen remains consistently have higher TBS. At approximately 100 ADD, only 20% of the paired samples scored in the same TBS range. Forty percent of the paired samples demonstrated that never-frozen human remains decomposed at a slower rate than previously frozen remains, while the other 40% of the pairs displayed the opposite pattern. At approximately 300 ADD, 40% of the paired samples scored in the same range, while the previously frozen bodies decomposed more rapidly than the never-frozen bodies in 60% of the pairs. At approximately 500 ADD, 20% of the paired samples had the same TBS range, but the never-frozen bodies had a greater TBS in 60% of the pairs.

This research provides evidence that freezing does have an effect on the decomposition rate of human remains, especially after more than 100 ADD. These observations indicate that after approximately 100 ADD, previously frozen human remains decompose faster than their never-frozen counterparts. While the pattern observed in this study differs from the previous study using pigs, the conclusion is the same.[2] That is, the rate of decomposition between previously frozen and never-frozen remains should not be compared in taphonomic studies examining the relationship between TBS and ADD.

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Decomposition, Frozen, Total Body Score

A103 The Use of Industrial Computed Tomography (CT) in Forensic Anthropology

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After attending this presentation, attendees will be familiar with the uses of industrial CT scanning in forensic anthropological examinations.

This presentation will impact the forensic science community by increasing awareness of alternative postmortem imaging approaches, as well as by demonstrating some of the uses of industrial CT technology in forensic anthropological casework and research.

The use of postmortem CT to examine skeletal remains is not new, but its use by forensic anthropologists is relatively rare. This may be due to limited resources for purchasing or accessing CT scanners, or unfamiliarity with CT options and their advantages. CT is used in a variety of industries, including not only medicine and dentistry, but also security, aerospace, automotive, manufacturing, and defense, among many others, and these industrial devices can be adjusted to parameters suitable for examining bone. Anthropologists may therefore be able to consider alternative technologies such as industrial CT, thereby increasing their potential access to it. Moreover, industrial CT scanners are highly versatile and offer several significant advantages over medical CT in the examination of skeletal remains.

For example, most industrial CT systems are self-contained and self-shielding, eliminating the need for specialized rooms for their operation, as well as individual shielding. Moreover, while medical CT systems are commonly limited to certain dosages (due to health concerns for living patients) or are tailored to specific diagnostic applications, industrial CT in postmortem imaging allows longer scan times and greater versatility in terms of the analytical parameters used. For example, the use of high intensity, micro-focus or nano-focus X-ray sources on industrial CT systems permit much greater magnification and resolution than a typical medical CT scanner. Industrial CT scanners are also capable of greater penetration of dense objects, such as dental fillings and surgical devices, than typical medical CT technology. If desired, most units can also be used for traditional 2D digital radiography. File formats generated from industrial CT scans are standard and compatible with most medical imaging viewers, analytical software platforms, and 3D printers.

Currently, the Federal Bureau of Investigation (FBI) Laboratory and the Forensic Anthropology Center at Texas State (FACTS) both utilize an industrial CT scanner in postmortem assessments of skeletal remains for forensic and research purposes. This unit is marketed primarily for non-medical, industrial applications but is readily adapted for use in anthropological investigations. Applications include documenting features for identification, analysis of skeletal trauma and disease, assessment and documentation of biological parameters, production of 3D printed replicas, and research on bone density and microstructure. Another significant advantage of this type of machine in a large forensic or academic laboratory is that, unlike a medical CT system, which is configured and dedicated to one or a few specific purposes, the machine has many forensic and research applications. In the FBI Laboratory, in addition to forensic anthropological applications, the device supports forensic examinations of manufactured products, explosive devices, electronics, and firearms. At FACTS, the system is used in forensic anthropological casework and research, as well as for imaging archaeological, paleontological, and geological specimens. This ensures a high utilization rate and more readily justifies the costs of its operation and maintenance.

CT is an extremely useful tool in postmortem forensic anthropological examinations, and the use of radiology for non-destructive documentation, examination, diagnosis, and preservation of skeletal remains is encouraged. Virtually any type of CT scanner can be adapted to effectively image skeletal material, so anthropologists are not limited to medical CT systems in their examinations. Laboratories can consider alternative CT scanners for purchase, but they may also be able to leverage resources that already exist in their community by developing relationships or partnerships with professionals in other offices or industries. The use of more versatile machines can result in greater imaging resolution and detail for skeletal examinations and may also be more cost effective for

a laboratory, especially in cases in which the device can serve multiple disciplines.

Forensic Anthropology, Forensic Radiology, Computed Tomography (CT)

A104 Prostheses and Medical Devices: Their Value in Forensic Anthropology

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After attending this presentation, attendees will better understand the value of prostheses for identification purposes.

This presentation will impact the forensic science community by providing relevant information on the multiple uses of prostheses and other medical devices.

Bodies in an advanced state of decomposition (e.g., skeletonized or burned) are a challenge for identification. Since identification of unknown human remains has major social, cultural, and economic implications, it is worthwhile to explore alternative means of identification. These can be particularly helpful in situations with multiple unidentified victims, such as mass disasters and human rights violations, or when the fragmentary nature or damage of the remains preclude the application of standardized methods. The primary techniques of identification may not always be applied in those contexts. The increase of life expectancy and the advances in medical knowledge are leading to a growing number of surgical implants that multiply the likelihood of finding a decedent with a surgical prosthetic device. Furthermore, manufacturers of these devices normally use a specific brand and serial number, which allow tracking of the individuals who possess them. Fortunately, these serial numbers are generally not affected by post-depositional processes, exposure to the environment, or heat-related events. In addition, several countries now possess national databases of implants. For forensic anthropology, the benefits of prostheses and other medical devices include: (1) they act as unique identity factors, disclosing information about a disease or injury afflicting the missing person, as well as his/her gait or posture, with which relatives and friends may be familiar; (2) in association with degenerative diseases such as osteoarthrosis or osteoporosis, they can supply data on age at death, primarily regarding elderly individuals; (3) since they imply the performance of surgery, they suggest that the victim may be from a socio-economic context or country that granted access to this type of procedure; (4) the type of material used as well as the technique performed can be relevant to assess ancestry and therefore be used as a biogeographical indicator as some cutting-edge techniques are only performed in developed countries; and, (5) they can provide an insight into the Postmortem Interval (PMI) since the device models are continuously being updated.

The goal of this presentation is to discuss these advantages through the medical devices found in the 21st Century Identified Skeletal Collection (CEI/XXI), housed at the Laboratory of Forensic Anthropology, University of Coimbra. A total of 43 cases of Portuguese-identified men and women, ages 29-98 years, with different types of prostheses and/or medical devices were analyzed.

The CEI/XXI collection is primarily composed of elderly individuals; as such, the prostheses found were useful to validate the biological profile, in particular age at death. Furthermore, all the analyzed prosthetic materials were applied in Portugal, corroborating the place at which the surgery was executed. In some cases, serial numbers and logos were found, leading to the identification of the individuals throughout the National Arthroplasty Observatory database, since it is obligatory to register all surgically added implants in Portugal. Materials used in the orthopedic prostheses were shown to be very resistant to time. Regarding PMI information, it was possible to detect a clear chronological sequence of prostheses utilization. For instance, while a Moore prosthesis points to an individual who underwent surgery a long time ago and who, most probably, died more than ten years ago, a ceramic prostheses points to individuals who died in the last 25 years.

In summary, the devices examined in this investigation proved to be of major value in providing key information regarding a positive identification or at least provided enough information regarding the individual to allow for a

narrowing of the list of potentially matchable missing persons. The prostheses and implants had severe consequences on the gait/locomotion and posture of the individuals under analysis, features identifiable by medical records and associates of the decedents.

Prostheses, Medical Devices, Identification

A105 Bones and Teeth as Osteological Signatures of the Identity of Human Remains Excavated From a 160-Year-Old Abandoned Well: A Forensic Anthropological Case Report From India

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After attending this presentation, attendees will better understand the outcomes of unscientific excavations of human remains from a confined location such as an abandoned well, preliminary identifications of exhumed remains from a forensic anthropological context, and future challenges to be faced by experts in their positive identifications.

This presentation will impact the forensic science community by encouraging forensic anthropologists to be extra cautious when excavating human remains from pre-existing structures such as wells, natural ravines, pits, and sewage channels and to make efforts to preserve the challenged forensic sites with the utmost care.

Mankind has witnessed several crimes against humanity in which disposal of human cadavers becomes problematic for the perpetrators to avoid international human rights attentions. The pre-existing structures, such as abandoned wells, potholes, natural ravines, roadside trenches, sewage systems etc., have remained preferred sites for the clandestine burial of such human remains. Post-conflict recovery of human remains has been reported from a number of countries, including Spain, Guatemala, Croatia, and Iraq; however, no such recovery was been reported from Indian soil from such sites, although in one incident, there was a written record of the presence of human remains from 282 individuals buried in a well under a religious structure at Ajnala, India.[1] This ignited the ethical conscience of various local people to excavate any such remains to corroborate or negate this reported case of a crime against humanity. As the entire excavation process was executed by amateur excavators, the bones of multiple individuals were recovered badly damaged and commingled. This study's primary goal is to identify whether the recovered human remains belong to victims of a reported mass-killing incident or to dead bodies disposed of prior to or after the reported incident in an attempt to solve the mystery of alleged crimes against humanity or human rights violations. The other objectives include: (1) determining whether the remains were modern or archaeological in nature; (2) if archaeological, determining whether the bodies date back to the mentioned period, or are older or younger; (3) discovering the minimum number of individuals present in the well; (4) establishing whether the remains were from North India (the local area of the site) or from Bengal, Bihar, and Uttar Pradesh, where the victims reportedly originated; and, (5) identifying the sex, age, ethnicity, and probable stature of the victims.

The shape, size, morphology, and proportions of different skeletal elements revealed these remains belong to adult male individuals. The articular ends of the fragmented long bones had no signs of osteoarthritis, implying that the majority of victims were less than 60 years of age. The overlapping, disorganized, and diverse positioning of individual skeletons and their stratigraphic sequences indicated that bodies were thrown into the well from the top at the same time. The retrieval of well-preserved teeth, hand and foot bones, vertebrae, a few intact skulls, femur heads, etc., from the commingled remains demonstrate that the present human archaeological site could have been processed more scientifically had it been excavated by trained anthropologists and archaeologists with expert knowledge in human osteology and odontology.

All intact skulls have traumatic injuries in the same region (i.e., the forehead region of the frontal bone), implying that some blunt weapon was used to inflict the same type of injury to the frontal head of the victims. Associated ballistic evidence in the form of cone-shaped stone bullets (a few still intact) was also found with the remains. The majority of seriously damaged skeletal remains were found unsuitable for forensic examinations and hence were not analyzed. Only teeth were found in fairly good conditions and are expected to facilitate forensic anthropological identification regarding the provenance, geographic origin, disease and health status, dietary habits, minimum number of individuals inside the well, etc. from their anthropological, molecular, and elemental analyses. Numerous personal artifacts, including copper and iron wrist bracelets, gold necklace pieces, coins and medals (with the Queen's photograph and year), and beaded arm bands, were also recovered with the human remains inside the well. The systematic and expert-mediated recovery of human remains using advanced archaeological techniques and instruments may have proved fruitful, not only in their precise and safe excavation, but also in the identification of these human remains in the laboratory. This presentation will highlight the consequences of both

the unscientific excavation of human remains and preliminary forensic anthropological identifications.

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Forensic Archaeology, Human Remains in a Well, India

A106 The First Case of a Mummified Face and Skull-Photo Superimposition in the United Arab Emirates

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After attending this presentation, attendees will better understand the first case of a mummified face and skull-photo superimposition in the United Arab Emirates and the importance of such a technique in the process of unknown person identification when there are no samples from relatives for DNA identification.

This presentation will impact the forensic science community by providing results from a mummified face and skull-photo superimposition case, which was possibly applied for the first time in the United Arab Emirates. This presentation will add to research being conducted in skull-photo superimposition by broadening the understanding of the usefulness and challenges of such techniques in connection with cultural differences, immigrant communities, unknown person identifications, intra-laboratory validations, and forensic investigations.

In 2012, an unknown mummified female body was discovered in salt marshes in the emirates of Abu Dhabi of the United Arab Emirates. Clothes located near the body were recovered. Documents found with the clothes indicated the unknown female was from a certain African nationality. Due to the effects of sea water, the photo in the document was unclear, damaged, and less useful in the identification process. The Criminal Identification department was successful in finding a passport of a missing female from nearby resident village. As it was not possible to reach the victim's relatives in their original country, a skull-photo superimposition examination was decided upon in order to check the connection between the passport photograph, the mummified face, and the skull X-ray photographs. One of the challenges was that the female's passport photograph had an Islamic hijab (a veil) covering the back the head, which can complicate the comparison process. In cooperation with Sheikh Khalifa Medical City in Abu Dhabi, a Computed Tomography (CT) scan of the unknown female skull was taken. The CT scan data was important in this technique to produce a 3D image of the skull to be used in skull-photo superimposition (in-house). To confirm and to adjust the in-house superimposition, the mummified face photographs, the CT scan Digital Imaging and Communications in Medicine (DICOM) data, and the passport photo were sent to the Center of Anatomy & Human Identification, the University of Dundee, for a separate mummified face and skull-photo superimposition report (intra-laboratories comparison and validation). The result of the in-house and intra-laboratories comparison confirm the presence of multi facial similarities between the mummified female face and skull and the passport photo.

In conclusion, this case study will be presented as the first case of a mummified face and skull-photo superimposition in the United Arab Emirates. Furthermore, the data and experiences gained from this case will be helpful for forensic experts faced with similar unknown person identifications that are due to cultural and technical challenges and limitations. This presentation also stresses the importance of intra-laboratory validation in such comparisons and superimposition techniques.

Mummified Body, Skull-Photo Superimposition, Unknown Person

A107 Documenting Fracture Time Since Injury (TSI) Through a National Archival Database of Antemortem Fracture Healing

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The goal of this presentation is to document anatomical variability in antemortem fracture healing to aid estimation of TSI in forensic abuse cases. This is accomplished through the creation and demonstration of a national archival database for antemortem fracture healing containing macroscopically, microscopically, and radiologically derived data.

This presentation will impact the forensic science community by providing forensic anthropologists and pathologists with an understanding of the variability inherent in antemortem fracture healing in relation to a number of key attributes. This presentation will aid in the determination of fracture TSI and ultimately provide evidence (and strengthen court testimony) relating to the determination of accidental vs. non-accidental etiology for peri-mortem fractures in forensic death investigations involving suspicions of pediatric, domestic, or elderly abuse.

Assessments of TSI for antemortem fractures heavily influence determinations of accidental vs. non-accidental origin of peri-mortem fractures in cases of suspected abuse. In this presentation, the anatomical basis for antemortem healing is reviewed, particularly in regard to dating of fractures and the factors that influence TSI determination.

Antemortem healing is a continuous process which does not lend itself well to rigid interpretation using a finite staging system.[1] Determining the timing of prior injury is difficult because bone repair is influenced by a number of important variables. These variables include an individual's age, sex, nutritional and disease status, fracture location, type, and severity as well as presence and nature of treatment and repetition of injury.[2,3]

This presentation illustrates the effect of these variables upon the healing rates of antemortem fractures. In this study, the antemortem healing process is chronicled through examination of a sample of more than 1,500 macroscopic, microscopic (from a digital light microscope at 5x-200x), and radiologic digital images representing 106 antemortem fractures from 11 forensic cases (six females, five males) involving pediatric, domestic, and elderly abuse.

These images are used in the creation of a digital archival database for antemortem fracture healing for use in forensic TSI estimation. This archive consists of a searchable, online (web-based) database of antemortem fracture imagery, including macroscopic, microscopic, and radiological modalities. The database is implemented as a web app through the Radford University Forensic Science Institute website, developed in python using a Flask microframework and a MySQL™ database; both the database and website are hosted on a single Linux® platform. This website allows users to search for antemortem fracture images across the three modalities and the variables discussed above (e.g., age, sex, bone, fracture location and type, healing status, and estimated TSI).

The database includes individuals ranging in age from 27 days to 79 years; 58% of all fractures derive from subadults. Fracture location overwhelmingly involves ribs (88%), but also long bones (primarily the diaphysis) and the clavicle. Fracture timing reflects the range of the healing process (early to late) and is categorized in terms of healing status based on criteria developed by Boyd et al.[1] In at least three cases, fairly precise healing times are known or inferred.

These macroscopic, microscopic, and radiological images and their associated metadata are used to illustrate differences in the healing process across the variables examined. It is noted, for example, that metaphyseal lesions, though less visible, manifest quicker healing times. Faster healing times are also documented in the younger subadults.

The database also provides users with the ability to upload additional antemortem fractures cases, including histology images. Future additions to the antemortem fracture database from the forensic anthropology and pathology community will result in larger, more diverse samples, enabling better understanding of fracture healing variability and more precise estimates of TSI in forensic abuse investigations.

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Antemortem, Fracture, Database

A108 Rediscovering the Unknowns of South Jersey: The Long-Term Initiative to Develop a Standard Methodology for Unidentified Remains at the Southern Regional Medical Examiner Office (SRMEO)

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After attending this presentation, attendees will have an understanding of the challenges involved with identifying cold unidentified cases without a standard methodology and how Rediscovering the Unknowns of South Jersey (RUSJ) is making an effort to allay these challenges for the SRMEO.

This presentation will impact the forensic science community by providing examples of the use of a formal qualitative algorithm on all unidentified cases. These examples will also highlight the importance of applying best forensic practices to cold cases.

RUSJ is a project that was undertaken by personnel at the SRMEO in 2010. The project's main goal was to better determine how forensic anthropology-related cases were handled by this office, historically and currently. RUSJ has evolved to include all unidentified cases within three county jurisdictions, the current oldest case being from 1972. RUSJ has encountered, and is likely to continue to encounter, a wide range of challenges. The challenges tend to focus primarily on gathering the missing information on the historical cases. Best forensic practices and technology have evolved immensely in the 40 years since many cold cases in the current database were investigated. Creating a comprehensive database of these cases revealed gaps in knowledge and documentation. These gaps ranged from DNA information to reports and photographs to the current locations of the actual remains. Research is still ongoing to ascertain any missing information. With the assistance of local law enforcement agencies, funeral homes, and various other state offices, this data is being gathered and verified when available.

This project is based on the premise that SRMEO is rediscovering cases that have not been actively investigated in more than 40 years and applying current forensic practice to them. RUSJ will work toward ensuring that best forensic practices, including, but not limited to, DNA testing and forensic anthropology consultation, are applied to historical data. The same testing and scrutiny will be applied to current and future unidentified cases. Creating a standardized method for handling unidentified remains and individuals can streamline the process. By adopting similar procedures, cooperating medical examiners offices could operate on a single reliable algorithm, making it possible to cross reference documentation for unidentified cases more efficiently. The implementation of best forensic practices in every office would be imperative to accommodating the standardized system. The hope is that a standard methodology can ensure consistency in all cases, unidentified and anthropological.

Since the creation of the database, the processes suggested in RUSJ have been applied to numerous cases. The results of this fact-finding mission have led to the positive of identification three individuals from 2001, 2010, and 2013. These previously unidentified individuals have since been reunited with family members, and the intention is for more identifications to follow.

The long-term goal is that implementation of this algorithm on current cases will mitigate the research time, costs, and manpower incurred by identifying historical cases. Utilizing a standardized procedure will ensure best forensic practices are used at the start of any case, thus improving the probability of identification in the future.

Outside the scientific community, the benefits of RUSJ are numerous and long term. The project provides the opportunity for more unidentified individuals to be identified and their remains returned to family members. It can also decrease the strain on county offices to process unclaimed individuals and give closure to loved ones long waiting for answers.

Unidentified, Identification, Cold Cases

A109 The Identify Indiana Initiative: A Collaborative Approach to Reinvestigating Cold Cases of Human Remains in the State of Indiana

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The goals of this presentation are for attendees to appreciate: (1) how these investigations require cooperation between multiple agencies; (2) how new technologies can be employed to further identification efforts; and, (3) the challenges faced in these reinvestigations within the context of an elected coroner system.

This presentation will impact the forensic science community by serving as an introduction to the coordination of multiple agencies and resources required for the reinvestigation of cold cases for those who may soon embark on a similar mission.

The Identify Indiana Initiative grew from the proposition that many unidentified cold cases in Indiana could benefit from the application of new technologies, such as missing persons databases, DNA profiling, and isotope analysis. A team of volunteer specialists, including forensic anthropologists, law enforcement personnel, DNA laboratory technicians, coroners, and National Missing Persons Data System (NamUs) administrators, was assembled to reopen these cases, which date back to 1974. The first step in this process was inventorying the unidentified cases in the state. Locating some of the unidentified individuals was straightforward since they are curated at the University of Indianapolis, while others require exhumation from cemeteries across the state. Osteological analyses were conducted on the exhumed remains that were buried before forensic anthropologists were practicing in the state. The information from the skeletal examination was combined with data from law enforcement records to create an entry in the Unidentified Persons section of the NamUs database. Skeletal samples were then collected for molecular investigations, including DNA profile generation and isotope analysis.

While locating the unidentified skeletal cases and implementing new technologies to aid in identification may seem rather straightforward, the team faces a number of practical challenges. For example, Indiana is a coroner system state, and the 92 coroners (who are usually not formally trained in either law enforcement or forensic science) are elected via the political process to six-year terms. According to state law, the coroner has the ultimate authority over found human remains and must therefore give advance permission for exhumations, analyses, database entry, and the release of case information to the press or public; however, many coroners operate in rural counties without formal office or morgue space in which files or evidence can be curated long-term. With the inevitable turnover in coroners over time, the team has encountered difficulty in obtaining basic case information and associating different case numbers from the various agencies that may have been involved in an investigation. This problem extends to the acquisition of family reference DNA samples; missing law enforcement reports may have included the names and addresses of possible relatives that were interviewed in missing persons cases. The ability to locate case files and case numbers is an instrumental step in furthering the identification of these individuals.

The collaboration process, utilization of new technologies, and challenges faced by the team will be discussed within the context of several examples in which unidentified remains were exhumed and reanalyzed for this initiative. Additionally, stable isotope analysis has been integrated into the Initiative to determine whether unidentified individuals are likely local or non-local to the location where their remains were discovered. Stable isotope analysis on one of these cases demonstrated that a deceased female, discovered in 1992 in central Indiana, was most likely from the Southwest (Arizona or New Mexico) but may have spent several months in western Indiana prior to her death. This information serves as an investigative tool to aid in narrowing search parameters for missing persons cases. As the scientific tools and technologies of forensic investigation evolve, unidentified human remains should be reinvestigated to increase the chance of identification.

Unidentified Individuals, NamUs, Isotopes

A110 Are They Full Siblings? The Distribution of the Sibship Index and Its Application in Forensic Identification

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After attending this presentation, attendees will understand the basic concepts of DNA profiling and the significance and challenges of using autosomal Short Tandem Repeat (STR) DNA in forensic identification.

This presentation will impact the forensic science community by providing a baseline dataset to assist in the interpretation of the sibship index to increase the chances of identifying missing people with no self-DNA references.

The Department of Defense has collected DNA samples for all American service members since 1992 to ensure reliable self DNA samples are obtained. Due to the absence of self DNA reference for the missing servicemen from the conflicts prior to 1992, the DNA sequences from bone samples are compared to the missing person's Family Reference Samples (FRSs). Mitochondrial DNA (mtDNA) has been most commonly used as the missing person shares the same mtDNA with his/her maternal relatives; however, an mtDNA sequence is not individual specific and can be shared by thousands of unrelated people. If the missing person has an mtDNA sequence common in a population, additional evidence may be required to establish identification. One such evidence is nuclear DNA, both Y-chromosome Short Tandem Repeat (Y-STR) and autosomal-STR (auto-STR). If the missing person is a male and has brother FRSs, the paternally inherited Y-STR is used for comparison. If the missing person is a male and has sister FRSs, auto-STR analysis is used. Unlike mtDNA or Y-STR, auto-STR is unique to an individual. The individual specific nature of the auto-STR poses a challenge for comparison because even full siblings do not have the same auto-STR profile. This is where the sibship index comes into play.

The sibship index is the likelihood that the auto-STR results obtained from a sample support the hypothesis that the sample and the included reference sample are biological siblings, rather than if the sample is a full sibling of an unrelated individual. For example, a sibship index of 100 means that it is 100 times more likely that the bone sample and the FRS are biological siblings, rather than if the bone is a full sibling of a random person. If the sibship index is in the millions, a full sibling relationship can convincingly be established as it is highly unlikely that two unrelated individuals have significant overlap in their auto-STR profile; however, a low sibship index may not necessarily indicate that the two individuals are unrelated due to the chance of two full siblings inheriting two complete different sets of auto-STR from their parents. The sibship index frequently falls into a gray area that neither supports nor refutes full sibling relationship. This is a problem because auto-STR is often the only way to identify a missing serviceman. The goal of this study is to document the distribution of the sibship index for: (1) full siblings; and, (2) unrelated individuals. The focus is on discovering the degree of overlap of the tales of these two distributions.

In this study, the auto-STR comparison data obtained from the following four scenarios were used to generate sibship index distributions (N = 1,000): (1) the bone of an identified soldier to the full siblings of the identified soldier; (2) the bone of an identified soldier to the excluded soldiers (unrelated individuals); (3) two known full siblings in the FRS database; and, (4) two unrelated individuals in the FRS database. The number of obtained auto-STR loci was also documented to see how significantly the sibship index can change based on the quality of the auto-STR data. The study found that when two samples are full siblings, the average sibship index was in the millions but indices lower than 0.05 were also observed (Negatively skewed curve; Mean 5,073,370; Median 10,635). When two samples are unrelated individuals, the average sibship index was in the hundredths but indices between zero and ten were also observed (Normal distribution curve; Mean 0.0598; Median 0.0005755). There was minimal overlap between the two curves. As predicted, the number of obtained auto-STR loci affected the sibship index significantly: the higher the number of loci, the stronger the sibship index to confirm/refute the sibling relationship. The current research will be expanded to increase the sample size as additional results are obtained from more bones and FRS samples.

Sibship Index, Autosomal STR, DNA Profiling

A111 A New Complex Investigation Model for Searching, Mapping, and Identifying Disappeared Persons in Argentina

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After attending this presentation, attendees will better understand the problems related to the identification of human remains of disappeared persons and, in particular, the importance of applying innovative frameworks of research such as, in this case, the combination of complex networks, Bayesian inference, and statistical evaluation tests in the systematization and analysis of the data.

This presentation will impact the forensic science community by providing a framework of complex networks and Bayesian inference to be applied to the identification of human remains belonging to disappeared persons during the last military dictatorship in Argentina (1976-1983).

During the past 32 years, the Argentine forensic anthropology team, Equipo Argentino de Antropología Forense (EAAF), has been using a multidisciplinary approach (archaeology, anthropology, dentistry, pathology, and genetics) to recover and identify the remains of thousands of disappeared in the country.[1] Typically, after their kidnapping, people were taken to illegal detention centers, tortured, and killed. Their unidentified bodies were buried in individual or common graves in official cemeteries or clandestine mass graves at military/police compounds.

The need to generate hypotheses of identity for the recovered remains triggered interest in applying an alternative model for the analysis of the information.

This new model has mathematically systematized non-genetic variables, resulting from information obtained by already solved identifications in specific events, in such a way that they can be used in the search, GIS mapping, and a generation of new hypotheses of identity for unsolved related cases. In other words, this dynamic model based on complex networks and Bayesian inference is able to "learn" from identified cases by generating a probabilistic ranking of candidates for unidentified related cases. The geographical and temporal systematized variables, using georeferenced data and image processing techniques, from the identified skeletal remains are analyzed within a Bayesian framework.[2] In a second stage, the data are included on a complex network, where connections are established between related cases by using rules based on the individuals' attributes.[3,4] Previous to this, the most effective rules in defining the network are evaluated. Once the network has been formalized, a ranking of candidates for unsolved cases (recovered skeletal remains) is produced.

The advantage of this complex model is, among others, to minimize bias in the investigation of related cases, providing probabilistic values to connected cases. This model has a high applicability in the mapping and search of burial sites, identification of human remains, and systematization of information, essential in the investigation of massive numbers of victims, as in crimes again humanity, conflict, or migration cases.

The developed software, which includes processing of raw data, calculations, and visualization, uses R language, a free software and open source environment for statistical computing and graphics.[5-7]

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Human Remains, Geographic Information, Complex Network

A112 Antemortem vs. Postmortem Measured Statures of Korean War Casualties

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After attending this presentation, attendees will better understand the relationship between antemortem and postmortem measurements of stature and how this impacts the reference samples used in the anthropological calculation of stature.

This presentation will impact the forensic science community by further illuminating the relationship between antemortem and postmortem stature measurements.

Forensic anthropologists estimate the stature of skeletal remains using regression models based upon various data sets. Some data sets include measured antemortem statures for individuals, while others include postmortem cadaver lengths; rarely are both available. Two of the largest data sets available are those collected by Mildred Trotter from United States casualties from World War II and the Korean War, for whom living, measured statures were recorded. Other data sets, such as many anatomical collections, lack antemortem measurements and substitute postmortem stature, or cadaver length. These two measurements are not the same. The stature of any living individual is not a constant: recorded statures can vary based upon when they were taken, the methods used, and how they were transcribed. Postmortem measurements exhibit similar methodological and transcription variation, but are generally longer than antemortem measurements, presumably because curvature of the spine relaxes to some degree.

The Individual Deceased Personnel Files of historically identified Korean War casualties provide a data set of postmortem lengths that can be compared with antemortem measured statures. Stature was an essential component of the wartime identification process and was recorded at different stages. Form 1042 (Report of Interment) was completed when remains were initially processed and interred in a temporary cemetery. Early in the war, the height field was generally only completed for unknown remains; later on, its completion came to be more standardized. These measurements were taken by Army Graves Registration personnel at the cemetery. Form 1044 (Identification Data) was completed when the remains were later exhumed for identification processing by a team that generally included an anthropologist. Per the Central Identification Unit Standard Operating Procedures (SOP), these teams recorded "table measurements" of all flesh-covered remains, and long bone measurements from all skeletal and semi-skeletal remains. While bone lengths were recorded in centimeters (cm), all heights were measured in inches.

What is the relationship between these two postmortem measured statures and the casualties' antemortem statures? Multiple studies indicate that postmortem measurements should be approximately 1" higher than antemortem. Furthermore, the table measurements recorded on Form 1044 should be more accurate than the cadaver lengths recorded on Form 1042, given the conditions under which each was taken. In fact, the cadaver lengths were subject to more rounding error than the 1042 or 1044 measures: of 239 cadaver lengths in the dataset, 7 (2.9%) ended in fractions, while 205/481 antemortem statures (42.6%) and 137/290 table measurements (47.2%) ended in fractions.

Comparing 235 cadaver lengths to antemortem statures, 10.6% were identical, 58.7% larger, and 30.6% smaller, and the mean difference was 0.73"; 49.4% of the cadaver lengths were less than 1" different from the antemortem heights, placing them within rounding error. Another 16.2% were at least 1" but less than 2" greater, placing them within the expected range for postmortem measures; 34.5% were from 2" or 8" larger. Some of these may well be the result of growth after enlistment, given that many of the men enlisted at 18 or even 17 years of age, but much is presumably the result of measurement or recording error in either the antemortem or postmortem measures.

Comparing 289 table measurements to antemortem heights, 17.3% were identical, 55.0% larger, and 27.7% smaller, and the mean difference was 0.45"; 57.1% were less than 1" different from the antemortem heights, 10.4% were 1" or more shorter than antemortem heights (with a maximum discrepancy of 4"), while 29.4% were between 1" and 2.75" taller, and 3.1% were between 3" and 5.5" taller.

Only 48 cases had both cadaver lengths and table measurements recorded. The mean difference between them was -0.37", with half of the cadaver lengths larger and 37.5% smaller; 55.3% of the table measurements were closer to the antemortem height than the cadaver length was. Several cases demonstrate clear error in the recorded cadaver length, such as an antemortem height of 74" with a table measurement of 73.5" and a cadaver length of 67".

A113 Stature Determination From the Adult Human Clavicle: A Medicolegal Autopsy Study on the Indian Population of the Northwest Region of India

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The goals of this presentation are to explore the relationships of the mean length of the clavicle and stature in the Indian population of the northwest region and to formulate regression equations for stature estimation from clavicular length for both sexes.

This presentation will impact the forensic science community by exploring methods to estimate stature from fragmentary remains of mutilated bodies to help establish individuality of a person in cases of terrorist attacks, war causalities, and train and airplane accidents in which body mutilation is likely. At times, criminals may intentionally destroy the remains of their victims to make their identification difficult or they may be destroyed by animals after death.

Left and right clavicular lengths from 256 adult cadavers (44 female and 212 male) without trauma-related fractures and mass lesions or deformities were collected between Jan 1, 2011, and December 31, 2012, at the Department of Forensic Medicine, Government Medical College Amritsar, Punjab, India. The cadaver stature was measured as distance from the vertex to the sole using a special osteometric board. The maximum clavicular length of both sides was measured using sliding digital Vernier calipers and the average length of bilateral clavicles was also calculated for each case.

The mean length of the clavicle in females on the right side was 139.68mm and 140.21mm on the left side. For males, the mean length of the clavicle on the right side was 150.71mm and for the left was 151.89mm. The mean stature of females was 157.47cm and was 168.51cm for males. Separate regression equations were calculated for both sexes in respect to the length of the right clavicle, left clavicle, and average length of bilateral clavicles.

In terms of gender, the male length of the clavicle was more correlated to the stature of a person as compared to females. In males, the average length of the bilateral clavicle (r=0.938) was more closely correlated to stature, followed by the mean length of the right clavicle (r=0.846). In females, the average length of bilateral clavicles (r=0.761) was also correlated with a comparatively fair degree as compared to the mean lengths of right (r=0.477) or left (r=0.424) clavicles. The standard of error was least for the average length of the bilateral clavicle.

In northwest India, in the same population, Singh and Sohal worked out a multiplication factor for the calculation of stature in the male population and concluded that it was possible to calculate stature from the length of the clavicle.[1] Jit and Singh, utilizing the same population, concluded that neither a multiplication factor nor a regression formula can be adopted that will estimate the height of an individual with a reasonable degree of accuracy.[2] Yashoda et al. also devised regression equations in a study on a population of north India and found that clavicle length was a good parameter for stature estimation. Per this presentation's research, the sample size in this particular study far exceeds those of other studies conducted on the similar population of northwest India. Together, this population-specific formula will provide an easy tool for stature estimation and can aid forensic research.

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Stature, Northwest Region, Clavicle

A114 The Role of Stigma in the Medicolegal Investigation of Unidentified Persons

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After attending this presentation, attendees will better understand factors that contribute to the persistence of unidentified persons in domestic casework, focusing on the role that stigma can play in the identification process.

This presentation will impact the forensic science community by illustrating how particular groups become devalued in death through the use of pejorative language in medicolegal reports and how this stigmatization can affect resources dedicated to identifying these people.

Forensic anthropologists are increasingly called upon to assist in the identification of decedents lacking name associations (or tentative names). The forensic science community and the public have benefited from the development of sophisticated tools (e.g., National Missing and Unidentified Persons System (NamUs)) to address this country's "silent mass disaster," which help investigators bypass jurisdictional boundaries and create central, accessible, and easily comparable repositories for antemortem and postmortem data. Still, unidentified persons cases persist. Previous research has noted that particular groups (e.g., the homeless, foreign nationals, etc.) are disproportionally represented among unidentified persons in the United States, the reasons for which are likely multifactorial. These may include deficiencies in the reporting of missing persons (e.g., due to social alienation, fear of legal retribution, lack of available antemortem data, lack of public awareness, incompetence of investigators, etc.) or deficiencies in the reporting of unidentified decedents (e.g., due to lack of available postmortem data, lack of investigator education, lack of funding for sophisticated methods, incompetence of investigators, etc.). One contributing factor that has received limited attention is the role of stigma, or the process by which certain populations are devalued in society.

Macro-level identity of decedents is frequently evaluated as a tool for generating new investigative leads. In forensic anthropology, this "biocultural approach" considers biological, cultural, and contextual indicators as a means to assess group affiliation of unidentified persons. In turn, recognition of group affiliation can help anthropologists cater their route of identification to a specific community. This approach has proven particularly effective in resolving cases of unidentified decedents suspected of being foreign nationals who die while clandestinely crossing the United States-Mexico border; however, the categorization of unknown decedents is not new, nor is it unique to anthropology. Those involved in the medicolegal investigation of death frequently (albeit informally) categorize decedent's macro-level identity, observable in written descriptions from medicolegal (e.g., police, investigative, and autopsy) reports.

While the recognition of group affiliation can be useful, the language used in investigations can both reflect and continue to construct particular groups as "deviant" or less valued in society. This study discusses case examples of written descriptions of unidentified decedents from medicolegal reports, focusing on various types of pejorative language used to describe decedents from particular groups (i.e., homeless, foreign nationals, sex workers, and Lesbian, Gay, Bisexual, Transgender, Questioning (LGBTQ) members). Through this review, the manner in which the construction of these individuals in death as "deviant" can negatively affect the outcome of identification attempts through the prioritization of casework and the unequal distribution of resources is analyzed. Structural inequalities that certain groups encounter in life can continue to marginalize them in death, and depreciatory language employed as descriptors of group affiliation reinforce the devalued nature of these decedents.

In conclusion, the assessment of a decedent's macro-level identity is inherently political. When considerately applied, this approach may provide incredibly useful investigative leads and innovative routes for identification; however, when objective assessments of decedents are replaced by subjective, morally weighted judgments of an individual's value in society, this can negatively affect the outcome of the investigation. As forensic anthropologists employed to help contribute to identifying unknown persons, it is the responsibility of professionals to recognize this process of stigmatization and devaluation in medicolegal settings and to prevent the continued marginalization of vulnerable populations.

A115 The Applications and Practicality of Dental 3D Scanning in Forensic Anthropology

Teresa V. Wilson, PhD, Louisiana State University, 227 Howe-Russell Geoscience Complex, Baton Rouge, LA 70803*

After attending this presentation, attendees will better understand how and if consumer-grade 3D scanning of teeth can be used to aid medicolegal investigations involving skeletal remains and assist in the teaching of forensic anthropology students.

This presentation will impact the forensic science community by demonstrating the possible advantages and disadvantages of incorporating the 3D scanning of teeth into forensic anthropological analyses, research, and teaching. This presentation will provide results pertaining to the accuracy and resolution of scans, minimization of scanning anomalies and artifacts, and ways in which scans can be displayed for demonstration and teaching.

The use of 3D scanning in macroscopic analyses is becoming more affordable and attainable by researchers and practitioners in forensic anthropology. The non-destructive nature of 3D scanners allows for the scanning of fragile and important specimens; however, consumer-grade scanners can create several logistical and quality problems, including excess noise and gaps in the scans. Teeth in particular are difficult to scan because of the glossy texture of the enamel interfering with the reflective angles of the lasers employed by 3D scanners. This research focuses on dentition because of the importance of teeth in positive identification of remains as well as the analysis of age, trauma, and overall health.

Twenty modern isolated teeth, four isolated teeth from a historic population, and five mandibles (teeth: n=24; mandibles: n =5) were chosen for this study. The teeth from the modern population were selected to represent all tooth types and various states of dental health (i.e., carious lesions, amalgam fillings, resin fillings, and natural). Each specimen was scanned using a NextEngine® 3D Ultra HD scanner and processed using the NextEngine® ScanStudio™ ProScan software. Each specimen was scanned using different resolution settings, lighting conditions, and scanning techniques. Each scan was compared for measurement accuracy and the precision in which the lesions or restorations were captured. Scans using the highest resolution (16k points/inch^2) showed a grainier texture to the surface and displayed large amounts of noise that created inconsistent measurements of the teeth. Scans could not be accurately replicated at the high resolution due to the noise and textured surface. Scans at low resolution (~2k points/inch^2) proved to be accurate in dimensional measurement; however, all of the details of the tooth surface were lost. Scans at the middle resolutions (~4.2k points/inch^2) provided an acceptable balance between measurement accuracy and surface detail retention. Scan resolution and noise reduction were achieved through manipulation of the ambient light during scans and the reduction of enamel shine through the use of surface powders and sprays.

Although inexpensive consumer-grade 3D scanners are not without their difficulties, 3D scanning of dentition can be useful to forensic anthropologists in both the laboratory and the classroom. Choosing the appropriate scan resolution and noise reduction techniques is dependent on the purpose of the scan. When the goal is to preserve accurate dimensions and surface detail of the teeth for presentation during trial or continued examination of the dentition after skeletal remains have been released, choosing a mid-level resolution and controlling for all lighting problems that may affect the surface integrity is imperative. Accuracy is less important when using these scans in a classroom or training setting and lower resolutions can be used. Although it is unlikely that 3D scanning of dentition would be utilized in every forensic case, this technology can be useful in the preservation of anomalous features (i.e., trauma, dental pathologies, and unique occlusal patterns) for use in the future or when another look at the teeth will be useful later.

3D Scanning, Dental Anthropology, Teeth

A116 The Curious Case of Cranial Dysraphism: How a Cranial Variant Appears as Trauma or Trephination

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After attending this presentation, attendees will be aware of a natural cranial variant rarely reported in archaeological or forensic literature. This presentation will describe the detail of the cranial variant, present supporting information from the clinical literature, and describe how to discern the variant from trauma or pathology. In particular, this presentation emphasizes the scope of knowledge required by skeletal anatomists and how the inclusion of clinical literature into skeletal analyses can broaden the understanding of natural cranial variation.

This presentation will impact the forensic science community by demonstrating the need for broad studies of pathology and normal skeletal variation. Highlighted is a rare case of cranial variation, known as cranial dysraphism, reported in clinical literature, but rarely reported in archaeological or forensic literature. This presentation, based on a case study of cranial dysraphism, provides fellow researchers the opportunity to observe and learn about this rare anomaly.

In order to locate a specific individual's remains from within a multiple interment in an indigent cemetery, human skeletal remains were recovered from a pauper's grave in Louisiana in 2015. Although the individual passed away in 2000 and should, theoretically, have been easy to locate, the destruction of records by Hurricane Katrina and other mismanagement by cemetery caretakers made this a complex recovery effort, requiring a combination of forensic archaeology, historical research, legal maneuvering, and some luck. Despite difficult recovery conditions owing to New Orleans' high water table, commingled remains, and the lack of meaningful documentation, the remains were well preserved.

Upon exhumation, an oddity was noted on the autopsied calvarium of a single individual. Initial speculation consisted of trauma or a pathological condition due to the position on the calvarium occurring at bregma. Only after cleaning of the skeletal material was it clear that the resulting defect at bregma was not the result of trauma, but rather appeared to be an unexplained cranial pathology or morphological variant.

The cranial defect was visible as a large perforating foramen occurring at bregma. Distinguishing the defect from trauma are the smooth borders and intact sutural lines — the coronal and sagittal sutures were completely present and descended the thinning bone into the foramen. The defect was widest on the external table and tapered to a five millimeter foramen, circular in shape, perforating the inner table of the cranium. Due to the perforation into the cranial cavity and the defect's smooth borders, trephination was considered as a cause; however, in this case, there was no exposure of the diploë and, based on the location at bregma, the expectation would be that a trephined section of the cranium would not retain the presence of the sutures.

This case study is proposed as an ideal example of cranial dysraphism, a relatively unknown morphological cranial variant. The detailed analysis of the defect, when researched and supported with clinical data, supports the diagnosis of cranial dysraphism and highlights the need to look beyond traditional sources of archaeological/anthropological and forensics literature when confronted with a seemingly unique morphological trait.

Skeletal Remains, Forensic Archaeology, Cranial Variation

A117 The Relationship of Palatal Suture Complexity and Fusion

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After attending this presentation, attendees will better understand how palatal suture complexity, palatal suture fusion, age, sex, and ancestry are interrelated.

This presentation will impact the forensic science community by demonstrating that sutural complexity and fusion are linked in such a way that complexity cannot be overlooked when producing an age estimate from the sutures of the hard palate.

Age estimation from the palatal sutures is based on the age-progressive synostosis of the incisive, median palatine (the Anterior Median Palatine (AMP) and Posterior Median Palantine (PMP)), and Transverse Palatine (TP) sutures. While closure of the palatal sutures is useful in categorizing unidentified skeletal remains into broad age categories, methods employing palatal sutures suffer from a lack of precision and exhibit only moderate correlations with known age-at-death. An investigation of other variables that potentially contribute to palatal suture fusion is important in order to better understand variation in suture closure and to improve current methods.

The palatal sutures are part of a complex biomechanical environment and play an important role in permitting strength and flexibility of the palate during mastication. Complex sutures, those with many interdigitations, have increased surface area along the sutural margin. These sutures thus have greater energy-absorption potential and strength in bending and are usually found where loading is predominantly compressive. Simple sutures signify a largely tensile loading environment, and they may resist fusion to a certain extent due to the continuous separation at the margins brought about by tensile forces.

Data on palatal suture fusion and complexity were collected from 762 male and female individuals of African, Asian, and European ancestry. The sample was stratified prior to data collection so that each sex, ancestry, and age group was evenly represented. The palate was photographed, and fusion, total sutural length, and suture chords were digitally measured for the TP, PMP, and AMP sutures using ImageJ and standardized landmarks; error introduced from digital measurement is believed to be negligible. Sutural complexity is the total suture length divided by the suture chord (suture length ratio; values at or close to 1 = simple suture; values increasing from 1 = greater complexity). The total amount of fusion along a given suture was measured, even if discontinuous, and this amount was divided by the total length of the suture (fusion ratio; 0 = absence of fusion; 1 = complete fusion). The relationship of sutural complexity and fusion was tested using Pearson's *r* and linear regression, and the relationships of complexity, fusion, and demographic group (e.g., age, sex, and ancestry) were tested using linear regression and Analysis of Variance (ANOVA).

Overall, the sutures of the palate in this sample are not complex (mean complexity values: TP suture = 1.53; PMP suture = 1.24; AMP suture = 1.25), and the correlation of fusion and suture complexity ratios is always negative (TP $r = -0.39$; PMP $r = -0.37$; AMP $r = -0.17$). The adjusted R^2 for regression of fusion on sutural complexity is 0.187, and TP and PMP suture complexity have significant effects on fusion while AMP complexity does not (TP, PMP $p < 0.001$; AMP $p = 0.292$). Sutural complexity shows little to no relationship with age (R^2 values from 0.001 to 0.006). Sutural complexity for all three sutures was significantly different between the sexes and among the ancestral groups ($p < 0.001$). Males show decreased complexity as compared to females. Asians have the most complex sutures for the median palatine suture, while Europeans are the most complex for the transverse palatine suture.

The negative correlation of sutural complexity and fusion indicates that sutures displaying increased complexity have a tendency to exhibit less fusion, while sutures that are less complex are more likely to display fusion. Since palatal sutures in this sample are simple, this indicates a largely tensile environment in the palate, which may explain the patency of palatal sutures even into late adulthood. These results also are interesting in light of sex and ancestral differences in fusion. Males have higher fusion ratios than females, but are less complex. Asian individuals have the most complex sutures for the median palatine but also the lowest fusion ratios. Future research will focus on including complexity data in age estimation methods.

Adult Age Estimation, Palatal Sutures, Sutural Complexity

A118 Elliptical Fourier Analysis of the Human Nasal Aperture

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After attending this presentation, attendees will appreciate the various biological and environmental factors that contribute to normal variation in human nasal aperture shape. The goals of this presentation are: (1) to discuss how age, sex, ancestry, and geography differentially contribute to the overall shape and size of the human nasal aperture; and, (2) to discuss how this information could be applied to modern forensic investigations.

This presentation will impact the forensic science community by quantifying and describing differences in shape of the nasal aperture across different population groups. Previous studies have focused on the nasal aperture as a qualitative trait. By quantifying the shape of the aperture, more rigorous statistical analyses can be applied to the data.

In the analysis of human skeletal remains, the nasal aperture is often used in discrete, non-metric analyses, especially in regard to ancestry assessment. The shape itself is complex, so the final assessment of the shape has traditionally been based largely on the subjective judgment of the observer. This means the final decision of the qualities of that shape are subject to the Gestalt of the observer, and do not necessarily accurately describe actual shape. Elliptical Fourier analysis can transform the complex shape of the nasal aperture into a numeric variable. Once transformed, not only can the complex shape be consistently and precisely recreated, but it can be quickly and easily compared against data from other nasal apertures.

A sample of 868 individuals from the Pretoria Bone Collection and the W.M. Bass Donated Skeletal Collection were utilized in this study. Data was collected only on individuals with a known age, sex, and ancestry. This study utilized both males and females, with ages ranging from 18 to 100 years, but only collected data on individuals identified as White or Black. Individuals were not included in the sample if there was any damage to the contour of the nasal aperture. Data was collected by photographing the nasal aperture on a standardized plane at a focal length of 55mm with the camera set to aperture priority with an f-stop of eight. All photos were taken from a distance of 30cm directly above nasion. After photography, all images were uploaded into Adobe® Photoshop® v 11. The outline of the nasal aperture was traced along the internal margin using the Magic Wand Tool. The shape was then filled using the Paint Bucket Tool. Traced and filled images were resized to 500 x 332 pixels and saved as a 24-bitmap file. Those files were subsequently uploaded into SHAPE v. 1.3 for elliptical Fourier analysis. Four principal components were identified using the SHAPE program. Univariate analyses were performed to evaluate which variables (age, sex, and/or ancestry) were contributing the most to the shape differences. An Analysis of Variance (ANOVA) test was performed to test for significant differences between group means. Even though four principal components were identified, only the first two significantly contributed to the overall shape of the nasal aperture. In this study, ancestry was the most powerful contributor to the overall shape and was found to be significant in Principle Component Analysis 1 (PCA1) (height-to-width ratio, p=.000) and PCA2 (inferior margin contour, I=.004). Sex contributed significantly to PCA1 (height-to-width ratio, p=.001) but also appeared to exhibit significant interaction with ancestry, which may confound significance. The biggest difference between the sexes is overall size, but the differences in the actual shape are minimal. Age did not contribute significantly to any principal component, suggesting that it does not systematically affect the shape of the nasal aperture.

Elliptical Fourier analysis was instrumental in quantifying a complex shape, which allowed this study to focus on statistical comparisons instead of qualitative descriptions. These results reify that ancestry is the strongest contributor to the shape of the nasal aperture. Although age and sex somewhat contribute to the differences in aperture shape, their effects are not systematic and therefore do not influence the overall mean.

Nasal Aperture, Elliptical Fourier Analysis, Shape Analysis

A119 Is Quantitative Ultrasound (QUS) a Useful Tool for Evaluating the Mechanical Properties of Infant Cortical Bone?

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The goal of this presentation is to compare Speed Of Sound (SOS) values measured from pediatric bone with mechanical properties derived from tibial specimens to determine whether SOS reflects bone strength.

This presentation will impact the forensic science community by introducing pilot data for biomechanical properties of infant bone, which is absent from clinical and anthropological literature, and by discussing the assessment of QUS as a technique for evaluating infant bone quality.

In pediatric cases with suspected non-accidental injury, bone quality/strength is of prime interest. Defense attorneys often argue that skeletal injuries in these cases could result from normal handling due to inherent bone fragility caused by various non-confirmed diagnoses (i.e., temporary brittle bone disease, osteogenesis imperfecta, etc.). These arguments are disputed using medical findings and opinions of expert witnesses; however, opinions regarding bone quality and fracture susceptibility are typically based on qualitative assessments. Currently, there are no validated quantitative methods available for the assessment of infant bone quality; however, QUS is a validated technique for osteoporosis diagnosis in adults.

This research tests the efficacy of QUS in assessing infant bone quality through the comparison of tibial SOS values with the mechanical properties of tibial bone planks. It was hypothesized that SOS would exhibit a significant positive relationship to ultimate strength and elastic modulus and a significant negative relationship to plastic toughness. These hypotheses were deduced from two research-based assertions: (1) bone mineralization increases with age in children and SOS is positively correlated with mineralization in adult studies; and, (2) degree of mineralization affects the mechanical behavior of bone.

This study sample consists of 19 decedents ranging from 0 to 10 months of age. For each individual, SOS was measured on the antero-medial aspect of the tibial midshaft with QUS. Subsequently, a 10mm x 2mm section of bone was excised from the tibia using a Dremel® tool, placed in 70% ethanol, and refrigerated at 3°C-4°C. In preparation for mechanical testing, the specimens were cut into planks using an IsoMet® wafering saw, rehydrated in phosphate-buffered saline prior to mechanical testing, and tested wet at room temperature. The specimens were loaded in 3-point bending according to the following protocol: specimens were preloaded to 0.2N for two seconds and loaded at a displacement rate of 0.1mm/s until fracture or until a strain of 20% was reached. Load and displacement data were captured at a rate of 40Hz using Bluehill® software. Statistical analyses were performed using SPSS 22.

Results reveal no significant associations between SOS and the intrinsic material properties of the tibial specimens. Age was significantly correlated with ultimate strength ($r = 0.48$, $p = .036$) and elastic modulus ($r_S = 0.48$, $p = .038$). There was no significant correlation between SOS and age in the pooled sample; however, SOS was significantly correlated with age ($r = 0.62$, $p = .017$) after removing infants less than one month of age from the analysis.

Results indicate a complex relationship between age and SOS. SOS did not significantly correlate with age during the first month of life nor did it significantly correlate with biomechanical variables. During the first month of life, there was notable variation in SOS, which was likely related to variability in bone composition and porosity. In contrast, the comparison between age and biomechanical factors performed as expected. Cortical bone stiffness increases with age along with the amount of stress the cortical bone could withstand prior to failure. Owing to the bone elasticity, mechanical loading to the point of fracture was difficult to achieve in some specimens requiring the predetermined total strain (20%) value as the failure point. Potential explanations for these findings include: small sample size; samples with substantial subperiosteal new bone formation, which may influence the acoustic impedance of SOS and the mechanical behavior (i.e., more crushing than bending); and the endosteal trabeculae were trimmed from the second batch, which was not performed on the first batch. The presence of trabeculae may

have introduced noise into the biomechanical data. Although the hypotheses were not accepted, these finding are preliminary. With additional data, a more definitive statement regarding the relationship between SOS and biomechanical properties in infants can be presented.

Speed of Sound (SOS), Biomechanical Properties, Infant Cortical Bone

A120 A Histomorphological Analysis on the Variability of the Entire Human Skeleton: Implications for Differentiating Human From Non-Human

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After attending this presentation, attendees will be better informed regarding bone histology of the entire human skeleton, not only the compact bones widely presented in literature. In addition, attendees will also understand the useful role bone histology may cover in forensics in general, specifically regarding species diagnosis.

This presentation will impact the forensic science community by informing attendees of the importance of continuing to investigate this particular field, which has become increasingly more important in anthropology for a variety of purposes.

Bone histology has always proven to be of great importance in anthropology, especially when dealing with age estimation, postmortem alterations, trauma analysis, and species identification. The latter (differentiating human from non-human) has acquired an essential value in forensics, and anthropologists often have to undertake species diagnosis conducted with very small bone fragments. Although bone histology has been deeply explored, there is not yet a full consensus regarding classifications and terminologies of bone microscopic structures. In addition, even if the literature provides numerous studies reporting the histological bone characterization within different species, it primarily regards the compact bones; thus, there is still a need for in-depth investigation of the variability of the bone tissue appearance within the entire skeleton and/or on the same bone.

This study seeks to histologically map the entire human skeleton in order to clarify the histomorphological variability by determining and comparing the diverse tissue typologies that can possibly belong to the human skeleton and to different portions of a same bone.

The histomorphological analysis conducted on 50 cross-sections from a human adult skeleton (comprising long, flat, and short bones) revealed the prevalence of secondary bone characterized by secondary osteons immersed in a lamellar matrix.

As per the literature, no histological sections exhibited fibrous bone, which is instead typical of initial growth (fetus and infant), bone healing, and response to pathological conditions. Regarding the Haversian tissue, the most frequent pattern was characterized by scattered secondary osteons with no organization.

Generally, excluding the trabecular bone (characterized by avascular or poorly vascularized lamellar tissue), long bones displayed a higher variability (alternation of tightly packed secondary osteons and scattered Haversian systems immersed in a lamellar matrix). On the contrary, flat and short bones seemed characterized by a greater uniformity, namely scattered osteons with no organization. In general, flat bones are formed by scattered secondary osteons and smaller Haversian systems with abundant interstitial lamellae; the occipital, rib, sternum, the superior border of scapula, and ileo-pubic ramus also exhibit a greater osteon density. The only exceptions regarding short bones are the middle portion of the metatarsal, in which the osteons are tightly packed without organization, and the middle portion and the distal end of the metacarpal, in which Haversian systems are organized in circular rows.

The difference in the Haversian system density between long bones confirms what has been reported in previous research regarding the relationship between the physical properties of bone and its histological structure. The femur and tibia displayed a higher osteon density when compared to the fibula, in which the Haversian systems are smaller with higher amounts of interstitial lamellae. A large number of small osteons and fragments make the tensile strength of a given amount of bone smaller if compared to areas with few large osteons and fragments. On one hand, Haversian systems reduce the tensile strength and increase the tensile strain of cortical bone; on the other hand, interstitial lamellae reduce the tensile strain while increasing the tensile strength. This results in a greater tensile strength and elasticity of the fibula as compared to the femur and tibia.

The observation of the histological sections by polarized light allowed identification of the presence of several

drifting osteons in the diaphysis of the ulna, clavicles, and rib's heads.

The finding of drifting osteons in the study sample is in accordance with literature since, although they are typical of young individuals, their presence in adult individuals is also verified.

Further research on the histomorphometric variability of human and non-human bones is paramount in order to have a complete understanding of bone histology and to improve all its application fields, such as the differentiation between human and non-human species.

Bone Histology, Human vs. Non-Human, Forensic Anthropology

A121 Introducing the Macromorphoscopic Databank (MaMD): A Data Collection and Analytical Tool for the Analysis of Macromorphoscopic Trait Data

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After attending this presentation, attendees will be familiar with the newly created MaMD, a data storage repository attached to a data collection and analytical tool for the analysis of macromorphoscopic trait data. Attendees will be better informed concerning the development of this project, the current state of the database, data contributors, and the research/analytical potential of these data.

This presentation will impact the forensic science community by addressing a significant gap in best practice in the forensic anthropological approaches to macromorphoscopic trait data, particularly as those data relate to the estimation of ancestry.

An integral part of the biological profile constructed by forensic anthropologists for unknown human skeletal remains is an estimation of the peer-perceived ancestry for that individual. This estimation is usually accomplished through visual inspection of morphological variants of the cranium and mandible (i.e., cranial non-metric, or macromorphoscopic, traits) and/or through an analysis of measurements of the cranial and postcranial skeleton; however, estimating ancestry using macromorphoscopic traits is not straightforward and often relies on a considerable number of years of experience and an extraordinary understanding of human variation.

The purpose of this presentation is to address this substantial gap in best practice in forensic anthropology. Very little reference data exist — and are publicly available — for the objective analysis of macromorphoscopic trait data. Consequently, forensic anthropologists rely on their own experience or outdated methods having very little empirical support. The fundamental flaw with these two approaches is that they offer no way of estimating error nor are they verifiable as a method. The result is *post hoc* trait selection, experience-based justifications, and anecdotal expert judgement with no empirical support.

This presentation addresses the gap in best practice through the introduction of the MaMD. The purpose of any databank is to serve as a repository of data and to make those data accessible to many end users, or practitioners. To that end, the MaMD serves as a repository for macromorphoscopic trait data obtained primarily from recent and well-documented forensic cases or donated skeletal material. To facilitate data sharing and to maximize analytical output, the databank comprises relational databases housing not only macromorphoscopic trait scores, but also demographic data on each decedent. These include but are not be limited to: age at death, sex, stature, ancestry, place of birth, occupation, self-identified ancestry (social race), etc. All data are maintained in these relational databases on a central server, using a database platform to perform the essential managerial and analytical functions necessary for data management.

The MaMD is populated using Macromorphoscopic Traits (v. 1.61), a newly developed data collection program for 17 macromorphoscopic traits. The end user provides provenience information after data are collected, retaining trait scores to a sample-specific database for subsequent submission to the MaMD. The MaMD currently contains macromorphoscopic trait data for 6,670 individuals. Examples of populations for which data are available include samples of modern American Black and White, Hispanic, Guatemalan, Colombian, Fijian, Thai, Japanese, Pacific Islander, and Peruvian.

The MaMD and a forthcoming analytical program utilizing classification algorithms appropriate for categorical data will be available for wider use following extensive beta testing, method validation, and inter- and intra-observer error tests. Forensic anthropology laboratories (applied and academic) are encouraged to assist in the validation and beta testing phase of this research through a data-sharing model similar in scope and function to the Forensic Anthropology Databank.[1] Further refinement of several classification algorithms and the user interface for the analytical program will be completed within the next year.

This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

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1. Jantz R.L., Moore-Jansen P.H. A data base for forensic anthropology. *Report of Investigations No. 47.* Department of Anthropology, University of Tennessee, Knoxville, TN. 1988.

Macromorphoscopic Databank, Ancestry, Macromorphoscopic Traits

A122 Postmortem Interval Estimation Using the Blackened Osteocyte Method

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After attending this presentation, attendees will be informed concerning the histological changes that occur in human bone during the decomposition process.

This presentation will impact the forensic science community by introducing a possible new method that may be further developed to estimate postmortem interval.

The ecology of human decomposition is a series of complex occurrences that are driven by biological and physiochemical processes and over the past decade has been at the forefront of forensic interest. The processes are responsible for leaving specific signatures in the landscape near a body, and in the body itself, including both soft tissue and bone. The documentation and identification of postmortem microstructural changes in bone are important and provide insight into both the peri-mortem and postmortem history of the body. Past histological studies focused on the microstructural changes that occur post-skeletonization but limited or no research has focused on pre-skeletonization conditions.

The focus of this study was to identify any histological changes in bone during the stages of decomposition, exploring any trends that could help estimate postmortem interval. Emphasis was placed on bones that have traditionally been used in past histological methods. The study was conducted at the Southeast Texas Applied Forensic Science (STAFS) facility, Sam Houston State University, Huntsville, TX.

Sixteen bone samples from the femur, tibia, humerus, and fourth rib were taken from human cadavers at each of the four stages of decomposition: fresh, early, advanced, and skeletonization. The samples were split into two sample groups: decalcified and undecalcified. The undecalcified samples were unaltered and had no chemical treatments. The decalcified samples were treated with decalcification reagents and stained. Thin sections of each bone were examined using light microscopy and submitted for analysis. The number of blackened osteocytes were counted and compared to the stages of decomposition. The fourth rib was ruled out due to difficulty acquiring clear imagery.

Preliminary results demonstrated a significant increase in the number of blackened osteocytes as the body progressed through the decomposition stages, not only in the stages of decomposition but also by bone type. For example, the femur exhibited an increasing number of blackened osteocytes, with the biggest changes occurring during the early and advanced stages. This method has the potential to be used in estimation of the postmortem interval. Blackened osteocytes were not visible in the decalcified samples. This may have been the result of the staining process or, more likely, that decalcification has removed part of the organic/mineral composition of the blackened osteocytes themselves. Additional research is underway, including increasing the sample size and collecting samples on bodies placed during various seasons of the year. If in these future studies the results continue to be reliable, the blackened osteocyte method may be a valuable tool for estimating the postmortem interval when skeletal remains are found in an advanced stage of decomposition and insect activity has long since ceased.

Forensic Science, Forensic Anthropology, Postmortem Interval

A123 A New Approach to Estimating Accumulated Degree Days (ADD): Using Binary Observations From Various Regions of the Body With Random Forest Modeling (RFM) and Geographic Information Systems (GIS)

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After attending this presentation, attendees will understand a new approach to estimating ADD to more accurately estimate the Postmortem Interval (PMI) by using binary observations from several regions of the body in conjunction with RFM and GIS.

This presentation will impact the forensic science community by offering an alternative approach to estimating ADD that will result in less-biased estimates by examining discrete features as opposed to components or arbitrarily weighted suites of characteristics, which leads to more accurate estimates of ADD.

Estimating PMI has long been a focus within the medicolegal community, particularly once rigor and algor mortis have passed as potential indicators of PMI. Currently, the most prominent method for estimating ADD is Megyesi and colleagues' Total Body Score (TBS).[1] Megyesi et al. have provided qualitative observations for three anatomical regions (head and neck; trunk; and limbs) that are assigned weighted scores and summed together to provide a quasi-continuous score that can be used to estimate ADD.[1] The model provided by Megyesi et al. resulted in an r^2 value of 0.84 for log 10 transformed ADD and squared total body score.[1] The current study seeks to improve ADD estimates by incorporating information from more anatomical regions and by using discrete binary (presence or absence) traits and advanced statistical models.

Ten donors received at the Forensic Anthropology Center were selected for the current study. For inclusion in the study, a donor must have had a known time of death, had not been subjected to an autopsy, and did not possess any overt trauma that might disrupt normal decomposition. Each donor was placed in a freezer for at least 24 hours before placement to ensure each donor was at the same temperature before placement. The ten donors were placed in a transect at the Anthropology Research Facility in a section designated for research. Donors were kept in body bags until they had reached ambient temperature.

A binary scoring list was developed for 16 regions of the body to be scored independently of one another. The 16 regions of the body scored include: (1) head and neck; (2) left upper arm; (3) right upper arm; (4) left lower arm; (5) right lower arm; (6) left hand; (7) right hand; (8) left upper leg; (9) right upper leg; (10) left lower leg; (11) right lower leg; (12) left foot; (13) right foot; (14) chest; (15) abdomen; and, (16) genitals. Each region was scored with the same list of 39 binary observations that examined bloating (6 observations), skin coloration (2 observations), purge (4 observations), skin appearance (14 observations), and insect activity (13 observations). Additionally, the head and neck region included two additional observations relating to retention of the scalp. In sum, each body was evaluated for the presence or absence of 626 traits each day from placement (at ambient temperature) through 2,000-averaged ADD.

Data were subjected to RFM regression using 1,000 trees and a maximum of five random variables tested at each node. The data were partitioned into a 70% training set, 15% validation set, and 15% test set. Each region was tested individually in addition to the entire body.

Known ADD was plotted against estimated ADD from the RFM regression, resulting in r^2values ranging from 0.74 (right foot) to 0.96 (all regions). Additionally, estimated ADD scores, from the regression, for each region were used in GIS to display heat maps showing the decomposition of each region for each donor and also to conduct hot spot analysis. Further, the individual heat maps were summed to create a general model displaying overarching trends in decomposition progression, which was also subjected to hot spot analysis. The heat maps reveal that the abdomen, genitalia, and upper leg decompose more rapidly than the rest of the body. Additionally, the right upper limb, chest, and feet exhibit significantly different hot or cold spots as compared to contiguous body regions.

Results indicate that using discrete observations from anatomical regions in conjunction with RFM provide excellent estimates of ADD that can be used to confidently estimate PMI.

Reference(s):

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Forensic Science, Forensic Anthropology, Time-Since-Death Estimation

A124 Validation of the Total Body Score/Accumulated Degree Days (TBS/ADD) Equation at 100, 300, 500, and 1,000 ADD on 30 Human Subjects With a Known Postmortem Interval (PMI) From Three Human Decomposition Facilities

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After attending this presentation, attendees will understand that the equation associated with the TBS method using ADD is ineffective as a means to estimate the PMI and becomes progressively less accurate as the decomposition process proceeds.

This presentation will impact the forensic science community by demonstrating the need for new regional-specific equations for calculating ADD from TBS.

Beginning in March, 2015, a validation study of the TBS and regression equation for calculating ADD to estimate the PMI was conducted at three human decomposition research facilities, each having different climates (subtropical-sub-humid, temperate, and subtropical-humid).[1] The study was conducted at the Forensic Anthropology Center at Texas State (FACTS) at Texas State University in San Marcos, TX, the Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville, TN, and the Southeast Texas Applied Forensic Science Facility (STAFS) at Sam Houston State University, Huntsville, TX. From March 2015 to June 2016, a total of 24 bodies were placed at each facility (total 72) during the spring, summer, fall, and winter seasons. Four subjects were placed on the ground surface at each facility each season; two in a shaded environment and two in the sun. Observations with TBS documentation and digital images were recorded daily. Data loggers captured daily temperatures and ADD was calculated.

The purpose of this study is to test inter-observer variability in the TBS and to examine the accuracy of the TBS/ADD equation developed by Megyesi and colleagues in the three environments.[1] A 30-subject subsample (ten subjects per facility) was selected for the validation study. The 30 subjects represent individuals placed in all four seasons and in both sun and shade. For the inter-observer test, photographs of ten subjects at different stages of decomposition were examined and researchers from each facility independently calculated the TBS. An Interclass Correlation (ICC) statistic test was used to assess the inter-observer error for the TBS as well as the score for each body portion. For the test of the TBS/ADD equation, photographs corresponding to 100, 300, 500, and 1,000 ADD were selected from each of the 30 subjects. Using the TBS/ADD equation ($ADD = 10^{(0.002*TBS*TBS+1.81)} \pm 388.16$), the mean and range of ADD for each subject was compared to actual ADD.

The inter-observer test exhibited no statistically significant differences between researchers. The average measures for absolute ICC for the head presented near perfect agreement between observers (.959). The trunk displayed even greater agreement (.975) and the limbs demonstrated the least agreement (.940), but still was not statistically different ($p<0.001$). Based on this study and a recent study conducted by Dabbs and colleagues, it is clear that assessment of TBS from digital images is reliable and consistent between observers.[2]

The results of the calculated ADD exhibited significant variation between actual and estimated ADD. The difference between the actual and estimated mean ADD increases as ADD increases. For example, at 100 ADD (TBS 7), the TBS/ADD equation mean for all 30 subjects is 80.9 with a range of 0 to 475 ADD. At 300 ADD (TBS 26), the estimated mean is 1,452 with a range of 1,064 to 1,840 ADD. In addition, there was a significant overlap of ADD ranges in more advanced stages of decomposition when TBS increased. Numerous individuals remained at the same advanced TBS for more than 1,000 ADD at each of the three facilities.

This validation study demonstrates that TBS can be reliably calculated for human remains, but the equation for

estimating ADD provided by Megyesi and colleagues is insufficient, especially as PMI increases, regardless of the decomposition environment.[1] Other means of estimating PMI have been suggested and are also being.[3] The current study is two years and validation of revised equations pertinent to each unique climate or a new PMI method will be performed in the second year.

This study was funded by the National Institute of Justice.

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Total Body Score, Validation Study, ADD Regression Equation

A125 Vulture Scavenging of Small-Sized Pig Carcasses in Central Florida: Utilizing Geographic Information Systems (GIS) to Analyze Site Variables Affecting Skeletal Dispersal

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After attending this presentation, attendees will have a better understanding of vulture scavenging of the Central Florida region and its impact on the scavenging of small-sized carcasses. This presentation will focus on vulture consumption, disarticulation, and dispersal of remains in two micro-environments (shaded and unshaded) in order to discuss regionally specific taphonomic modifications of carcasses and deposition sites.

This presentation will impact the forensic science community by describing how vultures scavenge small-sized carcasses in Central Florida and their collective ability to modify deposition sites and destroy forensic contexts in the process. The dispersal of remains will be analyzed using arcGIS® spatial analysis tools.

Scavengers can significantly alter a forensic scene and consume, modify, disarticulate, and disperse bodies. These scavengers can differ greatly between different geographic regions. In recent years, there have been a number of important vulture studies; however, little research exists regarding scavenging in Central Florida, specifically scavenging involving black and turkey vultures (*Coragyps atratus* and *Cathartes aura*).

The research site was located on the University of Central Florida (UCF) campus at the Deep Foundations Geotechnical Research Site, which consists of approximately two acres surrounded by a six-foot chain-link fence. The tall fence was optimal to primarily select for avian scavengers. The research sample consisted of four pig (*Sus scrofa*) carcasses weighing between 25kgs and 29.5kgs that were deposited individually during the month of March in two distinct micro-environments: two in shaded and two in unshaded locations. The shaded micro-environment consisted of a grove of long-leaf pines with a layer of pine needles over the ground surface. The unshaded micro-environment consisted of vegetation on the ground surface and was surrounded by taller vegetation and bushes. Two field cameras were placed at each site to record both video and time-lapse photography. The dispersal data was mapped in the field using the azimuth control method and analyzed using arcGIS® v. 10.2.2 spatial analysis tools.

A mass vulture scavenging event occurred over two days, beginning during the evening of the sixth day of the postmortem time period and ceasing at dusk. Scavenging then continued during the morning of the seventh day. The vultures reduced the small-sized carcasses to primarily skeletal elements, with large sections of skin containing adhered small bones of the lower limbs, in approximately eight to nine hours of feeding time combined over these two days. This mass scavenging event was accomplished by large numbers of vultures simultaneously feeding at each carcass, with a maximum total number of 43 observed on video at one time at a single carcass. While two bald eagles were also recorded contributing to consumption of the carcasses, they participated minimally. Vultures disarticulated the carcasses, usually by entering through the orifices of the caudal and cranial ends. It is important to note that as the vultures consumed soft tissue, the large bones were scattered away from the initial deposition site of the carcass, while the smallest bones (including epiphyses) were sometimes consumed. It was after the initial mass scavenging event that opossums began scavenging the remains at night, particularly any remaining sections of skin, including skin with attached skeletal elements from the lower limbs.

The spatial analysis results indicated a number of site ground variables impacted vulture dispersal, such as the site perimeter fence and the tall ground surface foliage. Vultures primarily dispersed skeletal elements in open areas, and the majority of elements were dispersed within 6m of the initial carcass deposition site, with a maximum distance of approximately 15m. Conversely, opossums dispersed remains directly into denser foliage, most likely to consume the remains in more secure locations, including modifying a number of bones. Additionally, the ground surface and foliage of the carcass locations were further modified by the mass vulture frenzy, including scratching the ground surface with talons and digging with beaks to consume all remnants of the carcasses. The scavenging observed in the current study illustrates the capability of vultures to consume, disarticulate, and disperse remains and to modify depositional sites in Central Florida. This study can hopefully provide a standard format for more appropriate comparisons among future vulture studies.

Avian Scavenging, Vulture Scavenging, arcGIS®

A126 Differential Temperatures of the Human Body and Fire

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After attending this presentation, attendees will better understand how temperatures external to the body in a fire can be more than 1,000°F-1,600°F, but the internal core temperature of the body does not rise by a single degree until a later interval of the fire (20-30 minutes).

This presentation will impact the forensic science community by providing documented time and temperature ranges in relation to heat-related changes of the body.

In taphonomic research, ambient temperatures are considered a key variable that affects the body's rate of decompositional changes over time. Temperature is also highly important in fire research since heat produces the burn patterns that are observed on the body at the crime scene or morgue. Aside from industry standard crematorium temperatures, there is scant data on temperatures at which the human body burns externally and internally.

Experimental fire research captured the temperatures around and inside the body using data loggers, Type K thermocouples, and digital photography. Three adult cadavers were placed inside of two structures and one outdoor fire with wood and ignitable liquids. Small incisions were made in the abdomen and a thermocouple wire was inserted a couple of inches into the liver. External temperatures of the interior structure along with internal liver temperatures were recorded. Fires burned for 8 to 50 minutes. For the structures, fire went through the stages of: incipient growth, development, fully developed, flash over, post flashover, and decay. Each of the stages has the capacity to inflict burn injury to the skin, fat, muscle, and later, bone. During the first five minutes of growth and development of the fire, temperatures increased to 1,500°F inside the structures with full involvement of the fire. The internal body temperature did not raise a single degree in less than ten minutes of burning.

The first structure fire (S1) burned for eight minutes with flashover occurring at four to five minutes and maintaining temperature for an additional three minutes prior to water extinguishment by the San Luis Obispo Fire Department in California. Temperatures climbed after three minutes up to 1,500°F at the floor and ceiling. Ignitable liquids were used to start the fire on the body as a suicide-by-gasoline in a room with a corner origin. After an eight-minute structure fire, the body was transformed into a charred mass of shrunken muscles with bone exposure of the extremities. The wrist fractured and the ankles broke away at the distal tibia and fibula. Exposed bones were charred and calcined for the feet, ankles, lower legs, distal arms, hands, face, and head. The internal body temperature did not change.

The second structure (S2) burned for 25 minutes with flashover occurring by three to four minutes with floor temperatures that peaked at 750°F and remained below 600°F for ten minutes until water suppression. Ignitable liquids were used to start the fire with a body inside of a shed full of combustible materials. At 22 minutes, the body had visible calcination of the face, head, and ribs during the fire. The suppression crew hit the body directly with a straight stream of water, which caused displacement and fragmentation of the fragile burned bones and superficial soft tissues. Sections of the calcined face, skull, and ribs were blasted off of the body, thus altering the final condition. There were no significant temperature changes in the internal body.

The outdoor fire (O1) burned for 50 minutes with temperatures peaking at 1,700°F after the first ten minutes, then hovered around 800°F for the duration of the fire. The body was on top of wood pallets, with more pallets placed on top along with other brush and started with ignitable liquids. The lower arms and legs were calcined, with charred and calcined bones of the head. The internal body temperature rose above 200°F after 18 minutes of burning and increased to 380°F by the end of the 50 minutes. By this point, the liver and other internal organs were exposed and charred. Bones of the head and extremities were exposed with patterned charring and calcination.

The results demonstrate that the external temperatures can reach 1,200°F-1,600°F or higher in the fire environment, but that the internal temperatures of the organs and soft tissues remain unaffected until after the first 20-30 minutes of burning. It also illustrates the variation in temperature for structure (ventilation-controlled) and outdoor (ventilation-driven) fire environments responsible for creating the burn patterns to bone that are examined by medical examiners and forensic anthropologists.

A127 A Multiple Fatality Response to Nine Indigenous Deaths in a Burned House in Pikangikum, Ontario: An Overview of the Procedures of the Hosts of the Triennial Meeting of the International Association of Forensic Sciences, 2017, the Ontario Forensic Pathology Service (OFPS)

Kathy L. Gruspier, JD, PhD, Ontario Forensic Pathology Services, 25 Morton Shulman Avenue, North York, ON M3M 0B1, CANADA*

After attending this presentation, attendees will learn about the host institution of the triennial International Association of Forensic Sciences 2017 meeting, the OFPS. The OFPS is housed in a new complex in Toronto that was purposely built to manage a multiple fatality response — up to a Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Level II. The OFPS has a multiple fatality implementation plan for its physical plant and is an integral part of the Provincial Mass Fatality Plan. The OFPS' response to a multiple fatality event occurring on a remote indigenous reserve will be shared.

This presentation will impact the forensic science community by illustrating that a death investigation system must draw upon its daily practices in order to successfully address a multiple fatality in its jurisdiction. If those regular practices do not include appropriate experts (or at least an established network of expert consultants) and good communication, then there is a real probability that an agency's response will be problematic. The use of inexperienced experts, the lack of a teamwork attitude, and the use of unfamiliar Disaster Victim Identification (DVI) tools would all have created problems with this multiple fatality response and would have delayed returning the decedents to the community.

A multiple fatality is defined as a single event in which two or more people die. A multiple fatality can occur with little or no warning and vary in its scope and nature. In Ontario, all death investigations fall under the jurisdiction of the coroner, regardless of the cause of the death, although there are often parallel investigations, most commonly criminal. The guiding principle for the implementation of the plan is: "Business as usual, just more business than usual." The OFPS provides qualified expertise in the areas of anthropology, pathology, odontology, medical imaging, and photography. For many years, anthropologists and sometimes pathologists have been integral members of scene examination and recovery teams when needed. This is business as usual. In addition to daily casework, a partnership with the Ontario Provincial Police (OPP) called the Resolve Initiative is maintained by the researchers. This is the vehicle used for comparing missing persons with unidentified remains. In the event of a multiple fatality, the OFPS has access to a clean copy of this functional relational database, in order to compare cases for DVI.

In the early morning of March 30, 2016, the OFPS forensic anthropologist was notified of a house fire on the Pikangikum Reserve. As the person responsible for the OFPS multiple fatality implementation plan, the anthropologist opened communications with partners in the Office of the Chief Coroner (OCC), the OPP, and the Office of the Fire Marshall (OFM) and also began planning responses with the staff of the OFPS, as well as expert consultants in anthropology and odontology. As the scene processing continued, she liaised with team members with regard to timing and records to accompany the bodies. Communication was often hampered by the lack of cell phone and internet access in the community. Communication with the OCC was also maintained with regard to transport of the remains to Toronto. The scene work was completed and the bodies were transported by April 2. Postmortems took place on April 3 and the identifications were completed and the bodies flown back to the community on April 4.

Multiple Fatality/DVI, IAFS 2017, ON Forensic Pathology Service

A128 A Mass Fatality Response and the Indigenous Community: The Intersection Between Science, Death, and Culture

Kona Williams, MD, Ontario Forensic Pathology Service, 25 Morton Shulman Avenue, Toronto, ON M3M 0B1, CANADA*

After attending this presentation, attendees will better understand the approach to death investigation in remote communities, the challenges faced by the death investigation team, and the indigenous people from socioeconomic, historical, and current viewpoints, as well as how to identify culturally appropriate and respectful outcomes when dealing with deaths in these communities.

This presentation will impact the forensic science community by examining how this coordinated effort can be used as an example for other members of the forensic community to build better relationships with indigenous people by fostering mutual understanding and respect and facilitating the process of death investigation. The outcomes of these investigations can also identify root causes affecting deaths in these communities and point toward preventative solutions.

Nine people of the Pikangikum First Nation were recovered from a house fire in March of 2016. There was a swift multi-agency and multi-disciplinary approach to the scene, identification of the bodies, postmortem examination, and analysis of the findings. All nine individuals were positively identified using scientific methods and the cause of death for all family members was "smoke inhalation." Despite the success of the death investigation team response, many challenges exist when deaths of this nature occur in indigenous communities.

Pikangikum is a remote reserve located in northwestern Ontario, Canada. It has a population of approximately 2,500 people of the Ojibwe First Nation. Historically, the interaction between the government, the health care system, and the police force with indigenous people in Canada has been fraught with racism, discrimination, and neglect. The death investigation system in Ontario encompasses all three institutions, leading to unique challenges when investigating deaths in indigenous communities. Combined with the remote location of many northern First Nation reserves, the lack of resources, such as running water, proper housing or adequate fire services, multiple socioeconomic factors as a result of intergeneration trauma, and the lasting effects of assimilation policies, tragedies such as these continue to occur; however, improved communication with families and community members, positive relationship-building, and mutual understanding between the death investigation team, the scientific community, and the indigenous population can serve to increase timely and culturally appropriate responses to these situations.

Multiple Fatality/DVI, Indigenous, Socioeconomic Impacts

A129 A Multiple Fatality Response to Nine Indigenous Deaths in a Burned House in Pikangikum, Ontario: The Scene and Its Challenges

Renee C. Kosalka, MA, Ontario Forensic Pathology Service, 25 Morton Shulman, Toronto, ON M3M 0B1, CANADA*

After attending this presentation, attendees will be informed concerning a house fire in a remote, indigenous community located in Pikangikum, Ontario, Canada, that resulted in nine fatalities and the subsequent forensic response at the scene. The response consisted of a multiagency provincial team who employed interdisciplinary methods to the background and witness investigations, including antemortem records collection, as well as to the search, recovery, and documentation of the scene, sets of human remains, and evidence of forensic interest. The agencies included representatives from the Office of the Chief Coroner (OCC), the Ontario Forensic Pathology Services (OFPS), the Ontario Provincial Police (OPP), and the Office of the Fire Marshall (OFM). This presentation is being presented as an illustration of the work that the hosts of the International Association of Forensic Sciences 2017 triennial meeting, the OFPS, undertake in a multiple fatality event.

This presentation will impact the forensic science community by explaining how a well-coordinated response with appropriate experts working systematically via a standard, interdisciplinary approach — implemented at the scene — serves to achieve a successful and expedient multiple fatality investigation of a significantly burned group of human remains.

The scene was located on the Pikangikum 14 Reserve in northwestern, Ontario, which was populated by ~2,500 members of the Ojibwe First Nation. Within the Canadian Shield, the Reserve was ~1,808 hectares in area, at an elevation of ~335m. In a rural neighborhood, the scene involved a private residence consisting of a one-story, six-room, wooden structure with side paneling. The fire was witnessed by neighbors at approximately 11:44 p.m. on March 29, 2016, and burned unsuppressed until at least 9:00 a.m. Multiple witness accounts, including police patrols, reported that the property owners were a married couple who were hosting a house party with extended family. The missing included six adults and three children who were each less than five years of age.

Forensic experts onsite included three OPP forensic identification officers, two OFM fire investigators, one OFM forensic engineer, and one OFPS forensic anthropologist. The ruins were such that there was significant thermal alteration to, and structural collapse of, approximately 95% of the structure. Systematic processing of the ruins took three working days, with the first 11 hours dedicated specifically to the exposure, documentation, and recovery of nine sets of human remains and associated artifacts. The cause of the fire could not be determined.

The main challenges included the remoteness of the location (more than 1,400km from Toronto) and the variable, cold weather (periods of snow with temperatures ranging from -15.5ºC to +3.8ºC and winds gusting up to 35km/h). These challenges affected general logistics, the initial response time by the forensic team, technical aspects of scene processing (e.g., the operability of an Unmanned Aerial Vehicle (UAV) and 3D site scanner in addition to the amount of time an expert could physically work), as well as the transport of cases, records, and information to the laboratory in Toronto for examination.

Multiple Fatality/DVI, Forensic Anthropology, Scene Recovery

A130 A Multiple Fatality Response to Nine Indigenous Deaths in a Burned House in Pikangikum, Ontario: Postmortem Investigations

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After attending this presentation, attendees will be informed concerning the approach to postmortem examination, the collection of information for identification, and how medical information from the dead can improve the quality of life for surviving family members in the event of a multiple fatality, presented by the hosts of the Triennial International Association of Forensic Sciences 2107 meeting, the Ontario Forensic Pathology Service (OFPS).

This presentation will impact the forensic science community by illustrating how a well-coordinated postmortem response with appropriate experts working systematically and as part of an interdisciplinary team in the morgue results in a successful and expedient multiple fatality investigation of a significantly burned group of human remains, as well as providing important information which may impact the health of surviving family members.

Nine family members were recovered from the rubble of a heavily damaged home following a fire on the Pikangikum Reserve, a remote Ojibwe community in Northern Ontario. A team of three forensic pathologists, two forensic anthropologists, six pathology assistants, two radiology technicians, and one forensic science technician worked alongside three forensic identification officers and three fire investigators to undertake the task of performing the postmortem examinations of this family in a single day. Communication and pre-planning for the postmortem examination of this multiple fatality allowed regular morgue business to continue uninterrupted. That the physical plant of the OFPS was designed to handle multiple fatalities also contributed to this successful single-day endeavor.

The purpose of the postmortem examinations was to collect information which would aid in identification and determination of the cause of death. The day began with a presentation from the scene forensic anthropologist that was attended by the entire team. Postmortems were assigned so any bodies with unresolved commingling would be autopsied concurrently. During the autopsies, there was ongoing communication among all members of the team, which was facilitated by the close proximity of the autopsy tables. The nine postmortems were divided among three teams with each forensic pathologist performing three autopsies and the forensic anthropologists circulating amongst cases that required their expertise.

All of the decedents had fire-related injuries and were in variable states of completeness. In particular, one of the children had extensive destruction to the pelvic organs that interfered with the recognition of the internal sex organs versus urinary bladder. The confirmation of the organ as a bladder was made by frozen histological section prior to the identification committee meeting the following morning. One adult had antemortem trauma and this was easily interpreted with the assistance of the scene forensic anthropologist. Samples for ancillary investigations included: blood collection for toxicological analysis, small tissue samples for histology, and samples for DNA. All nine individuals died from smoke inhalation. Biological information, such as approximate age and sex as well as natural diseases and other identifying features, were communicated to the identification committee. Any unexpected findings in the ancillary investigations were communicated to the other forensic pathologists prior to signing out the autopsy reports. All of the autopsy reports were audited by another forensic pathologist on the team to ensure completeness and similar content.

Two of the adult individuals were diagnosed with hypertrophic cardiomyopathy and referred to the cardiac pathologist for further examination. Genetic testing is currently underway in order to determine if a genetic mutation exists. Hypertrophic cardiomyopathy is typically an autosomal dominant condition and can therefore affect multiple members of a family and their extended families. The finding of this disease on postmortem remains allows for surviving family members to be screened, examined, and treated for this condition.

Multiple Fatality/DVI, Postmortem Examination, Hypertrophic Cardiomyopathy

A131 A Multiple Fatality Response to Nine Indigenous Deaths in a Burned House in Pikangikum, Ontario: The Interdisciplinary Approach to Dental Identification

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After attending this presentation, attendees will be informed concerning the approach to dental identification of the Ontario Forensic Pathology Service (OFPS) in the event of a multiple fatality.

This presentation will impact the forensic science community by demonstrating the limits of the use of antemortem dental records for identification. This presentation will also illustrate how a team approach utilizing forensic anthropology from scene to dental assessment ensures that the most information possible is available to the odontologist and is not lost through mishandling by non-experts. In addition, this presentation will disclose how other skills, such as age stratification, can be useful in the final identification of decedents of a multiple fatality.

The OFPS and the Office of the Chief Coroner were engaged to investigate a single house fire in the remote Ojibwe community on the Pikangikum Reserve. A team including a forensic anthropologist, the Ontario Provincial Police, and the Office of the Fire Marshall were dispatched to the isolated community to recover the fire-damaged remains of nine family members, which included six adults and three children of known and differing ages. The decedents were transported to the Provincial mortuary facility for the purposes of conducting postmortem examinations and establishing identifications. The dental identification team consisted of a forensic odontologist and two pathologists' assistants. Upon examination, the remains were noted to have extensive fire-related injuries, were heavily fragmented, and, in some cases, incomplete. The lack of material available for examination posed a challenge to dental identification despite having a closed population of individuals to identify in a multiple fatality incident.

The dental identification team was provided with antemortem records for the six adults and two of the children by the local community nursing station. The records received were in varying states of completeness and quality. Typically, these remote areas are served by a series of itinerant dentists that leave after a short time; thus, continuity of antemortem dental records can be problematic. Further, as it is rare for these records to be audited, there is little impetus to maintain them. Additionally, most of the individuals from these remote areas attend school outside of their communities for a time, so assembling a complete antemortem data set may require contacting multiple dentists. As a result, it was a requirement that the dental identification team cautiously review the entire antemortem record, including odontograms, billings, and chart notes from the past to the present, then recheck some of the records from the present to the past to verify that an accurate record of the dental conditions at the time of death was available for comparison. Following this review, the team developed a list of identifiable conditions and created an antemortem odontogram for each individual.

The available dentition of each decedent was recovered, restored, and reapproximated by the forensic anthropologists during the postmortem examinations. The dental identification team then positioned and exposed numerous intraoral radiographs of the teeth and jaws; however, the incinerated, fragmented, and incomplete nature of the available tissue made it impossible to create complete postmortem dental charts and odontograms for each individual.

The dental team had to determine points of concordance and no points of discordance between the partial or complete antemortem records and the partial postmortem records. Identifying features that differentiated between people were used as much as were unique identifiers. A total of six positive dental identifications were provided to the Identification Committee as well as three dental age-at-death estimates for the children. Although antemortem dental records were not available for one of the children, investigators had accounted for every person in the community; therefore, having a closed population of the missing allowed the use of age stratification techniques to assist in the final identification of the three children.

Multiple Fatality/DVI, Forensic Odontology, Dental Identification

A132 A Multiple Fatality Response to Nine Indigenous Deaths in a Burned House in Pikangikum, Ontario: Forensic Anthropology

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After attending this presentation, attendees will better understand the contributions of forensic anthropology in a multiple fatality response to a house fire in a remote Ojibwe First Nation community located on the Pikangikum 14 Reserve in northwestern Ontario, Canada. This presentation serves as a demonstration of the work of the Ontario Forensic Pathology Service (OFPS), the hosts of the International Association of Forensic Sciences 2017 triennial meeting.

This presentation will impact the forensic science community by illustrating how integrating forensic anthropologists into daily practice, as well as into multiple fatality responses, serves to achieve successful and expedient death investigations of significantly burned human remains by maximizing recovery, minimizing commingling, and preserving context. This presentation will also demonstrate how Computed Tomography (CT) scans can be useful for osteological age-at-death estimations.

In the OFPS, forensic anthropology is part of the daily business of death investigation. Since the 1980s, forensic anthropologists have been utilized in ever-expanding roles in death investigation. There is one full-time Forensic Anthropologist (FA) at the OFPS, and five fee-for-service FAs who can be called upon as necessary. In daily practice, OFPS FAs are involved in most death investigations in which bones are found and the species is unknown and/or human remains are visually unrecognizable and/or fragmentary, partial, or burned. In addition, the full-time FA manages long-term unidentified remains and is responsible for the OFPS Multiple Fatality Plan. Forensic anthropology is always utilized at scenes of found remains that are not fresh or intact. Forensic anthropology is an expected and integrated discipline at the scene of such found remains where the anthropologist most often leads the recovery of human remains. Forensic anthropology is also engaged in postmortem examinations, ancillary testing (such as tool mark analysis), and human identification. Forensic anthropology is considered an essential service in the Ontario government.

During the evening of March 29, 2016, a single-story home on the Pikangikum 14 Reserve burned unsuppressed into the morning, taking the lives of an extended family, including six adults and three children. Forensic anthropologists played principle roles in the multiple fatality response throughout the duration of the investigation, from March 30 to April 4. Initially, at the laboratory, the full-time FA coordinated the effort and deployed a consultant FA to the scene to integrate into the field team and be responsible for the search, exposure, documentation, and recovery of human remains and associated artifacts, as well as antemortem records collection and transport.

Specifically relating to the laboratory setting during April 3-4, the FAs integrated with every discipline while contributing to the postmortem examinations and identifications. Prior to any laboratory work, the FA interpreted preliminary scene findings and photos and created maps in order to lead a briefing to nearly 40 at-the-ready staff (no other field team members attended the postmortems). During the postmortems, which were held three at a time by three forensic teams, contributions by the FAs included: the interpretation of postmortem CT scans; removal of debris; anatomical positioning and skeletal inventory; dental reconstructions; burn-pattern interpretation; traumatic injury interpretation; estimation of biological profile (age at death and sex); analysis of unique skeletal features for identification purposes via antemortem radiographs; 3D virtual reconstruction (for skeletal inventory and juvenile age-at-death estimation); and the resolution of commingling.

Both FAs sat on the Identification Committee and collated summary findings from the antemortem and postmortem records. The full-time anthropologist led the reconciliation meeting through the comparison of antemortem/postmortem case profiles for final identification. The consultant anthropologist wrote the final forensic anthropology report, which was comprehensive, from scene recoveries to identifications.

Multiple Fatality/DVI, Forensic Anthropology, Recovery/Postmortem

A133 A Multiple Fatality Response to Nine Indigenous Deaths in a Burned House in Pikangikum, Ontario: Disaster Victim Identification (DVI)

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After attending this presentation, attendees will be better informed concerning the approach to DVI in a multiple fatality and will be aware of lessons learned from its application to a specific event from the hosts of the Triennial International Association of Forensic Sciences 2107 meeting, the Ontario Forensic Pathology Service (OFPS).

This presentation will impact the forensic science community by illustrating that a team or committee approach to identification in multiple fatalities utilizing evidence-based methods can be accomplished in a short timeframe. Early communication with all members of the team will ensure that the appropriate antemortem information is collected as soon as possible after the event. Finally, blinding of the postmortem team to existing antemortem information ensures that unbiased information is collected.

Nine family members died in a house fire on the Pikangikum Reserve, a remote Ojibwe community in Northern Ontario. While commingling of the remains at the scene was minimal, and what commingling existed was resolved by the forensic anthropologist at the scene and at postmortems, the remains were extensively thermally damaged and fragmented. Antemortem medical and dental records were only available at the reserve's nursing station on the reserve and had to be collected while the scene was being processed in order to be transported with the remains to Toronto for the purposes of identification.

The OFPS maintains a Multiple Fatality Plan with a section devoted to undertaking DVI. In large multiple fatalities, a clean copy of the Resolve Initiative database will be used. This database is used on a daily basis to compare unidentified remains and missing persons in Ontario and is a shared relational database used by members of the Resolve Initiative team at the OFPS and the Ontario Provincial Police (OPP). In smaller multiple fatalities, it has been practice to utilize Excel® spreadsheets, one each for antemortem and postmortem information. The spreadsheets contain available information and identifications are confirmed by a meeting of an Identification Committee prior to the release of the bodies to the families.

In this multiple fatality case, the anthropologists and pathologists provided information on age, sex, and antemortem medical findings to populate the postmortem spreadsheet. Another team carefully examined all of the medical records for each presumed decedent and populated another spreadsheet with pertinent information for identification. Police investigation in the community prior to removal of the bodies from the scene had confirmed that the decedent group was a closed population, as all other community members were accounted for. Although there was an attempt to blind the collectors of the postmortem information to the antemortem information, some was shared, which resulted in an interesting case of confirmation bias. This was easily resolved, but it illustrated that it is a good practice to separate the antemortem information team findings from the postmortem team findings until they are formally reconciled by the Identification Committee.

When all of the postmortem and antemortem information had been compiled, the Identification Committee, which consisted of all members of the team, met to determine the final identifications. A combination of dental, medical, group biological, and exclusionary criteria was utilized for the evidence-based final positive identifications. Having a closed population permitted the use of exclusion criteria based on age so the decedents could be returned to the community in a timely manner without having to await DNA identification results.

Multiple Fatality/DVI, Identification Committee, Confirmation Bias

A134 The Use of Laser Scanning Confocal Microscopy in Distinguishing Between Peri-Mortem Trauma and Postmortem Damage Using Histotaphonomic and Histochemical Techniques

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After attending this presentation, attendees will better understand the techniques used to implement laser scanning confocal microscopy in assessing whether bone damage occurred peri- or postmortem. While this pilot project used bone damaged approximately 24 hours after death and five years after death, this presentation will demonstrate that the methods introduced can be used to distinguish peri-mortem from early postmortem damage occurring just days after death.

This presentation will impact the forensic science community by adding to the body of knowledge on modern histological techniques used in forensic science and anthropology. This study will also add to the body of knowledge used in timing bone damage as either peri-mortem or postmortem. Present techniques in timing bone damage relies on macroscopic variations, particularly with regard to break margin morphology and color variation. By understanding the microstructural and histochemical variation between bone fractured at the time of death and bone damaged after death, one can better reconstruct the death and depositional events pertaining to human remains.

The purpose of this research was to determine if it is possible to distinguish bone damaged peri-mortem from damage occurring five years postmortem using microstructural and histochemical techniques. This research used laser-scanning confocal microscopy to quantify the degradation of two non-collagenous proteins, osteocalcin and osteopontin, as well as the presence of osteoclasts at margins of peri-mortem fractures and postmortem breaks. Additionally, this project analyzed the microstructural aspect of the fracture and break margins using qualitative and semi-quantitative methods and further quantified the natural autofluorescence.

The peri-mortem sample for this research derived from bone sections excised from commercially reared domestic pig (*Sus scrofa*), while the postmortem sample originated from elements from purpose-bred *S. scrofa* left over from a decompositional research study conducted five years ago with a known date and time of death, and documented environmental factors. The break margins were excised and sectioned to 10µm using a Buehler® IsoMet® low-speed saw. After sectioning, the samples were divided into three approximately equal sections and differentially stained for visualization of the specific proteins and the osteoclasts. The "protein sections" were stained using basic fuchsin as a base stain and specific Enzyme-Linked Immuno-Sorbent Assay (ELISA) kits to quantify their differential presence. The osteoclast sections were stained using Alexa Fluor® 488 and an osteoclast antibody kit. Imaging was conducted using a Carl Zeiss™ LSM-510 META® Laser Scanning Confocal and Multi-Photon Microscope with a 4',6-Diamidino-2-Phenylindole/Green Fluorescent Protein (DAPI/GFP) base setting and an Argon 2 and Helium/Neon (HeNe) 1 and 2 lasers. Image acquisition was achieved using the Carl Zeiss™ Zen 2009® Image software that was tied to the microscope, with image analyses conducted using ImageJ v1.50i, and Volocity® v6.3. A one-way Analysis of Variance (ANOVA) was conducted, with the levels of osteopontin, osteocalcin, and the ratio between the two set as the dependent variable, and the subject class as the independent variable. Further, a linear regression analysis was conducted to determine the relationship of osteopontin:osteocalcin ratio to time. A one-way ANOVA was conducted to evaluate the variance between the levels of osteoclasts present in the "peri-mortem" and postmortem samples. Lastly, a 3D analysis was conducted from the z-stack results in order to ascertain the surface structural variation between the two sample groups.

The results of this study found that laser scanning confocal microscopy can be used to distinguish between the peri-mortem trauma group and the postmortem group. In particular, higher protein levels were seen in the peri-mortem trauma group as opposed to the postmortem, and a higher osteoclast level was also observed. Additionally, the postmortem group generated a higher amount of natural autofluorescence. Lastly, the margin of the peri-mortem group appeared more jagged than the postmortem group.

Laser Scanning Confocal Micros, Taphonomy, Skeletal Trauma

A135 Skeletal Trauma Resulting From Falls Involving Stairs: An Analysis of Fracture Patterns and Morphologies Using Postmortem Computed Tomography (PMCT)

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After attending this presentation, attendees will better understand the skeletal fracture patterns and fracture morphologies that result from fatal falls involving stairs.

This presentation will impact the forensic science community by providing forensic anthropologists with an improved evaluation of skeletal fractures resulting from falls involving stairs. This presentation will also demonstrate the value of PMCT analyses in the context of detailed National Coronial Information System (NCIS) documentation for providing comprehensive, contextual, and accurate analyses and interpretations of skeletal trauma.

Falls are the second-leading global cause of unintentional injury and death.[1] Understanding the skeletal Blunt Force Trauma (BFT) resulting from falls, both in terms of the resulting facture patterns and morphologies, is of value to forensic anthropologists when interpreting circumstances of death in medicolegal cases involving skeletal BFT; however, the skeletal BFT resulting from falls involving stairs in particular is poorly understood. Currently, a small number of forensic pathology and clinical medicine studies have investigated trauma patterns following falls involving stairs and have illustrated that all areas of the skeleton are susceptible to skeletal fracturing, given the "tumbling" nature of this mechanism.[2] As these disciplines are predominantly focused on the soft tissues as well as the skeletal structure, unfortunately the level of skeletal trauma detail is typically not sufficient for forensic anthropologists to use when forming interpretations regarding the circumstances of death from only the skeletal material. To address this deficit, this study has investigated the skeletal fracture patterns and morphologies (size and shape) resulting from falls involving stairs using PMCT data in the context of detailed NCIS data.

A search of the NCIS database for fatal falls involving stairs in Victoria, Australia, between 2005 and 2014, identified 89 cases. For each case, the variables known to influence an indivdiual's fall, and to subsquently influence how his/her skeleton fractured, were recorded. These variables were comprised of sex, age, body mass index, number of stairs, landing/stair surface, manner of fall, and the presence of psychoactive drugs and preconditions (medical and/or physical). Skeletal trauma for each case was analyzed using the associated full-body PMCT scan (undertaken as part of the routine autopsy process at the Victorian Institue of Forensic Medicine using a 128-row helical CT – SOMATOM® Definition Flash). The data were analyzed using the *syngo*.via imaging software. Skeletal trauma recording for each indivdiual was comprised of the skeletal element and anatomical location traumatized, fracture/s classfication, descriptions of the fracture/s (including measurements and angles where appropriate), and a schematic of the fractures. The skeletal fracture patterns and morphologies were investigated in the context of the NCIS variables.

The 89 cases of fatal falls involving stairs was comprised of indivdiuals between the ages of 42 and 103 years (mean 78±11 years) with a relatively equal sex distribution of 47% females and 53% males. Almost all falls were mechanical, the location of the stairs were a combination of indoor and outdoor environments, and the number of stairs involved varied from 1 to 15 steps. Preliminary results indicate all regions of the skeleton, except for the hands and feet, were susceptible to fracturing following falls involving stairs, and that the fracture pattern resulting from the fall is largely determined by the associated variables. Skeletal trauma was present in 62% of individuals (55 cases) and within those cases, 75% exhibited trauma to one skeletal region while the remaining 25% exhibited poly trauma. Fracturing was primarily associated with the skull (45%) with the majority of these fractures sited to the vault, and typically comprised single linear fractures, depressed fractures, and/or hinge fractures. The cervical vertebrae (25%), ribs (20%), and femora (13%) were also frequently fractured. Almost all cases of rib fractures were associated with trauma to other skeletal regions.

This research will provide improved qualitative evaluations of skeletal fractures resulting from falls involving stairs and, subsequently, augment forensic anthropologists' interpretations of circumstances of fatal falls in medicolegal contexts.

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Stair Falls, Computed Tomography, Skeletal Trauma

A136 Concentrated Four-Point Bending and Fracture Behavior in the Human Femora

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After attending this presentation, attendees will be aware of fracture outcomes in controlled, four-point bending tests on fresh human femora.

This presentation will impact the forensic science community by linking a known set of loading conditions with an expected range of bone behavior and fracture morphology.

Case studies and medical literature associate long bones failed in bending with butterfly fractures. It is expected these occur in a tension wedge configuration, with fracture initiating as a transverse crack at the tensile surface of a bent bone and branching toward the compressed or impact surface. Wedge configuration is forensically significant because analysts often cite the presence and orientation of butterfly fractures as diagnostic of impact direction.

Experimental research indicates this practice is problematic. Wedge fragments are not always produced in long bones failed in bending.[1-3] Furthermore, butterfly fractures may occur in a compression wedge configuration, with the transverse crack on the impact surface.[1,4]

Recently, researchers have reported on specific fracture characteristics better suited for reconstructing bending direction. Fenton et al. documented a consistent suite of incomplete fractures in dry human femora failed under three-point bending.[2] Isa et al. confirmed these features and documented consistent fracture surface features in experiments with fresh human femora.[3] These features could be used to reliably reconstruct failure in tension and compression and subsequently determine impact direction (anterior or posterior) within an experimental bending set.

Most experimental forensic research focuses on a three-point model of bending. Though compression wedges have been reported, they are observed less frequently than tension wedge-type fractures; however, Martens' engineering research team reported near-exclusive production of compression wedges in posteriorly loaded femora failed under a concentrated four-point bending configuration.[1,4] It is forensically relevant to understand if this type of bending produces a different range of fracture characteristics than three-point bending.

The objectives of the current study were: (1) to execute controlled, Martens-type four-point bending tests of fresh human femora; (2) to identify fracture outcomes, including characteristics of the complete and incomplete fractures and fracture surfaces; and, (3) to compare these fracture outcomes with those of three-point bending experiments.

Four unembalmed human femora were failed under four-point bending, with the supports at 60% bone length and the inner probes set at 10% bone length, using a servo-hydraulic testing machine. Failure was achieved via controlled displacement at a rate of 1Hz over a 20mm displacement of the inner probes, loading the posterior bone surface. All impacts were filmed with a high-speed camera at 40,000 frames per second. Following each experiment, specimens were examined for complete and incomplete fractures and fracture surface morphology.

Controlled bending tests produced compression wedge-type fractures in two specimens and distally oriented oblique fractures in two specimens. In all impacts, video footage confirmed fracture initiation on the bone's tensile side. Failure loads were 6,567N ± 1,323N. The displacement of the bone to failure was 7.99mm ± 1.80mm, and the energy input into the system until failure was 31.10J ± 15.77J.

Incomplete fracture characteristics observed in previous three-point bending impacts were not observed in long bones failed under this four-point bending configuration; however, for the two specimens with compression wedges, fracture surface characteristics were consistent with those described in previous 3-point bending impact experiments.[3] The fracture surface of the non-impact (tension) side was billowy with shallow topography. The surface of the impact (compression) side was jagged with steep topography.

The experimental results indicate there may be some differences in fracture characteristics of long bones failed under three-point and concentrated four-point bending. Regardless of the specific bending conditions, when wedge fractures are present, fracture surface characteristics are most useful for assessing failure in tension and compression and thus reconstructing impact direction.

Supporting the work of Martens et al., compression wedges and distally oriented oblique fractures were noted in this study. In all four experiments, fracture initiated on the tensile side and propagated to the compression side.

This presentation communicates the outcomes of experimentally controlled loading conditions in fresh/unembalmed human femora. The data contributed here provides a set of fracture characteristics observed in "known" cases of blunt trauma that may help experts better reconstruct the specific loading conditions in an injury scenario.

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Trauma Analysis, Fracture Patterns, Biomechanics

A137 Differentiating Between Sharp Force Trauma (SFT) Defects and Insect Invasion of the Skin of Human Cadavers Throughout the Decomposition Process in a Terrestrial and Fluvial Environment

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After attending this presentation, attendees will understand that SFT skin defects, induced by a variety of sharp instruments, can be recognizable and distinguished from insect invasion defects throughout the human decomposition process into the late phases of the advanced stage. Issues that affect the margins of SFT will also be addressed.

This presentation will impact the forensic science community by providing results of SFT to skin from two controlled but natural environments where no current human taphonomic research exists. This presentation will acknowledge the need for the collaboration of human decomposition studies with trauma studies on both hard and soft tissue and will also broaden the forensic taphonomy research arena.

SFT in bone and cartilage is well documented in forensic literature and recent literature documents SFT evidence on hair and fabric; however, there is no literature on the effects of taphonomy on SFT of the skin and whether it is identifiable and distinguishable from insect activity throughout the decomposition process.[1-11] It is not known whether particular sharp instruments that produce a certain pattern on the body in the fresh stage of decomposition will maintain that pattern in the late stages of decomposition in either a terrestrial or fluvial environment. In addition, do sharp instruments produce a particular pattern when used in any area of the body, and is the pattern visible throughout decomposition? If the patterns are recognizable, can they be distinguished from insect activity?

This study was conducted at the Physical Anthropology Skeletal and Soft Tissue Trauma (PAST) Institute at Sam Houston State University.

SFT defects of human skin, how those defects changed through decomposition in a terrestrial and fluvial environment, and how they were distinguished from insect defects were examined. Eight sharp instruments were used to induce SFT on six human subjects at ten areas of the body. Each subject was in a fresh stage of decomposition and unclothed at the time of the SFT induction. Subjects were protected from scavenging by wire cages. Subjects in the fluvial environment were placed in a fiberglass mortuary vault filled with well water and local soil to simulate a regional water environment. Bodies were accessible to the outdoor environment, including insects. Instruments used included a Ryobi® reciprocating saw, a Marshalltown® trowel, an Estwing® axe, a Condor® Crocodilian machete, an HDX® clawed hammer, a Dexter Russell® knife, a Cold Steel® serrated edge knife, an Ace® shovel, and an HDX® screwdriver.

Length and width measurements of each wound were taken each day until advanced decomposition was reached. As expected, SFT wounds increased in length and width as decomposition progressed. For SFT measurements that could be taken at both the fresh and advanced stages for each instrument, percentage length increase ranged from 2% to 83%, with the clawed hammer having the largest average percentage length increase (57%). Percentage width increase ranged from 14 – 88% with the axe having the largest average percentage width increase (76%). All SFT wounds on terrestrial subjects were still evident into the advanced stage of decomposition and distinct from insect activity defects. Insect defects were predominantly circular in appearance and measured 1mm-4mm in diameter.

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Human Decomposition, Sharp Force Trauma, Entomology

A138 Constricting Structures: A Critical Analysis of a Past Forensic Anthropology Case Through the Systematic Examination of a Neck Organ Block Traumatic Injury in the Present

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After attending this presentation, attendees will understand the frequency of trauma to the hyoid, thyroid cartilage, and cricoid cartilage that results from hanging, with special consideration paid to the age, sex, and ancestry of the decedent as well as the type of ligature used. In addition, this presentation reinforces observations made by forensic anthropologist William R. Maples, PhD, concerning a controversial international 1991 case involving neck trauma.

This presentation will impact the forensic science community by demonstrating how the intellectual merit of this study is grounded in the knowledge that the medical examiner's determination of cause and manner of death may not be reliably determined when the influences of the variables mentioned above are not taken into account.

With these analysis needs in mind, this study conducted macroscopic and radiographic forensic anthropological examinations of trauma at the request of the medical examiners from three medical examiner districts in Florida.

Over the past two years, 52 medical examiner cases (25 = with evidence of peri-mortem trauma; 27 = no evidence of peri-mortem trauma) were macroscopically (with a 300x dissecting scope) and radiographically (AGFA CR30-X machine at 40kvp and 1/30 exposure) examined in each medical examiner's facility. Each medical examiner removed the neck organ block from the decedent without cracking the thyroid cartilage along the midline. Cartilage and bone traumatic discontinuities (complete or greenstick fractures) were photographed and mapped (to scale) onto anatomical figures. The sample comprised 37 males and 15 females between the ages of 16 and 99 years (mean age = 42 years) who were of European (42), African (4), Asian (1), and Hispanic (5) ancestries, with statures ranging from 4'4" to 6'2". These cases involved 28 ligatures (rope, electrical cord, belt, etc.) and one instance of manual strangulation. Most of the hangings involved partial suspension while only two evidenced full suspension.

The analyses revealed that 25 individuals (48%) presented with fractures and that greenstick fractures (60%) predominated. Interestingly, 53% of the individuals presented with thyroid cartilage fractures, a finding that was statistically significant ($x^2 = 22.354$, $p < .001$). There was no significant association between ligature type and frequency of thyroid fracture ($x^2 = 10.69$, $p > 0.05$); however, there is a significant association between ligature type and the frequency of hyoid fracture ($x^2 = 23.69$, $p < 0.05$). No statistically significant difference in the fracture frequency of males and females was noted ($t = -0.876$, $p = 0.385$). These findings helped in the heuristic validation of a forensic anthropology case analyzed by Dr. William R. Maples that was part of more than 7,000 slides, notes, books, bones, and casts comprising the William and Margaret Maples Special Collection at Florida Gulf Coast University.

During Dr. Maples' 29-year career, which involved 1,000 forensic anthropology cases, one international case brought public emotions to the forefront. Ms. Flor Contemplacion's family and Filipino human rights activists fought to have her death sentence by judicial hanging overturned. Ms. Contemplacion, a housekeeper in Singapore, was found guilty of the homicides of Master Nicholas Huang (the 4-year-old son of their employer) and Ms. Della Maga (the 34-year-old Filipino coworker). On May 4, 1991, Master Huang's father came home to find his son's head in a pail of water and Ms. Maga strangled in the bathroom. Ms. Contemplacion was hanged on March 17, 1995 for her crimes. The incident triggered an emotional outrage by some who indicated that Contemplacion was denied a fair trial and that new evidence was off-handedly dismissed. Two years later, the Philippine National Bureau of Investigation put together a team of forensic scientists from United States, which included Maples, to review the exhumation records and reports in order to provide opinions on the validity of the new evidence, cause, and manner of death. Maples and his colleagues found Della Maga died as a result of asphyxiation due to ligature strangulation; thereby supporting the original autopsy while asserting that the evidence of the exhumation was fundamentally flawed.

Peri-Mortem Trauma, Hyoid, William R. Maples

A139 Searching for the Unidentified in South Texas: The Forensic Border Coalition (FBC) Cemetery Survey Project

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After attending this presentation, attendees will understand the difficult task of locating burials of unidentified human remains along the South Texas border.

This presentation will impact the forensic science community by providing methods for locating burials of unidentified human remains for identification purposes.

In 1995, Texas passed laws specifying explicit procedures for processing unidentified human remains found within a forensic context. In 2005, additional state laws concerning missing and unidentified persons were passed, based on new technological advances using federal databases and DNA to help identify missing and unidentified persons. Currently, many counties in Texas follow these state laws. Unidentified human remains that are part of a medicolegal investigation and are processed through a medical examiner's office have a final disposition that is carefully tracked; however, the majority of Texas counties do not have a medical examiner's office and human remains found in these counties have historically been less carefully tracked.

Prior to 2005, most unidentified human remains found in South Texas representing presumed migrant deaths were buried without identification efforts. In Texas, there is no centralized recordkeeping system to address how many unidentified human remains have been found and buried statewide or along the South Texas border. Knowing the number of unidentified remains likely to correspond to migrants would allow for a better understanding of the humanitarian crisis at the South Texas border and across the United States-Mexico border region. Further, knowing the number of deaths and burial locations would allow for strategic planning for exhumation, analyses, and identification efforts of the long-term dead.

In an effort to count the number of migrants who have died crossing the Texas/Mexico border, the FBC conducted a cemetery survey project to address the following questions: (1) How many migrants have died crossing the Texas/Mexico border per county per year?; (2) Where are they buried?; and, (3) What are the county protocols regarding unidentified deceased persons? The FBC employed several different methodologies, including searching county records, soliciting public information from funeral homes, cemeteries, law enforcement, and medicolegal agencies, and surveying cemeteries for unidentified burials. A total of seven counties were targeted based on their close proximity to the border and accounts from journalistic sources detailing high numbers of migrant deaths.

Based on a lack of county records, even with two counties having an indigent burial program, it is difficult to address the question of how many unidentified deaths have occurred per county per year for the past 20 years. Unidentified remains buried in counties without a medical examiner (n=5) or indigent burial service (n=5) had little-to-no records regarding the number of unidentified deaths or locations of burials. A total of 70 burials were located through pedestrian survey of cemeteries, and an additional 62 burials were located through conversations with medicolegal death investigators, funeral homes, and cemetery directors. Burials with associated information were mapped using the Global Positioning System (GPS). Through information obtained from funeral homes, additional potential burial sites were located; however, these potential burials are unmarked with no existing records. Each of the seven counties had different approaches to processing unidentified human remains and not all complied with Texas state laws. Only one county, with a medical examiner, tracked the exact burial location and associated case information.

Since the survey for these seven counties is now complete, strategically planned, funded exhumations will take

place. Further, collectively the FBC will work toward identification of the exhumed remains. It is crucial that local jurisdictions secure pathology and/or anthropology exams, submit DNA samples, and track the final disposition of unidentified human remains and all associated case information. Without tracking final disposition or associated case numbers, unidentified remains will be difficult to locate and identification may never take place.

Forensic Anthropology, Identification, Migrant Deaths

A140 The Forensic Border Coalition (FBC): Collaborations in Forensic Sciences, Human Rights, and Public Policy

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After attending this presentation, attendees will understand: (1) the multiple barriers to identifying and repatriating the remains of migrant border crossers; (2) the benefits of collaboration between forensic scientists, human rights organizations, cultural anthropologists, geographers, and others to address these barriers; and, (3) the need for changes in public policy to improve outcomes.

This presentation will impact the forensic science community by introducing the FBC and its strategies to count the number of migrants who have died or disappeared crossing the United States-Mexico border, to locate burials of unidentified remains, to identify the dead, and to improve practices and protocols for future identifications of the dead.

The mission of the FBC is to support families of missing migrants searching for their loved ones and to address myriad barriers related to the identification of human remains found near the United States-Mexico border. The FBC formed in the summer of 2013 following the high number of border deaths in 2012. According to official data from the United States Customs and Border Patrol, the number of border deaths reached 471 in 2012. The coalition is comprised of forensic scientists and other scholars together with human rights activists to comprehensively address the significant barriers to identifying the remains of border crossers. This presentation will focus on the work of the FBC in Texas to provide one example of the benefits of collaborations.

Border deaths in Texas occur in more than 20 counties at or near the U.S.-Mexico border, some of which are among the poorest counties in the nation. The majority of these counties lack medical examiners or forensic pathologists, some have failed to collect DNA on unidentified remains as required by state law, and many cases of unidentified remains fail to be entered into databases such as the National Missing and Unidentified Persons System (NamUs) that allow them to be compared against missing persons reports. Because many remains are severely decomposed or skeletonized, family reference samples are often required to make identifications via DNA. Some family members of migrant border crossers reside outside the United States (primarily in Mexico and Central America) and others are undocumented individuals who hesitate to visit law enforcement offices to file a missing persons report. In either case, families may be prevented from presenting missing persons reports or from entering their family reference sample into the Combined DNA Index System (CODIS).

Even locating the site of burials of unidentified remains in many South Texas counties is difficult due to lack of standardized recordkeeping, the lack of coordination between county officials, the large number of burials sites and funeral homes that process remains, the failure to clearly label unidentified remains in many cemeteries, and the failure of cemeteries to keep and share records.

In order to address barriers related to locating and identifying human remains, the FBC relied on the methodology of requesting and analyzing public records regarding migrant deaths and unidentified remains and accessing death certificates and indigent burial records to locate the final disposition of the death. In addition, the FBC collaborated with Missing in Harris County Day so migrant families could safely present cases and submit family reference samples, resulting in a number of identifications.

Non-governmental organizations and other FBC collaborators have been key in making recommendations and meeting with policymakers to improve protocols to identify, locate, and repatriate the dead in both Texas and

Arizona. The multidisciplinary nature of these collaborations is instrumental in exposing the gaps within these protocols and thus in making them a powerful humanitarian voice for pushing forward policy change at the local, state, national, and international levels.

Migrant Deaths, Human Rights, Policy Reform

A141 The Role of the Anthropologist in the Identification and Repatriation of Deceased Migrants Along the United States-Mexico Border

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After attending this presentation, attendees will better understand the role of the anthropologist in the identification and repatriation processes of deceased migrants located along the United States-Mexico border.

This presentation will impact the forensic science community by highlighting how the role of an anthropologist varies in identification and repatriation processes depending on local and state laws and how this role may extend well beyond skeletal analysis.

Often law enforcement may be responsible for identification efforts while an anthropologist provides a report to a medical examiner, law enforcement agency, coroner, or Justice of the Peace (JP); however, in other circumstances, responsibility for identification may fall to the anthropologist. Depending on jurisdiction, these responsibilities vary, with the anthropologist taking on new roles.

These varied roles will be examined through a survey of three partner organizations of the Forensic Border Coalition (FBC): the Argentine forensic anthropology team, Equipo Argentino de Antropología Forense (EAAF), the Pima County Office of the Medical Examiner (PCOME), and the Forensic Anthropology Center at Texas State (FACTS). A generalized description of identification and repatriation processes is given for migrant deaths in Arizona, while case studies will be discussed to illustrate the complexities of these processes in Texas.

The EAAF investigates migrant deaths along both sides of the United States-Mexico border. Regarding cases of unidentified remains recovered in the United States, EAAF facilitates the collection of family reference sample DNA for comparison to DNA samples from remains likely to correspond to migrants. When DNA analysis suggests an identification, the EAAF works with forensic data banks on missing migrants or other mechanisms within the migrant's country of origin to compare all antemortem and postmortem records to confirm an identification. The EAAF writes an identification report and works with the appropriate local United States officials to legally recognize the identification. In recent years, EAAF has helped identify 65 migrants who perished within the United States. Repatriation is then handled by a Consulate's office, overseen by the Foreign Affairs Ministry from the decedent's home county.

In Arizona, forensic anthropological investigations of presumed migrants take place at the PCOME. Between 2001 and 2013, the PCOME received the remains of 2,203 presumed migrants and successfully identified 1,463. For cases requiring skeletal analysis, anthropologists at the PCOME construct both a biological and cultural profile. Once an identification hypothesis is made, the anthropologist compares all antemortem and postmortem data, writes an identification report, and briefs the medical examiner, who legally signs off on an identification. If the decedent is to be returned to their country of origin, the local Consulate's office manages the repatriation of the remains, a process that can take weeks to months.

Since 2013, Operation Identification (OpID), housed within FACTS, has received the remains of 87 presumed migrants, many through exhumation efforts carried out by Baylor University and the University of Indianapolis. DNA analysis, in addition to anthropological analyses of the skeletal remains and personal effects, are the primary sources for identification hypotheses. Once a DNA association is reported, anthropologists associated with OpID compare all antemortem and postmortem data, write an identification report, and brief the appropriate JP, who legally approves the identification. Since 2014, OpID has helped facilitate nine identifications; however, only five of those individuals have been successfully repatriated. The repatriation process for counties without a medical examiner requires the coordinated efforts of Consulate offices, the funeral home handling the repatriation, the funeral home that originally filed the death certificate, and the JP, which can all vary by case. Unlike the Arizona model in which these efforts are centralized, in Texas, these parties can be vastly separated by geography, culture, and available resources, which can result in a breakdown of communication and stagnation of the repatriation process. In the

most egregious example, an individual identified by OpID in August 2014 has still not been repatriated as of July 2016.

This unfortunate reality has led to anthropologists in Texas adopting new roles as de facto case managers and stewards of identification and repatriation processes. Anthropologists associated with OpID, with help from other FBC partners, are now facilitating/mediating communication between funeral homes, law enforcement, JPs, medical examiners, and the decedent's family members. It is believed these expanded roles of the anthropologist will help streamline and hasten the repatriation of remains.

Migrant Deaths, Identification, Repatriation

A142 Addressing the Challenges of Migrant Death Investigations Through Non-Governmental Organization (NGO) and Government Agency Collaborations

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After attending this presentation, attendees will better understand how collaborations between NGOs and government agencies are essential to unidentified human remains investigations throughout Central and North America, particularly along the United States-Mexico border.

This presentation will impact the forensic science community by highlighting challenges that medicolegal systems face along the United States-Mexico border and by presenting how those challenges are often overcome through collaborations with NGOs.

The purpose of this study is to: (1) explore how three NGOs have partnered with different government agencies in unique ways to facilitate investigations of deceased migrants in specific regions; (2) compare the similarities and differences between the different strategies they have employed; and, (3) discuss how collaborations between these NGOs create a unified model for addressing a humanitarian crisis. Data analyzed include: the protocols for each NGO/government agency collaboration, the number of cases investigated, the number of family reference DNA samples collected, and the number of identifications through collaborations. Additionally, case studies will illustrate general challenges and how these obstacles are overcome.

Migration across Central America and the United States-Mexico border is a dangerous and often deadly process. Investigations of migrant deaths are complicated by sociopolitical and geographical factors, resulting in challenges for the government agencies responsible for properly investigating these deaths. For example, many families live in countries far from the location of the death of their loved one, making it difficult for investigating agencies to obtain family reference DNA samples for comparisons to unidentified human remains. Furthermore, many families are afraid to meet with law enforcement, let alone submit a biological sample to a feared agency. Consequently, many of these investigations are incomplete.

In Central America and Mexico, the Argentine forensic anthropology team, Equipo Argentino de Antropología Forense (EAAF), facilitates the creation of forensic data banks on missing migrants, with case files containing all background and antemortem data, as well as DNA profiles from direct relatives for genetic comparisons. These forensic data banks are composed of governmental and non-governmental institutions, an unprecedented development in the region, allowing direct participation from the NGO sector, particularly committees of relatives, to monitor and inform work of government officials. Forensic data banks currently exist in Honduras, El Salvador, and Chiapas and Oaxaca in Mexico. As of July 2016, EAAF has 929 missing migrant cases, 2,520 family reference DNA samples, and has made 117 identifications through these data banks.

In Pima County, AZ, the Colibrí Center for Human Rights' process relies on building strong relationships with both families of the missing and the officials tasked with the investigation of unidentified remains. Colibrí is based in Arizona and works most closely with the PCOME, which has provided in-kind support to Colibrí in the form of office space and equipment, allowing the collaboration to be a regular part of the daily investigation process at this county office. Colibrí supports the investigation of migrant cases in Pima County by managing all incoming inquiries from families of missing migrants, providing detailed antemortem data to forensic practitioners, and producing identification hypotheses that can then be followed up scientifically. Colibrí has assisted with the investigation of hundreds of cases at the PCOME and several cases in Texas and in California. Colibrí's database includes records for more than 2,000 missing migrants last seen crossing the United States-Mexico border.

In Brooks County, TX, the South Texas Human Rights Center (STHRC) has established a Memorandum of Understanding (MOU) with the Brooks County Sheriff's Office (BCSO) that allows the STHRC to investigate both missing persons and unidentified human remains cases on behalf of the BCSO. As a result, the STHRC, in collaboration with other organizations, has assisted with the investigation of more than 200 missing persons cases, the collection of family reference samples from more than 40 families, the identifications of more than 10 deceased

individuals, and the finding of more than 40 missing individuals, some whose lives were saved by search and rescue efforts.

Collaborations between NGOs and government agencies are essential to the investigations of missing migrants and unidentified human remains found throughout Central and North America. Both reported statistics and presented case studies illustrate the strengths of involving NGOs in these investigations, as they combine diverse, multidisciplinary perspectives while working with families to resolve these investigations.

Migrant Deaths, Regional Collaborations, Non-Government Organizations

A143 Missing Migrant Data Managed by the Forensic Border Coalition (FBC)

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After attending this presentation, attendees will understand the current state of the data regarding missing migrants managed by members of the FBC as well as strategies in data comparison and the latest figures on the minimum number of missing migrant cases in the United States-Mexico border area.

This presentation will impact the forensic science community by highlighting the unique challenges in collecting and sharing data about missing migrants along the United States-Mexico border and by providing a methodology for the collaborative management of such data as developed by the FBC.

The transnational nature of migration in the Americas poses unique challenges to the tracing of missing persons. Migrants may pass through multiple countries or states before reaching their final destination, facing extreme vulnerability at all points during their journey. Migrants are dying and disappearing at alarming rates throughout Mexico as well as along the United States-Mexico border. Relatives of the missing may live in Central American countries, Mexico, or the United States and reach out to a variety of governmental and non-governmental organizations in their search for information. These organizations in turn may or may not have access to comprehensive and accurate data about unidentified human remains discovered along migrant routes. The United States has a public, non-genetic, national database, the National Missing and Unidentified Persons System (NamUs), that is intended to centralize information regarding missing persons and unidentified remains, as well as a separate DNA-index system, the Combined DNA Index System (CODIS), operating at local, national, and state levels that has the potential to facilitate genetic comparisons; however, families of missing migrants face significant obstacles to participation in either system for a variety of reasons, particularly when family members reside outside of the United States and/or are living in the United States without official documentation. The consequence is that data regarding missing migrants is scattered throughout the Americas, with both duplication of efforts between organizations and inconsistency of responsiveness to relatives. In turn, experts trying to identify the dead struggle to obtain antemortem information about missing migrants that is accurate, organized, and complete.

Three non-governmental organizations within the FBC manage missing migrant data: (1) the Argentine forensic anthropology team, Equipo Argentino de Antropología Forense (EAAF); (2) the Colibrí Center for Human Rights; and, (3) the South Texas Human Rights Center (STHRC). Each organization differs slightly in geographical focus, with EAAF covering regions throughout Central America, Mexico, and the United States, Colibrí encompassing the entire United States-Mexico border, and STHRC focusing on those who disappear crossing into Texas. Despite these differences in geographical focus, there is overlap between these organizations' databases.

To compile a complete and non-overlapping list of missing migrant reports, cases were compared based on name, age, country of origin, and last known whereabouts. Common cases (appearing in two or more databases) were counted only once, and the organization with the most complete data was assigned the status of main point of contact, with the other organization(s) serving as secondary contacts. Between the three organizations, there is a total of 3,332 missing migrant cases, 929 of which are managed by EAAF, 2,118 by Colibrí, and 285 by STHRC. Matching cases is a crucial component in the prevention of duplicating efforts as well as clarifying who is responsible for a case, thus ensuring that all the necessary documentation, from antemortem to postmortem data, reaches the proper organization.

Obtaining reliable and comprehensive information regarding minimum numbers of migrants likely to have gone missing in the United States-Mexico border region has traditionally been a challenge. The FBC has addressed this need through case data collection and case data comparison processes that respect the unique needs of each organization's mandate while still facilitating large-scale comparison of case data to determine minimum numbers. These numbers represent the first comprehensive effort to obtain an estimate of those who have disappeared crossing the United States-Mexico border.

A144 Identification Notifications and Their Applicability to Families of Missing Migrants

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After attending this presentation, attendees will understand the relevance of the protocol designed and successfully applied by the Argentine forensic anthropology team, Equipo Argentino de Antropología Forense (EAAF), for identification notifications to families of missing or disappeared people, particularly in missing migrant cases.

This presentation will impact the forensic science community by: (1) highlighting the gaps in documentation and communication that have led to insufficient or inappropriate notifications; (2) offering suggestions to improve this critical moment for families receiving information; and, (3) discussing considerations of a wider role for forensic scientists in the notification process. The goal of EAAF's notification protocol utilized in cases of disappeared people and missing migrants is to significantly improve the information, documentation, and circumstances in which notifications of remains identifications take place so family members can end their uncertainty regarding the fate of their loved ones.

In many countries, forensic specialists are typically not involved in the notification of identifications to the respective family. Further, in EAAF's experience with families of victims of human rights violations and, more recently, with families of missing migrants, it has been apparent that in numerous countries, whether forensic experts are involved or not, there are no specific protocols regarding how to conduct notifications. Yet, the notification of identification is a critical moment that may jeopardize excellent forensic work if not performed properly. In the migrant context, the absence of an identification report, the communication to families over the phone of identification from one country to another, the delivery of sealed coffins with the instruction to not open them, among other practices reported by families of missing migrants, often leave them with an open, severely painful doubt regarding identification results instead of the identification ending their uncertainty.

The Border Project, coordinated by the EAAF beginning in 2009, seeks to create a regional forensic mechanism throughout the Central America, Mexico, and United States migrant corridor to significantly improve both the identifications of missing migrants among unidentified remains in the region and the response of governments to families searching for missing migrant relatives. As part of this initiative, the project addresses the need to alleviate the doubts regarding an identification; such doubts are increased in the context experienced by families of missing migrants due to death occurring in a foreign country where they typically cannot afford to travel and often occurring in unknown circumstances. To truly evaluate the effectiveness of the work of forensic scientists on the impact of identifications on affected families, the significance of the notification process must also be evaluated, particularly the importance of forensic specialists' involvement. The Forensic Data Banks on missing migrants and related mechanisms that are currently part of the Border Project in El Salvador, Honduras, and parts of Mexico and Guatemala are applying a notification protocol that derives largely from EAAF's experience in other types of cases, such as enforced disappearances, to the migrant context.

EAAF's notification protocol involves several phases, including: the compilation, data verification, and review of all case materials prior to scheduling a notification; the completion of an integrated, multidisciplinary identification report in the family's language; risk assessment of the family receiving a notification, be it health related or threat from other persons; conducting the notification in person with the family; providing psychological and medical support; and explaining the repatriation process, among others.

Collaborative and centralized efforts involving forensic, governmental, and non-governmental institutions internationally are crucial to reaching the point of identification notification with all available information in place and to facilitating its delivery in the best way possible. The benefit of EAAF's protocol model is that it affords families clear and accessible forensic and circumstantial information on the death of their loved ones, assisting directly in their mourning process; it helps to standardize a critical moment in the identification of remains process and can be widely adapted in ongoing humanitarian efforts. It is through this tested mechanism that families can be ensured of their right to information, truth, and reparation. Conducting forensic work involves responsibilities not

only to the dead but also to the living and, as a result, requires that the Border Project serve the families of missing migrants by ending their uncertainty in cases in which their missing relatives have died.

Forensic Anthropology, Identification Notifications, Migrant Deaths

A145 The Evolution of Forensic Anthropology and the Influence of Court Rulings, Legislative Actions, and Social Trends

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After attending this presentation, attendees will better understand the impact of court rulings, legislative actions, and social trends on the evolution of forensic anthropology. Several recent Supreme Court rulings, legislative actions, and social trends will be reviewed and the ways in which each impacts the field of forensic anthropology will be investigated.

This presentation will impact the forensic science community by emphasizing the importance of awareness of court rulings, legislative actions, and social trends that influence the practice of forensic science. Some of these influences may appear ancillary, but each has the potential to directly impact laboratory policy, admission of forensic testimony, and available funding.

Forensic anthropology has experienced significant growth during its relatively short history. In the past 45 years, since the first meeting of the Physical Anthropology section of the American Academy of Forensic Sciences, the field has experienced great change often driven not by forensic anthropological research and technical development, but by court rulings, legislative actions, and social trends. Some external influences are well known by practitioners (e.g., *Daubert v. Merrell Dow Pharmaceuticals, Inc,* the 2009 National Academy of Science Report *Strengthening Forensic Science in the United States: A Path Forward* (NAS Report), and The Innocence Project), yet others are less familiar (e.g., *Bullcoming v. New Mexico*).[1-3] Despite the low level of awareness, each of these influences affects the practice of forensic anthropology.

The NAS Report showcased systemic weaknesses in the field of forensic sciences. The Report emphasized the fact that few forensic science methods adequately measured inherent uncertainty or accuracy of inferences made by forensic scientists. Further, the Report presented concerns for contextual bias, drawing into question what "working in the blind" means for forensic science and how it can strengthen or limit analyses. As a response to the Report, anthropologists have focused research on defining method error rates, with specific attention on non-metric methods. Yet, the field has done little to explore error rates associated with analyst inference. Further, the trending concern for contextual bias has influenced some anthropology laboratories to require their analysts to perform analyses without contextual information; however, the field has not responded by investigating errors stemming from this approach.

Bullcoming v. New Mexico focused on the Sixth Amendment Confrontation Clause giving the accused the right to confront the witnesses against him. During the trial, the forensic analyst who completed, signed, and certified the toxicology report was on unpaid leave and unavailable. The State called another analyst to validate the report. Bullcoming's counsel objected, asserting that introducing the report without the opportunity to cross-examine the analyst was in violation of the Confrontation Clause. Ultimately, the Supreme Court ruled that the laboratory report was out-of-court testimony; therefore, it could not be introduced unless the analyst was available at trial or the accused had prior opportunity to confront the witness. This ruling should directly impact a laboratory's policy for co-signing reports.

Recent media coverage of court cases has brought forensic science under increased public scrutiny. For example, ProPublica, collaborating with PBS's *Frontline* and NPR, reported on the conviction of a Texas man for the homicide and sexual assault of a 6-month-old child. During the trial, the medical examiner and hospital workers provided testimony describing the injuries the child sustained. The Texas Court of Criminal Appeals overturned the conviction after hearing testimony from a different pathologist who concluded the child had been afflicted by a severe blood-clotting disorder. This type of media coverage increases the public's scrutiny of forensic science, in return causing prosecutors to seek more analyses, leading to a trickle-down effect as pathologists reach out to anthropologists for consultation on difficult cases. Popular culture has also affected the public's opinion of forensic scientists. Juries now disregard arguments not based on solid forensic evidence or they believe that the information provided on television shows (e.g., *Bones*) and in films accurately depicts the work of forensic scientists; therefore, conflicting information can be legitimately (in their minds) disavowed.

The NAS Report, *Bullcoming v. New Mexico,* and the ProPublica report are three of many examples of external influences that impact the direction of change in forensic anthropology. Understanding these influences is important to ensure the field's responses are appropriate and deliberate. Without such an understanding, forensic anthropologists may follow practices and develop policies that are detrimental to the field.

Reference(s):

1. *Daubert v. Merrill Dow Pharmaceuticals, Inc.* 509 U.S. 579, 113 S. Ct. 2786, 125 L. Ed. 2d 469, 1993 U.S.
2. National Research Council of the National Academies, *Strengthening Forensic Science in the United States: A Path Forward.* Washington, DC: National Academies Press, 2009.
3. *Bullcoming v. New Mexico.* 564 U.S. ___, 131 S.CT. 2705, 180 L.Ed.2d 610 (2011).

Forensic Anthropology, Court Rulings, Legislation

A146 Virtual Skeletal Data — The Ethical, Technical, and Legal Questions Anthropology Faces When Working With Virtual Skeletal Remains

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After attending this presentation, attendees will better understand the challenges the anthropological community is facing and will be facing in the future concerning ethical and legal questions regarding virtual skeletal remains data. Currently, there is no legal framework anywhere in the world that governs the gathering and storing of analysis or the storage of data that represents virtual skeletal remains. There are also no ethical guidelines or technical standards for setting up a virtual skeletal collection. Attendees will be presented with the open questions, the issues caused by a lack of answers, and possible solutions to a number of the issues.

This presentation will impact the forensic science community by starting a discussion about several of the numerous questions that need to be addressed if virtual skeletal analysis is to have a productive place in anthropology. Ethical, technical, and legal question regarding virtual skeletal remains need to be addressed soon or anthropological research and forensic anthropological casework will soon suffer from a lack of answers. Virtual skeletal anthropology cannot exist in an ethical, technical, or legal vacuum.

For more than a century, anthropologists have collected skeletal remains and built up collections. These collections have helped develop and test numerous methods in physical anthropology and without them, forensic anthropology would not be where it is today. In recent years, a new type of skeletal collection began to emerge: the virtual skeletal collection. Here, electronic 3D models of bones are stored, rather than the physical original. Such virtual data can be tomographic or topographic, can come with "skins" or without, and can be produced in various file formats, sizes, and resolutions. This is a very promising new research area, but one that is more or less entirely void of rules, regulations, guidelines, and laws.

There are three areas in which a multitude of questions need to be addressed: ethical, technical, and legal. The legal questions concentrate primarily on the issue of anonymity and on intellectual property rights. While the first of these two issues is familiar to anthropology and fairly easily dealt with, the second becomes more difficult. Who owns a virtual skeletal bone? The person whose bone it is if the person is alive, their family if the person is dead, the institution that is housing a physical skeletal collection that is being scanned, or the institution that put all the time, work, and effort into scanning and/or processing the bones? Can one institution share the bones with one or more third parties? There are many questions and so far very few answers.

The ethical questions may turn out to be more easily answered. It is likely that, in many ways, virtual bones can be treated just like physical bones; however, while in many parts of the world physical remains are being reburied, there appears to be no need for that to happen with virtual bones. Should communities that request the burial of physical remains have the right to have virtual ones deleted? Does anthropology have the right to keep the data? Such questions can be ethical ones, but they can quickly turn into legal ones as well.

Finally, there are technical issues to address. One of the greatest arguments for those in favor of virtual skeletal collections is their potential size. I f the legal/ethical issues of sharing data can be solved and enough institutions cooperate, virtual skeletal collections could be thousands or tens of thousands of individuals large. If medial Computed Tomography (CT) scans are included, there could be millions of samples and the size would increase steadily; however, numerous technical standards for 3D model files exist and there is no specific technical standard agreement among those anthropologists who work with them. Should one of the existing file standards be used or should there be a particular anthropological file standard that can contain information on pathologies, trauma, etc. on top of the already existing xyz coordinates, texture, etc.? If so, detailed standards would have to be developed and that is a very considerable task.

Some of these questions can be solved by the international anthropological community, if they are willing to address them. Others questions need to be addressed by governments or international organizations but anthropologists will still need to lobby for change.

Virtual Skeletal Data, Ethics, Standards

A147 A Review of Statutes in Place to Stem the Commodification of Human Skeletal Remains in the United States

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After attending this presentation, attendees will better understand the laws in place relating to the commodification of human skeletal remains. This presentation categorizes and compiles all of the current legal statutes in order to provide a review of the protection of human skeletal remains from commodification in the United States. In particular, this presentation considers how the law protects cemeteries, archaeological sites, and even criminal sites, and further considers the extent to which the laws may protect human skeletal remains when removed from their final disposition.

This presentation will impact the forensic science community by demonstrating the need for statutory language that can stem the trade in human skeletal remains commodification. Highlighted is the unique problem with the commodification of human skeletal remains that may result from a modern version of grave robbing. This presentation provides an opening platform for the discussion regarding the commodification of human skeletal remains, regardless of the context from which the remains are derived (i.e., anatomical specimens, archaeological sites, sites of forensic significance, or cemeteries).

Damage to cemeteries, particularly above-ground vaults or unmarked cemeteries so common in the Southeastern United States, is inevitable in most locations. In South Louisiana, where bodies are often entombed in above-ground vaults, the effects of both natural events and intentional damage result in relatively easy access to human skeletal remains. Below-ground interments and archaeological sites are equally vulnerable, particularly when located on private lands. Legal protection at the state level varies greatly, not only in scope but in language. The language that addresses the law concerning the dead as it relates to interment spaces is of particular concern in Louisiana; however, each state has its own specific challenges.

Because federal law (aside from the Native American Graves Protection and Repatriation Act) regarding the protection of human skeletal remains is incomplete at best and negligible at worst, it is up to each state to put that legislation in place and to monitor the sales of human skeletal remains; otherwise, the commodification of human skeletal remains is ignored. Because highly visible platforms for human skeletal remains sales are dwindling (in particular, eBay® shut down the sale of human remains in 2016), it will become increasingly difficult to track the location of remains. To swiftly intercept human skeletal remains, state officials need laws in place as well as working protocols to implement rapid responses to recover such threatened remains. Louisiana serves as one example of working to fill in gaps in the legislation, which involves multi-agency cooperation to ensure swift action and proper documentation/investigation of the human skeletal remains.

While enforcement is presently lacking, researchers should be made aware of what is illegal for human skeletal remains trade in their respective states. Cooperation among local forensic anthropologists, legal entities, state archaeologists, and law enforcement is important in identifying and creating protocols for human skeletal remains trafficking.

Skeletal Remains, Forensic Archaeology, Commodification

B1 The Development and Initial Evaluation of a MicroRNA (miRNA) System for Forensically Relevant Body Fluids Using Capillary Electrophoresis

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After attending this presentation, attendees will better understand the utility of miRNAs for Body Fluid Identification (BFID) in a forensic setting. Recently, the development of molecular-based assays has shown some improvement over conventional biological screening methods through increased body fluid specificity, sensitivity, the identification of a greater number of body fluids, and less consumption of the evidentiary sample.

This presentation will impact the forensic science community by demonstrating a possible confirmatory method of BFID for venous blood, menstrual blood, semen, vaginal material, and saliva using miRNAs that utilizes similar techniques and instrumentation already in place in crime laboratories.

BFID can be of great importance during the course of an investigation and in the courtroom. Determining the body fluid origin of a stain may provide probative information about the events that took place during the commission of a crime. Current methods for BFID, such as chemical tests, microscopy, enzymatic activity, and immunological tests, do not offer the level of specificity the forensic field requires. In recent years, miRNAs have been suggested as a viable biomarker for BFID and have shown considerable body fluid specificity. MiRNAs are small, typically 18-25 nucleotides in length, which makes them ideal for analyzing highly degraded forensic samples. In addition, miRNA interrogation allows for the co-analysis of DNA for individualization and miRNA for BFID from a single sample.

A common strategy for miRNA profiling systems is to analyze relative expression (delta Cycle Threshold (ΔCT)) values of various miRNAs compared to an endogenous reference gene using Real-Time quantitative Polymerase Chain Reaction (RT-qPCR); however, most instrumentation for qPCR can only detect up to five different fluorescent dyes, which would limit the amount of markers that can be amplified simultaneously. Additional reactions would be required to analyze multiple markers, which increases sample consumption, the risk of contamination, cost of reagents, and time of analysis. To address these issues, a previously reported linear primer system was expanded in order to incorporate additional miRNA markers by forming a comprehensive four-dye multiplex system using capillary electrophoresis.

In this study, a new ten-marker system for BFID was designed to differentiate between venous blood (miR-451 and miR-142-3), menstrual blood (miR-144 and miR-412), semen (miR-891 and miR-10), vaginal material (miR-124 and miR-617), and saliva (miR-205 and miR-658). Each marker was tested in singleplex reactions to assess marker viability in the multiplex as well as evaluate cross-reactivity. It was observed that the vaginal markers interacted during reverse transcription and were removed from the multiplex at this time. All other primers amplified the correct miRNA targets. Five samples each of venous blood, menstrual blood, semen, vaginal material, and saliva were evaluated with the multiplex. Although further work is needed, the miRNA system was able to generate an STR profile (from the DNA extract) and distinguish between venous blood, menstrual blood, and semen (RNA extract) from a single sample.

microRNA, Body Fluid Identification, Capillary Electrophoresis

B2 The Development and Evaluation of Real-Time Polymerase Chain Reaction (PCR) Methods for DNA Methylation-Based Identification of Semen and Blood

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After attending this presentation, attendees will understand how the methylation status of the semen-specific unmethylated DNA region and the blood-specific methylated DNA region are analyzed using a real-time PCR technology. Attendees will also understand the usefulness of these methods for semen and blood identification.

This presentation will impact the forensic science community by demonstrating methods that can accurately and sensitively identify semen and blood from various samples, including highly aged body fluid stains. In addition, these methods can be readily established in forensic laboratories and are simple to perform.

The identification of body fluids provides important evidence in forensic investigation to prove the existence of criminal events, such as murder, injury, and sexual assault. Recently, DNA regions that are specifically methylated or unmethylated in different types of body fluid have been reported as novel markers for body fluid identification. The stable nature of DNA offers an advantage for forensic use because forensic samples have often been exposed to severe conditions before laboratory analysis.

Previous preliminary work reported the development of a MethyLight-based-method for analyzing the —C—phosphate—G— (CpG) sites in the DACT1 gene, which was previously reported as a semen-specific unmethylated region.[1,2] MethyLight is a method for quantitating the methylation ratio of a targeted region using a real-time PCR device and a pair of TaqMan® probes that are designed for methylated or unmethylated status.

In this presentation, further evaluations of the method are described to enable detection of the DACT1 region for semen identification. A MethyLight-based method was also developed and evaluated for detecting the methylation ratio of cg06379435 and its neighboring CpGs, which were previously reported to be specifically methylated in blood. To set the threshold methylation ratios for semen or blood identification, DNA from various body fluid samples (blood, semen, saliva, and vaginal fluid), which were bisulfite-converted using an EpiTect Bisulfite Kit, were analyzed by the two methods, each using a pair of TaqMan® probes, the EpiTect MethyLight PCR Kit and the Smart Cycler II system. The results revealed almost exclusive non-methylation of the DACT1 region in semen and high methylation levels of the cg06379435 region in blood, which is consistent with previous studies. Based on these data, the threshold methylation ratios were set for semen and blood identification. For semen identification, another threshold ratio to detect semen mixed with other tissues could be set near the average methylation ratio of DACT1 in non-semen samples, because the DACT1 region was almost fully methylated in non-semen samples. Use of these thresholds can accurately identify semen or blood from other samples, including highly aged (29-year-old) semen, blood stains, and mixture samples containing a smaller amount (20%) of semen DNA. The sensitivity of these methods was evaluated by analyzing pooled samples of semen and blood DNA and demonstrated the necessary amounts of DNA for bisulfite conversion to be 1ng for both methods.

In conclusion, these methods can accurately and sensitively identify semen and blood. Because a real-time PCR device is common in forensic laboratories, this method can easily be introduced without the purchase of further equipment. Moreover, because a real-time PCR-based method requires no substantial work beyond preparing the PCR reaction mixture, simple and quick analysis may be realized with a reduced risk of contamination. Therefore, these methods could be better suited to forensic work compared with other methods of methylation analysis, such as bisulfite sequencing methods.

Reference(s):

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2. Eads CA et al., MethyLight: a high-throughput assay to measure DNA methylation. *Nucleic Acids Res.* 2000; 28(8): e32.

Body Fluid Identification, DNA Methylation, Real-Time PCR

B3 Investigating the Identification of Vaginal Material Using Histological, Spectroscopic, and Molecular Methods

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The goal of this presentation is to inform attendees of the recent advancements in three emerging techniques for the identification of vaginal material in a forensic context.

This presentation will impact the forensic science community by providing insight into multiple techniques — histological, spectroscopic, and molecular — to identify and distinguish vaginal material from other biological fluids. Vaginal material does not yet have its own validated, specific, and reliable test for identification.

Body fluid identification is a crucial component in the forensic investigations of sexual assault. The recovery and identification of vaginal material has been a significant challenge for forensic scientists for decades. Forensic Short Tandem Repeat (STR) DNA profiles can be used to identify the donor; however, a crucial facet is to identifying the body fluid source of that DNA, thereby elucidating the circumstances of the case. The identification of vaginal material present on penile swabs, hand swabs, or foreign objects can be used to confirm or refute a victim's or suspect's statement. Several methods have previously been proposed, but none have garnered widespread acceptance. Traditional methods such as histological staining face challenges due to similarities in the cellular makeup of vaginal, buccal, and skin epithelial cells. In recent years, Raman spectroscopy has gained much attention for its use in the identification of body fluids; however, vaginal material has to date been overlooked. Finally, microbial profiling of vaginal material has most recently been proposed as a potential molecular biomarker; however, this work is still very much in its infancy. Further investigation of both traditional and emerging techniques to identify vaginal material is absolutely necessary to address the challenges faced in the industry today.

Following informed consent, this study investigated three techniques: histological, spectroscopic, and molecular methods. Histological methods involved staining skin (n=10), buccal (n=10), and vaginal (n=10) epithelial smears with six histological stains: hematoxylin and eosin, crystal violet, Lugol's iodine stain, Csaba stain, Dane stain, and the Ayoub-Shklar stain. While some of these stains are not considered novel, the last three have recently been identified as having the potential to identify vaginal epithelium. The DXR Raman microscope was used to analyze the spectra for vaginal material, buccal cells, skin epithelial cells, venous blood, menstrual blood, saliva, and semen (n=10 for each). Molecular methods involved relatively quantifying the expression of *Lactobacillus gasseri* and *Lactobacillus crispatus*, two common vaginal bacterial species. Real-time Polymerase Chain Reaction (PCR) was used with TaqMan® Universal PCR MasterMix and a primer-probe pair designed for each bacterial species. Cycle threshold values were used to determine expression levels.

For the histology test, skin cells were easily distinguished from vaginal and buccal cells when stained using any method due to the contrast in morphology; however, the Dane stain was found to be superior for the differentiation of all three cell types, as each cell type resulted in a different color, thereby reducing subjectivity. Using Raman spectroscopy, averaged spectra for each body fluid were taken and significant peaks were noted and identified. The resulting peaks from the previously researched body fluids such as venous/menstrual blood, semen, and saliva, were as expected, while the resulting spectra from the vaginal, buccal, and skin epithelial cells showed variation in peak presence and intensity. Preliminary results obtained from the molecular investigation of the vaginal bacteria indicate higher expression levels in vaginal material when compared to other body fluids; however, further experimental investigation and data analysis is required.

This research has highlighted the value of traditional methods as well as the merit of emerging methods for the identification of vaginal material in forensic investigations. Each method has been shown to have potential for use in casework, albeit each with their own shortcomings, thereby reinforcing the need for continued research and validation in this field.

Vaginal Material, Body Fluids, Sexual Assault

B4 The Use of Proximity Ligation Assays Coupled With Real-Time Polymerase Chain Reaction (PCR) as a Detection Method for Proteins Within Blood for Forensic Analysis

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After attending this presentation, attendees will better understand how Proximity Ligation Real-Time PCR (PLiRT-PCR) can be utilized as a rapid confirmatory assay for the presence of blood in a biological sample.

This presentation will impact the forensic science community by demonstrating that PLiRT-PCR can be a very effective tool for throughput body fluid identification. Furthermore, the assay utilizes technology already present in forensic laboratories; thus, no new equipment is necessary. This research effort focuses on the identification of blood and is meant to complement, eventually in a single assay, PLiRT-PCR-based confirmatory assay for sperm/semen and saliva.

Blood evidence can be found at the scene of many different types of violent crime, including murder, rape/sexual assault, robbery, assault, domestic and stranger violence, and crimes involving injuries. Presumptive tests for the presence of blood performed at the crime scene are often followed by confirmatory tests in the laboratory, not only to confirm the sample is blood, but also that the blood is human. Presumptive tests are generally very sensitive but lack in specificity, while confirmatory tests, although very specific, are generally less sensitive. PLiRT-PCR possesses both characteristics.

PLiRT-PCR combines the specificity of an immunological reaction with the sensitivity of PCR and only requires a minimal amount of sample in order to be effective. A small cutting of the sample or small drop of diluted sample can be utilized for the assay, minimizing sample consumption for downstream processes (i.e., generating a DNA profile) should it prove to be human blood. An additional advantage of PLiRT-PCR is the potential for high throughput sample processing. In fact, this can be performed on a 96-well plate format and plate set up could potentially be performed by an automated liquid handling platform.

Samples are first cut from a swab or diluted from a sample of neat suspected human blood and lysed in a prepared lysis buffer to expose the proteins within the cells. Once lysed, the sample is exposed to specific antibodies that are targeting the proteins of interest. This binding reaction allows the antibodies to bind with the desired proteins for detection. The antibodies have different DNA segments attached to them to create proximity probes. After the binding step, the sample goes through a ligation period. Probes close to each other and with the complementary oligonucleotide strand will be hybridized. This new DNA strand is then amplified and can be measured by quantitative PCR (qPCR).

This presentation will demonstrate how PLiRT-PCR has the potential to be successfully implemented in the laboratory workflow of forensic laboratories for the identification of human blood. This presentation will reveal how sensitive and human specific PLiRT-PCR can be through the analysis of known samples provided by The George Washington University. This method can be used with samples that have a mixture of fluids or only the suspected blood.

Although there are other confirmatory tests for the presence of blood, PLiRT-PCR is a powerful alternative as it can be performed in as little as a few hours and with such sensitivity and specificity that it can replace other methods of detection. PLiRT-PCR consumes only a small amount of the original sample and can utilize many instruments already in use in forensic laboratories.

Blood, Proximity Ligation PCR, Hemoglobin

B5 The Effect of Washing and Blood-Enhancement Reagents on the Use of Raman Spectroscopy for Human Blood Identification

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After attending this presentation, attendees will gain insight into both the advantages and disadvantages of Raman spectroscopy in reference to its use in the identification of human bloodstains under a variety of conditions frequently encountered in a forensic setting. These conditions include blood present on a number of fabrics, varying dilutions of blood, and the effect of washing and enhancement reagents on the analysis.

This presentation will impact the forensic science community by disseminating results of novel research regarding the effect of washing and subsequent enhancement of bloodstains, which according to this study has not yet been reported. This research also highlights alternative approaches to the methodology and the importance of investigating the effect of variables.

In forensic investigations, determining the identity of an unknown biological stain can aid both in reconstruction and identification of an individual. Human blood is commonly found at crime scenes, which is first presumptively identified at the scene, then confirmed in a laboratory setting; however, many of the tests used, both presumptive and confirmatory, consume the sample in question, preventing further analysis, namely DNA profiling. Raman spectroscopy has been gaining interest as a new method of body fluid identification, partly due to its non-destructive nature. Prior research has demonstrated that Raman spectroscopy provides a unique spectrum for blood, while also preserving the sample for DNA analysis. The goal of this study was to further investigate the use of Raman spectroscopy for human blood identification in simulated crime scene samples, including bloodstains on a variety of fabrics, at varying dilutions, following washing, and finally post-enhancement.

After obtaining informed consent from volunteers, venous blood was collected in sterile vacutainer EDTA vials. Using a 780nm wavelength laser and a controlled laboratory setting, Raman spectroscopy was performed on samples of blood under various conditions. These conditions included five fabrics (black and white cotton, black and white polyester, and denim), a series of dilutions (1:10 to 1:10^6, both wet and dry), and after washing and treatment with three enhancement reagents (Leuco Crystal Violet (LCV), Coomassie blue, and luminol).

A method of extraction of the stain from the fabrics was also tested. Baseline corrections for fluorescence were performed as necessary.

The results obtained from the bloodstains on a variety of fabrics illustrated that by using spectral subtraction, a signal similar to blood could only be recovered from the white cotton and white polyester samples. The results obtained from the diluted bloodstains revealed that only the neat blood gave a signal while wet. When dried, the neat blood, as well as the 1:10 and 1:100 dilutions, gave a signal with peak shapes similar to the blood reference; however, the peaks became significantly less intense after each successive dilution. The results obtained from the bloodstains that were washed and subsequently enhanced revealed luminol to have no effect or interference on the ability to obtain a clear blood spectrum following spectral subtraction; however, LCV and Coomassie blue introduced interference, giving indeterminate results. The results obtained by utilizing an extraction method of the stain from the fabric revealed a spectrum with similar peak shape and location, but lower intensity, resulting in a weak match to a library reference for blood, regardless of the substrate from which the stain was extracted.

This study demonstrated the capabilities of Raman spectroscopy as a means of identifying human blood in a variety of situations common to forensic investigations. The impact of washing and blood enhancement reagents reveals the importance of the choice of method and its bearing on subsequent Raman analysis.

Raman Spectroscopy, Blood, Enhancement

B6 A Comparative Analysis of Commercially Available Protein and Peroxidase Reagents for Blood Detection and Enhancement on Laundered Clothing of Varying Fabric Types

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After attending this presentation, attendees will better understand the use of a variety of protein and peroxidase reagents that are commercially available to forensic professionals. Attendees will also gain insight into the advantages and disadvantages of these reagents, particularly when used with a variety of colored fabrics and following laundering.

This presentation will impact the forensic science community by providing a comprehensive comparative analysis of various blood detection reagents, their sensitivities on laundered fabrics, and their best use with particular fabric types, thereby providing a useful resource for forensic professionals.

Human blood is commonly encountered in forensic investigations, particularly on clothing following a violent incident. In certain circumstances, clothing may have been laundered prior to seizing to eliminate any traces of human blood. A number of methods and commercially available products have been introduced in recent years for the detection and enhancement of dilute bloodstains, including both protein- and peroxidase-based reagents. While each product has been researched individually in the literature, to date, these products have not yet been simultaneously and comparatively analyzed on a variety of laundered fabric types. The aim of this study was to investigate six commonly used protein and peroxidase reagents, which are commercially available from crime scene supply companies, and determine their sensitivities and most appropriate utilization on varying colored fabric types post-laundering.

Following informed consent from volunteers, venous blood was collected in sterile vacutainer EDTA vials. Five fabric types were selected: white cotton, black cotton, white polyester, black polyester, and blue denim. Three protein-based reagents and three peroxidase-based reagents were selected and purchased from Sirchie®: Hungarian red, Coomassie blue, amido black, luminol, Leuco Crustal Violet (LCV), and Bluestar® Forensic Magnum. One hundred microliters (µL) of human blood was deposited onto each fabric type in a range of seven dilutions from neat to 1:1 million. Each sample was performed in triplicate and photographed prior to laundering and enhancement. Following laundering, each sample was photographed and subsequently enhanced following the manufacturer's instructions provided with each of the six reagents. The results of each reagent, dilution, and fabric type were compared, using a scale from 0-4 (0 = no reaction; 4 = strong positive reaction).

The results of the post-laundering enhancement of neat blood and blood dilutions on the varying fabric types revealed the peroxidase-based reagents (luminol, LCV, and Bluestar® Forensic Magnum) to have the greatest sensitivities on the natural fabric types (white cotton, black cotton, and denim) as they all reacted positively on these fabrics down to 1:1,000; however, when the protein reagents were tested on the dilutions and varying fabric types, they revealed the greatest sensitivities (1:10) on the white polyester when compared to the peroxidase reagents, which only produced positive reactions on the laundered neat blood. As the protein-based reagents are color reactions and are not based on chemiluminescence, their use on dark fabrics revealed indeterminate results. The results of this study suggest peroxidase-based reagents to be the superior method for use on natural fabrics and all dark fabrics. Protein-based reagents have previously been shown to have merit for the enhancement of blood detail on non-absorbent surfaces, while in this study, their use, albeit inferior in most cases to peroxidase-based reagents, was shown on absorbent fabrics.

This study highlights the variety of commercially available blood detection and enhancement reagents offered and reveals their advantages and disadvantages in certain settings and on difficult types of evidence. The results of this research provide a much-needed comparative analysis of these reagents and could be used in the decision-making process for forensic investigators evaluating fabric evidence.

Blood Enhancement, Laundered, Fabric

B7 Rapid and Reliable Validation of Body Fluids Using Paper Microfluidic Device (µPAD) Chips

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After attending this presentation, attendees will understand the development of a new procedure for the determination of the presence of body fluids at crime scenes using µPADs. Paper microfluidics permit the simultaneous analysis of multiple sources of body fluids from a variety of substrates using multiplexed colorimetric detection. The result is a fast, simple, and reliable analytical tool for screening body fluids found at a crime scene.

This presentation will impact the forensic science community by demonstrating the practical application of µPADs in forensic serology. The design and developmental validation of the system will be discussed, including sensitivity, specificity, and long-term stability.

In recent years, there have been a number of new developments in the field of forensic serology; however, these methods involve lengthy laboratory testing (genomics, proteomics) and are not applicable to direct measurements at the crime scene. In contrast, field tests are simple, fast, and presumptive. Unfortunately, these analyses generally involve multiple testing procedures for each body fluid and are inherently destructive (with the exception of Raman and alternate light sources), wasting precious samples. With the advent of genetic testing, it becomes critical to determine all potential sources of human DNA at the scene to assist collection and sample analysis. These include blood, semen, saliva, vaginal fluids, urine, and sweat. To test each suspected body fluid with all these different procedures clearly would be uneconomical. Thus, a multiplexed presumptive body fluid screening procedure was proposed using paper microfluidics.

The µPADs utilize sheets of chromatographic paper and thermal wax to create hydrophilic channels that are bounded by hydrophobic barriers that direct a liquid sample to multiple test wells, each with a different sensor. In this project, various bodily fluids have been detected through the development of a µPAD chip. The device is designed like a tree. A single sample is placed at the base of the device and the sample flow is divided into several branches. A different colorimetric reagent is placed at the terminus end of each branch, each of which is capable of detecting a different body fluid. Currently, four different fluids can be simultaneously detected on a single device.

In this project, a variety of colorimetric sensing systems have been developed and modified for the presumptive determination of blood, urine, saliva, and semen. Reagents for detecting blood were prepared using sodium perborate as a longer-lasting oxidizing agent for the phenolphthalein-based Kastle-Meyer test. This compound becomes oxidized when sodium perborate comes in contact with water, generating hydrogen peroxide. A colorimetric change is produced in a minimum of ten seconds. To detect urine, a second test site utilizes the hydrolysis of urea via urease. The ammonia that is released interacts with the Nessler's reagent (mercuric iodide) and produces a color change in less than a minute. The reagents used for saliva detection utilize the amylase-based hydrolysis of the a-1,4 glyosidic linkages in starch/iodine, resulting in the loss of the initial colored complex. Reagents used for semen involve the reaction of sodium a- naphthyl phosphate with acid phosphatase. Upon analysis, the paper microfluidic chip permits the determination of sub-microliter volumes of samples.

Test results with mixtures and single-source samples demonstrate clear and distinct signals for the presence of each body fluid type. Further interference testing and validation is currently underway. Overall, this paper microfluidic presumptive testing method can serve as a cost-effective and field-able analytical method for screening unknown fluids found at crime scenes.

Colorimetric, Microfluidic, Serology

B8 The Detection of MicroRNAs (miRNAs) in DNA Extraction Methods Commonly Used for Forensic Casework

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After attending this presentation, attendees will understand how miRNAs function and why they can be of significant value in forensic analyses. Recent forensic literature has focused on molecular markers for forensic body fluid identification, including miRNAs, messenger RNAs, methylation, and proteomics. Each has been proposed as a supplement to or replacement of the current serological methods. The miRNA and RNA work for forensic body fluid identification thus far has been performed using RNA extraction of samples, which requires an additional step in the evidence analysis process. Not only is analyst time lost in the secondary extraction process, but it could also consume additional sample, which is problematic when the sample is of limited size. Although two reports have shown that miRNAs can potentially be detected within silica column-based DNA extracts, only one type of silica column isolation method was tested and other extraction methodologies have not been explored for miRNA co-isolation and detection. Attendees will understand the importance and limitations of miRNA detection using common forensic DNA extraction methods and be encouraged to promote the necessary research for implementing miRNAs into forensic casework.

This presentation will impact the forensic science community by illustrating how forensic research on miRNAs continues to build evidence for their utility in forensic casework.

MiRNAs are small non-coding RNAs that regulate gene expression by binding to messenger RNA in the cytosol to prevent further translation. Their short length of 18-22 nucleotides, cellular function, and resistance to degradation allow for easy detection in highly degraded samples, as is often the case in forensic casework samples. This study was conducted to explore detection of miRNAs in a variety of DNA extraction methods. Liquid donations of semen, blood, and saliva were collected from three individuals, and 100µL was aliquoted onto cotton swabs. Seven of the most common DNA extraction methods used by forensic laboratories were performed on all samples, with a total RNA isolation method for each sample as a control. A portion of all DNA and RNA extracts were DNase-treated to ensure that the data was a reflection of miRNA detection rather than genomic DNA contamination. MiRNA presence was evaluated using Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) analysis of miRNAs let-7g and let-7i because research has shown that they have relatively similar expression levels across multiple body fluids and between individuals. DNase treatment of extracts had little effect on miRNA expression levels between untreated and DNase-treated samples. Subsequent work revealed that multiple silica columns as well as other non-silica-based DNA isolation methods yielded detectable miRNA levels consistent with those found in the RNA extracts of the same samples. Based on these data, miRNAs are present at detectable levels in DNA extracts when using common DNA extraction methods. Co-extracting miRNAs with DNA would be beneficial for forensic investigations since it could provide probative information about an evidence sample without a separate RNA isolation, thus consuming less sample and reducing the amount of hands-on time for biological evidence analysis.

microRNAs, DNA Extraction, RT-qPCR

B9 Y-Screening and Direct Amplification of Casework Samples

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After attending this presentation, attendees will be able to evaluate and implement both a Y-screening protocol for processing sexual assault samples and a direct amplification process of casework samples in their laboratories.

This presentation will impact the forensic science community by streamlining and simplifying the processing of DNA casework samples.

Current serological screening of sexual assault samples is time consuming and limited in sensitivity, especially when compared to amplification-based methods for the detection of male DNA. Current quantitative Polymerase Chain Reaction (qPCR) and Short Tandem Repeat (STR) amplification chemistries display significant improvements over previous chemistries due to the robust performance in the presence of PCR inhibitors commonly found in casework samples. This makes it possible to perform direct amplification from such samples without the need for a DNA purification step. Elimination of this purification step reduces both cost and the potential for loss of DNA during purification, especially with low-level DNA samples, and it streamlines the laboratory workflow.

The Casework Direct Kit provides a method for the rapid generation of lysates from casework samples, which may be subsequently used in amplification-based assays, such as the PowerQuant® System, to quantify the abundance of human DNA, determine the male/female DNA ratio, predict PCR inhibition, and assess degradation of the DNA. If preferred, the lysate can be used directly in an STR amplification assay to generate an STR profile.

In the case of sexual assault samples, the lysates are used in the PowerQuant® System to screen for the presence of male DNA. Based on this information, the analyst can decide to either perform a differential extraction of the sample, take the lysate directly into an STR amplification reaction, or choose to stop processing the sample.

Unlike direct amplification from a punch, quantification results from the lysate can be used to normalize template DNA in downstream STR amplification reactions. Processing samples with the Casework Direct Kit facilitates the generation of high-quality laboratory results by directing workflow decisions and minimizing repeat assays and/or sampling. The method is fast (less than one hour) and requires minimal hands-on manipulation due to the use of new and improved spin baskets during the incubation step. Data will be presented from Y-screening and mock casework applications, including touch DNA samples, an example of the ability to detect the presence of male DNA, and a full PowerPlex® Y23 System profile in a 96-hour post-coital sample.

Y-Screening, Sexual Assault Samples, Direct Amplification

B10 Evaluating the Success of DNA Analysis and Latent Print Examinations on Submitted Firearms

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After attending this presentation, attendees will be informed regarding statistical data compiled from casework performed at the Iowa Division of Criminal Investigations Criminalistics Laboratory involving latent print examinations and DNA analysis of firearms evidence.

This presentation will impact the forensic science community by encouraging attendees to draw conclusions from the presented data that may alter or change methods and/or procedures regarding submitted firearms evidence at their laboratories across the country.

A request for multiple examinations on firearms evidence has become more and more common across the country. The development of latent prints has been a reliable method of human identification for decades, while touch DNA is a newer investigative tool that is employed in forensic casework. Latent print examinations date back to the early 20th century, while touch DNA dates back to 1999 in the United Kingdom and 2003 in the United States. Nationwide, laboratories have seen approximately a 10%-15% success rate with both latent print examinations and touch DNA analysis on firearms. Other studies have confirmed that latent print and DNA methods have even lower success rates on ammunition.

This presentation analyzes the types of cases that were submitted to the Iowa Division of Criminal Investigation Criminalistics Laboratory. Included are 104 closed cases from 2015 in which firearms evidence was submitted in addition to requests for analyses other than traditional firearms testing. First, cases were sorted into the type of crime that was committed (crimes against a person, weapon offenses, drug related crime, and property crime) and the type of request (latent print examination, DNA analysis, or both) beyond the firearms examination. Of the 104 cases, it was found that 58% were subjected to latent prints only, 26% to DNA only, and 16% had both examinations performed. Crimes against people constituted 45% of the total cases; property crimes had the least amount of case submissions.

One hundred pieces of firearms-related evidence were sent for latent print examination. The majority of the evidence was either a pistol or a magazine from a pistol. Fifty latent prints were developed, and 39 were considered suitable for identification on 24 firearms. This resulted in a 24% success rate. Blood was found on eight firearms and one bullet; the DNA analysis resulted in nine complete profiles suitable for identification, resulting in a 100% success rate for DNA analysis on blood-related firearms evidence. Sixty-seven swabs from 41 firearms and four ammunition types were analyzed for touch DNA. Of these 67 swabs, 11 profiles resulted in the identification of a person, providing a 16% success rate for touch DNA analysis. The most common outcome was a mixed DNA profile that was too weak to interpret. This result was seen nearly 50% of the time.

This research suggests that similar studies be performed over several years to determine if the rate of success is maintained and also to determine if the percentages are consistent in a larger data set. Studies like these are imperative since there is an increasing demand nationwide regarding turnaround time due to backlogs; therefore, laboratories need to be aware of and use the most efficient identification methods available.

Latent Prints, DNA, Firearms

B11 The Persistence of Ignitable Liquids on Laundered T-Shirts

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The goal of this presentation is to illustrate the patterns of ignitable liquids on clothing (a commonly collected item in suspected arson cases) that has been washed and dried.

This presentation will impact the forensic science community by presenting samples to demonstrate the importance of noting whether or not clothing has been washed and dried prior to analysis.

When attempting to set fire to a material that is relatively difficult to ignite, a perpetrator may turn to an ignitable liquid to accelerate the growth of the fire. These ignitable liquids may potentially be spilled onto the clothes of the person who is pouring it. It has been questioned whether or not these ignitable liquid residues could be washed off in the course of laundering, as would be the case if someone was trying to eliminate evidence of having committed arson. This study sought to determine if ignitable liquids could be detected on cotton, polyester, and nylon T-shirts after they have been cleaned in a conventional residential washing machine.

Different volumes ranging from 10mL to 100mL of a 1:1 mixture of gasoline and diesel fuel, a Heavy Petroleum Distillate (HPD), were added to one-half of a T-shirt. This T-shirt fragment was inserted into the washing machine with another T-shirt fragment without ignitable liquids to test how efficiently ignitable liquids would transfer from one shirt to another. One detergent was investigated to determine if detergent has an effect on the retention of ignitable liquids on the T-shirts, relative to simple water immersion and agitation. A subsection of the test shirts was dried in a dryer to evaluate the extent to which any ignitable liquids remaining after washing with detergent would evaporate during the drying process. The presence of ignitable liquids was determined using passive headspace extraction with activated charcoal strips and Gas Chromatography/Mass Spectrometry (GC/MS).

Gasoline and/or an HPD could be identified on all of the samples spiked with 10 mL of the Standard Accelerant Mixture (SAM) for all washing conditions (water only, detergent added, and detergent with subsequent drying). At the 1mL spike level, at least one of the ignitable liquids in the SAM was potentially identifiable for the different washing conditions, but the fabric type had an observable effect on which ignitable liquid was identified. At 100µL, there were some indications of ignitable liquids on the cotton and nylon fabrics; however, for the polyester samples, ignitable liquids could not be identified once detergent was used and were detected at an even lower abundance once drying was incorporated.

The different fabric types, use of detergent, and volume of ignitable liquid each had an observable impact on the final appearance and identifiability of the ignitable liquids. These variables also affected the extent to which the components of the ignitable liquids transferred to secondary pieces of fabric. The transfer to secondary pieces of fabric and the surprising retention of ignitable liquids through laundering has potentially important implications.

This research was funded by the University of California, Davis Forensic Science Program and the Bureau of Alcohol, Tobacco, Firearms, and Explosives.

Ignitable Liquids, Laundry, GC/MS

B12 Trace Chemical Analysis of Potassium Permanganate Hypergolic Mixtures Analyzed With Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES)

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After attending this presentation, attendees will understand the types of chemical variation among commercial sources of potassium permanganate that can be used to create incendiary mixtures. Attendees will also be introduced to the utility of trace element differences and their potential to determine the source of arson or explosive residues recovered as evidence.

This presentation will impact the forensic science community by presenting a new chemical signature system based on elemental profiles that may be used for the attribution of arson/explosive residues.

Hypergolic mixtures are self-igniting reactions that have been used for various types of criminal mischief as well as higher-profile terrorism-related incidents. They are a particular concern for law enforcement and the forensic science community as the ingredients are easily accessible and the reactions are simple to execute. Many current forensic techniques for analyzing hypergolic residues can identify the primary components, but provide little other information pertaining to their source. Few strategies exist for differentiating mixtures that have the same bulk chemical composition but are derived from different source ingredients (i.e., brands, commercial sources of the same product).

Therefore, the goal of this research was to characterize variations in trace element concentrations in the post-reaction residues of hypergolic reactions made with potassium permanganate and test whether different commercial sources of permanganate yielded distinct elemental profiles. Four different commercial sources of potassium permanganate were examined as well as three different mixing ratios of potassium permanganate with automotive brake fluid or glycerin as the fuel: 1:1; 2:1; and, 3:1 (grams:milliliter). Four replicate reactions were produced for each source-mixing ratio combination. The residues were then extracted using 2% trace metal-free nitric acid and analyzed using Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES) to determine the concentration of 24 different elements. The quantitation range was between 5ppb and 100ppm. Discriminant Function Analysis (DFA) was used to analyze the resulting chemical profile for each mixture sample and identify a subset of variables that optimized multivariate differentiation of each sample group. These included Na, Mg, Zn, Pb, Ca, Al, and Ba.

Results show that ICP/OES profiles of the two post-reaction residues from the analytical-grade permanganate sources could be differentiated by the concentrations of magnesium and calcium (~10 ± 0.1 ppb vs. ~6 ± 0.1 ppb and ~100 ± 2 ppb vs. ~50 ± 1 ppb, respectively). DFA of the trace metal profiles also exhibited clear separation between the two analytical-grade sources. Standardized function coefficients indicate that the elements that had the highest contribution to the differences were magnesium and calcium and, to a lesser extent, aluminum and sodium. The abundance of many elements decreased as the permanganate ratio increased. For example, the concentration of magnesium varied between ~14ppb ± 0.1ppb, ~8.6ppb ± 0.1ppb, and ~5.8ppb ± 0.1ppb for 1:1, 2:1, and 3:1 mixture samples, respectively. Furthermore, each mixing ratio group was resolved with two discriminant functions. This indicates that trace elemental profiles may be influenced by the source of oxidant as well as other individualizing aspects of the reaction, such as the mixing ratio. This research suggests that various brands of potassium permanganate can be differentiated by their trace elemental variations and that elemental profiles may be useful for characterizing the source of hypergolic mixture residues recovered during an investigation.

Hypergolic Mixtures, ICP/OES, Trace Metals

B13 Updates From the Drug Enforcement Administration National Forensic Laboratory Information System (NFLIS): Benzodiazepines Reported in the NFLIS — 2009-2015

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After attending this presentation, attendees will understand the breadth of information the NFLIS provides to the forensic science community.

This presentation will impact the forensic science community by providing specific knowledge of national and regional trends for selected benzodiazepines reported to the NFLIS between January 1, 2009, and December 31, 2015.

The NFLIS is a program of the Drug Enforcement Administration (DEA), Office of Diversion Control, that collects drug identification results from cases analyzed by federal, state, and local laboratories. The NFLIS participation rate, defined as the percentage of the national drug caseload represented by laboratories that have joined NFLIS, is currently more than 97%. Presently, the NFLIS includes 50 state systems and 101 local or municipal laboratories/laboratory systems, representing a total of 277 individual laboratories. The NFLIS database also includes federal data from DEA and United States Customs and Border Protection (CBP) laboratories. Results from the NFLIS are regularly used to support drug scheduling efforts and to aid drug initiatives, including the identification and tracking of emerging drugs of abuse.

National annual estimates for selected benzodiazepines, regional alprazolam, clonazepam, and diazepam trends, and selected benzodiazepines reported by federal laboratories are presented. This presentation will also highlight selected benzodiazepines of interest that are not approved for therapeutic use in the United States, counts of alprazolam reported with other drugs in the same item, and reports of clonazepam, phenazepam, and etizolam by state in 2009 and 2015.

Based on previously published NFLIS 2009-2014 data, a total of 369,738 benzodiazepine reports were identified by state and local laboratories. Alprazolam, clonazepam, and diazepam were the most commonly reported benzodiazepines during this period, with alprazolam accounting for 66% of all selected benzodiazepines. Phenazepam was first reported to the NFLIS in 2005 and had a total count of 302 reports between January 2009 and December 2014, and etizolam was first reported in 2012 and had a total count of 254 reports during that same period. Diclazepam was first reported in 2014 and by that year's end had a total count of two reports.

The NFLIS publicly shares data that can benefit management decisions of crime laboratories through various reports throughout the year, including special reports on such drug classes as presented in this presentation. The NFLIS provides a resource for the community to identify and respond to drug trends.

NFLIS, Benzodiazepines, Regional Trends

B14 Hand Odor Volatiles and Drug Abuse: A Pilot Study Using a Chemical-Dependent Target Group

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After attending this presentation, attendees will better understand the usefulness of employing hand odor volatiles as an alternate bio-specimen for monitoring drug use using a criminal justice population.

This presentation will impact the forensic science community by providing a proof-of-concept study that uses a novel bio-specimen, such as hand odor, which has proven to be a valuable forensic tool with human scent canines, by extracting further traits (i.e., drug use bio-markers) from a single odor sample. Odor sampling could become another favorable sampling technique for use in forensic laboratories due to its non-invasive character as well as its low adulteration possibility during routine treatment programs mandated by court systems.

Different types of drugs are commonly sampled and detected in urine, sweat, blood, hair, and other biological materials of the human body; however, little or no information has ever been available on the physiognomies of drug excretion and detection in human hand odor under any controlled or designed drug admission.

The main purpose of odor sampling in this study was to obtain a chemical representative of the odor source in order to monitor drug use by the subjects. This pilot study was designed to "chemically fingerprint" different hand odor samples, their concentration, variability of the drug metabolite excreted, and dose dependency by human subjects undergoing court-ordered drug treatment programs at the Lubbock County Community Corrections Facility/ Court Residential Treatment Center (CRTC). Human odor was collected using Solid Phase Micro-Extraction (SPME) -gas chromatography and kept for 24 hours to allow maximum headspace volatilization before analysis. Due to the organic origin by nature, human odor contains Volatile Organic Compounds (VOCs) that are generated by the body and excreted through superficial pathways, such as skin. The collection method was a passive, contact-surface source (i.e., the mass flow from the cotton gauze into the headspace through volatilization of odor to achieve equilibrium distribution.) Gas Chromatography/Mass Spectrometry (GC/MS) was the main technique used to analyze the odor as carrier gas aids in the transfer of odor from the headspace into the analyzer. There were various types of drugs excreted in odor depending on the individual cases observed for each subject in this study.

A total of five male individuals receiving drug abuse treatment at the CRTC were monitored on a bi-weekly basis to obtain the chemical odor profile as a function of rehabilitation time. Detailed histories and subjective reports of chemical dependency of the individuals' substance use patterns were gathered for comparison with collected samples. To further obtain a baseline as to the types and amounts of volatiles observed, the same number of non-drug users were sampled to evaluate a drug free hand odor profile. It was hypothesized that a small number of drugs or their metabolites would be detected from odor samples following drug administration and would constantly increase upon repeated administration or lengthy abuse periods. Hence, due to the cessation of drug administration and use by the subjects at the facility, this resulted in decreased excretion and detection of drug-related volatiles and/ or their metabolites in the excreted hand odor over time.

Odor excretion can become an important mechanism that can be used to monitor drug use in forensic settings, other than its extensive detection by canine use. The data from this pilot study could be implemented to shift the already established drug testing sampling methods. Thus, hand odor can serve as an important non-invasive, low-adulteration method to monitor drugs of abuse among individuals in criminal justice settings.

Hand Odor, Drug Abuse, SPME-GC/MS

B15 Forensic Drug Analysis by Thermal Desorption and Pyrolysis Combined With Direct Analysis in Real Time-Mass Spectrometry (TDP/DART®-MS)

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After attending this presentation, attendees will understand the value of a TDP device combined with DART®-MS for the rapid identification and screening of forensic drugs in biological and autopsy specimens (e.g., urine and blood).

This presentation will impact the forensic science community by explaining how DART®-MS can be effectively applied as an identification and screening technique for the forensic drugs present in biological and autopsy specimens.

Drugs present in biological and autopsy specimens cannot be detected without first selecting the pretreatment and analytical conditions appropriate for the drugs. Thus, it is extremely important to investigate the analytical conditions suitable for specific compounds and samples; however, recently several new substances that threaten society have appeared one after another, including New Psychoactive Substances (NPS). It is very difficult to individually examine the analytical conditions that are appropriate for each new substance. Thus, a comprehensive analysis system for drugs that requires minimal investigation of pretreatment and analytical conditions is greatly desired. This study investigates an analytical technique for directly analyzing drugs in blood and urine that does not require any pretreatment.

The samples were standard drug mixture solutions and drug mixture-loaded blood and urine (i.e., blank blood and urine samples with several types of drug mixture added). Mass spectra were obtained by using a quadropole Time-Of-Flight (qTOF) mass spectrometry equipped with a DART® ion source and a TDP unit. The TDP unit was mounted between the DART® ion source and the mass spectrometry. This study assessed whether drugs could be detected by using this analytical system. Mass spectra were measured in positive-ion mode as the samples were heated from ambient temperature to 600°C at a rate of 100°C per minute.

Each drug was separated and detected through thermal gradient heating for all samples, and thermal desorption profiles were highly reproducible for individual drugs. The detected ions were correctly identified according to their measured accurate mass and product ion spectra. Moreover, this analysis system was deemed to have the potential of quantitative analysis for drugs, as the drug's ion intensity was increased with increasing drugs concentrations in the samples; however, the drug's ion intensity was decreasing in the order of standard drugs solution > urine-added drugs > blood-added drugs, even at the same drug concentration, with the drug's ion intensity being markedly reduced in blood in particular. Accordingly, the blood samples that were pretreated by using dispersive solid-phase extraction for removing phospholipid were analyzed, whereupon the drug's ion intensity was dramatically improved. Future research should continue to investigate pretreatment conditions and mass spectrometry conditions in order to further improve the detection sensitivity, with eventual application in real samples.

Forensic Drugs, DART®-MS, Thermal Desorption

B16 Semi-Quantitation of Trace Drug Residues on Fabric by Direct Analysis in Real-Time (DART®)

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After attending this presentation, attendees will better understand the sample introduction methods currently available for DART® systems and the recent progress toward obtaining semi-quantitative results for drug residues on fabrics. Fundamental aspects of the DART® source ionization and signal optimization will be reviewed along with options for extraction of drug residue from a variety of fabrics. Several examples of applications will be provided, including the analysis of trace amounts of methamphetamine and cocaine.

This presentation will impact the forensic science community by providing a sound fundamental description of currently available methods for sample introduction into DART® systems. While DIP-It™ glass tips and QuickStrip™ cards provide rapid analysis options for some types of samples, additional options based on membranes and paper disks will be presented. The potential advantages these options offer for several types of samples, including drug residue on clothing, floor coverings, and upholstery, will be described.

The general approach is to add an internal standard solution to cloth and re-absorb the solution using an absorbent material in the shape of a disk. The amount of internal standard solution added to the fabric is matched to the diameter of the absorbent disk to maximize recovery of traces of drug that dissolve in this applied internal standard solution. Initial screening studies were performed with ink-jet printer paper, cellulosic membrane material and glass fiber membranes. Conditions were developed that resulted in reasonable recovery with all types of absorbent disks. After re-absorption, the disks were allowed to dry and were placed between the DART® source and the mass spectrometer entrance. For rapid analysis in the DART® instrument, the disks must allow interaction of the energized gas from the DART® source with the extracted trace drug residue and internal standard. So, prior to use, a portion of the disk was removed using a hole punch or a craft cutter to allow gas to flow through the membrane. Removal of 20%-40% of the disk had a minimal effect on the amount of drug and internal standard recovered, but greatly enhanced the magnitude of the DART® signal. Also, the patterned membrane disks gave signal intensities that were much greater than found with the commercially available sample introduction systems, namely the DIP-It™ glass tips, which are dipped into liquid solutions, and QuickStrip™ cards, which consist of a metal wire mesh.[1] Presumably, the surface area of the patterned membrane is much larger than the surface area of either the glass tip or wire mesh, allowing ionization of much more drug and internal standard.

Comparison of the peak heights of protonated-parent ions associated with several drugs of interest (i.e., methamphetamine and cocaine) with the peak height of the internal standard allowed quantitation of the amount of drug traces in cotton fabric at the five-microgram level. Studies were performed using a Simplified Voltage and Pressure (SVP) ion source interfaced to an LTQ XL™ linear ion trap mass spectrometer with data analysis using the Thermo Xcalibur™ software. Custom holders were constructed and placed inline between the DART® source outlet and the ceramic tube leading to the Vapur™ flange before the inlet to the mass spectrometer. Key operating parameters, including the DART® source temperature, were characterized over the range of 200°C-500°C.

In summary, the extraction of drug residues from fabric with patterned absorbent disks in addition to the use of an internal standard in the extraction solution is a promising approach for semi-quantitation of small amounts of drug residue in a variety of fabrics.

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1. Musselman, Brian D. Membrane for holding samples for use with surface ionization technology. U.S. Patent No. 8,481,922. 9 Jul. 2013.

DART®, Drug Analysis, Trace Analysis

B17 Microcrystalline Tests for Emerging Drugs of Abuse

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After attending this presentation, attendees will be able to describe the steps used to develop and validate new microcrystalline tests. Attendees will be informed of the development of a database of microcrystalline tests for emerging drugs.

This presentation will impact the forensic science community by providing a detailed study of microcrystals observed for an array of emerging drugs of abuse. This presentation discusses performance characteristics of the tests, such as precision and selectivity. The forensic science community will be able to assess the tests for practical applicability.

Forensic laboratories have used microcrystalline tests both as preliminary and confirmatory tests when presented with unknown substances. A microcrystal representative of the substance being studied results from the formation of a temporary complex between the precipitating reagent and the substance. The crystal shape, habit, and optical activity are often used as parameters to identify the substance. Tests for cocaine, heroin, and amphetamines are very well documented. The tests are simple and rapid, requiring minimal sample manipulation. Creating an ideal environment for reproducible crystal growth has its challenges. Factors such as pH, concentration, humidity, and interfering compounds can all play a role in determining the quality of crystal growth. When these factors are studied and described, microcrystalline tests can be very valuable as screening tests and, with expertise, serve as confirmatory tests.

Recently, numerous substances with psychoactive properties have emerged as drugs of abuse. Despite the advantages discussed for microcrystalline tests, they have not been studied extensively for these substances. A recent compendium of microcrystals for various drugs and pharmaceutical substances describes tests for piperazines and crystal growth in mixtures.[1] More such studies are needed to understand the behavior of other emerging drugs with traditional microcrystalline test reagents.

This presentation describes a systematic evaluation of microcrystalline tests for 32 emerging drugs of abuse categorized into the following classes: cathinones, phenethylamines, opioids, piperazines, and others. A previous presentation discussed the feasibility of the study and demonstrated crystal formation for some compounds.[2] This follow-up presentation is an expanded study and discusses the performance characteristics of the individual tests. Each substance was studied with seven reagents to determine tests that provide reliable crystal formation. Effects of pH, concentration, temperature, and interferences on the crystal habit are presented. Mixtures of closely related substances affect the crystal formation in a concentration-dependent manner. Understanding crystal deformations in mixtures of two or more compounds is useful when heterogeneous samples are presented. All of this information is used to create a database of microcrystals for several emerging drugs of abuse.

Reference(s):

1. McCrone Research Institute. *A Modern Compendium of Microcrystal Tests for Illicit-Drugs and Diverted Pharmaceuticals.* 2015.
2. Brady S, Joshi M. A Study of Microcrystal Tests for Emerging Psychoactive Substances. *Proceedings of the American Academy of Forensic Sciences*, 68th Annual Scientific Meeting, Las Vegas, NV. 2016.

Microcrystalline Tests, Psychoactive Substances, Emerging Drugs

B18 The Development of a Characterization Scheme for Emerging Synthetic Phenethylamines

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After attending this presentation, attendees will be familiar with the development of a characterization scheme that can be used to assign new synthetic phenethylamine analogs according to structural class. The scheme is based on mass spectral data and uses isotope patterns, neutral losses, and mass defects to define the structural class.

This presentation will impact the forensic science community by providing a tool that can be used to screen emerging analogs to determine the likely designer drug class. While this initial work focuses on synthetic phenethylamines, similar characterization schemes can be developed for other designer drug classes.

The identification of synthetic designer drugs by conventional Gas Chromatography/Mass Spectrometry (GC/MS) methods is challenging due to the high degree of structural similarity among members of a given class. For example, the 2C-phenethylamines consist of a core 2,5-dimethoxyamphetamine structure, but compounds within this series differ in the position and identity of substituents on the aromatic ring. Such modifications to the core structure create compounds that have similar psychoactive effects but may not be regulated under current legislation. As a result, new analogs rapidly appear on the market to circumvent current legislation and no reference standards are immediately available to aid in the identification.

The objective of this study was to develop a characterization scheme to assign emerging analogs to a designer drug class such that more resources can be focused on definitive identification, as necessary. This initial study uses synthetic phenethylamines as the model class to develop such a characterization scheme. A set of 2C-phenethylamines that included alkyl, halogen, and nitro substitutions on the aromatic ring was analyzed by Gas Chromatography/Time-Of-Flight/Mass Spectrometry (GC/TOF/MS). This high-resolution instrument uses electron ionization, insuring that the fragmentation patterns obtained are similar to those obtained by the more conventional GC/MS instruments currently used. Further, the exact mass of each molecular (when detected) and fragment ion is determined from which elemental formulas can be assigned with a high degree of confidence.

To begin developing a suitable characterization scheme, the mass spectral data for each 2C-phenethylamine were probed to identify common features. These features included isotope patterns, neutral losses, and mass defects of characteristic ions. Isotope patterns in the mass spectra were used to identify the presence of chlorine and bromine. Neutral losses from each molecular ion were determined and losses common to all 2C-phenethylamines in the training set were identified. These losses included CH_3N, C_2H_6N, and C_2H_6NO, which were measured as neutral losses of 29 Da, 44 Da, and 60 Da, respectively, from the molecular ion. Finally, the Kendrick mass defect of the fragment ion remaining after each neutral loss was calculated. For example, for the loss of $CH_3N\bullet$, the Kendrick mass defect filter based was defined as 85.96 mDa ± 0.7224 mDa, at the 95% confidence level.

The characterization scheme was then applied to a prediction set containing additional 2C-phenethylamines, other synthetic phenethylamines, and synthetic cathinones, which are structurally similar to the phenethylamines. Spectra for the test set were interrogated first for isotope patterns and, second, for the characteristic neutral losses previously identified. For those test set compounds that displayed the targeted neutral losses, the Kendrick mass defect of the remaining fragment ion was calculated and tested against the previously developed filter. Overall, the characterization scheme showed promise for initial screening of 2C-phenethylamines, despite some false positives and negatives.

This presentation will demonstrate the characterization scheme in the form of a flow-chart and further discuss the development and application of the scheme for the characterization of 2C-phenethylamines.

Synthetic Phenethylamine, High-Resolution MS, Mass Defect

B19 Portable Trace Vapor Sampling With Field-Capable Porous Layer Open Tubular (PLOT) -Cryoadsorption

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After attending this presentation, attendees will be able to apply a portable (briefcase-mounted) vapor sampling instrument based on PLOT-cryoadsorption to trace vapors of explosives, fuels, drugs, fuels, pollutants, and so forth.

This presentation will impact the forensic science community by enabling the use of a very rapid, yet very sensitive, dynamic trace vapor sampling method that facilitates comprehensive rapid vapor collection for qualitative identification and quantitative determinations of low uncertainty.

Building on the successful application in the laboratory of PLOT-cryoadsorption for collecting trace vapor samples, a robust portable instrument suitable for field conditions is presented.[1-4] The unit mounts in an aluminum briefcase, easily transported by vehicle or by air. The instrument functions entirely on compressed air, making it suitable for use in locations lacking electrical power, and for use in flammable and explosive environments. The three major functional aspects of the instrument will be discussed: (1) the field-capable PLOT-capillary platform; (2) the supporting equipment platform; and, (3) the interface with the necessary peripherals. The PLOT-capillary platform used in the portable unit differs from the lab unit in that it uses a high-flow multipath module that is highly robust. Vapor sampling can be conducted with either a wand-like hand piece (containing the PLOT module) for close sampling or with a special standoff module for more remote sampling. In both cases, the PLOT module can be heated and cooled to facilitate vapor collection and subsequent vapor sample removal. The interface (between the support platform and the sampling units) features a unique countercurrent approach to minimize thermal loss. Several types of PLOT-capillary elements and sampling probes are described, and applications to a variety of samples relevant to forensic analysis will be discussed.[5]

To demonstrate the capabilities of the portable unit, trace vapors were sampled from coumarin (a drug), the explosive 2,4,6-trinitrotoluene (solutes used in initial development of PLOT-cryo technology), naphthalene, aviation fuel, and diesel fuel on a variety of matrices and test beds. It was demonstrated that trace vapors from these analytes can be easily detected and reliably identified using the portable unit. By leveraging efficiency-boosting temperature control and the high-flow-rate multiple capillary wafer, very short collection times (three seconds) yielded accurate detection. For diesel fuel spiked on glass beads, a method detection limit below 1ppm was determined.

Current work in which vapor sampling inside of a large volume, closed chamber shows that quantitative performance is somewhat more uncertain than the lab version of PLOT-cryo (approximately 1%, mass/mass), but the portable unit nevertheless provides lower uncertainty than conventional purge and trap methods.

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1. Bruno T.J. Simple, quantitative headspace analysis by cryoadsorption on a short alumina PLOT column. *J. Chromatogr. Sci.*, 2009. 47: p. 5069-5074.
2. Lovestead, T.M., Bruno T.J. Trace Headspace Sampling for Quantitative Analysis of Explosives with Cryoadsorption on Short Alumina Porous Layer Open Tubular Columns. *Anal. Chem.*, 2010. 82: p. 5621-5627.
3. Bruno T.J. Field Portable Low Temperature Porous Layer Open Tubular Cryoadsorption Headspace Sampling and Analysis Part I: Instrumentation. *J. Chromatogr.* A, 2016. 1429: p. 63 – 71.
4. Bruno T.J. *Sampling system and process for sampling.* Patent Applied For, Patent Application No 15-014,0673. 2015, Secretary, United States Department of Commerce.
5. Harries M.E., Bukovsky-Reyes S., Bruno T.J. Field portable low temperature porous layer open tubular cryoadsorption headspace sampling and analysis Part II: applications. *J. Chromatog.* A, 2016. 1429: p. 72 – 78.

Headspace Analysis, PLOT-Cryoadsorption, Trace Vapor Sampling

B20 An Examination of Hypergolic Mixtures Involving Potassium Permanganate and Select Fuels

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After attending this presentation, attendees will better understand what types of chemicals can be used to create hypergolic mixtures, the types of evidence that a hypergolic reaction may leave at a crime scene, and how changing a single variable, such as the type of fuel added to the mixture, can change both the reaction and the post-combustion material (residue).

This presentation will impact the forensic science community by providing a framework for the examination and analysis of specific hypergolic mixtures, the threat they present to the public, and possible avenues of analysis to obtain as much relevant information as possible from hypergolic residues.

Bombings due to criminal and terrorist activities are in the news on a weekly or daily basis. The heightened alertness of law enforcement and security personnel does not seem adequate to prevent the destructive actions of terrorists when explosive materials are so easily obtained. Hypergolic mixtures are chemicals useful for retail or commercial purposes but, when mixed, create a type of oxidation-reduction reaction that results in spontaneous combustion, without the need for external ignition. Hypergolic mixtures can be created with a number of different oxidant and fuel combinations. Based on a literature search, little research has been published exploring the time to reaction, temperature, and the variety of commercial products involved in initiating these reactions. These mixtures are very simple to construct and are made with materials that are readily available for purchase, such as pool chlorine, pool shock, water softening chemicals, and fuels, such as brake fluid or antifreeze.

Typically, a delay in the combustion reaction occurs after the reactants are mixed. This delay can allow a person to leave the area before the reaction occurs. After combustion, the post-burn products may include the remnants of the containment vessel and the post-burn hypergolic products. Original ingredients may also be present.

This study involves the creation and examination of the hypergolic mixture of potassium permanganate (a strong oxidizer) and selected fuels, including polyethylene glycol, ethylene glycol, and glycerin. Potassium permanganate can be found in select water softening chemicals. Polyethylene glycol has several uses in cosmetics, pharmaceuticals, commercial products, and industry. Ethylene glycol can be used as antifreeze and in the fiber industry in the manufacture of polyester fibers. Glycerin has uses in the food, pharmaceutical, and chemical industries.

Hypergolic mixtures were created and recovered on a small scale (approximately 0.5g of powdered potassium permanganate and approximately 0.25mL of a chosen fuel) in open Pyrex® glass petri dishes (100mm x 15mm) in a fume hood (with a flow of at least 100fpm) to explore the reactions and analyze the residues. After a brief period, typically less than one minute, a small flame with white smoke occurred that moved across the mixture. After the reaction, the resulting products were examined visually, with the aid of a stereo light microscope, with a diamond-attenuated total reflectance Fourier Transform Infrared (FTIR) spectrometer and by a Gas Chromatograph with a Mass Spectrometer (GC/MS) detector.

The small-scale reactions were varied to determine optimal ratios. The resultant ratio of 2:1 weight/volume (g/ml) of potassium permanganate to liquid fuel consistently produced combustion. Still images and video recordings captured the reactions. The video recordings showed differences in both the speed and duration of combustion.

Preliminarily, the results of hypergolic mixture residues analyzed by FTIR, which has been used as a discriminatory technique for explosive residues on other types of explosives, may not have the discriminating power necessary to identify post-combustion products to determine original reactants. The GC/MS data will also be presented.

Hypergolic, Potassium Permanganate, Alkyl Glycols

B21 A Comparison of Headspace Cannabinoid Profiles Detected From Different Structures of Dried Cannabis Inflorescences

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After attending this presentation, attendees will better understand the variations in cannabinoid profiles detected from different structures of dried cannabis inflorescences as well as the effect of homogenizing the plant matter on intra-sample variation.

This presentation will impact the forensic science community by providing evidence that homogenization of plant material samples for marijuana yields profile results with less variation when generating headspace cannabinoid profiles.

Marijuana is one of the most common drugs analyzed in a forensic laboratory. When analyzing marijuana, which may be composed of dried plant materials of *Cannabis sativa*, there are several structures of the plant that can be sampled for chemical analysis. The leaves, stems, and buds are the main structures present in the majority of both legal and illegal marijuana samples; however, not all of these dried cannabis plant materials have the same concentration of cannabinoids, such as Δ9-tetrahydrocannabinol (Δ9-THC), Cannabinol (CBN), and Cannabidiol (CBD). Marijuana in its natural state is not a homogenous sample. Sampling from different plant structures within the same marijuana sample may lead to various cannabinoid profiles for that sample. A solution to reduce intra-sample variation is to make the plant material more homogenous by grinding the sample.

In this research, reference marijuana samples (N=14) obtained from the National Institute of Drug Abuse (NIDA) with known concentrations of Δ9-THC and CBD were tested. Headspace cannabinoids of marijuana were extracted by Heated Headspace/Solid-Phase Microextraction (HHS-SPME) and headspace cannabinoid profiles were then obtained by using Gas Chromatography/Mass Spectrometry (GC/MS). Each reference marijuana sample was divided into two groups. Group A was analyzed by HHS/SPME-GC/MS without any sample preparation. Group B samples were homogenized using an herb grinder before HHS/SPME-GC/MS. Using a stereomicroscope, samples in group A were first divided into sub groups of stems and leaves. Each of these structures was documented and verified. Plant materials containing only one type of structure collected from different structures of cannabis inflorescences were weighted (10mg) and transferred to the headspace vials for HHS/SPME-GC/MS. The peak area of major cannabinoids, Δ9-THC, CBD, and CBN, were recorded for comparison. Finally, each sample in group A was also run as a mixture of structures (leaves, buds, and stems) and the cannabinoid profiles of each were compared to the profiles of group B.

The results of the experiment illustrate that samples collected from different plant structures in the same batch of dried cannabis inflorescences exhibited variations of headspace cannabinoid profiles. The whole sample containing both stems, leaves, and other structures presented a higher level of intra-sample variation in Δ9-THC peak areas compared to the samples containing only one type of plant structure. The ground sample registered the lowest level of intra-sample variation in Δ9-THC peak areas when compared to the single-structure samples and the multiple-structure samples. In order to obtain more consistent headspace cannabinoid profiles for marijuana by the HHS/SPME-GC/MS approach, the selection of single structures of cannabis inflorescences from marijuana samples will be discussed in this presentation.

Marijuana, Homogenization, Headspace SPME

B22 Validation of Various Fingerprint Processes in a Medium-Size Municipal Laboratory

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After attending this presentation, attendees will better understand the processes for validating fingerprint methods for a medium-sized municipal laboratory while trying to complete the quality requirements necessary to achieve the International Organization for Standardization (ISO) accreditation.

This presentation will impact the forensic science community by providing a benefit for the Huntington Police Department Forensic Investigations Unit (HPD FIU) that will lay the foundation for the laboratory's workflow and the product it generates.

According to the 2009 National Academy of Sciences Report, Recommendation 7, laboratory accreditation should be mandatory and follow the standards published by the ISO. There are many different types of methods utilized to visualize latent prints, and they all need to be tested in order to see which is best suited for the HPD FIU.

This project includes non-porous, porous, adhesive, and blood processes, with many different methods for each process. Each process must be used in the proper sequence of a series of development techniques or risk the possibility of destroying certain matrix components that react with subsequent processes. If one method is performed out of order, the fingerprints could be underdeveloped or even destroyed. The HPD FIU has to be very careful in order to preserve a possible fingerprint and allow it to be detected. Validating these methods allows the HPD FIU to know if the method works for its intended use or if there is another that is better suited.

Validations of fingerprinting processes and methods will allow this medium-sized municipal laboratory to become accredited through ISO 17020 standards. Five data sets were generated for each method and results were evaluated based on the method's ability to be visualized. A result of a positive (able to see ridge detail) or a negative was noted for each to ascertain if any ridge detail was seen. To offer proof that these tests function accordingly or produce inadequate results, pictures or possible prints were kept in order to be accredited through the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB) by the ISO 17020 for crime scene response.

Three validations were to be produced for each method, but unforeseen circumstances developed. Unfortunately, the unit lost some employees, resulting in a problem getting tests validated twice more in a timely fashion, due to casework. Once the two other validations are completed, those results will create a new decision as to whether or not the methods are valid to use in this laboratory.

Additionally, new methods were tested and validated for implementation in the workflow of the medium municipal laboratory. Some of the new methods, such as 1,2-indanedione and acid fuchsin, are now validated for utilization in the laboratory. The saying, "You always get the good with the bad," came into play here, as there were four methods that were found to be no longer useful for this laboratory, which will save the laboratory money. These methods include Coomassie Blue, 5-MTN, ThermaNin, and Oil Red O. D.F.O did not work well either, but future use is being evaluated by the laboratory.

Nearly all of the fingerprint methods produced results that were expected. The few that did not meet expectations were D.F.O., 5-MTN, Oil Red O, and ThermaNin; however, 5-MTN, Oil Red O, and ThermaNin were new products to this laboratory and did not produce the results the products' manufacturers stated they would. The validation of these methods helped the HPD FIU in the accreditation process as well as discovering which methods produce better results.

These and other details of the study will be discussed.

Fingerprints, Validation, Accreditation

B23 An Appropriate and Practical Sample Preparation Procedure for Clean-Up and Analysis of Explosives Residues

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After attending this presentation, attendees will better understand an appropriate and practical Solid-Phase Extraction (SPE) procedure for the analysis of explosives residues.

This presentation will impact the forensic science community by establishing streamlined clean-up procedures for the recovery of explosives residues.

The detection of trace explosive residues from post-blast scenes has always been challenging in the forensic field, due to possible high matrix interference from the background of the scene, trace amounts of explosive residues left after the blast, and the wide range of possible compounds used by the perpetrator. Thus, there is a compelling need to develop an efficient extraction procedure for clean-up and analysis of samples.

An optimized single-step SPE procedure was reported by Nopporn Song-im et. al. involving four target organic explosives and two inorganic anions.[1] In this study, an SPE method will be utilized for sample clean-up and analysis of a wide range of analytes. A suitable solvent system and SPE adsorbent will be determined to effectively retain the target organic explosive analytes while allowing inorganic explosive analytes to be eluted. The target organic explosive analytes will then be eluted in subsequent steps. Other parameters of the SPE method will also be optimized. Liquid Chromatography coupled to high-resolution accurate mass Orbitrap mass spectrometer (LC-Orbitrap) and Gas Chromatography coupled to a Mass Spectrometer (GC/MS) will be used for the analysis of organic explosive analytes while Ion Chromatography (IC) will be used for the analysis of inorganic analytes. This SPE sample preparation method will provide efficient clean-up of the samples. The resultant effluents are compatible with multiple types of instrumental analysis for a wide range of explosive analytes.

Reference(s):

1. Nopporn Song-im et. al, Establishing a universal swabbing and clean-up protocol for the combined recovery of organic and inorganic explosive residues. *Forensic Science International*. 223 (2012) 136–147

Explosives, Extraction, SPE

B24 Seeing Through Noise: Investigating Information Sampling and Weighting in Fingerprint Recognition

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After attending this presentation, attendees will better understand the visual processes at the root of fingerprint examination and comparison. Understanding the perceptual processes that underlie pattern recognition in fingerprint examination can lead to improvements in recruitment, training, and examination guidelines, as well as error mitigation.

This presentation will impact the forensic science community by improving understanding of the perceptual processes that underlie pattern recognition in fingerprint examination.

The present research seeks to model and compare the manner in which fingerprint examiners and naïve observers sample and weight information across different fingerprint regions. This research also investigated whether any differences in information sampling and weighting are due to: (1) differences in the information available in the print; (3) learned differences in expertise; or, (3) general perceptual biases.

Artificially generated fingerprints were presented as experimental stimuli to control for low-level cues of identity (e.g., luminance, contrast, and/or size information). Fingerprint stimuli consisted of a central core and a peripheral region. Each region could be independently manipulated, meaning that the core could be drawn from a different fingerprint identity than the periphery. The boundary of the regions was masked to avoid completion cues. Additionally, Gaussian noise masks were used to simulate the loss of detail typically encountered in latent prints. In a 2-Alternative Forced-Choice (2AFC) recognition task, a target and probe display were presented serially, for one second each, with a one-second inter-stimulus interval. To examine information sampling across the core and periphery regions, the relationship between the target and distractor was manipulated using five conditions: (1) only the core region is visible (core-only); (2) only the peripheral region is visible (periphery-only); (3) only the core region changes (core-change); (4) only the peripheral region changes (periphery-change); and, (5) both regions change (both-change). Additionally, three core-to-periphery size ratios were used to assess information weighting across the fingerprint regions. Participants, consisting of naïve observers as well as fingerprint examiners at different levels of experience, indicated their recognition response (match/no match) and response confidence (six-point scale) after every trial.

Initial results were analyzed using Signal Detection Theory, and models describing the weighting and integration of information from the core and peripheral regions will be presented. Additionally, the potential impact of these findings on fingerprint training, recruitment, and examination guidelines will be discussed.

Fingerprint Examination, Visual Processing, Information Sampling

B25 Gold Nanoparticles (AuNPs)/Aptamer-Based Paper Microfluidic Devices Developed for the Detection of Cocaine

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The goal of this presentation is to describe the development of AuNp/aptamer-based paper microfluidic devices for the presumptive determination of cocaine in acid and base form. Information provided will include the optimized design of the paper chip and the validation of this device.

This presentation will impact the forensic science community by demonstrating the application of this newly designed paper microfluidic device in the presumptive detection of cocaine. This new method is rapid, inexpensive, and specific toward the cocaine molecule.

Over the past ten years, a number of specifically targeted aptamers have been developed to identify illicit drugs. The most common detection methods involve Ultraviolet/Visible (UV/Vis) spectroscopy or gold nanoparticles. These tests were developed as rapid solution-based tests, similar to immunoassays; however, these tests are less convenient for field use. Many of these kits require infrastructure to keep and store the reagents because they can be sensitive to temperature, light, and age. An alternative platform for AuNPs/aptamer detection based on paper microfluidic devices has been investigated. A new chip design has been created that adapts the AuNPs and aptamers to a ready-to-use format. In the field, samples are dissolved in a carrier solvent in vials and then applied to the paper just before analysis. The devices can be used at crime scenes, in laboratories, and at any other locations where the suspected powders may occur. The paper chips are easy to prepare and inexpensive to operate. Furthermore, they can be conveniently stored for later uses.

The paper microfluidic devices are prepared using a wax-ink printer, thermal laminator, chromatography paper, aptamers, and gold nanoparticles. The wax-ink printer and a thermal laminator produce hydrophilic channels defined by melted wax on the paper. Next, gold nanoparticles and aptamers are prepared for the channel. Cocaine samples in acid/basic form are dissolved in solutions, then transferred to the chips. Cocaine next travels down the channel via capillary action, interacting with the aptimers and causing a color change to occur due to the aggregation of the nanoparticles. When cocaine is not present, the nanoparticles cannot aggregate and no color change occurs as aptimers are then free to bind gold. The entire process takes approximately five minutes. The applied aptamer is specific for cocaine. This presentation will also report a preliminary validation of this device, including tests for sensitivity, specificity, and stability against a variety of potential interferences.

The use of paper microfluidic devices permits the development of rapid, inexpensive, and easily operated tests for cocaine samples. These devices present a safe and convenient presumptive tool that can be used in the field.

AuNPs/Aptamer, Cocaine, Paper Microfluidic Device

B26 The Analysis of 2,5-Dimethoxy-N-(N-methoxybenzyl)phenethylamine (NBOMe) Isomers Using Traditional and Fast Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will better understand NBOMes and their analysis via traditional and fast GC/MS. The concepts of retention time, retention index, relative ion abundance, and characteristic ion ratios will be discussed, as well as an assessment of the relative contribution of various factors on the relative ion abundances of the different isomers.

This presentation will impact the forensic science community by providing attendees with characteristic ion ratios for the ortho, meta, and para isomers of 25C- and 25I-NBOMes. Additionally, the effect of tune file on each analysis as well as the assessment of the variability of relative ion abundances through repeated analyses will assist attendees through a more comprehensive understanding of their casework. Finally, the conserved nature of the characteristic ion ratios between traditional and fast GC will further validate the use of fast GC as a means of seized drug analysis.

It is hypothesized that each NBOMe isomer has a unique retention time (and therefore retention index) and Electron Ionization (EI) fragmentation pattern that can be used to successfully identify each isomer from a series of possibilities. Additionally, it is proposed that although the tune profile will have an impact on the data, it will not be statistically significant in practice. Finally, it is hypothesized that the developed characteristic ion ratios will be conserved between both traditional and fast GC/MS.

In this project, the analysis of NBOMe isomers was conducted using both traditional and fast GC/MS. NBOMes are synthetic phenethylamine derivatives that have become increasingly popular throughout the world. Due to their hallucinogenic potency being derived from their structure, the ability to differentiate NBOMe isomers is becoming increasingly important.

For the purpose of this research an Agilent GC/MS was used for both the traditional and fast analyses. The traditional analyses were performed with a 30m HP-5 column in less than 30 minutes, while the fast GC analyses were performed with a 10m VF-5MS column in less than seven minutes. Standards of the ortho, meta, and para isomers of both 25C- and 25I-NBOMe were obtained from the Drug Enforcement Administration (DEA). The retention times, retention indices, relative ion abundances, and characteristic ion ratios were determined using both traditional and fast GC/MS. Furthermore, the variability of the relative ion abundances was assessed through repeated analyses of each NBOMe isomer standard. Finally, an evaluation of the effect of the tune profile on each analysis was performed.

One-way Analysis of Variance (ANOVA) was performed to evaluate the significance of different factors on the within-factor to between-factor variance. The factors that were assessed include positional isomers, day of analysis, week of analysis, tune profile, and speed of GC. The variables that were used to assess these factors include the retention time, retention index, absolute peak areas, relative ion abundances, and characteristic ion ratios.

It was determined that each NBOMe isomer had a different statistically significant (α=0.05) retention time, retention index, and characteristic ion ratios. Additionally, the variability of relative abundances through repeated analyses of each NBOMe isomer standard was captured. Finally, it was determined that the tune profile does not have a statistically significant effect on the variables assessed

Seized Drugs, Fast GC, EI Fragmentation

B27 Profiling the Use of Explosives in Automated Teller Machine (ATM) Robberies in Brazil

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The goal of this presentation is to describe the first attempt to create a database of the use of explosives in ATM robberies in Brazil. This presentation endeavors to shed light on possible solutions to the epidemic use of explosives in ATM robberies.

This presentation will impact the forensic science community by presenting actual case evidence of the use of explosives in Brazil and by introducing the first relevant data on which types of explosives are commonly used, in addition to exploring regional tendencies of different explosives.

The Explosives and Post-Explosives Section of the Central Laboratory of the Brazilian Federal Police has routinely analyzed samples collected at post-blast scenes of ATM robberies in Brazil and has used the data available to try to create a more concrete picture of which explosives are more commonly used, where they are used, and the *modus operandi* of these criminals.

Since 2007, Brazil has experienced an exponential growth of the use of explosives to breach ATM cash machines. Although statistics are unreliable as there is no unified bomb data center, some states have reported an increase of more than 200% in the number of attacks since 2010. According to the Brazilian Bank Federation (FEBRABAN), in the three-year span from 2009 to 2012, approximately 2,500 Brazilian ATM's were targeted with explosives, an average of 833 per year. As a result of this increase, banks have adopted various measures to rectify this situation, such as stronger vaults, spray painting the bank notes in case of an explosion, and even an agreement with the army to provide a convoy to protect the transport of explosives (it is estimated that 1,000kg of explosives are stolen every year from mining companies). Unfortunately, none of the proposed solutions have generated positive results.

In the past three years, the Explosives and Post-Explosives Section of the Central Laboratory of the Brazilian Federal Police has analyzed 49 cases of post-blast events related to ATM robberies. In 2016 alone, 14 reports were written and another 11 cases are pending analysis. The first glaring problem is that such a number represents only about 0.5 percent of post-blast cases in Brazil during the same period, so it is clear that most cases either were not sent to a forensic laboratory for determination of the explosive or, even worse, were not processed at all.

The analyses were performed using a Dionex™ ICS-5000 ion chromatographer for inorganic residues, an AB Sciex API 3200™ system coupled with an APCI probe for organic explosives, and an energy-dispersive X-ray spectroscopy with an FEI Quanta 200 3D to detect metals, primarily aluminum, in the composition of the explosives.

In 61% of the cases (30 events), the thieves used chlorate/perchlorate-based explosives as the main charge, whereas emulsions, mainly Ammoniun Nitrate Fuel Oil (ANFO), was used in 25% of the cases (12 events). Only twice were organic explosives detected. In one case, a Triacetone Triperoxide (TATP) booster was recovered unexploded and, in another case, a small sample of a Pentaerythritol Tetranitrate (PETN) cord was recovered from the crime scene. These results are preliminary and do not represent an accurate portrayal of the problem, since nearly half of the cases (47%) were sent from only one state, Minas Gerais. Hopefully, in time there will be sufficient data from other states to be able to provide more accurate statistics.

The Explosives and Post-Blast Section has implemented technical training for forensic experts to increase the number of ATM explosions that are adequately processed in Brazil. Some patterns are beginning to emerge and, hopefully, with more information, a realistic database will be created to help investigators better understand the *modus operandi* of these criminals.

Explosives, ATM Robbery, Chemical Analysis

B28 The Development and Preliminary Validation of an App for Recording and Interpreting Colorimetric Drug Test Results

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After attending this presentation, attendees will be aware of the availability and capabilities of a new smartphone application to aid in recording and interpreting colorimetric drug test results.

This presentation will impact the forensic science community by demonstrating the ability of an app for Apple® and Android™ devices and software that runs on a Personal Computer (PC) and a Raspberry Pi that aids in the interpretation of colorimetric drug test results and also standardizes and secures testing records.

Millions of suspected drug-related crimes are reported annually in the United States. Colorimetric drug tests are used to aid in the identification of the substances, based upon their reactivity and the colors rendered with the test reagents.

A database of Red, Green, and Blue (RGB) three-point numerical colors for over 3000 colorimetric test-drug combinations has been recorded and uploaded to the app. Assigning numerical RGB values to the colors standardizes the color record and enables the use of an algorithm to search for the color in the database. The application searches the database and matches the results using a Euclidian distance algorithm or another algorithm designated by the examiner. The display lists the results. The suggested matches based upon the closest set of RGB values in the database are listed in rank order with accuracy of prediction for evaluation by a technical expert. The crime laboratory or another examiner can be consulted about the results in real-time through the secure server.

This study investigated colorimetric drug test results for 800 unique drug/test combinations. Three versions of the software were on a PC and a Raspberry Pi. The software has been tested on Samsung Android™ and Apple® iPhone® devices, a Dell™ PC and a Raspberry Pi B+. The Raspberry Pi, purchased from the Raspberry Pi Foundation, runs a Linux-based operating system, "Raspbian." The RGB value is determined using the ColorAssist app with the device camera light or using a mini spectrometer connected via the USB port. ColorAssist was demonstrated to outperform other similar apps for this purpose in a recent study. The software is a technical solution to limitations of visualizing color at crime scenes and due to constraints of human vision differences, training, and experience of the examiner. The apps also allow the user to view previous test results by date and time and to add case notes. Interpretation of controlled substances and drug standards via tests performed on different replicates, different days, by different examiners, and with different devices will be discussed. The functionality of the apps and results of a preliminary validation of seized substances using the software will be presented.

Smartphone App, Apple® iOS®, Android™

B29 Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Detection of Cocaine in Packaging Residue

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After attending this presentation, attendees will understand the potential for LC/MS/MS to be used as a fast and accurate method to detect the presence of cocaine from very small amounts of adulterated powder residue.

This presentation will impact the forensic science community by illustrating the ability of LC/MS/MS to be used in forensic investigations as a fast and reliable method to analyze powder samples that may contain illicit drugs, such as cocaine.

In modern forensics investigations, common evidence of drug possession comes in the form of powder residue from packaging materials. Previous research has shown LC/MS/MS can be used to detect and quantify trace amounts of methamphetamine from packaging residue. This study proposes that Liquid Chromatography coupled with TOF Mass Spectrometry can also be used to detect minute amounts of cocaine within powder residue. Common drug packaging materials, such as zip-lock bags, may only have very small amounts of residue on them, and the drug itself may be impure. Powdered street drugs like cocaine are almost invariably mixed with adulterants, which reduce the amount of drug present in the powder residue and could make drug detection more difficult. The primary objective of this study was to determine lower Limits of Detection (LOD) and lower Limits of Quantitation (LOQ) for cocaine from bag residue when cut with different adulterants.

In order to test the efficacy of this method, cocaine hydrochloride powder was cut with nine commonly used adulterants, creating samples with cocaine amounts ranging from 0.1% to 4% by mass. One gram of each sample was loaded into a 1in-square zip-lock bag, then emptied, leaving behind approximately 10mg of residue. The bags were rinsed out with 1mL 18MΩ nanopure water, which was then diluted 100-fold and mixed with an internal standard of 10ppm D3-cocaine. Each sample was run using an Agilent® 1100 series binary pump liquid chromatograph coupled to a Bruker® Compact quadropole Time-Of-Flight (qTOF) mass spectrometer. Analysis was performed using Bruker® QuantAnalysis 2.2, and results were compiled in Microsoft® Excel®.

To unambiguously confirm the presence of cocaine, MS/MS was used to obtain fragmentation mass spectra of all ions with the *m/z* of cocaine and D3-cocaine, which were matched with literature fragmentation mass spectra of cocaine and D3-cocaine. LOD and LOQ were obtained for each of the nine adulterants tested. Calibration curves were created for each adulterant by correlating the cocaine: internal standard ratios with the percentage of cocaine in the samples. LOD ranged from 0.11% to 0.65% by mass, while LOQ ranged from 0.37% to 2.16% by mass.

This study explored the ability of LC/MS/MS to detect cocaine from packaging residue. Cocaine could be detected down to a purity as low as 0.11% by mass, from residue amounts averaging 10mg. Most real-world cocaine samples contain a far purer drug than the obtained LOD values, qualifying this method as a sufficiently sensitive cocaine detection tool. Adulterant properties such as water solubility and affinity for the inside of the bags appeared to influence the sensitivity of this method, but all LOD values were below 1%.

Trace Drug Detection, Cocaine, LC/MS/MS

B30 Direct Thermal Desorption Coupled to Gas Chromatography/Mass Spectrometry (GC/MS) for the Characterization of Organic Firearm Discharge Residues

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After attending this presentation, attendees will understand how thermal desorption may be used to characterize organic Gunshot Residue (GSR).

This presentation will impact the forensic science community by providing an overview and method validation data for a new way of characterizing organic GSR using GC/MS instrumentation.

The development of powerful new hybrid mass spectrometry systems has led to renewed interest in targeting the organic constituents of Firearms Discharge Residue (FDR). Several methods have been published in the last decade reporting on the analysis of organics found in FDR using Liquid Chromatography/Mass Spectrometry (LC/MS). The mass spectrometers used include Time-Of-Flight (TOF), quadrupole Time-Of-Flight (qTOF), and Triple quadrupole (QqQ) designs. For LC/MS analysis, a substrate such as a hand swab is typically extracted, concentrated, and introduced into the chromatographic system and analytes such as Diphenylamine (DPA), ethyl and methyl centralites, and dinitrotoluenes and other diphenylamines are detected. Forensic laboratories are adopting advanced LC/MS instrumentation, but in most cases these instruments are devoted to toxicological assays, which presents real-world limitations for adaptation to routine casework. Conversely, forensic laboratories often have greater access to GC/MS, but the detection limits of even the best of these systems for extracted samples is often too high to be used with the same type of extracts often used for LC/MS. This problem is difficult to alleviate procedurally, even using multiple extractions, drying, and selected ion MS; however, thermal desorption has already been shown to be useful as an inlet for FDR types of analysis using ion mobility spectrometry and pre-concentration using Solid-Phase Microextraction (SPME) and similar techniques.

This study reports on the development of a direct thermal desorption method as an inlet for GC/MS analysis of hand swabs collected on clean room wipes. A commercial Thermal Separation Probe (TSP) was used to hold swabs as they were placed directly into the injection port of the GC. Desorption proceeded over ~30 seconds while the GC column was held at room temperature. Samples were then eluted using a temperature program and detected using Selected Ion Monitoring (SIM) of three to four ions per target compound. An internal standard and surrogate spikes were utilized for semi-quantitative analysis and recovery monitoring, respectively. Ethyl centralite and diphenylamine were the most frequently detected compounds, with swab recoveries estimated at >65% based on surrogate spike data. Limits of detection corresponded to µg quantities, which is comparable to what is expected to be deposited by realistic forensic shooting scenarios. The results for several shooting experiments using different weapons, ammunition, and number of shots will be presented, along with method development and validation, figures of merit, and instrumental parameters.

Organic Gunshot Residue, Thermal Desorprtion, GC/MS

B31 Fabric Phase Sorptive Extraction (FPSE) Media: A Novel Forensic Sample Collection and Storage Device

Abuzar Kabir, PhD, Florida International University, 11200 SW 8th Street, AHC4-215, Miami, FL 33199*

After attending this presentation, attendees will have a thorough understanding of the fabrication, working principle, and advantages of FPSE media as a forensic sample collection and storage device for accumulating fluidic samples from different crime scenes as the potential evidence of the crime, storing them for a prolonged period of time, if necessary, and analyzing them using chromatographic separation and mass spectrometric identification in order to obtain chemical profiles of the collected samples.

This presentation will impact the forensic science community by assisting those who are actively looking for a better alternative to conventional approaches of collecting fluidic samples from crime scenes. Future adaptation of the proposed forensic sample collection device as a standard practice will potentially minimize the majority of the sampling-related errors that often compromise the quality of the forensic evidence and consequently brings into question the admissibility of the evidence to a court of law.

Although recent decades have witnessed an exponential growth in modern analytical equipment possessing superior sensitivity, higher resolution, portability, and exceptional selectivity that synergistically help in solving forensic criminalistics cases faster with higher confidence, little progress has been conducted in standardizing a universal forensic sample collection protocol and tool thereof. Due to the lack of such a universal protocol and a sample collection media, crime scene sample collection teams often use a non-standard sample collection medium, such as cotton gauze, to collect fluidic samples from the crime scene as the potential chemical evidence. These non-standard sample collection tools often fail to collect pertinent chemical information from the collected sample in order to complement the criminal investigation and may be thrown out by the court of law due to inferior quality as forensic evidence.

FPSE media, developed by Kabir and Furton, have eloquently addressed a majority of the hurdles often experienced by the forensic sample collection team, such as variability between different collection media, the inability to retain chemical information for a prolonged period of time, and a lack of standard sample collection protocols, among others.[1] FPSE sample collection media are prepared using flexible fabric substrate (cellulose/polyester/fiber glass) coated with high-efficiency, sol-gel hybrid inorganic-organic sorbents. Due to the ultra-thin film of the robust sorbent system, the FPSE media retains its flexibility. The chemical bonding between the substrate and the sol-gel sorbent network offers unprecedented mechanical and chemical stability to the sample collection device. As such, the FPSE media can be used to wipe any fluidic forensic sample from the crime scene, even from uneven surfaces. The sponge-like porous architecture of the sol-gel coating on the FPSE media allows rapid permeation of the fluid through its body for effective analyte-sorbent interaction, leading to the successful accumulation of the chemical information of the suspect sample. Due to the strong interaction between the analytes and the FPSE sorbents, the FPSE media containing the chemical information can be stored at ambient conditions under proper chain of custody without risking any potential loss of the chemical information. A small volume of organic solvent can be used to desorb collected analytes for chromatographic profiling.

Analytical data obtained from a number of real-life applications of FPSE sample collection and storage media mimicking different crime scene samples, including blood and saliva, will be presented, showcasing the advantages of FPSE forensic sample collection and storage media.

Reference(s):

1. Kabir A., Furton K.G. F*ield Sampling Kit for Chemical Recovery, Storage, and Profiling, Method of Making and Using the Kit, and a Dynamic Fabric Phase Sorptive Extraction (DFPSE) Media.* Patent pending. USPTO Serial Number 61/954,293. March 17, 2014.

Forensic Sampling, FPSE, Sample Collection

B32 The Identification and Quantification of Sexual Lubricant Degradation Pathways From Exposure to the Vaginal Bacterial Environment

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After attending this presentation, attendees will understand how the vaginal bacterial environment can degrade sexual lubricants and change their chemical composition prior to laboratory analysis. The goal of this presentation is to educate the forensic trace evidence community on how lubricants collected can differ from the known source over time based on bacteria and how to address that issue in casework.

This presentation will impact the forensic science community by bridging the gap in knowledge regarding the characterization of residual lubricant degradation and by describing how degradation from microbial exposure affects the identification and comparison of condoms and sexual lubricants for forensic and judicial purposes.

Due to the use of DNA analysis for identification in criminal acts, an increasing number of offenders are using condoms to mask their identity from law enforcement. During the act of coitus, lubricant found on the condom can be transferred to the victim. In forensic lubricant analysis, the major components of lubricants are used as indicators of the presence of sexual lubricants. The major components of lubricants can include Polydimethylsiloxane (PDMS), Polyethylene Glycol (PEG), and Nonoxynol-9 (N-9). These normal alkane and aromatic compounds can be degraded, into smaller components, by the bacteria that is natural to the vaginal cavity.[1,2]

In order to maintain a healthy environment, the female genital tract will maintain stable conditions using bacteria with the ability to produce lactic acid. This lactic acid-producing bacteria, which includes members of the *Lactobacillus, Pseudomonas*, or the *Megasphaera* species, keep the pH at the desired levels and keeps out any disease-causing microorganisms. The lactic acid production from these bacteria can use residual lubricants that remain in or near the vagina as a possible energy source, thus leading to sample degradation, which can change the original chemical composition. The degradation caused by microbial exposure makes it necessary to understand how the microbes degrade the condom lubricant components and how the degradation changes the overall chemical profile of the lubricant.

The degradation of a common lubricant, PEG, was studied using a common vaginal microbe, *Pseudomonas putida* (*P. putida*). PEG was inoculated with *P. putida* and the resulting samples were collected over a four-day period. Chemical degradation was determined with the use of a Direct Analysis in Real Time-Time-Of-Flight/Mass Spectrometer (DART®-TOF/MS). The DART®-TOF/MS was selected for this analysis primarily based on the direct MS analysis. This instrument can analyze individual components in the sample simultaneously with high resolution and accurate mass measurements, which provides a high degree of discrimination between samples. Coupled with the short run time and minimal sample preparation of the DART®-TOF/MS, the instrument is ideal for analysis of degraded and non-degraded lubricant samples.

Preliminary data suggest the *P. putida* bacteria will degrade the lubricant component over a period of three to four days before the lubricant chemical profile is no longer detectable. Based on this information it is necessary to determine how other common bacteria affect PEG, PDMS, or the condom lubricant as a whole. This information can provide the forensic science and sexual assault investigation communities with a new analytical timeline for vaginal samples collected after a sexual assault.

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DART®, Microbial, Degradation

B33 Application of Gas Chromatography-Vacuum Ultraviolet (GC-VUV) Spectroscopy in the Analysis of Synthetic Cathinones

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After attending this presentation, attendees will understand characteristics of synthetic cathinones, problems surrounding current methods of analysis for these compounds, principles of VUV spectroscopy, and the application of this instrumental analysis to synthetic cathinones.

This presentation will impact the forensic science community by providing an additional and complementary method of analysis for emerging drugs, such as synthetic cathinones. VUV methodology will be particularly valuable for previously problematic analysis of positional isomers of synthetic cathinones.

Gas Chromatography/Mass Spectrometry (GC/MS) is a standard method of analysis for synthetic cathinones; however, GC/MS, as well as alternative Liquid Chromatography/Mass Spectrometry (LC/MS), often show limitations in the differentiation between isomers with the same molecular weight (positional isomers and diastereomers) that may co-elute and/or have identical mass spectra.

In order to address this limitation, new detectors have begun to emerge, including the introduction of VUV. This instrument rapidly measures absorption in the range of 115nm to 240nm. One distinct advantage to VUV is the unique ability to probe the excitation energy associated with electrons forming most single bonds (σ à σ^*) as well as monitor some π à π^* bond transitions. VUV also has the ability to deconvolute multiple co-eluting compounds when the spectra are different and properly added to the working library. This is extremely valuable for screening and confirmation purposes and could lead to faster separations where resolution is sacrificed. GC-VUV, which combines the high resolving power of GC with a detector that can provide a unique spectrum for a given compound, appears well suited for the analysis of emerging drugs, such as synthetic cathinones.

Comparisons of 35 spectra taken from multiple classes of synthetic cathinones were examined, including spectra from ten sets of positional isomers. Synthetic cathinones provide a unique UV spectrum. This was particularly valuable for the identification of positional isomers. For solutes differentiated by MS, VUV provides useful complementary information. VUV also allows for unique overall VUV patterns for different classes of synthetic cathinones, proving to be very useful in the analysis and identification of unknown compounds. In some instances, VUV can also tell substitution in the aromatic position. The potential for quantitative analysis, the repeatability of VUV detection, and the effect of concentration on spectral identification will be described.

GC-VUV, which provides excellent peak shapes and high specificity of detection, is well suited for the analysis of synthetic cathinones. In order to accomplish good chromatographic performance, de-salting of the analytes is required, which is easily accomplished by the addition of sodium bicarbonate to the methanolic sample solution.

GC-VUV, Synthetic Cathinones, Positional Isomers

B34 Our Future Reflects Our Past: The Evolution of Criminalistics

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After attending this presentation, attendees will gain deeper knowledge and insight on the evolution of the practice of criminalistics from the perspectives of experienced and knowledgeable forensic scientists. This presentation will engage attendees in discussions regarding the current state of criminalistics. Experienced forensic scientists on the panel will share their views of the past development of criminalistics, their views on current practices, and their visions for the future evolution of this field.

This presentation will impact the forensic science community by increasing awareness of present issues in the field that developed from the past and by offering insights into possible tracks that may lead to the future evolution of criminalistics.

Criminalistics has evolved on many fronts, resulting in an increase in the demand for its services, in the number and size of forensic laboratories, and also in the number of academic programs offering undergraduate and graduate degrees in forensic science. Efforts in the area of laboratory accreditation, expert certification, and proficiency testing have contributed to improving the quality of the analytical work being conducted. These, along with continuing technological advances, are obviously perceived as positive developments. The services delivered have progressed from microscopical comparisons of trace evidence items, through application of advancements in science, such as wet chemistry analytical and serological techniques, to sophisticated instrumental and digital analyses, which allow for greater and greater sensitivity and specificity. The result has been a massive increase in the reliability of the results presented to the criminal justice system. The introduction of DNA and the related developments has been the paradigm shift in this era. What is the future of criminalistics? Who does define the path of criminalistics — criminalists themselves or outsiders from the field? How can one learn from the past of criminalistics to define its future?

Criminalistics was seen as multifaceted by pioneers at the turn of the 20th century. The focus was on tiny details, trace materials that could provide clues to a hidden and uncertain past, otherwise inaccessible. As highlighted by the historian Carlo Ginzburg, who noticed the change of paradigm that occurred with Freud's psychoanalysis, Morelli's art studies … and Sherlock Holmes, a philosophical and historical movement was underpinning the views of pioneers who saw the perspectives offered and opened by the study of "signs." Whereas some, like the Austrian magistrate Hans Gross, saw the tactical aspects offered in the form of an investigative science (which he incidentally called "criminalistics") as well as the value of these signs in the form of evidence, others, such as Alphonse Bertillon, saw their value in classifying, identifying, and creating databases, leading to police files, or Edmond Locard and later Paul Kirk, as a form of clinical science with laboratory support. These categories were not rigid; even if they reflected each pioneer's background, knowledge, and vision. During the 20th century, this was taken up by practitioners with little vision or power to develop the astonishing potential of this new "science" and it is only now that science is reclaiming its ground over test providers and other administrative organizations to offer new perspectives in detecting crime phenomena through detection, intelligence, and databasing (strategic and tactical dimension), as well as support for evidential purposes. The future, in that perspective, may not be what law enforcement administrators want or what the legal profession perceives. Currently, isn't the forensic laboratory conceptualized as a mere testing facility focusing exclusively on associative evidence problems rather than more general scientific problem-solving with the total physical evidence record? Isn't there too much emphasis on the question of "who" being addressed? Wouldn't notions such as transfer, persistence, and alterations coupled with an appreciation of background, contamination, and preservation of the recovered trace materials be needed for the evaluation of the significance of findings?

This special session will engage attendees in discussions regarding the current state of criminalistics.

Foundations, History, Current Practice

B35 Implementing 3D Virtual Comparison Microscopy Into Forensic Firearm/ Tool Mark Examinations

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After attending this presentation, attendees will better understand the validation method used to evaluate 3D instruments for virtual comparison microscopy, the method used to incorporate these technologies into traditional forensic firearms/tool marks examinations, and the capabilities of using 3D technology for training, examination verification, and blind verification/testing.

This presentation will impact the forensic science community by demonstrating the effectiveness of 3D technologies for integration into forensic firearms/tool marks examinations. The topics covered will include how the Federal Bureau of Investigation (FBI) Laboratory Firearms/Toolmarks Unit (FTU) has been tasked with evaluating 3D technologies for validation and incorporation into casework.

The FTU has been conducting a validation study for the incorporation of various 3D platforms into operational casework as another tool to assist in the examination of ammunition components. It is anticipated that having these technologies available will enable efficiency for cases with a high number of submitted components, provide additional training aids, and also provide larger-scale views of information captured for the examiner's examination and review. A portion of the validation study was to determine if a qualified firearms/tool mark examiner could successfully determine, using virtual comparison microscopy, the correct answers to previously distributed proficiency tests and consecutively manufactured test sets. The FBI Laboratory has a repository of Collaborative Testing Services® (CTS), Inc. firearms proficiency tests previously distributed to evaluate the FTU for quality assurance. Ten of these proficiency tests were selected, with test distributions spanning from 2003 to 2012. Some of the test participants had previously taken these proficiency tests using traditional optical comparison microscopy. All proficiency test samples, including the three knowns submitted for evaluation, were provided random identifiers for virtual microscopic comparison. Additionally, three test sets were assembled using consecutively manufactured slides from the FBI Laboratory Consecutively Manufactured Slides and Barrel Collection (CMSBC), which is used for training and research. Consecutively manufactured Ruger®, SR9 slides were selected using Winchester® ammunition to create test samples. Each consecutively manufactured test set consisted of ten cartridge cases with randomly assigned identifiers for virtual comparison. The participants contributing to this study ranged in years of experience and included qualified examiners and examiner trainees.

The Cadre® Forensic TopMatch-GelSight instrument uses the Bidirectional Reflectance Distribution Function (BRDF) to acquire 3D surface images and was acquired by the FBI Laboratory in 2014. The system's software allows for side-by-side evaluation of surface topographies and matching algorithm search capabilities for topography similarities. For this virtual comparison microscopy evaluation study, test participants did not have access to the matching algorithm to assist in reaching a conclusion. Test participants were given operating instructions on GelSight prior to conducting test examinations. Results were recorded by the individual test participant on an answer key and they were encouraged as well as the incorporation into the Firearms/Toolmarks Unit's standard operating procedures and implementation into casework as an alternative to traditional comparison microscopy.

3D Technology, Firearms, Virtual Comparison

B36 A Framework for Firearm Tool Mark Population Statistics

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After attending this presentation, attendees will better understand a potential pathway toward developing firearm tool mark population statistics.

This presentation will impact the forensic science community by providing a potential statistical framework for reporting error rates in firearms and tool mark identifications.

The population statistics describe the frequency distributions of a similarity score for, respectively, same-source comparisons and different-source comparisons of ballistic samples, such as cartridge cases and bullets. Similar to DNA analysis, these distributions are needed to establish a statistical foundation for the estimation of identification confidence limits and error rates. An important component of the proposed research is to systematically evaluate, for different firearm populations, the effects of key processing parameters on the comparison score distributions and associated error rate estimates. Conducting this research will play an important role in determining the extent to which the comparison protocol, population statistics, and error rate estimation need to be tailored to a particular firearm subpopulation.

Firearms from five manufacturers were chosen for this research. These include a minimum of 50 firearms each from Sig Sauer®, Ruger, Smith and Wesson®, Glock®, and Colt®. The breechface impression on 568 test-fired cartridge cases were measured from 284 firearms. Distributions for known match and known non-match comparison scores were generated for all the samples, for each firearm model, and for each breechface class characteristic. Furthermore, the effects of key processing parameters on these distributions were evaluated. The distributions were applied to estimate the respective cumulative false positive and false negative error rates. The proposed framework will enable evaluation of other similarity metrics and the dynamic update of distributions when new data becomes available.

Using the improved processing parameters, the National Institute of Standards and Technology (NIST) will be able to continue entering new batches of firearm comparison results into each of the established populations to create continually growing "living distributions." These can be used to estimate cumulative false positive and false negative error rates for each subpopulation and will play an important role in assessing the strength of the evidence for a particular comparison. Results provide guidance for the collection of new data and enable further research into whether or not groups of firearms or class characteristics converge to the same statistical model and processing parameters. The data and framework can be applied to generate distributions for other tool mark identification metrics and guide their development. The conclusions drawn from this research serve as a stepping stone to answering important questions regarding subpopulations and the role they play in firearm identification error rates and data processing parameters. While not covered in this research, the protocol used can be applied to other tool mark regions of interest, such as firing pin and land impressions. There is also a potential for this research to be applied in other pattern evidence disciplines, such as fingerprint and shoe print identification.

Firearms, Tool Marks, Populations

B37 Performance Evaluation and Calibration Artifacts for 3D Ballistic Imaging

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After attending this presentation, attendees will understand the fundamental metrological requirements for a smooth transition of 3D ballistic imaging from research to application in crime laboratories.

This presentation will impact the forensic science community by providing a roadmap to practitioners for obtaining accurate and traceable surface topography measurement results of ballistic samples in crime laboratories. A new effort at the National Institute of Standards and Technology (NIST) is working toward providing instrument performance evaluation specifications, performance evaluation procedures, and custom reference artifacts tailored to objective tool mark identification.

A promising approach to improving objective tool mark identification is the direct optical measurement of 3D sample surface topography; however, forensic laboratories seeking to implement this new approach are faced with several challenges. There is no guidance on instrument performance specifications that address the challenges and requirements of tool mark identification. Consequently, when a crime laboratory compares instrument specifications, it is difficult to determine which system is most appropriate for its measurement needs. Furthermore, there is no guidance on the type and frequency of tests that the laboratory needs to conduct to ensure that the instrument performs to specification and to establish traceability of measurement results. Finally, many physical reference artifacts for testing surface topography measurement devices are not compatible with the instruments and sample mounts designed for forensic applications and are time consuming to apply.

NIST is addressing these challenges by first developing a detailed performance-specification document, explicitly defining instrument performance parameters relevant to forensic tool mark metrology. This document will enable a laboratory to make informed decisions when specifying and comparing 3D optical instruments for ballistic imaging purposes. The specification will address relevant performance parameters, such as the instrument transfer function, lateral and vertical measurement accuracy, stitching performance, maximum measurable slope, and environmental conditions.

The main part of the project is focused on the design of a set of ballistics-oriented test procedures and physical reference standards that enable a forensic laboratory to validate the specified instrument performance. The standards are complementary to the NIST Standard Reference Manuals (SRMs) 2460 (standard bullet) and 2461 (standard cartridge case) and are envisioned as having the shape and dimension of a cartridge case to facilitate their application. The process control and calibration artifacts will be made available in one set, calibrated at NIST and International System of Units (SI) traceable. The set will likely include roughness standards, step height standards, 2D grids, and a reference flat. The specifications, test procedures, and reference artifacts addressed by this project are critical in establishing traceability of tool mark measurement results in forensic laboratories.

This presentation will outline the primary components of the performance evaluation specification detailed above and will present initial designs of the new ballistics-oriented physical reference standards.

Tool Mark, Topography, Standards

B38 Determining the Angle of Impact From the Analysis of Bullets Following Perforation of Glass

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After attending this presentation, attendees will better understand how applying different methodologies in analyzing a bullet can assist investigators in determining the position of a suspect in the reconstruction of a shooting incident, specifically in situations where that bullet has perforated a glass target.

This presentation will impact the forensic science community by providing a quantitative measure in analyzing and interpreting bullet evidence for future use in the courtroom.

When two objects come into contact with one another, there is a potential for the transfer of material between those objects. The goal of this research was to develop statistical models to aid investigators in the reconstruction of a shooting incident. Specifically, the determination of the direction of fire from the bullet's angle of impact was addressed by assessing the deformation of the bullet and the transfer of glass onto the bullet.

Transfer of material to bullets is an underexploited area of trace evidence. Current research has mainly been observational, and no attempt has been made to provide a quantitative measure to the results.

Four aspects of bullet deformation after perforation of a glass target were studied during this research: (1) the shape of the bullet holes; (2) the side view of bullet deformation; (3) the frontal view of bullet deformation; and, (4) the distribution of glass onto the bullets. A Ruger SR9 9mm pistol was used to fire 100 cartridges at individual glass targets at angles of 45º, 50º, 60º, 75º, and 90º using Winchester® 115 grain, 9mm, Luger® full metal jacket factory ammunition and reloaded lead round nose ammunition consisting of 115 grain Missouri Bullet Company bullets, 4.1 grains of Hodgdon® Titegroup powder, and Sellier & Bellot® 4,4 small pistol boxer primers.

The following methodologies were employed for image capture and analysis: (1) focus stacking using Zerene Stacker to generate high-quality images of the frontal view of the bullet; (2) analysis of the bullet holes in the glass targets using HemoSpat; and, (3) analysis of bullet deformation and distribution of glass onto bullets using ImageJ. Regression modeling and principal component analysis were performed on the data. The research found that examining bullet holes in glass is not a viable method for determining angle of impact. It also found that the side view deformation of full metal jacket bullets can be used to distinguish between some impact angles, for example, 90º and 65º, but cannot be used for lead round nose bullets. Furthermore, the front view deformation and distribution of glass on full metal jacket bullets can be used to distinguish between some impact angles, for example, 75º and 50º, but cannot be used for lead round nose bullets.

Bullets, Angle of Impact, Shooting Reconstruction

B39 The Determination of Critical Angle in Automobile Windshield Glass Using 9mm Luger® Ammunition

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After attending this presentation, attendees will better understand the characteristics of automobile windshield glass and how it reacts to bullet impacts. Attendees will also be aware of the type of ammunition used in these experiments and the factors influencing bullet perforation or bullet ricochet off a windshield.

This presentation will impact the forensic science community by exploring the angle necessary for a bullet to perforate or ricochet after impacting a windshield, which has implications in examining a shooting scene involving an automobile.

An understanding of bullet ricochet is a vital part of deconstructing shooting scenes; however, a bullet's ricochet depends on many factors, the totality of which may not be replicated from one scenario to the next. This presentation deals with bullet ricochet off windshield glass. All variables were held constant with the exception of incident angle, which is the angle at which a projectile impacts the target surface. The angle at which the projectile leaves the substrate is the ricochet angle. When a bullet strikes a target, it may perforate or penetrate the substance, fragment into pieces or ricochet off the surface. Ricochet is the change in direction of a bullet's trajectory after impact with a surface. Each surface has an angle called the critical angle, below which a projectile will ricochet. The critical angle is dependent upon each type of surface. The inability to generalize is due to the fact that there are many factors that affect the ricochet of a bullet, such as incident angle, impact velocity, bullet shape, bullet weight, bullet hardness, bullet center of gravity, the hardness of the impact surface, and the response of that surface to the bullet's impact. It is important to note that not only do the characteristics of the bullet have an effect on the ricochet, characteristics of the impact surface do as well. Both need to be analyzed in cases of ricochet.

Laminated glass, which is used on automobile windshields, is composed of a thin polyvinyl plastic layer sandwiched between two pieces of glass. The glass is bonded to the plastic by heat and pressure. The layers are then secured to the auto with a gasket material. This type of glass is the strongest available for its particular thickness of glass according to the National Glass Association.[1] It is also more resistant to shattering because the plastic middle layer will remain bound to glass pieces that have been broken. This prevents the glass from breaking up into large sharp pieces after impact. In the United States, laminated glass must be installed in the windshields of vehicles while tempered glass is required in the side and rear windows according to the United States Department of Transportation.[2]

Because there are many variables associated with bullet ricochet in general and specifically with windshield glass, the firearm, ammunition, and substrate were kept consistent throughout the course of the experiments. This study used 9mm Luger® ammunition with a Full Metal Jacket (FMJ) bullet. According to the manufacturer, the muzzle velocity is 1,150 feet per second, which was confirmed using a chronograph to determine the velocity of the specific lot of ammunition used.

Initial test shots at 5°, 10°, and 20° incident angles were performed to determine the effect the bullet had on the windshield. Five degrees yielded a clean ricochet with minimal damage to the windshield and a fairly intact bullet. This was not the case with 20°, where the bullet perforated the windshield. High-speed photography elucidated the mechanism of the bullet-glass interaction. Shots fired at 10° resulted in a ricocheted bullet that retained a substantial portion of its initial mass. Ten degrees was then bracketed by shots at 9° and 11° to compare the damage caused by the bullet and the associated damage caused to the bullet. A system was devised to capture and recover bullet fragments from both the surface of the windshield and from underneath the windshield, which was used to determine the percent of post-ricochet weight loss. Witness papers were used downrange to determine the ricochet angle using trigonometry. Incident angles of 5°, 10°, and 20° had ricochet angles of 3°, 8°, and 15°, respectively.

Reference(s):

1. Industry Resources. Ask the Expert. Retrieved 30 July 2016 from https://glass.org/industry-resources-ask-the-expert-faq.html.

2. Code of Federal Regulations (CFR). *Federal Motor Vehicle Safety Standards, Glazing and Window Construction.* 49 CFR 571,205, 1972.

Ricochet, Windshield, Critical Angle

B40 Improved Total Nitrite Visualization (TNV) for Gunshot Distance Determination

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After attending this presentation, attendees will better understand the improved TNV technique used to detect nitrites in gunshot distance determination casework. This technique has been shown to significantly increase the ability of analysts to detect nitrites on the clothing of shooting victims and has an increased potential for providing useful distance determination information.

This presentation will impact the forensic science community by illustrating that the TNV method is the best practice for Gunshot Residue (GSR) muzzle-to-target distance casework. This could potentially allow for the detection of residues at farther distances and the development of more accurate nitrite residue patterns, which can expand the value and reliability of cases in which gunshot distance determinations are conducted.

Visualization of residues is an essential part of gunshot distance determination. Typically, the current protocols include the Modified Griess Test (MGT) for nitrites and the Sodium Rhodizonate Test for lead. One problem with gunshot distance determination is that the nitrites that are tested for using the MGT are typically not stable in the environment. In addition, nitrites will only be deposited when partially burned gunpowder particles come into contact with the target, which is less likely at long distances. Unburned gunpowder particles may travel farther than vaporous residues or partially burned gunpowder, but they are not detectable using current MGT protocols. Glattstein et al. demonstrated the ability of alkaline hydrolysis to convert nitrates into nitrites to allow visualization of unburned gunpowder particles using the MGT.[1,2] This is referred to as TNV. This method involves using an adhesive lift of the residues, as well as a one-hour incubation time; however, in a laboratory setting, this method proved to be ineffective on soiled evidence items due to the inability of the adhesive to remove substantial residues. Also, the long incubation time proved to be inefficient in a casework environment.

A validation research study was conducted at the New York City Police Laboratory, which sought to improve upon and apply the TNV method to casework samples. Upon completing this study, it was found that effective results could be obtained using a five- to ten-minute incubation time. This is a significant improvement to the one-hour incubation from the original research. In addition, a direct application method of the hydrolysis reagent was found to be effective on soiled items, eliminating the need for an adhesive transfer. When the TNV method was compared to samples processed using the traditional MGT method, a significant increase in the abundance of nitrite residues was observed.

The TNV procedure was implemented in Gunshot Distance Determination casework at the New York Police Department (NYPD) Police Laboratory. To evaluate the effectiveness of the TNV procedure, reports released before and after the method's implementation were compared. This evaluation found that after the implementation of the TNV method, the percentage of analyzed holes in which nitrite residues were detected increased from 24.7% to 50.9%. In addition, the percentage of cases that contained residues that would allow for a muzzle-to-target distance determination increased from 9.9% to 18.6%.

The results of the validation study and the casework evaluation of the TNV method indicate that it is the best practice for GSR muzzle-to-target distance casework. This could potentially allow for the detection of residues at farther distances and development of more accurate nitrite residue patterns. Implementing this method can positively influence the criminal justice system by expanding the value and reliability of cases in which gunshot distance determinations are conducted.

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1. Glattstein B., Vinokurov A., Levin N., Zeichner A. (2000). Improved method for shooting distance estimation. Part 1. Bullet holes in clothing items. *Journal of Forensic Sciences*, *45*(4), 801-806.
2. Glattstein B., Vinokurov A., Levin N., Zeichner A. (2000). Improved method for shooting distance estimation. Part 2. Bullet holes in objects that cannot be processed in the laboratory. *Journal of Forensic Sciences*, *45*(5), 1000-1008.

Gunshot Distance Determination, Nitrite, Total Nitrite Visualization

B41 Using Clonal Massively Parallel Sequencing (MPS) to Characterize Heteroplasmy in the Mitochondrial DNA (mtDNA) of Human Head Hair, Pubic Hair, and Buccal Samples

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After attending this presentation, attendees will better understand how MPS better characterizes heteroplasmy in the mtDNA profiles of head hair, pubic hair, and buccal samples.

This presentation will impact the forensic science community by contributing to an understanding of the heteroplasmy levels that are present in forensically relevant tissues, how heteroplasmy affects the interpretation of mtDNA profiles, and how MPS can be applied to forensic mtDNA analysis.

MtDNA is a useful target for analyzing forensic samples when nuclear DNA (nDNA) profiles are difficult to obtain (for example, when a hair sample does not have follicular tissue attached). Obtaining mtDNA does not often depend on the presence of follicular tissue because mtDNA is present in the hair shaft; however, mtDNA analysis presents challenges that nDNA analysis does not. One such challenge is interpreting heteroplasmy, which is an mtDNA mutation phenomenon that causes more than one mtDNA sequence to be present in an individual. Heteroplasmy has the potential to be useful in forensic mtDNA typing because the presence of heteroplasmy at identical sites in both a casework and a reference sample could not only help confirm a match but also increase the significance of the match.

MPS can overcome the challenges of forensic mtDNA typing. MPS methods are highly sensitive approaches for clonally amplifying and sequencing many samples at once. MPS methods can resolve complex mixtures (from ≥3 contributors) and can also quantify mtDNA mutations. The level of sensitivity achieved with MPS allows for better detection of low-level mutations unobtainable with Sanger sequencing, which is the current forensic mtDNA typing method. The goals of this study were to use an MPS method to better characterize low levels of heteroplasmy across tissues and to compare MPS to Sanger sequencing.

In this study, the Roche® 454 GS Junior MPS platform was used to sequence the mtDNA Hypervariable regions I and II (HVI/HVII) from human head hair, pubic hair, and buccal samples. The MPS sequencing results were compared to the Sanger sequencing results for different tissues from the same sample sets. Point heteroplasmy was detected in more samples when MPS was used than when Sanger sequencing was used. The frequency of heteroplasmy was highest in head hairs, followed by pubic hairs, then by buccal swabs. The 454 was able to quantify levels of heteroplasmy, which cannot be achieved with Sanger sequencing. The 454 detected low levels of heteroplasmy better than Sanger sequencing by reporting levels as low as 1.14% (with at least 500X coverage). With Sanger sequencing, only instances of heteroplasmy at approximately 10% or above could be confidently reported. The 454 also detected more somatic mutations and more heteroplasmy differences within and between different tissue types. Roughly equal numbers of somatic and germ-line heteroplasmy were observed, as well as four heteroplasmic "hot spots" at positions 150, 183, 185, and 189 in the HVII. Finally, there was no significant difference in the frequency of heteroplasmy with an increase in an individual's age.

In conclusion, this study improves understanding of forensic mtDNA sequencing by better characterizing the mutations inherently present in mtDNA. This study also further argues for the use of MPS platforms for forensic mtDNA sequencing.

Massively Parallel Sequencing, Mitochondrial DNA, Heteroplasmy

B42 The Forensic Value of Processed Human Hair Extensions

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After attending this presentation, attendees will better understand the probative value of processed human hair extensions in forensic casework. Specifically, this presentation will address the inability to identify a hair as an extension using microscopic and genetic techniques and the associated forensic implications.

This presentation will impact the forensic science community by indicating the forensic value of processed human hair extensions and emphasizing the implications associated with not identifying a human hair as an extension in casework.

The human hair extension industry has grown immensely with revenues exceeding nine billion dollars each year. Statistics indicate that more than 60% of women have at some point invested in hair extensions and that they are even becoming popular with men.[1] In spite of the expansion of the extension consumer market, human hair extensions have never been evaluated for their evidentiary value in a forensic case. A human hair extension collected from a crime scene would be evaluated as a shed human telogen hair and analyzed using microscopic techniques and mitochondrial DNA (mtDNA).[2] Sequencing of mtDNA from the extension would place the hair donor, not the suspect, at the scene. Although it is not likely that the mtDNA sequence would be matched to the donor's maternal lineage, the evidence would be misleading. Hair extensions collected from a crime scene would misdirect an investigation and result in a misuse of time and resources. The ability to identify a human hair as an extension would be invaluable during an investigation and might exclude the hair as probative evidence.

In this study, three brands of processed human hair extensions were evaluated microscopically and genetically for their probative value in forensic casework. Microscopic analysis of hair morphology by transmitted light microscopy and Scanning Electron Microscopy (SEM) determined that the internal and surface characteristics of the human hair extensions were consistent with human head hair and failed to identify any distinguishing features (pitting, striations, indentations, or internal variations) that differentiated the extensions from natural human hair. Chemical analysis by an Energy Dispersive X-Ray (EDX) detector in conjunction with an SEM identified carbon, oxygen, sulfur, aluminum, and calcium as the main elemental components of the processed human hair extensions, which is consistent with human hair. No elements unique to the extensions were detected.

MtDNA extracted from the hair extensions was sequenced and compared to the revised Cambridge Sequence (rCRS) to identify Single Nucleotide Polymorphisms (SNPs).[3] SNPs were used to assign haplotypes and distinguish regional affiliations associated with the extensions in an attempt to establish the ethnicity of the hair donor's maternal lineage.[3] Haplotype assignments for the hair extensions were based on Hypervariable region 2 (HV2) genetic polymorphisms and represented multiple geographic regions and a large portion of the population. HV2 sequences were not restrictive enough to determine regional affiliations for particular extension brands or processed human hair extensions as a whole. More definitive haplotype assignments would be possible with Hypervariable region 1 (HV1) SNP discrimination. Also, sequence variation between hair extensions of the same brand indicated that the hair within a single package of extensions was from multiple donors. This has significant implications in forensic analysis.

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3. Allocco D.J., Song Q., Gibbons G.H., Ramoni M.F., Kohane I.S. (2007). Geography and Genography: Prediction of Continental Origin Using Randomly Selected Single Nucleotide Polymorphisms. *BMC Genomics*, 8: 68.

Mitochondrial DNA, Haplotype, Hair Microscopy

B43 A Proteomic Analysis of Human Hair From Various Body Sites

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After attending this presentation, attendees will better understand that human hair is a biological link between its source and the forensic context in which it is found. This presentation will inform attendees regarding the proteomic information found within hair and how it can be exploited to render additional data regarding the anatomical location of human hair on the body.

This presentation will impact the forensic science community by informing attendees that forensic science is in critical need of new laboratory-based methods that provide a quantitative statistical association between an individual and a forensic context. If DNA is not available or is degraded, genetic information in protein may provide additional data.

Recent methodological advances in proteomic analysis offer the prospect of considerable improvement in the use of hair evidence for individual identification. An advantage is the supplementation of subjective findings from microscopic hair comparison with quantitative values amenable to probability estimates and experimentally determined error rates. Results to date are promising with respect to distinguishing among individuals by profiling protein expression levels in the hair shaft. Variations in expression level reflect variations in transcription factor levels, their affinities for response elements, and possibly epigenetic as well as genetic variation. Another form of variation is the presence of Genetically Variable Peptides (GVPs) that offer the prospect of even more discriminating analysis. GVPs are a consequence of non-synonymous single nucleotide polymorphisms that are translated as single amino acid polymorphisms. Increasingly well described by the database of single nucleotide polymorphisms in human DNA, the known non-synonymous mutations among them permit detection of GVPs in digests of hair proteins.

To maximize the usefulness of proteomic technology, two factors merit further exploration. First, optimization of hair processing, now very time consuming, is envisioned to increase the speed and sensitivity of the method so that eventually a single hair fragment will suffice for analysis. Progress in this optimization is occurring. Second, the relative value of hair from different anatomic sites remains to be assessed. The initial study indicated that the protein profiles of hair classes from four sites (axillary, beard, pubic, and scalp regions) are distinguishable. Current work addresses the relative discriminating ability of protein profiling of hair samples from these sites within and among individuals. Whether the profiles differ in a reproducible fashion according to site could be useful in assessing individual comparisons when a hair sample to be identified is of uncertain site origin. Complementing this direction, current work also addresses the yield of GVPs from hair from the different sites. Since GVPs from less abundant proteins are more likely to be observed with increased efficiency of hair digestion, optimization of the processing is envisioned to increase the number of GVP identifications and therefore increase the level of discrimination possible by this route.

Body Hair, Proteomics, Genetically Variant Peptides

B44 Validation of a Method to Sequence the Mitochondrial Genome of High-Quality Reference Samples Using the Illumina® MiSeq® FGx Platform

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After attending this presentation, attendees will better understand the practical considerations in implementing next generation sequencing methods into a forensic laboratory.

This presentation will impact the forensic science community by presenting a method to sequence the entire mitochondrial genome of high-quality reference samples as well as detailing the validation studies performed in order to implement this method into a forensic laboratory.

The Armed Forces DNA Identification Laboratory (AFDIL) has recently validated a Next Generation Sequencing (NGS) method for degraded specimens using hybridization capture enrichment of the mitochondrial genome (mitoGenome) and multiplexed sequencing on the Illumina® MiSeq®. The capture procedure was shown to be robust and reliable for the generation of mitoGenome data from degraded DNA samples. The sequencing of the complete mitoGenome enables enhanced discriminatory power compared to Control Region (CR) sequence data obtained with traditional Sanger techniques. To take full advantage of the increased genetic information now generated from casework samples and to facilitate comparison with reference samples, it was necessary to validate a suitable NGS mitoGenome method for high-quality samples.

Recently, the AFDIL, in conjunction with the Federal Bureau of Investigation (FBI) Laboratory, performed an extensive evaluation of an NGS protocol for generating mitoGenome data from high quality samples.[1] The procedure begins with a target enrichment step that uses Long Range (LR) Polymerase Chain Reaction (PCR) to amplify the mitoGenome in two overlapping 8,500-bp amplicons. Libraries of the amplicons are prepared by the Nextera® XT DNA Library Preparation kit. This user-friendly kit involves enzymatic tagmentation to fragment the large amplicons followed by a limited cycle PCR step to incorporate indexed adapters necessary for massively parallel sequencing on the MiSeq® FGx. Single-end sequence reads are analyzed with the CLC Genomics Workbench using an optimized workflow that includes a custom mitochondrial DNA (mtDNA) analysis tool. The overall simplicity and rapid speed of this method make it ideal for high-quality sample processing.

For the present validation study, nearly 200 case-type samples and controls were processed following AFDIL's Nextera® XT mitoGenome sequencing and analysis protocol. The Scientific Working Group on DNA Analysis Methods (SWGDAM) validation guidelines were followed for a developmental validation of a novel mtDNA NGS procedure. The LR amplification, Nextera® XT library preparation, and Illumina® MiSeq® sequencing procedures were shown to generate robust sequence data at DNA inputs as low as 100pg. Stochastic artifacts due to amplification errors were observed when the input was less than 100pg, and in crude Bloodstain Card (BSC) extracts regardless of the DNA input (from 3.5ng to 19.5ng). The low template effects observed in these high quantity BSCs was likely attributable to the presence of inhibitors that reduced the amplification efficiency. Extracts that generated sufficient LR amplification product of approximately 4ng/μL produced concordant and reproducible mitoGenome profiles. Moreover, all control DNA samples generated high-quality sequence data consistent with the expected profiles. Only one of the 30+ negative controls was contaminated, which was likely introduced during library preparation due to the lack of detection of an amplicon peak and sequence data consistent with a neighboring sample. Based on quantitative metrics from the validation, interpretation guidelines using variant quality scores and percent of the mitoGenome covered were developed to ensure the reporting of reliable, single-source profiles. Validation testing of this NGS method demonstrated its sensitivity, reproducibility, specificity, stability, mixture detection capabilities, and reliability. As a result, the Nextera® XT mitoGenome sequencing method on the MiSeq® FGx platform was deemed suitable for implementation into casework at the AFDIL.

Reference(s):

1. Peck M.A., Brandhagen M.D., Marshall C., Diegoli T.M., Irwin J.A., Sturk-Andreaggi K. Concordance and reproducibility of a next generation mtGenome sequencing method for high-quality samples using the Illumina MiSeq. *Forensic Science International: Genetics*. Vol. 24, p103–111.

Mitochondrial DNA, Next Generation Sequencing, Validation

B45 Analyzing DNA Mixtures Using Polymerase Chain Reaction/Capillary Electrophoresis (PCR/CE) Compared to Next Generation Sequencing (NGS)

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After attending this presentation, attendees will better understand the contribution that NGS can make to the analysis of DNA mixtures when compared to typical PCR/CE methods.

This presentation will impact the forensic science community by providing a comparison of DNA mixture analyses from a study of two-, three-, and four-person mixtures using both standard PCR/CE methods and NGS to analyze Short Tandem Repeat (STR) profiles.

NGS or Mass Parallel Sequencing (MPS) is a newer technology that can simultaneously sequence multiple genomic regions. This technology has been widely used to study human genetics and testing has now begun on forensic samples. Current forensic DNA analysis methods use a combination of PCR and CE to produce informative results. Variable genomic regions known as Short Tandem Repeats (STRs) are amplified using PCR and the variable PCR fragment lengths are measured using CE. Alleles at multiple loci are used to produce highly informative DNA profiles.

NGS technology adds the ability to sequence the STR region to distinguish alleles of the same length but differing sequence, permitting some mixtures with shared alleles to be resolved. In addition, NGS technology can be more sensitive than CE, making it possible to detect alleles that CE misses.

This study examined some of the advantages of NGS. DNA mixtures were prepared using purified DNA extracted from saliva of known individuals. Two-, three-, and four-person mixtures were prepared at 1:1, 1:3, 1:10, 1:1:1, 1:1:3, 1:1:10, 1:1:1:1, 1:1:1:3, and 1:1:1:10. For CE DNA analysis, the mixtures were amplified using Identifiler® Plus or GlobalFiler™, run on a 3130xl Genetic Analyzer CE instrument, and analyzed with GeneMapper® ID-X software. In parallel, the mixtures were prepared for sequencing using the PowerSeq™ NGS library preparation kit, sequenced on a MiSeq®, and analyzed using ExactID® software.

The results show an increase in total alleles due to sequence variation of shared alleles and an overall increase in sensitivity by the NGS instrument compared to the traditional techniques. A comparison of corresponding loci from each kit showed that the Promega® PowerSeq™ had more total alleles called compared to Identifiler® Plus (418 to 496) and GlobalFiler™ (629 to 682). Of these additional alleles, 46 were due to sequence variation not seen in the PCR/CE based techniques. The increase in allele number enhanced the ability to determine the number of contributors at some loci. The additional sequence variant alleles led to 17 and 19 more loci with the correct number of contributors called, compared to Identifiler® Plus and GlobalFiler™, respectively. Overall, NGS technology improved the ability to interpret mixtures.

NGS, DNA Mixtures, Next Generation Sequencing

B46 Comparative Tolerance of Short Tandem Repeat (STR) and Massively Parallel Sequencing (MPS) Chemistries to Inhibited Samples

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After attending this presentation, attendees will better understand the difference in tolerance to common Polymerase Chain Reaction (PCR) inhibitors between the chemistries of a commercial DNA amplification kit for Capillary Electrophoresis (CE) -based STR typing and a Single Nucleotide Polymorphism (SNP) -based identity kit sequenced via MPS with the Ion Personal Genome Machine® (PGM™) platform.

This presentation will impact the forensic science community by demonstrating how common inhibitors found in challenging bone and tissue samples, such as those that may be recovered during a mass disaster or missing person's case, affect different genotyping successes employing conventional STR typing used in forensic laboratories and newer MPS methods.

Victim identification is one of the most important goals after a mass disaster event or in a missing person's case. Oftentimes, these human remains are very challenging samples to identify as they may be highly degraded and fragmented, burnt, decomposed, or containing inhibitory substances. CE-based STR markers and mitochondrial analyses are traditionally used for DNA identification, but MPS has more recently emerged as an alternative approach for identifying human remains. The purpose of this study is to compare the tolerance of a commercial STR kit and a MPS-based system to common inhibitors that are frequently encountered in skeletal and decomposed tissue samples requiring identification in forensic and missing persons' casework.

DNA (1ng and 0.1ng) was spiked with various concentrations of five inhibitors (humic acid, melanin, hematin, collagen, and calcium). Samples (*N*=150) were amplified with the GlobalFiler® PCR Amplification Kit and genotyped on the ABI 3500 Genetic Analyzer. A subset of samples (*N*=25) was also sequenced using the Human Identification (HID) -Ion AmpliSeq™ Identity Panel with the Ion PGM™.

In general, STR results showed a decrease in the number of alleles being amplified and detected as inhibitor concentrations increased. As expected, the average peak height and average heterozygote peak height ratios showed a decreasing trend as inhibitor concentration increased. Samples with 0.1ng DNA input resulted in considerably poorer STR profiles than 1ng DNA samples at all inhibitor concentrations, suggesting that samples amplified with less DNA template are more susceptible to the effect of PCR inhibition.

MPS sequencing results suggest that the HID-Ion AmpliSeq™ Identity chemistry may not be as tolerant to PCR inhibitors as STR amplification kits. Samples with the same inhibitor concentrations generated considerably worse results via MPS. The lowest inhibitor concentrations for humic acid, melanin, and hematin resulted in complete STR profiles but performed poorly when the samples were sequenced via MPS; however, the highest inhibitor concentrations for collagen and calcium resulted in poor STR profiles but performed well with MPS, resulting in complete SNP profiles.

Data also showed that when highly inhibited samples were pooled for library amplification and loading onto the same chip for sequencing, carry-over inhibition from highly inhibited samples affected the performance of samples with little or no inhibition. In fact, when samples with the highest amounts of inhibitor were processed, library amplification failed completely. Overall, the chemistry in commercial STR kits is more tolerant to most of the common inhibitors found in biological samples than the MPS sequencing chemistry tested in this study.

Massively Parallel Sequencing, STR Typing, PCR Inhibitor

B47 Fetal DNA Detection in Pregnancy Serum Using the Ion Personal Genome Machine® (PGM™) System With an Identity Single Nucleotide Polymorphism (SNP) Panel

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After attending this presentation, attendees will understand the operability of a Next Generation Sequencing (NGS) system in Low Template DNA (LT DNA) analysis, specifically in fetal DNA detection in maternal serum, and the basic principle of the Ion PGM™ platform with the Human Identification (HID) -Ion AmpliSeq™ Identity Panel and its utility in practical casework samples.

This presentation will impact the forensic science community by analyzing DNA obtained from challenging sources, such as degraded DNA and mixed DNA.

NGS systems offer new possibilities in genotype analysis using established forensic markers for identification. The results of the analysis of fetal DNA in maternal plasma or serum is often similar to LT DNA and mixed DNA.

Serum samples were taken from eight anonymous pregnant women. There was no detailed information about the fetus due to the fact that miscarried pregnancies were included and no gender information was available. DNA was isolated using the QIAamp® DNA Mini Kit. DNA quantification was performed using real-time Polymerase Chain Reaction (PCR) and the Quantifiler® Duo Kit. The levels of sequence coverage and mixed DNA patterns were evaluated through HID-Ion AmpliSeq™ Identity Panel with 124-markers designed for the Ion PGM™ system.

Partial Y-chromosome Single Nucleotide Polymorphisms (Y-SNP) markers were typed. Negative Y-STR profiles (no single STR locus) were obtained from serum samples taken from two pregnant women who were assumed to carry male fetuses. Using those particular two Y-SNP samples, partial fetal autosomal SNP profiles were implicitly inferred via retrospective mixture analyses in all of the eight samples.

If additional information, such as portion and degree of mixtures, were available, this platform could be used for non-invasive prenatal testing to detect the mixture samples. This study also suggests that if relevant template chromosomal data are provided, NGS can be readily used to analyze mixed DNA samples. The findings also indicate that NGS will be practical for processing LT DNAs and mixed DNA.

Next Generation Sequencing, Fetal DNA, Mixed DNA

B48 A Preliminary Sensitivity Assessment Comparing Two Next Generation Sequencing (NGS) Laboratory Workflows for Forensic Analysis

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The goal of this presentation is to educate the forensic science community concerning the progress of NGS and the performance of two specific workflows in regard to use in forensic DNA analysis.

This presentation will impact the forensic science community by sharing information about the potential of these two specific workflows in forensic crime laboratories.

DNA evidence recovered from a crime scene is rarely ample in quality or quantity. Therefore, it is vital to know the limits of any assay used for forensic DNA analysis. NGS DNA sequencing technology is now being implemented and investigated in forensic laboratories. Integration of NGS into forensic DNA analysis workflows provides value by its ability to: (1) successfully process degraded samples; (2) reduce the cost of materials, labor, and time by multiplexing a large number of DNA markers into one sequencing run; (3) better distinguish individual profiles from mixtures; and, (4) use phenotypic markers to identify persons by physical characteristics. It is important to perform a sensitivity assessment to ultimately validate this new technology.

The Scientific Working Group on DNA Analysis Methods (SWGDAM) recommends various studies be conducted in order to evaluate the limits of an assay. With these evaluations having been performed for current technology (i.e. capillary electrophoresis) in the forensic setting, SWGDAM guidelines are serving as a guide for the assessment and validation of the NGS technology.

For this experiment, the Promega® PowerSeq™ Auto/Y System Prototype and Illumina® ForenSeq™ DNA Signature Prep kits were evaluated as both use the MiSeq® instrument platform. Initially, two high-quality DNA samples were assessed at different titrations, ranging in amplification input from 500pg to 15.6pg. The same samples were processed with both laboratory workflows being compared according to the manufacturer's instructions. Following this study, eight mock casework samples were tested with both workflows on separate sequencing runs. Data were analyzed using default analytical threshold settings using software platforms recommended in the manufacturer's instructions. Preliminary results indicated full DNA profiles were obtained for autosomal Short Tandem Repeats (STRs) at lower amplification input levels in samples processed with the PowerSeq™ workflow than samples processed with the ForenSeq™ DNA Signature Prep kit. Similar trends were observed with the mock casework samples. Genotypes for each marker were deemed concordant when the correct allele or alleles were the most abundant for homo- and heterozygous loci, respectively. Correct alleles were obtained from capillary electrophoresis data previously generated.

It is recommended that more sensitivity and forensic casework-like studies be performed within the community to further supplant these initial findings. This assessment will be valuable for the forensic casework and academic research communities in order to demonstrate the capability of NGS to assist in their selection of an appropriate methodology. This preliminary study can assist laboratories that are seeking to integrate this new technology to gain an initial idea of which kit and workflow are best suited for their needs.

Next Generation Sequencing, Sensitivity Assessment, Validation

B49 Optimization of a Droplet Digital™ Polymerase Chain Reaction (ddPCR™) Assay for the Quantitative and Qualitative Analysis of Illumina® MiSeq® Massively Parallel Sequencing (MPS) Libraries

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After attending this presentation, attendees will better understand ddPCR™, a cutting-edge technique used to quantify nucleic acids. Focus will be placed on the use of ddPCR™ to accurately quantify prepared MPS libraries. Additionally, the use of ddPCR™ as a way to determine the average length of fragments in an MPS library will be discussed.

This presentation will impact the forensic science community by describing a newly optimized method that enables laboratories to fully assess prepared MPS libraries. This level of library assessment results in the generation of large amounts of high-quality data without sample or sequencing run failures. Furthermore, the assay described has been optimized to reduce the potential for contamination.

MPS methods are quickly being adopted by the forensic community for analysis of precious evidentiary samples. These methods are capable of generating an unprecedented amount of data, particularly when analysis is performed using commercially available highly multiplexed panels designed to target hundreds of loci per amplification. Accurate qualitative and quantitative assessment of prepared MPS libraries is of paramount importance for obtaining maximum yield of high-quality data from a sequencing run. Many vendor-recommended protocols suggest assessment of the final library using fluorometric methods, agarose gel or chip-based electrophoresis, or quantitative PCR (qPCR); however, these methods can be problematic because they often: (1) result in over- or underestimation of library DNA concentrations; (2) do not enable estimation of the size of DNA fragments in the library, which can lead to incompatible kit selection; and, (3) are not typically specific for those fragments that are MPS-ready.

A ddPCR™ is similar to qPCR in that target-specific primers and 5' nuclease probes are utilized for detection following an end-point PCR reaction; however, due to the nature of the method, no standard curve is needed for estimation of DNA concentration. With ddPCR™, a 20µL aqueous PCR reaction is emulsified into 1nL uniformly sized droplets. Each droplet is then counted as fluorescence positive or negative and a Poisson correction is applied to estimate the starting copy number of DNA fragments in the sample. In addition, previous literature has shown that droplet florescence intensity is dependent upon the average length of the fragments being assessed.[1] Longer fragments tend to result in lower droplet fluorescence intensities than shorter fragments due to the kinetics and stoichiometry of the PCR reaction. As a result of this observed fluorescence intensity:fragment-size correlation, Laurie et al. have developed a ddPCR™ assay that allows for simultaneous quantitative and qualitative assessment of MPS libraries.[1] The assay specifically targets MPS platform-specific library modifications (i.e., flow cell adapter sequences) to enable quantification of only those fragments that are sequenceable. The assay also includes the use of a series of size standards to facilitate estimation of average length of fragments in the prepared library; however, the standards are derived from a commercially available agarose gel electrophoresis ladder. The reported preparation is time-consuming, labor intensive, and may give rise to low-level contamination evident in MPS data.

This presentation reports an optimization of the aforementioned assay using synthetically prepared size standards. Initially, a series of oligonucleotides with known sizes ranging from 25bp to 700bp was designed to consist of PhiX DNA with Illumina® MiSeq® sequencing primer flanking regions. The oligonucleotides were designed using PhiX to reduce possible run contamination from exogenous sources. PhiX is supplied for use as an MPS control, and data generated from any part of the PhiX genome is easily identified and bioinformatically filtered from raw data. The sequencing primer region serves as a primer binding site to allow for additional incorporation of barcoding indices and flow cell adapters into the synthetic oligonucleotide during the limited-cycle PCR step. Final products are then normalized for reaction input of 10,000 copies to avoid fluorescence intensity variability due to copy number and not length. Average observed droplet fluorescence intensities range from 13,157RFU (+/- 203.3) for the 25bp standard to 3,860.4RFU (+/-352.3) for the 700bp standard. The standard series appears to be efficient at predicting the average size of Illumina® MiSeq® libraries while avoiding quantification of adapter dimers and other artifacts often generated during library preparation. This increases first-pass Illumina® MiSeq® run success.

Reference(s):

1. Laurie M.T., Bertour J.A., Taylor S.D., Burton J.N., Shendure J.A., Bielas J.H. Simultaneous digital quatification and fluorescence-based size characterization of massively parallel sequencing libraries. *Biotechniques* 2013; 55:61-67.

Quantitative PCR, Droplet Digital™ PCR, Massively Parallel Sequencing

B50 Separating Complex DNA Mixtures Containing Related Individuals Using TrueAllele® Mixture Interpretation Software

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After attending this presentation, attendees will better understand an analysis method that reliably separates (deconvolutes) familial DNA mixtures of two to five individuals using TrueAllele® technology. Furthermore, this presentation demonstrates the difficulty of deconvolution of mixtures with high levels of allele sharing at both optimal and low levels of DNA.

This presentation will impact the forensic science community by demonstrating the effectiveness of a reliable tool that can be used for the interpretation of complex familial DNA mixtures, as well as the benefits of automated DNA analysis.

According to the preliminary 2015 report from the Federal Bureau of Investigation (FBI), violent crimes have increased 1.7% from the reported 1,165,383 violent crimes in 2014.[1,2] Major devastating events such as the Boston Marathon bombing and incest sexual assaults are only some of the recent occurrences that have bombarded laboratories with biological evidence. In the eyes of forensic scientists, many of these crimes have two matters in common: related individuals and complex DNA mixtures. DNA mixtures can pose multiple analysis issues; some of these issues stem from degraded DNA, small amounts of DNA (Low-Template (LT-DNA)), and the number of contributors in the mixture.[3,4] Using manual interpretation, the complexity of DNA mixtures often leads to inconclusive reports.[5]

The use of computer technology in the past ten years has successfully simplified DNA mixture interpretation. Innovative forensic Short Tandem Repeat (STR) commercial software available today is primarily used to separate contributors in a mixture. Recent studies are being conducted to validate these systems, especially for deconvoluting mixtures of more than two contributors; however, complex mixtures limit this new technology, especially when contributors share genetic material as a result of being from the same biological family.[3,4] There has been limited published research on how familial DNA mixtures affect automated interpretation.

This research focuses on the analysis of DNA mixtures containing related individuals and the prospects of using the continuous probabilistic STR analysis software TrueAllele® for statistical calculations in complex mixtures containing up to five individuals. It is proposed that TrueAllele® technology will resolve complex DNA mixtures containing three to five related individuals, generate Likelihood Ratio (LR) match statistics suitable for court, and reproduce those match results to show reliability in automated analysis.

Buccal swabs were collected from individuals in three separate families to create single-source DNA profiles. These samples were extracted using the DNA IQ™ System and quantified using Real-Time Polymerase Chain Reaction (RT-PCR) with Applied Biosystems® Quantifiler® assay. Based on the family pedigrees and the quantification results, 25 mixtures of two to five contributors were created using related individuals from those families. These mixtures were then replicated and diluted to provide mock LT-DNA samples. After amplification using the PowerPlex® Fusion STR kit and genotyping on Applied Biosystems® 3130 genetic analyzer, the mixtures were analyzed using TrueAllele®. The statistics produced across all mixtures were compared to determine if TrueAllele® could separate mixture contributors, regardless of family origin. Reproducibility among triplicate runs was assessed within each mixture set to ensure the software was correctly separating the contributors and producing similar match statistics. Finally, the degree of shared alleles in a mixture (in relation to the produced match statistics) was compared.

Analysis of familial DNA mixtures provides an insight to the complexity of interpretation in a situation in which allele sharing is at its highest. Obtaining accurate and unbiased results with the help of automated analysis could lead to further advancements in other methods, such as kinship analysis and paternity testing. Conclusions from this study may not only provide a reliable tool for forensic scientists to analyze complex familial mixtures, but may also standardize the analysis process and decrease the number of inconclusive reports.

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DNA Mixtures, Familial DNA, TrueAllele®

B51 Microhaplotypes (MHs): A Possible Biogeographic Ancestry Predictor for Forensic DNA Evidence

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After attending this presentation, attendees will understand MHs and the potential that MH analysis has in predicting the biogeographic ancestry of an individual.

This presentation will impact the forensic science community by suggesting how MHs can enhance mixture interpretation and ancestry prediction capabilities, potentially providing investigators with an extra tool for identifying and/or eliminating suspects.

MHs are short regions (<250 base pairs long) that have two or more Single Nucleotide Polymorphisms (SNPs) with three or more allelic combinations. Given the short distance between SNPs in MHs, recombination is highly unlikely. Furthermore, the mutation rate is significantly less than that of Short Tandem Repeat (STR) markers, which makes them a better candidate for familial testing. Contrary to STRs, the amplification of MHs is not affected by polymerase slippage; given the absence of a short sequence repeated multiple times, this feature eliminates the presence of a common STR analysis artifact: stutter. With conventional SNP typing methods, such as Sanger sequencing, TaqMan®, and SNaPShot®, haplotypes cannot be determined. Using Massively Parallel Sequencing (MPS) methods, the *cis/trans* relationship between SNP alleles within the same amplicon (i.e., haplotype detection) can be accomplished. MPS-based MH analysis can be a valuable forensic tool for parentage testing and mixture deconvolution, particularly in mixtures where the minor contributor (or contributors) is in the stutter range of the major. The small amplicon size also makes MHs a good candidate for typing degraded DNA samples. Although initially selected based on the highest effective number of alleles across populations, MH allele frequencies vary significantly in different populations. The goal of this project was to evaluate if a selected panel of MHs, together with individualization, could also effectively predict biogeographic ancestry.

A panel of 33 MHs was used on 98 individuals who self-identified as European-Americans. Samples were sequenced on an Ion S5 MPS platform using a 530 chip. The initial library preparation was conducted manually but the templating step was carried out on an Ion Chef™. Allele frequencies from a database of 58 different populations were used to calculate the Random Match Probability (RMP) of each profile in each individual population. A biogeographic prediction was performed by evaluating the raw RMP values for each population and whether or not the highest RMP was in a European population. A second method to evaluate biogeographic inference was by calculating a Likelihood Ratio (LR) determined by dividing the probability of the highest (as in most common) RMP in question by the RMP of the population chosen for comparison.

Of the 98 individuals, only nine were found to have their highest RMP in a non-European population. All nine were within one order of magnitude or less. For these nine individuals, the highest RMP was calculated in a Middle Eastern population. European populations and Middle Eastern populations are generally considered admixed.

The LR was calculated for all 98 individuals by placing the European-American RMP in the numerator and RMPs of Yoruba, African-American, Cambodian, San Francisco Chinese, and Arizona Pima populations in the denominator as proxies for the major United States populations. The value of the LR obtained represents how much more likely it is to observe that profile if the individual is of European descent versus one of the other populations. The average LR was ~3 E^{18} for European-Yoruba or -Pima, only a magnitude greater for European-Cambodians, but three orders greater for the European-San Francisco Chinese at E^{21}. The European-African-American was the smallest range from 556 to 5.5 E^{14} while the European-San Francisco Chinese was the greatest from 208 to 6.3E^{23}. The European-Pima and European-Yoruba LRs were similar from the 10,000s to E^{20}. For the 98 samples, 94 LRs were above the arbitrary level of confidence of 1,000. The lowest value in the four remaining was 208 (European/-San Francisco Chinese), and the second lowest value was 556 (European-African-American). These four profiles also had high frequencies in other non-European populations, suggesting they may have originated from admixed individuals.

Further research is necessary to identify more effective ancestry informant MHs. Yet, these results support the hypothesis that MHs can be used for biogeographic ancestry prediction, which can provide investigators with

useful information in cases in which the STR profile from crime scene evidence does not match any suspects or database profiles.

Microhaplotypes, Forensic, Ancestry

B52 Rapid DNA Amplification of Forensic Samples

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After attending this presentation, attendees will better understand various principles of genetic analyses on forensic samples and the challenges related to this type of investigation, especially concerning bone remains.

This presentation will impact the forensic science community by highlighting the importance of reducing the forensic DNA-typing workflow time through the possibility of dramatically speeding up the Polymerase Chain Reaction (PCR) amplification procedure. In this regard, the rapid PCR protocol described was shown to be effective for single-source forensic samples, allowing the procurement of reliable Short Tandem Repeat (STR) profiles, not only from saliva and blood collected at the crime scene, but even from challenging bone samples recovered in extremely critical environmental conditions.

Forensic DNA typing typically involves a multi-step workflow, including DNA isolation, quantification, amplification of a set of STR markers, separation of PCR products by Capillary Electrophoresis (CE), and DNA profile analysis and interpretation. Considering all these steps, the process can take approximately ten hours. The PCR amplification portion of the workflow typically takes approximately 1.5 to 3 hours with standard thermal cycling protocols. For many peculiar and critical forensic caseworks, it would be extremely helpful to speed up this process and allow the generation of an interpretable, DNA profile-suitable DNA database search within a few hours.

Through the use of a faster DNA polymerase coupled with the use of a rapid thermal cycler, the amplification cycling times were reduced down to as few as 16 minutes using PCR primers from the commercially available multiplex STR typing kit AmpFℓSTR® Identifiler® Plus. This rapid protocol employs the commercially available TaKaRa Z-Taq™ DNA Polymerase and the Philisa® Streck Thermal Cycler. A previously described three-step thermal cycling protocol was evaluated on single-source DNA extracts from saliva, blood, and bone samples. In addition, a comparison among different DNA extraction methods (i.e., FTA™, Chelex®, Promega® DNA IQ™ System, and MP Biomedicals Geneclean® For Ancient DNA Kit) was conducted due to the constantly negative typing results obtained by samples extracted with the Chelex® method, then subjected to the rapid PCR protocol.

Capillary electrophoresis characterization of the PCR products indicated good peak balance between loci, strong signal intensity, and minor adenylation and PCR artifacts. Genotyping results were concordant with standard amplification conditions utilizing a standard thermal cycling procedure with a GeneAmp® 2720 PCR System. Assay conditions were robust enough to routinely amplify 250pg to 500pg of template DNA.

Forensic DNA Typing, Rapid PCR Amplification, Forensic Samples

B53 Using Rapid DNA Analysis to Obtain DNA Profiles From Improvised Explosive Devices (IEDs)

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After attending this presentation, attendees will better understand the potential for using Rapid DNA analysis to obtain DNA profiles from low-level samples. This presentation compares the performance of traditional versus Rapid DNA analysis on low-level samples. This presentation presents data from both traditional and Rapid DNA methods regarding quantity and quality of DNA recovered from various components of IEDs pre- and post-deflagration.

This presentation will impact the forensic science community by determining whether Rapid DNA analysis can produce detectable DNA profiles from low-level samples and by determining the limitations of Rapid DNA analysis. Using Rapid DNA analysis, it is possible to produce a DNA profile in less than two hours. Therefore, this presentation will assist analysts in determining whether it is appropriate to use Rapid DNA analysis methods on low-level samples when time is of the essence, especially when generating investigative leads.

DNA recovery from IEDs was evaluated using traditional and Rapid DNA analysis to compare the performance of both methods. IEDs were chosen because the DNA recovered is low level by nature (touch), often degraded (heat and pressure), and time may be critical when obtaining a profile due to the potential danger of further detonations. In the first stage of the study, mock IEDs were assembled using previously cleaned materials to assess each method's potential for successfully generating a Short Tandem Repeat (STR) profile. The external surfaces and wire twists of the mock devices were swabbed using a double-swab technique (moistened and dry). In addition, DNA samples from tape were collected using a single swab moistened with QIAGEN® ATL tissue lysis buffer. Duplicate swabs were used and processed using traditional DNA analysis and Rapid DNA analysis methods. All samples were produced at least in triplicate. In the second stage of the study, the first stage was repeated using actual IEDs that were deflagrated by professionals in a controlled setting rather than using assembled mock IEDs.

For traditional DNA analysis, standard methods utilized in forensic laboratories including extraction (QIAamp® DNA Investigator), quantification (Investigator® QuantiPlex™), and amplification (PowerPlex® Fusion) were used. Samples were separated and detected using a 3130*xl* and analyzed using GeneMapper®ID 3.2.1 software. For Rapid DNA analysis, the NetBio DNAscan (BioChipSet™ Cassette) was used. Materials that were cleaned for mock IED assembly were checked for background DNA prior to assembly. Each component swabbed was assessed by the average recovered yield of DNA from the entire surface area of the component. Additionally, sample collection from tape was assessed by the average recovered yield of DNA per cm^2. DNA profiles produced by both methods were then generated and analyzed by comparing sample profiles to reference profiles obtained from the known device assemblers (mock and real) and by comparing sample profiles to each other. Profiles were assessed by examining the total number of alleles out of the possible 45 and the total number of complete loci out of 23. Upon analyzing the data for the samples collected from the mock devices, traditional methods yielded complete and concordant profiles from the tape and the external surfaces. Also, traditional methods yielded either no results or partial concordant profiles from the wires. Rapid DNA analysis yielded either no results or partial profiles from the components. Initial results with the DNAscan on mock IEDs indicated that samples collected from deflagrated devices may yield reduced information when processed with Rapid technology instruments.

The goal of this research was to determine whether Rapid DNA analysis can be used on time-sensitive evidence that most likely yields low-level DNA rather than using traditional DNA analysis in order to decrease the time it takes to produce a DNA profile and potential investigative lead. Overall, traditional DNA analysis performed better on the low-level samples; however, Rapid DNA analysis did produce partial profiles on some samples.

Rapid DNA, Improvised Explosive Device, Low-Level DNA

B54 The Characterization and Differentiation of 21 Fentanyl Analogues by Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Mass Spectrometry (LC/MS), and Nuclear Magnetic Resonance (NMR) Spectroscopy

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After attending this presentation, attendees will gain a deeper knowledge regarding the analysis of fentanyl analogues. This analysis includes the separation and identification of the structural and geometric isomers of fentanyl analogues and the unusual behaviors exhibited by several fentanyl analogues evaluated by NMR spectroscopy.

This presentation will impact the forensic science community by providing the analytical data of 21 fentanyl analogues, including the structural and geometric isomers, by GC/MS, LC/MS, and NMR spectroscopy.

Fentanyl is a powerful synthetic analgesic developed in the 1960s. To date, a wide variety of fentanyl analogues, such as α-methylfentanyl and 3-methylfentanyl, have been abused all over the world. In the present study, 21 compounds of fentanyl analogues, including structural and geometric isomers, were analyzed by GC/MS, LC/MS, and NMR spectroscopy.

GC/MS conditions: column, capillary column coated with 5% phenyl methylpolysiloxane (30m × 0.25mm i.d.); oven temperature, 120°C (1min hold) and programmed up to 300°C at a rate of 15°C/min; injection port temperature, 250°C; carrier gas, helium; sample injection, splitless mode; ionization, Electron Ionization (EI); electron energy, 70 eV. LC/MS conditions: column, ODS column (2.1 × 150mm); mobile phase composition, 10mM ammonium acetate (A) and methanol (B); linear gradient mode, 80% A and 20% B to 10% A and 90% B over 18min, followed by a 2min hold at 10% A and 90% B, and returned to 80% A and 20% B in 0.1min; flow rate, 0.20ml/min; MS interface, positive Electrospray Ionization (ESI). NMR conditions: proton resonance frequency, 600MHz; solvent, $CDCl_3$ or CD_3OD; internal standard, tetramethylsilane.

In GC/MS, fentanyl analogues, except for fentanyl and acetyl-α-methylfentanyl, were separated on the Extracted Ion Chromatograms (EIC) of the characteristic fragment ions of each compound. Fentanyl and acetyl-α-methylfentanyl were separated by slowing the rate of the temperature increase of the column to 3°C/min. Geometric isomers (*cis-trans* isomers) of 3-methylfentanyl analogues were completely separated by GC. The thermal decomposition of β-hydroxyfentanyl should be closely monitored: a high-injection port temperature (>250°C) induced the cleavage of the C-N bond of β-hydroxyfentanyl to form norfentanyl. The thermal decomposition of β-hydroxyfentanyl was completely suppressed by Trimethylsilyl (TMS) derivatization of the hydroxyl group.

In LC/MS, most of the fentanyl analogues were separated on the EICs of the protonated molecule of each compound. It is notable that the two diastereomers of β-hydroxy-*cis*-3-methylfentanyl were separated by LC, but not by GC; however, it was difficult to separate the diastereomers of β-hydroxy-*trans*-3-methylfentanyl by GC and LC in this study.

Several 3-methylfentanyl analogues exhibited unusual behavior when analyzed by ^{1}H-NMR. Specifically, the proton signal of the 3-methyl group of acetyl-*cis*-3-methylfentanyl hydrochloride split at a ratio of 3:2, when this compound was dissolved in $CDCl_3$. The same phenomenon was observed in the cases of *cis*-3-methylfentanyl hydrochloride and *cis*-3-methylthiofentanyl hydrochloride. In contrast, when these compounds were dissolved in CD_3OD, the proton signal did not split. This phenomenon may be explained as follows: the proton of the hydrochloride coordinated to the nitrogen of the piperidine ring when the hydrochloride salts of the 3-methylfentanyl analogues were dissolved in a non-polar solvent, such as $CDCl_3$. A pair of stereoisomers were formed by the coordination, and the split of the proton signal of the 3-methyl group was observed.

Fentanyl, Isomer, Analysis

B55 An Investigation Into the Preservation and Storage Conditions for Extracts of Ignitable Liquid Residues

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After attending this presentation, attendees will understand how the storage conditions of activated charcoal strips can affect the preservation of sample extracts of ignitable liquid residues from fire debris samples. Attendees will be provided with a short review of past documented preservation techniques and ideas as to what practitioners can do to create a consensus in the field.

This presentation will impact the forensic science community by providing information on storage and reanalysis conditions of carbon disulfide extracts of residues preserved on activated charcoal strips.

Fire debris analysis presents a constant challenge to those who investigate the scene. Practitioners responsible for determining what ignitable liquid may have been used to intentionally set a fire have a difficult challenge. It is crucial for practitioners to not only correctly identify which ignitable liquid is present, if any, but to also preserve extracts for future analysis and peer review. Forensic scientists commonly use passive headspace to sample and concentrate ignitable liquid residues onto activated charcoal strips. There have been some studies conducted on this topic, but little work has been reported on the preservation and reanalysis of activated charcoal strips that have been previously extracted with carbon disulfide. Presently, there is no accepted standard practice for storing and preserving these extracts. The American Society for Testing and Materials (ASTM) E2451-13 states "… changes to a preserved sample extract and the length of time it remains viable under storage conditions are unknown".[1] Clear standards and validation of the conditions for preservation of the extracts should be documented and universally accepted within the field.

In this study, Gas Chromatography/Mass Spectrometry (GC/MS) was used to analyze passive headspace extracts to study the changes of a Standard Accelerant Mixture (SAM) adsorbed onto activated charcoal strips and preserved under different storage conditions. The SAM consisted of a mixture of 1:1:2 ratio of gasoline, kerosene, and diesel fuel. Two different types of chromatographic vials were compared, screw cap vials and snap cap vials. Four different storage conditions for the extracts were studied: (1) room temperature (25°C); (2) refrigeration (4°C); (3) freezer (-20°C); and, (4) freeze and thaw cycles. The study examined three different conditions in regard to the preservation of the sample in a chromatographic vial during analysis: (1) no change to the septum after each injection of multiple injections; (2) septum replaced after each injection; and, (3) cap removed and carbon disulfide evaporated in order to reconstitute with carbon disulfide once the strip is dried. Area normalization of peak abundances was used to calculate recovery and reproducibility of GC patterns. Chromatographic peaks used for the quantitative comparison were validated through mass spectral analysis and comparison to library standard reference spectra.

The results indicate ignitable liquid residue passive headspace-activated carbon strip samples extracted with carbon disulfide may be dried and reconstituted at least two times with no loss of sample integrity or diagnostic chromatographic peaks used for identification. After three evaporation/reconstitution cycles, lighter constituents began to evaporate and affect the chromatographic validity of the data. Sample vial, storage temperature, and conditions are crucial aspects of fire debris sample preservation. Further studies are necessary to understand how the sample extracts should best be stored and preserved in order to test them in the future.

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Fire Debris, Ignitable Liquid Residues, Sample Extract Preservation

B56 Optimizing a Sequence of Methods for the Development of Latent Fingerprints on Thermal Paper

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After attending this presentation, attendees will be more knowledgeable regarding the best techniques that are currently available to process fingerprints on thermal paper. Attendees will understand the difficulties of working with thermal paper, how well current methods work, and how methods compare to each other.

This presentation will impact the forensic science community by illustrating what current techniques work the best with thermal paper substrates. This presentation will provide information on how well current thermal paper processing techniques work, separately and in sequence, as well as whether any methods stand out in terms of accuracy, consistency, and ease of use.

Thermal paper has been known to be a tedious substrate in latent fingerprint laboratories. Although it is considered a porous substrate, techniques that are commonly used to develop fingerprints on porous items have shown to be unsuccessful on thermal paper. A major issue is that chemicals used in these processes, as well as the common application of heat, can interact with the components of the paper, activate it, and darken the entire surface. The darkening of the paper makes the visualization of existing latent fingerprints a difficult task. Recently, numerous procedures have been created to successfully develop fingerprints on thermal paper evidence without interacting with the thermal properties. For other porous substrates, a sequence of methods may be followed to ensure that all existing fingerprints have been found' however, since thermal paper requires special techniques, a known sequence does not currently exist and laboratories may only utilize one method. If a latent fingerprint examiner solely uses one method for thermal paper evidence, the examiner may be unaware of fingerprints that were present but failed to develop. The examiner may also be unaware that a combination of these methods may yield better results than one method alone.

The goal of this study was to determine if a sequence of current processing techniques (1,2-indanedione with zinc chloride, PDMAC® paper, muriatic acid fuming, application of heat, and ThermaNin®) could be optimized to allow an analyst to state with confidence that all existing fingerprints have been found.

Nine sequences were created from the combination of the five methods previously mentioned. Each sequence was performed on known fingerprints that were 4 weeks, 3 weeks, 2 weeks, 1 week and 24 hours old. It was found that three out of the five techniques (1,2-indanedione with zinc chloride, PDMAC® paper, and ThermaNin®) developed fingerprints on both the thermal and non-thermal sides of the paper. It was found that 1,2-indanedione with zinc chloride and PDMAC® paper developed the highest number of fingerprints consistently. In many cases, treatment with PDMAC® paper directly after 1,2-indanedione with zinc chloride seemed to allow visualization of additional prints and enhanced fluorescence. This modified method was then applied to real receipt samples that were treated as mock evidence. In most cases, the same results occurred after treatment with 1,2-indanedione with zinc chloride and PDMAC® paper, but there were some samples where an additional latent print was seen after PDMAC® paper treatment. Lastly, it was studied whether magnetic powder, a common starting point in latent print processing sequences, interferes with other techniques. Fingerprints were developed with either plain black magnetic powder or fluorescent magnetic powder, followed by additional processing by either 1,2-indanedione with zinc chloride, PDMAC® paper or ThermaNin®. The only method that showed additional fingerprint development was 1,2-indanedione with zinc chloride on non-thermal side fingerprints previously developed with plain magnetic black powder; however, the black magnetic powder hindered the fluorescence.

In conclusion, the use of 1,2-indanedione with zinc chloride and PDMAC® paper allowed the highest number of prints to be visualized. In some cases, combining these two methods allowed previously missed fingerprints to be visualized. These worked best when other techniques were not previously used. These techniques were simple, required little preparation, and could be left alone to develop while performing other tasks. The use of either of these two methods alone would be sufficient on thermal paper samples, but using them in sequence may increase the likelihood that all fingerprints present have been found.

B57 Shooting Distance Estimation Using Gunshot Residue (GSR) on Mammalian Pelts

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After attending this presentation, attendees will understand how GSR is used for shooting distance estimation and the complications presented by mammalian fur. The various methods that are used to overcome interferences created by fur will be explained. These methods include some of the traditionally used visual and chemical methods, a few of which have been adapted from their traditional form.

This presentation will impact the forensic science community by providing methods that are useful in the field of veterinary forensic science when animals are subject to crimes involving firearms. This presentation will also provide additional methods of enhancing GSR patterns on surfaces that interfere with the commonly performed GSR tests.

GSR is produced from the discharge of a firearm and, for the purpose of this research, GSR includes any residue originating from the propellant, primer, projectile, cartridge case, residues from previous shots, and cleaning agents or lubricants present in the barrel that travel with the bullet to create a pattern on the target. The patterns, both visible and enhanced, may permit an estimation of muzzle-to-target distance to aid in reconstruction of firearm-related events. In cases involving animals, visualization of GSR is complicated by fur color and length. This requires visual and chemical techniques to be adapted so enough contrast is created to assist with muzzle-to-target distance estimation.

Initial photography of the pattern permits visualization, but the periphery shows little contrast. The use of a high-intensity, tunable-wavelength light source may excite some GSR, resulting in fluorescence, thereby increasing contrast between the fur and GSR. Infrared (IR) light can also be used to enhance GSR patterns using a specialized IR camera or an IR viewer. Radiography can be employed to detect the presence of radiopaque metallic particles surrounding the entrance hole. Once all methods of visual enhancement are complete, the Modified Griess test (MGT) can be used to detect the presence of nitrites.

Cow and rabbit hides were shot from a range of distances from contact to three feet with jacketed and unjacketed ammunition using handguns of various calibers, including a .38 Special, a 9mm, and a .45 Automatic Colt® Pistol (ACP). Visualization with white light and IR light shows patterns increasing in size as muzzle-to-target distance increases. In some instances, the presence of a radiopaque ring, presumably metallic lead, around the entrance hole was detected with radiography. The ability to visualize this ring can be enhanced by adjusting characteristics of the image, allowing the radiopaque ring to be visualized at greater distances. As expected, both observable features begin to fade with increasing muzzle-to-target distance. The MGT was conducted using filter paper rather than photographic paper and applying the heat to cheesecloth on the backside of the filter paper. Results of the MGT are consistent with the visual methods, as the patterns increase in size and become more dispersed with increased firing distance.

Gunshot Residue, Mammalian Pelts, Shooting Reconstruction

B58 The Development of an Infrared Microspectroscopy Method for Microcrystals

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After attending this presentation, attendees will better understand the combined role of infrared microspectroscopy and microcrystalline tests for the analysis of drug evidence.

This presentation will impact the forensic science community by creating a deeper understanding of the reliability and validity of an infrared microspectroscopy method for the analysis of drug microcrystals. This presentation describes the challenges and solutions associated with growing microcrystals on substrates suitable for transmission and reflectance infrared analysis.

Microcrystalline tests have long been used by forensic chemists for the analysis of drugs, but there is continued debate regarding the extent of their discriminatory power. The debate lies in the fact that the chemistry of the microcrystals is not completely understood, although characteristic crystals are obtained for some substances. Lack of tangible instrumental data to confirm the microcrystal identity has steered analysis schemes away from these tests; however, microcrystalline tests are advantageous for several reasons. They are easy to perform, require virtually no sample preparation, use negligible amounts of solvents, require microgram quantities of test samples, are cost-effective, very sensitive, are non-destructive and, most importantly, very rapid. Analytical techniques that can combine the simplicity of the microcrystalline tests and molecular structure information would be very useful.

Infrared spectroscopy is widely used in forensic laboratories for the analysis of drug evidence and is classified as a category A technique in the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) analytical techniques, affording the highest discriminating ability. Infrared spectroscopy is a valuable tool for differentiating between isomers based on spectral differences. Combining infrared microspectroscopy with drug microcrystals will provide unambiguous confirmation of the crystal identity, thereby removing the subjectivity often seen as a weakness of microcrystalline tests.

Infrared microspectroscopy has been previously demonstrated for the analysis of drug microcrystals; however, there are challenges associated with obtaining quality spectra with this technique.[1,2] Three modes of Fourier Transform Infrared Spectroscopy (FTIR) are commonly used: transmission, reflectance, and Attenuated Total Reflectance (ATR). ATR is destructive to the crystals and spatial resolution is poor. The microcrystalline reagents are often acidic and are incompatible with the traditional infrared substrates used for transmission and reflectance studies. A detailed study to improve the understanding of this technique will help analysts adopt this technique in their analytical schemes for drug evidence.

This presentation describes the challenges associated with analyzing microcrystals in the different infrared spectroscopy modes. This presentation compares the quality of spectra obtained with gold-coated slides to the MirrIR low-e microscope slides in the reflectance mode. In the transmission mode, barium fluoride windows are compared to the novel Amorphous Material Transmitting Infrared Radiation (AMTIR) windows. Differences in the vibrational bands of the drug-reagent microcrystal are observed when compared to the drug-only spectra. These differences are documented and quantified. In addition, procedures to reduce interference from the reagent in solution around the microcrystal are presented. These steps improve the quality of spectra obtained for the drug-reagent microcrystals. This presentation discusses the validity and reliability of the infrared microspectroscopy method for microcrystals analyzed in different modes.

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2. McCrone Research Institute. *A Modern Compendium of Microcrystal Tests for Illicit-Drugs and Diverted Pharmaceuticals.* 2015.

Infrared Microspectroscopy, Microcrystals, Emerging Drugs

B59 Ion Trap Mobility Spectrometry Nuisance Alarm Threshold Analysis for Illicit Narcotics Based on Environmental Background and a Receiver Operating Characteristic (ROC) -Curve Approach

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After attending this presentation, attendees will understand the effect environmental background has on the sensitivity and specificity of Ion Mobility Spectrometry (IMS) narcotics trace detection.

This presentation will impact the forensic science community by demonstrating the discriminative potential of an IMS for trace detection of illicit narcotics relative to the environmental background.

The rapid and sensitive detection of illicit narcotics remains vital to a multitude of law enforcement, corrections/prisons, customs and border protection, and transportation agencies. Laboratory-based analytical techniques including Thin-Layer Chromatography (TLC), Gas Chromatography and Liquid Chromatography (GC and LC), Capillary Electrophoresis (CE), and Mass Spectrometry (MS) have been developed and utilized for the detection of a wide range of narcotic compounds; however, many of these techniques require additional laboratory infrastructure and are not conducive to field deployment or point-of-measurement screening. Colorimetric methods for the detection of narcotics have been developed and provide rapid detection in the field; however, these presumptive tests are subject to screener interpretation of the resulting color(s) and require onsite reagent mixing. In recent years, IMS has surged forward as a robust field-deployable analytical technique for the trace detection of a wide range of compounds, most notably explosives, Chemical Warfare Agents (CWAs), and illicit narcotics.

The discriminative potential of an ion mobility spectrometer for trace detection of illicit narcotics relative to environmental background was investigated with a Receiver Operating Characteristic (ROC) curve framework. The IMS response of cocaine, heroin, methamphetamine, 3,4-Methylenedioxymethamphetamine (MDMA), and Δ^9-Tetrahydro-Cannabinol (THC) was evaluated against environmental background levels derived from the screening of incoming delivery vehicles at a federal facility. More than 20,000 samples were collected over a multiyear period under two distinct sets of instrument operating conditions, a baseline mode and an increased desorption/drift tube temperature and sampling time mode. ROC curves provided a quantifiable representation of the interplay between sensitivity (True Positive Rate (TPR)) and specificity (1 – False Positive Rate (FPR)). A TPR of 90% and minimized FPR were targeted as the detection limits of IMS for the selected narcotics. MDMA, THC, and cocaine demonstrated single nanogram sensitivity at 90% TPR and <10% FPR, with improvements to both MDMA and cocaine in the elevated temperature/increased sampling mode. Detection limits in the tens of nanograms with poor specificity (FPR ≈ 20%) were observed for methamphetamine and heroin under baseline conditions; however, elevating the temperature reduced the background in the methamphetamine window, drastically improving its response (90% TPR and 3.8% FPR at 1ng). On the contrary, the altered mode conditions increased the level of background for THC and heroin, partially offsetting observed enhancements to desorption. The presented framework demonstrated the significant effect environmental background distributions have on sensitivity and specificity. The implementation of ROC curves demonstrated the significant effect environmental background has on the sensitivity and specificity of IMS trace detection. The particular distribution of environmental background for each screening arena must be considered when evaluating the detection and discriminative potential of a methodology.

IMS, ROC Curve, Narcotics

B60 Primary Ion Middle Ion Structure Analysis (PIMISA®) -Enabled Direct Analysis in Real-Time (DART®) QDa Mass Spectrometer (MS): The Latest Development in Forensic Analysis Efficiency

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The goals of this presentation are to: (1) describe the reverse search data analysis software, PIMISA®; (2) understand the capabilities of the DART® source, Waters Acquity QDa mass detector, and PIMISA® software; and, (3) recommend workflow that allows scientists to produce high-quality, efficient, and economic drug screening analyses in minutes.

This presentation will impact the forensic science community by introducing an innovative and cost-effective drug screening approach, PIMISA®-enabled DART® Acquity QDa® mass detector, and by recommending a potential workflow for increased efficiency.

The new combination of the reverse search library program, PIMISA®, and DART® -equipped compact mass detector offers an efficient, highly selective, and cost-effective drug screening approach for compound identification in forensic laboratories.

PIMISA®-enabled DART® QDa MS is evaluated for implementation into forensic workflow. The instrument exploits Collision Induced Dissociation (CID) to collect four spectra with varied fragmentation. The PIMISA® program analyzes these spectra, identifying analytes through library matching. The library contained spectra for 170 certified reference materials. This approach was evaluated through accuracy, specificity, limit of detection, experienced versus inexperienced users, and correlation studies. In all studies, samples were analyzed in triplicate with an analysis time of two to five minutes per sample, including data analysis.

Accuracy studies were performed through analyses of 70 adjudicated case specimens (pills, powders, plant materials, etc.) over a 4-day period by an experienced user. Samples were previously analyzed by traditional methodologies (visual identification, microscopy, gas chromatography/mass spectrometry, etc.) and reported to contain up to four drugs. The specificity study was evaluated for six sets of analytes, two pairs in three categories, at concentration ratios ranging from 10:1 to 1:10. Categories were determined by molecular ion differences of 1, 2, and 10s of daltons. Analyte pairs included methadone/alprazolam, codeine/temazepam, methamphetamine/CMP, hydrocodone/oxymorphone, hydrocodone/acetaminophen, and cocaine/procaine. The Limit Of Detection (LOD) study was performed for six analytes representing the Alabama casework population with varying polarities. Two scientists, a novice user (<1 year experience) and an experienced user (5^{+} years experience), and two sample introduction methods (semi-automated versus manual) were compared for 20 randomly selected, adjudicated cases (not included in the previous 70) in the blind study. The semi-automated sample introduction method utilized wire mesh consumable (QuickStrip®) cards, while the manual method used fused capillaries. The correlation study was designed to compare a DART® Time Of Flight (TOF) /MS with the PIMISA®-enabled DART® QDa MS. An experienced user re-analyzed 20 of the 70 adjudicated case samples on the DART® TOF/MS and compared the data.

Results of the selectivity studies were calculated as percentages of "analyte detection" versus "negative" and ranged from 81% to 100%. In the specificity study, analyte identification of molecular ions within 1 and 2 daltons was not observed at certain concentration ratio differences and this ratio varied between analyte pairs. For the 1 dalton separation pairs, observed interference began at concentration ratios of 2:1 for methadone/alprazolam and 4:1 and 1:10 for codeine/temazepam. Concentration ratio interference for methamphetamine/CMP was observed at 2:1, while hydrocodone/oxycodone was observed at 5:1. No concentration ratio interference was observed for cocaine/procaine; however, hydrocodone/acetaminophen was observed at 4:1. The LOD study included analysis of methamphetamine, diphenhydramine, cocaine, alprazolam, methadone, and THC. The cutoff concentration results varied between 0.5µg/mL and 10µg/mL. The robustness of the approach was demonstrated by both sample introduction and user experience during the blind study. Results for the experienced user were consistent between both sample introduction methods with a screening acceptability (accuracy and false positive) of 90%. Results for the novice user ranged from 70% to 80% screening acceptability with manual sampling preferred. Data comparisons

of the PIMISA®-enabled DART® QDa MS to the DART® TOF/MS resulted in 89% accuracy.

The DART® Waters Acquity QDa mass detector enabled with PIMISA® demonstrated capabilities for detection of multicomponent mixtures in minutes. With screening acceptability exceeding 70% and selectivity results above 80%, the approach is determined to be robust and highly selective. Additionally, the platform is shown to be comparable to existing DART® TOF/MS workflows at half the capital investment. Therefore, the DART® QDa MS and PIMISA® approach is an efficient, highly selective, and economic platform for forensic drug screening.

DART® MS, PIMISA® Software, Forensic Drug Screening

B61 Latent Print Development on Duct Tape Using Rhodamine 6G/Tween® 20 Solution on Simulated Evidence Samples

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After attending this presentation, attendees will have an opportunity to investigate this process and, in the future, possibly bring to their laboratories a latent print developing process that will both reduce the processing time and the materials needed.

This presentation will impact the forensic science community by offering a possible alternative latent print developing process for adhesive tape with results of equal if not better quality to two currently used processing methods for gray duct tape.

Forensic examiners are able to obtain multiple forms of information from gray duct tape submitted as crime scene evidence. While this can include DNA and trace evidence, it is also possible to apply one of the oldest forensic disciplines: the examination and development of latent fingerprints. While there are multiple techniques used to process adhesive tape for latent prints, currently different procedures are employed for processing the adhesive side versus the non-adhesive side of the tape. A parent study to this research optimized a rhodamine 6G/Tween® 20 solution that was successful in allowing both sides of adhesive gray duct tape to be simultaneously developed for latent prints after cyanoacrylate fuming, therefore shortening the overall processing time and materials. The results obtained from the former study were dependent on the use of pristine tape samples not often received as evidence, whereas the focus of this research involves validating this optimized solution for use on tape samples representative of the conditions commonly found in real-life crime scenes.

The research presented summarizes the results obtained when samples of gray duct tape were prepared to simulate real-life evidence samples, separated, and subsequently processed for latent fingerprints. Tape sample preparation involved attaching sample adhesive side to adhesive side, adhesive side to non-adhesive side, and simulations meant to represent the binding of a victim. Tape separation techniques included the use of adhesive neutralizer un-du®, a freezing technique, and the application of liquid nitrogen. After separation, each tape sample was processed using one of three processing techniques. These included either the optimized rhodamine 6G/Tween® 20 solution, crystal violet, or P-Methoxybenzylamino-4-nitrobenz-zoxa-1,3-Diazole (MBD). Three troubleshooting studies compared how the rhodamine 6G/Tween® 20 solution interacted with seven different brands of gray duct tape, two different fuming techniques, and three different alternative light sources.

Results obtained supported the conclusion that the optimized rhodamine 6G/Tween® 20 solution is a successful method for developing latent fingerprints simultaneously on both sides of duct tape samples representing the condition often encountered as evidence to a crime.

Latent Prints, Duct Tape, Rhodamine 6G

B62 Do Hygiene Products Cause False Positives in Arson Investigations?

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After attending this presentation, attendees will better understand the frequency with which male hygiene products contain compounds that can interfere with Ignitable Liquid Residue (ILR) analysis. Through this presentation, attendees will learn that although several classes of hygiene products are very unlikely to contain traces of ignitable liquids, other products do contain potential interference that can complicate the interpretation of ILRs found on a suspect's clothing.

This presentation will impact the forensic science community by presenting information that may lead to the ability to help arson investigators arrive at better conclusions about the presence of ILRs on a suspect's clothing.

Arson investigators often search for the presence of ILRs in fire debris samples that are collected from suspected arson scenes and suspects. Such residues most frequently involve gasoline but can include other petroleum distillates, such as the kerosene. As described in the American Society for Testing and Materials (ASTM) E1618, fire debris analysts typically use Gas Chromatography/Mass Spectrometry (GC/MS) data to analyze samples for compounds that are common in ignitable liquid residues. These compounds include aromatics, naphthalenes, straight chain alkanes, branched alkanes, and cyclic alkanes. During an investigation, a suspect's clothing is sometimes collected and analyzed for the presence of ILRs. Previous research has shown that certain matrices — including clothing, fabric and footwear — contain compounds similar to those found in ILRs. In these cases, it is therefore important to distinguish between "innocent" background residues of ILRs from possible transfer during the crime. Although examples of matrix interference have been quite well documented, there are fewer studies that assess the likelihood of interference from common products that suspects are exposed to on a daily basis.

This study surveys the frequency of occurrence of ILRs in more than 27 men's personal care products. After headspace analysis, GC/MS analysis enabled the different components of ILRs to be elucidated through the use of extracted ion chromatograms. The various samples were analyzed using common extracted profiles, such as m/z 43, 91, and 142. The data were analyzed for the presence of toluene, ethylbenzene, p/o-xylene, n-alkanes, cycloalkanes, and branched alkylnaphthalenes. The results revealed that of the hygiene products tested, all samples were negative for each of the compounds in question. The absence of these compounds indicates that men's hygiene products are very unlikely to result in false positives for ILRs. Therefore, it is very unlikely that in an arson investigation, a male hygiene product would lead to a false positive in the suspect's clothing. In contrast, several hand cleaners and wipes designed for mechanics and construction workers were found to be formulated with petroleum distillates. These products have the potential to interfere with the determination of ILRs.

The results of this study may lead to the ability to help arson investigators arrive at better conclusions about the presence of ILRs on a suspect's clothing.

Arson, Ignitable Liquid Residues, Interferences

B63 Rapid Analysis of Peptides and Proteins Utilizing Matrix-Assisted Inlet Ionization Mass Spectrometry

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After attending this presentation, attendees will be aware of a recently developed mass spectrometric technique capable of analyzing a vast array of peptides and large biomolecules with minimal-to-no sample preparation prior to analysis.

This presentation will impact the forensic science community by demonstrating a simple method for the analysis of peptides and large biomolecules that can be easily implemented in drug identification laboratories, often utilizing pre-existing equipment.

The number of internet vendors selling performance-enhancing peptides and cosmetic peptides to the public is staggering. These vendors allow customers the convenience of purchasing peptides online at minimal cost with no questions asked. Received as lyophilized powders, these products are reconstituted in bacteriostatic water with the intention of being injected into the body to promote muscle growth or cosmetic augmentation. Though the sale of peptides may not necessarily be illegal, it is a major concern. Counterfeit peptide sales are known to be fairly common, especially those reportedly containing recombinant Human Growth Hormone (rHGH). This leads to questions regarding the authenticity of the peptides being sold and, more importantly, concerns regarding health and safety.

Many drug identification laboratories do not have protocols in place for successful identification of peptides and large biomolecules. Forensic drug analysis typically relies on Gas Chromatography/Mass Spectrometry (GC/MS) for identification; however, this technique is limited to relatively small molecules that are readily vaporized at the inlet. Due to the increasing presence of high-resolution ambient ionization mass spectrometers in crime laboratory settings, protocols can be easily implemented that utilize the analytical method discussed in this presentation. More importantly, this technique requires no external ion source or additional equipment aside from the mass spectrometer.

Various peptide standards and case samples were successfully analyzed utilizing matrix-assisted inlet ionization MS. To determine the molecular mass of the peptides, samples were dissolved in 1:1 acetonitrile:water with 1% formic acid. The matrix compound, 3-Nitrobenzonitrile (3-NBN), was added to the sample solutions until a noticeable amount of solid accumulated at the bottom of each sample vial. Approximately 5µL of liquid sample, including some of the solid 3-NBN, was drawn up into a microliter pipette. The sample mixtures were introduced directly to the mass spectrometer inlet to initiate ionization. In a matter of seconds, Electrospray Ionization (ESI) -like spectra were obtained. Molecular masses were calculated using a mass spectral interpretation software package.

To further characterize the peptides, a simple enzymatic protein digestion procedure was utilized. Matrix-assisted inlet ionization was utilized to analyze the resulting peptide fragments. Possible identifications for each peptide were assigned by comparing each digested spectrum against an online peptide database. Combined with the molecular mass attained from analysis of the intact peptides, identification of each peptide was made at a reasonable level of certainty.

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Peptides, Mass Spectrometry, Inlet Ionization

B64 The Effect of Environmental Conditions and Substrate Material on the Weathering of Gasoline and Light Petroleum Distillates

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After attending this presentation, attendees will understand how different environmental conditions and substrate materials affect the chromatograms of weathered ignitable liquids and how this affects their class identification.

This presentation will impact the forensic science community by offering attendees a better understanding of the consequences various conditions have on the chromatograms of weathered ignitable liquids and the effects these conditions have on their classifications.

In fire investigations, it is often crucial for analysts to determine if an ignitable liquid was present at the scene, as these liquids are often used as accelerants. A wide variety of ignitable liquids exist that can be used to help start or maintain a fire. The most commonly used is gasoline, in part because it is widely available and cost effective. Light petroleum distillates, such as paint thinners, lighter fuels, and cleaning fluids, are also a commonly used class of ignitable liquids because they too are easily obtainable and highly flammable. Both of these classes of ignitable liquids contain many highly volatile compounds and are particularly susceptible to weathering. Weathering is the effect of evaporation on the chemical makeup of an ignitable liquid. Samples recovered from fire scenes rarely show the characteristics of unweathered materials, so criminalists must be capable of recognizing ignitable liquids in their weathered forms.

It has long been stated that a wide variety of conditions may have effects on the weathering process of ignitable liquids. These conditions can include extent, temperature, air composition, airflow, exposure to sunlight (specifically Ultraviolet (UV) light), the chemical makeup of substrates, and several others. Various studies have sought to use pattern recognition software to either classify the ignitable liquids and/or determine the original conditions under which a sample was weathered. Few published studies have investigated how these conditions affect the presence of the various different compounds detected in ignitable liquids.

In this research, samples of three different ignitable liquids were studied: gasoline, lighter fuel, and a simulated ignitable liquid mixture made up of ten compounds from classes commonly found in ignitable liquids (alkanes, aromatics, and condensed ring aromatics). The simulated ignitable liquid mixture serves as a simpler system in which the effects of the different conditions on specific homologous chemicals can be studied before looking at the more complex mixtures that make up actual ignitable liquid samples. All samples were weathered to 25%, 50%, 75%, and 90% extents by volume while varying the following conditions: container size, temperature (a greater range of temperatures than in prior studies), exposure to UV, and substrate identity. Samples of each were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), and the resulting chromatograms compared to determine any notable differences. Results indicate that extent and temperature have the greatest impact on the pattern of the weathered chromatogram, while other conditions have limited effects. Although the differences in the chromatograms from samples of the same ignitable liquids weathered to the same point are not significant enough to result in one concluding that they come from different classes, the different conditions can cause them to appear as if weathered to different extents. Consequently, these conditions must be considered and included in calculations if mathematical modeling of weathered ignitable liquids is to have meaningful and successful real-world application.

Arson, Ignitable Liquids, Weathering

B65 The Potential of Geochemical Soil Surveys for the Discrimination of Soil Traces From Remote Bushland Sites

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WITHDRAWN

B66 Thermochemical Characterization of Cannabinoids by Porous Layer Open Tubular-Cryoadsorption (PLOT-Cryo) and Nuclear Magnetic Resonance (NMR)

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After attending this presentation, attendees will understand the importance of characterizing thermophysical properties (both vapor pressure and enthalpy of association) of two important cannabinoids, Cannabidiol (CBD) and Δ^9-Tetrahydrocannabinol (Δ^9-THC), for the development of useful, in-the-field vapor phase detection devices to aid law enforcement personnel in determining recent illicit drug use.

This presentation will impact the forensic science community by providing insight into the complexities surrounding the two most important questions that need to be addressed to develop an in-the-field vapor phase detection device to determine cannabinoid intoxication: (1) what is the correct "catch" material and how is it made selective for the compounds that will indicate intoxication; and, (2) what is the correct way to "release" these compounds for accurate analysis. This presentation will provide the chemical foundation necessary to begin to answer these non-trivial questions.

Decreased criminalization of cannabis has led to a surge in both medical and recreational use. Different plant cannabinoids impart either a therapeutic or psychoactive effects (e.g., CBD and Δ^9-THC, respectively). The physical and chemical properties of cannabinoids are difficult to investigate because cannabinoids are unstable in the presence of oxygen and heat. It is also difficult to detect recent use/abuse of cannabis with a chemical or biological test. While methods do exist to detect Δ^9-THC in the blood, urine, saliva, breath, and hair, these tests cannot indicate either recent use or intoxication/impairment.

In this study, the vapor pressure and enthalpies of association of Δ^9-THC and CBD were characterized by use of the ultra-sensitive, quantitative, trace headspace analysis technique, PLOT-cryo, and a 600MHz NMR spectrometer.[1-3] In the first experiment, PLOT-cryo was used to make thermodynamic measurements of the enthalpy of vaporization of select cannabinoids. The mass collected in the vapor phase is presented in the form of a Van't Hoff equation plot, which plots the concentration collected as a function of headspace collection temperature. A linear relationship of the recovered mass as a function of inverse collection temperature reveals the predictive capability of the methodology employed here.

In addition to PLOT-cryo techniques, NMR has been used to examine minute structural changes of Δ^9-THC and CBD in the presence of selected modifiers (such as Gas Chromatography (GC) stationary phases) and solvents. The ability to monitor intermolecular interactions of specific protons in biologically active molecules provides a more in-depth understanding of the thermodynamic and kinetic data associated with intermolecular interactions than is usually possible and is important in evaluating physiochemical properties and reactivity parameters. These parameters, such as association constants and enthalpies of association, have successfully been used to model thermodynamic and kinetic data. The resulting data were used to develop solvation models, which are used to guide research in more effective and rapid separation and detection methods and for the development of future field-ready detection devices.

Measurements on the vapor pressure and enthalpy of sublimation for pure Δ^9-THC and pure CBD will be presented as well as the enthalpies of associations of these compounds with different GC stationary phases. In conclusion, these important thermochemical data will inform the most useful materials for catch and release of cannabinoids for in-the-field law enforcement detection device development.

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B67 The Applicability of Vacuum Ultraviolet (VUV) Spectroscopy as a Gas Chromatography (GC) Detection Technique for Synthetic Cannabinoids

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After attending this presentation, attendees will understand some of the detection principles of Gas Chromatography-Vacuum Ultraviolet (GC-VUV) spectroscopy; in particular, the ability to differentiate synthetic cannabinoids by their absorption spectra. Attendees will gain insight into whether GC-VUV should be used by forensic chemists to identify and detect emerging drugs.

This presentation will impact the forensic science community by presenting a new instrumental technique that has the potential to enhance the ability to screen or confirm the presence of synthetic cannabinoids. This information would be beneficial in assisting the analyst in determining which controlled substance is present and in distinguishing between a controlled and a non-controlled emerging synthetic cannabinoid.

The recently introduced detection method of VUV spectroscopy provides for rapid and relatively low limits of detection via absorption in the wavelengths range of 115nm and 240nm, a region in which almost all chemicals absorb.[1] The detector is capable of both qualitative and quantitative analysis and requires minimal maintenance.[1] VUV spectroscopy can be particularly helpful in the identification of positional isomers through distinctive absorption spectra, which can be particularly difficult to distinguish by Mass Spectrometry (MS) techniques. The VUV detector can also de-convolute co-eluting compounds by matching the measured signals to distinctive library reference spectra.[1] The library spectra can be updated with standards of compounds, to have an expanding library for screening and confirmation purposes. This is particularly useful in aiding in the detection and differentiation of synthetic cannabinoids and their isomers, many of which have the same exact mass and similar retention times.

In this study, VUV spectroscopy was investigated for the detection and differentiation of synthetic cannabinoids. Separation was performed using GC with a VUV detector. The synthetic cannabinoids studied consisted of 22 synthetic controlled drugs and at least 8 non-controlled isomers of controlled JWH-018 were analyzed using GC-VUV. Although all 22 solutes could not be distinguished via retention time, unique UV spectra were obtained for the target solutes.

Although some co-elution was observed for the controlled synthetic cannabinoids and non-controlled isomers of controlled JWH-018, the co-eluting compounds could be differentiated from one another by their individual spectrum. For the most part, unique UV spectra was obtained for the individual isomers. It is possible to determine the basic structure of the controlled synthetic cannabinoids from the shape of the spectra. Furthermore, the work demonstrates that GC-VUV is particularly valuable for the detection of controlled and non-controlled isomers of synthetic cannabinoids and the spectra can be used in conjunction with the retention times and MS spectra from runs using GC/MS or other relevant technique to identify positional isomers and diastereomers.

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Synthetic Cannabinoids, Isomers, VUV Spectroscopy

B68 Profiling Reaction Intermediates and By-Products in Illicit Synthetic Drug Samples Using Direct Analysis in Real Time (DART®) and an Ion Trap Mass Spectrometer

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After attending this presentation, attendees will better understand the detection of impurities present in synthetic drugs for the purpose of sourcing the drug being analyzed. Attendees will be aware of a method being developed for this purpose based on DART® ionization coupled with an ion trap mass spectrometer.

This presentation will impact the forensic science community by demonstrating a new approach for sourcing synthetic drugs. DART®-ion trap Mass Spectrometry (MS) is a rapid technique and the Tandem Mass Spectrometry (MS/MS) capabilities can determine the presence of minority components in samples that are primarily synthetic drugs.

Profiling of excipients has been previously used to source "cut" drugs. Characterization of impurities in methamphetamine samples can be an effective tool to determine the synthetic pathway used to make the drug. Typically, these profiling approaches have been conducted with Gas Chromatography/Mass Spectrometry (GC/MS). The goal of this work is to explore rapid drug sample profiling using DART® MS and to focus on contaminants in newer synthetic drugs. Recently seen designer drugs, such as the synthetic cannabinoids, are produced through multi-step reactions that are significantly more involved than methamphetamine production. Clandestine facilities are unlikely to have rigorous quality control and may contain some amount of impurities due to insufficient sample clean-up. These containments (even when present at low levels) are likely to originate from intermediate products in the multi-step reaction and could potentially be used to source the synthetic batch from which the drug came.

Using DART® to profile the impurities in these samples imparts the advantage of a fast and simple analysis with little sample preparation. The use of an ion trap capable of MS/MS analysis will allow detection of impurities present at low concentrations in a sample with large amounts of the drug product. This type of analysis allows for isolation of a known impurity ion and further fragmentation of that ion to yield a selective signal with high sensitivity.

Initial validation experiments were performed using an IonSense® DART® source coupled with a Thermo™ LTQ XL™ ion trap mass spectrometer. Diphenhydramine was used as an uncontrolled analog drug. The synthesis reaction was conducted using a commonly employed pathway and fractions from various points in the reaction were analyzed. Several reaction intermediates were observed in the final product and identified using MS/MS. Current work is focused on the synthesis of alkyl indoles, which are intermediate products used to make the naphthylindole class of synthetic cannabinoids (i.e., JWH-018, AM-2201). Additionally, a flow system was constructed and coupled with the DART® source for real-time analysis of the reaction products during synthesis.

Near-future work will focus on several areas: (1) determining the relative dynamic range of the DART®-ion trap instrument; (2) comparing this parameter with GC/MS analysis; and, (3) analyzing actual illicit synthetic drugs. The purity of clandestine synthetic drugs is likely to be relatively high, such that any signal from minority contaminants may be masked by the signal from the drug itself. The initial goal is to determine at what level the minority components can be determined in the presence of overwhelming signal for the majority component. The signal-to-noise for low-level contaminants is significantly enhanced through the use of MS/MS. The levels of minority components determined using DART®-MS/MS will be compared with the signal achieved using GC/MS. Finally, actual synthesized cannabinoids will be tested to determine if the presence of reaction intermediates or byproducts can be determined in these samples.

DART®, Impurity Profiling, Synthetic Cannabinoids

B69 A New Method for Non-Contact Recovery of Footwear Mark Impressions Using a 3D Structured Light Scanner

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After attending this presentation, attendees will be informed regarding a new method for the recovery of footwear mark impressions in a totally non-contact manner using a 3D structured light scanner.

This presentation will impact the forensic science community by providing attendees with the ability to recover footwear mark impressions from crime scenes in a non-contact manner, which is faster than the traditional methods. The evidence can then be analyzed and shared electronically.

Footwear marks are one of the most common forms of evidence to be found at a crime scene and can potentially offer the investigator a wealth of intelligence. It is vital that this evidence is collected and preserved. Currently the combined techniques of photography and casting the mark using a product such as dental stone are used. The casting of the mark is a destructive process and, in the event of improper casting, potentially the evidence is lost. The goal of this research is to highlight a new and improved technique for the recovery of footwear impressions using 3D structured light scanning. This new approach is a non-invasive, safer method and is fast, reliable, and extremely accurate. Further, it is capable of recovering more detail than traditional casting techniques and does not require any fixatives to be used. The system used is portable and, after initial training, is relatively easy to operate. The system needs to be calibrated before use — this is a key stage — as it determines the accuracy of the scanned data.

To date, it has been possible to collect evidence from a number of different substrates including fine and coarse sand, soil, compost, and is currently being trialed on snow. After the data have been processed, which can easily be conducted at the crime scene, it is easy to share electronically. This will undoubtedly speed up the investigation process. If the need were to arise, the scanned footwear mark can be 3D printed so that investigators would have a physical model. An important phase of this research will be the validation stage, in which experts will be asked to compare the 3D models against casts of the same impression. The purpose of this is to obtain expert opinions as to whether the 3D scanned images capture at least the same amount of information as the traditional method of casting the mark. The second stage of the research is to look at the potential of using computer software to produce automatic comparisons of shapes and patterns to enable class matches. Using the software, it is also possible to overlay scanned items of footwear with the scanned impressions to aid in the identification process. Another important area of this research is to determine how the new method could be introduced to the courtroom.

It is envisaged that this research will provide investigators with a complete package, which includes the recovery of impression evidence along with the tools to conduct the comparisons of the evidence.

Footwear Marks, 3D Scanning, Crime Scene

B70 Service Implementation for the Identification of Gunshot Residue (GSR) in Rio de Janeiro, Brazil

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After attending this presentation, attendees will better understand how to validate their methodologies and consequently improve laboratory analysis.

This presentation will impact the forensic science community by providing reliable information in criminal investigations and in the analyses of evidence to be presented before a court.

Metrology has become an indispensable tool for improving products and services to today's world where competitiveness demands more quality. The trinomial, reliability, credibility, and quality primarily serves the needs of justice, which uses laboratory tests for these reasons. In Brazil, there is no culture of research and development of forensic sciences, which causes a serious disparity in relation to global scientific advances. The Institute of Metrology, Quality and Technology (Inmetro) of Brazil has responsibility for standard measurements and is therefore the executing agency of public policies in this area.

The primary objective of this work was to validate methodologies for GSR analysis by a Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) technique according to the American Society for Testing and Materials (ASTM) 1588/2010 to meet the demands generated by Brazilian forensic institutes and to provide metrological traceability of all analyses. The results and protocols are available for the forensic community through specific publications and personal training. To this end, Inmetro has established a cooperation with the civil police of the state of Rio de Janeiro (PCERJ).

The observation of inconsistencies in the results obtained in the analysis of the Reference Material (RM), the analysis of samples obtained using different media, as well as problems in detecting submicroscopic particles, showcased the need to validate different methodologies. The first validated analysis of GSR was by SEM microscope. A synthetic GSR RM was developed and used in accordance to the International Organization for Standardization (ISO) Guide 34. For quality control, a sample of LaCe (Fe) particles were used, which simulate GSR particles in morphology, size and atomic number contrast. This method is qualitative, but the microscope verification using reference material is also quantitative. The validation protocol included: (1) selectivity, to verify if the presence of interfering particles in the matrix/support intervenes at analytes identification; (2) sensitivity, to evaluate the response variation due to the variation of the analyte concentration; (3) Detection Limit (DL), the smaller amount of analyte present in the sample that can be detected by the method, regardless of the background (DL is related to particle size and not to its concentration); (4) accuracy, to evaluate the agreement between the result of a test and the accepted reference value; and, (5) precision, results repeatability and reproducibility.

The data revealed that a random laboratory has a 79% probability of detection and a 76% chance of identifying a particle of 1µm in diameter. On average, 90% of 1.5µm particles are detected and 90% of particles ≥ 1.6µm are identified. The acquisition of a Quanta FEG 450 microscope allowed precision tests to be performed, resulting in acceptable values of repeatability (<1.3). The equipment has resolution to identify particles smaller than 0.5µm, generating more consistent results in all areas, especially the GSR analysis.

The results presented suggest that it is possible to perform more precise forensic analyses, improving forensic services and thus emitting more reliable reports. The GSR identification service was implemented in mid- 2013 and in April 2014, the first official test report was produced. Since then, 194 samples have been analyzed, resulting in 82 test reports. The service offered by Inmetro to the PCERJ has a direct impact on public safety as it provides essential information in criminal investigations and the analysis of evidence to be taken before a court.

Forensics, Metrology, Validation

B71 Flow Injection Analysis-Triple Quadrupole (FIA-QQQ) Characterization of Organic and Inorganic Constituents of Firearms Discharge Residue (FDR) Using a Single Injection: The Potential for Rapid Screening of FDR

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After attending this presentation, attendees will be familiar with a GSR screening method using FIA-QQQ.

This presentation will impact the forensic science community by demonstrating a novel and simple screening protocol for FDR that works on an instrument found in many, if not most, forensic laboratories.

One of the intrinsic limitations to the forensic characterization of Gunshot Residues (GSR) is the lack of a quick and reliable screening test. Color tests have been used in the past on skin swabs, but most focus on nitrites and nitrates, which are nearly ubiquitous in the environment and thus of limited value for screening. Other methods used in studies, such as distance determinations, are not amenable to skin sampling or skin swabs. An ideal method would be designed to work with skin samples collected in the same or similar manner as stubs currently collected for traditional Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) characterization using the American Society for Testing and Materials (ASTM) 1588 methodology. In addition, such a method would have to be non- or minimally destructive to insure that subsequent confirmation analysis could be conducted without limitations. This presentation will present one option that is based on stubs, skin sampling, flow injection analysis, and mass spectrometry. The methodology is capable of detecting elemental and organic constituents (diphenylamine, ethyl centralite, and 2,4-dinitrotoluene), as well as lead, barium, antimony, and copper from a single extraction and in the same injection. The method presented here utilized QQQ mass spectrometry and Electrospray Ionization (ESI), but the approach could be adapted to any ESI-MS system. With FIA, no chromatography is needed.

For this project, typical SEM collection stubs were covered with a tacky adhesive-free pad used to mount posters. In a forensic setting, this stub sample would be collected using the same dabbing method used for GSR. The GSR stub would be collected first to insure no compromise. The sticky surface of the second stub collects remaining traces of both organic and inorganic residues. The pad is transferred to a plastic tube for extraction in dilute (~ 0.4%) nitric acid/water/methanol with sonication and mild heating. An equal volume of a complexing agent (15-crown-5-ether) at 200ppm is added to complex metal cations, such as Pb^{2+} and Ba^{2+}. The solution is centrifuged before injection using FIA. The QQQ detection is based on transitions (Multiple Reaction Monitoring (MRMs)) focusing on precursor ions and collision by-products. For example, diphenylamine forms an MH+ ion with a mass of 169.9m/z; collisions result in the formation of ions with m/z values of 92.6 and 65.4. To detect metal ions, the precursor ion is a complex between the metal, the ligand (crown ether), and nitrate ion. The crown ether did not interfere with any of the organic constituents. Samples from two weapons (0.38 revolver and 9mm semiautomatic) were characterized using skin swabs collected from shooters discharging one to five rounds in controlled firing events; lead, copper, and ethyl centralite were the most common constituents detected.

This presentation will present method development and validation data in addition to the results from authentic shooting scenarios. This method shows promise for forensic applications as it uses instrumentation available in many forensic toxicology sections but avoids the need for chromatographic analysis, reducing the barriers to routine use, given that method validation is simplified.

Firearms Discharge Residue, Flow Injection Analysis, Rapid Screening

B72 A Single Extraction, Consecutive Detection of Organic and Inorganic Firearms Discharge Residue Using Thermal Desorption Gas Chromatography/ Mass Spectrometry (TD-GC/MS) and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)

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After attending this presentation, attendees will be familiar with a method that allows for characterization of both organic and inorganic Gunshot Residue (GSR) using a single sample.

This presentation will impact the forensic science community by presenting an alternative method for the analysis of Firearms Discharge Residue (FDR) using one hand swab sample.

FDR consists of unburnt and partially burned particles and particulates arising from the primer, cartridge, propellant powder, grease, and lubricant. GSR (an inorganic fraction of FDR) is currently associated principally with the primer and consists of particulates with a characteristic size, morphology, and chemical composition. GSR is detected by Scanning Electron Microscope Energy coupled to Dispersion X-ray (SEM/EDX) analysis with a methodology described by the American Society for Testing and Materials (ASTM) method E-1588-16a. Samples for this type of analysis are collected using a small adhesive stub. Some of the known limitations of this methodology include secondary transfer and concerns arising from newer primer formulations that minimize heavy metals, such as lead. These concerns, coupled with the development of new methods for sample introduction and advanced MS, have led to increased interest in the organic constituents of FDR or Organic Gunshot Residue (OGSR). These residues arise from the propellant, grease, and lubricant and include compounds such as stabilizers (diphenylamines), dinitrotoluenes, and methyl and ethyl centralite. The energetics (nitrocellulose and nitroglycerin) are not routinely used as target analytes for post-firing characterization. While the ability to characterize OGSR could at some point become viable for forensic laboratories, an even better analytical approach would facilitate characterization of both GSR and OGSR from a single sample. Here, a method is described using Thermal Desorption-Gas Chromatography/Mass Spectrometry (TD-GC/MS) to characterize OGSR, followed by acid extraction of the same swab and elemental analysis using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). The advantage of this method is that a single swab is used for both analyses; this is countered by the loss of morphological information obtained from SEM.

For this project, hand swabs were collected under an approved Institutional Review Board (IRB) protocol using isopropanol and clean room wipes. The collection area was ~1cm x 1cm and all surfaces of both hands were sampled and concentrated into this small surface area. The swab was placed in a thermal desorption probe and inserted directly into the injection port of the GC/MS. A selected ion mode analysis was employed targeting 2, 4-di-tert-butylphenol, 2, 4-dinitrotoluene, diphenylamine, methyl centralite, ethyl centralite, and dibutylphthalates, all common OGSR target compounds. After desorption, the swab was removed and subjected to a single 2% nitric acid extraction, followed by ICP/MS analysis. The method was developed using standards and swabs collected after shooting 10-20 rounds of a variety of weapons and ammunition. Subsequently, samples were collected from known shooters and non-shooters for analysis. Overall, diphenylamine, ethyl centralite, lead, barium, and copper were detected in most cases with three or more shots discharged; the results from smaller caliber weapons were variable with ethyl centralite, lead, and copper predominating. This presentation will describe method development, validation, experimental conditions, and results across a range of authentic sampling situations.

Firearms Discharge Residue, OGSR, GSR

B73 An Evaluation of the Repeatability, Reproducibility, and Uncertainty of Retention Indices and Electron Impact Spectra of Selected Novel Psychoactive Substances (NPS)

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After attending this presentation, attendees will be familiar with ongoing work related to the characterization and identification of NPS.

This presentation will impact the forensic science community by disseminating current results related to forensic identification of NPS and uncertainties associated with retention indices and Electron Ionization (EI) ion abundances.

The analytical challenges presented by synthetic cannabinoids and cathinones (NPS) include the lack of reference materials and the rapid appearance of new synthetic variants. Consequently, the traditional forensic identification tools cannot keep pace with the flow of new structures and compounds appearing in physical and toxicological evidence. The structural similarity among families of NPS may afford tentative identifications or classifications, but laboratories often must report inconclusive results and new variants may go unrecognized for some time. This can have implications in law enforcement as well as public health, given the range of toxic effects being associated with ingestion of many of these drugs.

A long-term goal of this project is to develop algorithms capable of classifying novel synthetics based on structure and to create similarity measurements that can characterize new NPS as compared to existing and known structures; however, before this goal can be achieved, verified and validated data and figures of merit must be established for the necessary measurands: retention time/retention index and mass spectra; specifically, relative ion abundances. There is no point in attempting to build a quantitative predictive model for mass spectral identification without knowing how much relative ion abundances vary across time and instruments. Reported here are results to date of a selected group of NPS compounds (JWHs, UR-144, and cathinones, as well as methamphetamine and amphetamine) analyzed using three different GC/MS systems over time. All instruments are models commonly used in forensic science laboratories. Variables recorded included tuning conditions, elapsed time since tuning, time since inlet change, time since column maintenance, and all relevant instrumental settings. Retention indices were calculated relative to a C7-C30 alkane ladder. Three levels of sample concentrations were examined: near the detection limit, mid-range, and near the upper end of the linear dynamic range of the instrument. DB-1 and DB-5 columns of different lengths were used. Sample concentrations were at low (close to the method detection limit), mid-range, and high concentrations. Ion repeatability and reproducibility were determined based on the ratios of the ten most abundant ions in the spectrum. In cases in which ions were critical for identification but not one of the top ten abundant, these ions were included and characterized. Figures of merit to date for all instruments will be presented and initial estimates of uncertainty will be presented.

Novel Psychoactive Substances, Uncertainty Estimation, GC/MS

B74 Comparing the Response of Portable Hydrocarbon Detectors to Laboratory Analysis of Household Substrates

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After attending this presentation, attendees will better understand the effectiveness of electronic hydrocarbon detectors (often referred to as sniffers or electronic noses).

This presentation will impact the forensic science community by informing attendees of the appropriate use of hydrocarbon detectors, which should be used with great caution and as a presumptive tool only.

Electronic hydrocarbon detectors have been commonly used in the field of fire investigations to aid in the possible location of ignitable liquid residues. These devices alert to the presence of volatile hydrocarbons to indicate a potential sampling location. In this study, the selectivity and sensitivity of two different brands of hydrocarbon detectors were examined. Sixteen common household substrates and building materials were tested, including foams, wood, flooring, carpet, and roofing material. Each substrate was tested in triplicate, using both hydrocarbon detectors, and the results were compared to laboratory analysis. Each substrate was tested in unburned and burned conditions to evaluate the effect of the addition of pyrolysis and combustion products. No ignitable liquids were spiked on substrates in this study; however, several substrates known to inherently contain petroleum products were intentionally chosen to determine detector efficacy in detecting such ignitable liquids.

Inherent petroleum products were identified by Gas Chromatography/Mass Spectrometry (GC/MS) in 5 of 16 substrates chosen. For each of these unburned substrates, both hydrocarbon detectors gave negative or inconclusive responses. These were considered false negatives. A sharp increase in the number of positive responses by the hydrocarbon detectors was observed for the burned substrates; however, only pyrolysis or combustion products were identified by GC/MS. These were considered false positives.

Overall, both detectors showed numerous false positives, false negatives, and inconclusive results. For both unburned and burned substrates, the hydrocarbon detectors yielded wrong or inconclusive results ranging from 31% to 56% of the tested samples. Additionally, consistent use of the detectors proved to be difficult as the sensitivity varied greatly during use. On each detector, a knob or dial was used to set the sensitivity, which is expressed as an audible chirp. Instructions regarding these devices require that they emit an audible chirp every one to two seconds, with the chirp increasing in frequency when volatile components are detected. During the study, the frequency of the chirp increased and decreased without any apparent cause. As this study was conducted in a static, climate-controlled environment, the variable sensitivity and poor selectivity could be extremely problematic on a fire scene. As such, these on-scene instruments should be used with great caution and as a presumptive tool only for sample location and selection and should not replace an investigator's training and experience.

Fire Debris, Fire Investigation, Ignitable Liquids

B75 Identification of Potential Target Compounds in Fire Debris

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After attending this presentation, attendees will better understand the chemical composition of fire debris from the identification of compounds within ignitable liquid residues and pyrolysis/combustion products from substrate materials.

This presentation will impact the forensic science community by providing information that can be utilized in determining the presence and classification of ignitable liquid residues by identifying potential target compounds.

Fire debris analysis is conducted to determine whether an ignitable liquid residue is present in the fire debris collected from the scene. One challenge to making this determination is the presence of decomposition products from the pyrolysis and combustion of substrate materials at the scene. Some of these decomposition products are extracted along with ignitable liquid residues to form a complex mixture of compounds from both. In 1991, Keto and Wineman developed a method for the analysis and identification of selected target compounds.[1] They identified target compounds from the American Society for Testing and Materials (ASTM) E 1618 classifications of gasoline, medium petroleum distillates, and heavy petroleum distillates.[2] They obtained semi-quantitative Gas Chromatography/Mass Spectrometry (GC/MS) peak areas for these target compounds to construct Target Compound Chromatograms (TCCs). The study determined that TCC for gasoline, medium petroleum distillates, and heavy petroleum distillates were different from those of plywood and carpet with padding. TCCs enable the analyst to visualize low concentrations of ignitable liquid residues in high concentrations of decomposition products.

The study being presented is based on the identification of potential target compounds in all ASTM E 1618 classes and in decomposition products from substrate materials.[2] The data consists of 660 neat ignitable liquids and 106 burned substrate materials from the Ignitable Liquids Reference Collection (ILRC) and the Substrate Databases.[3,4] A library containing retention time and mass spectra of 255 standards has been developed for identification of major peaks within ignitable liquids and substrates in the databases.[3,4] Identification of these compounds within the 766 samples is performed by Target Factor Analysis (TFA), which calculates a correlation between the spectrum of the sample and the 255 test spectra. Logistic regression is performed to convert the correlations to probabilities. When a probability is larger than a specific threshold, that compound is identified as being present in the sample. A maximum likelihood frequency of occurrence in ignitable liquids and substrates is tabulated for each of the 255 compounds. Compounds with a high frequency of occurrence in ignitable liquids and a low frequency of occurrence in decomposition products from burned substrate materials are potential target compounds. This concept can also be applied for each ASTM E1618 class of ignitable liquid.[2]

This work was supported by the National Institute of Justice, Office of Justice Programs. The content of this publication does not necessarily reflect the position or the policy of the Government and no official endorsement should be inferred. Support is also acknowledged from the University of Central Florida, National Center for Forensic Science, a State of Florida Type II Research Center.

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2. ASTM, *E1618-11 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry*. 2014, ASTM International: West Conshohocken, PA.
3. The Ignitable Liquids Reference Collection Database can be found at the universal resource locator. Available from: http://ilrc.ucf.edu.
4. The Substrate Database can be found at the universal resource locator. Available from http://ilrc.ucf.edu/substrate/.

Fire Debris, Target Compounds, Ignitable Liquids

B76 Probabilistic Assertions in Fire Debris Analysis Based on Chemical Compound Occurrence

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After attending this presentation, attendees will better understand one way to arrive at a probabilistic assertion regarding the presence or absence of ignitable liquid residue in fire debris based on knowledge of the frequency of occurrence of chemical compounds.

This presentation will impact the forensic science community by providing information that can assist in the development of statistical methods in trace analysis.

Ignitable liquids are often complex mixtures of many different chemical compounds. Similarly, the pyrolysis and partial combustion of common household furnishings and building materials often produce many different chemical compounds. The frequency of occurrence of 255 compounds has been determined in the Ignitable Liquids Reference Collection (ILRC) and the Substrate Database.[1,2] An analysis of the compounds in both databases demonstrates that some of the compounds are only observed in ignitable liquids, some are only observed in substrate pyrolysis samples, and some are observed in both types of samples. Failure to see a compound in ignitable liquids or in substrate pyrolysis does not mean that it will never be observed in these sample types. It is possible to estimate the frequency of occurrence in a population for compounds that were not seen in a sample of the population.[3] The approach of Good and Turing uses the maximum likelihood approximation for compounds that occur frequently and an approximation for the frequency of occurrence of compounds that are observed rarely or unseen in a sample.[3] This approach has, for the first time, allowed the determination of the frequency of occurrence of 255 compounds in ignitable liquids and substrates.

The frequencies of occurrence can then be used to classify a sample as positive or negative for ignitable liquid residue. It is also possible to calculate a likelihood ratio for a fire debris sample being positive or negative for ignitable liquid residue. Both of these calculations rely on the identification of a subset of the 255 compounds in a fire debris sample, the frequencies of occurrence of those compounds, and the naïve Bayes approximation. The naïve Bayes approximation assumes independence of the frequencies of occurrence of the 255 compounds. The naïve Bayes assumption is often not true, as is the case here; however, models based on the assumption often work very well. Analysis of 129 fire debris samples from large-scale test burns were performed. The results allow the calculation of likelihood ratios and the subsequent formulation of probabilistic assertions regarding the presence of ignitable liquid residue in each sample. The results will be discussed and evaluated using conventional methods, such as Receiver Operator Curves (ROCs) and Tippett plots. This method will be compared to previously published methods based on the total ion spectrum.[4]

This work was supported by the National Institute of Justice, Office of Justice Programs. The content of this publication does not necessarily reflect the position or the policy of the Government and no official endorsement should be inferred. Support is also acknowledged from the University of Central Florida, National Center for Forensic Science, a State of Florida Type II Research Center.

Reference(s):

1. The Ignitable Liquids Reference Collection Database can be found at the universal resource locator. Available from: http://ilrc.ucf.edu.
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B77 A Quantitative Approach to Differentiating Mixtures of Gasoline and No. 2 Fuel Oil From No. 2 Fuel Oil Using Gas Chromatography/Mass Spectrometry (GC/MS) Target Compound Analysis Ratios

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After attending this presentation, attendees will better understand the significance and fundamental importance of a quantitative approach to the analysis of ignitable liquids and fire debris samples.

This presentation will impact the forensic science community by demonstrating the application of a quantitative approach and methodology to characterize ignitable liquids and mixtures of ignitable liquids that can be implemented to facilitate the interpretation of analytical data.

The investigation of a suspected arson intimately involves the forensic laboratory. Ignitable liquids, in particular gasoline, are commonly used as accelerants. It is critical to an arson investigation that the presence of a trace amount of an ignitable liquid be detected and correctly identified by the laboratory. The laboratory detection, identification, and proper classification of ignitable liquids recovered from fire debris can be critical to an investigation.

In the United States, many homes and establishments are heated with No. 2 fuel oil. Gasoline, a common fuel and a common accelerant, is detected in many arson cases. A complication can arise when the fire scene involves a home or establishment that is heated with No. 2 fuel. The No. 2 fuel oil product inherently contains the compounds common to gasoline. The task of determining if gasoline is absent or present as a mixture with a No. 2 fuel oil can be both challenging and problematic under certain conditions.

The American Society for Testing and Materials (ASTM) E1618-14, Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography/Mass Spectrometry, is a test method utilized in many forensic laboratories. E1618-14 does not address or provide guidance in the identification and classification of complex ignitable liquid mixtures.

The data presented here include samples of gasoline, home heating fuel oil, and diesel fuel oil collected twice from selected distributors over an eight-month period. The collected samples were analyzed as neat liquids, combinations of neat liquids, liquids at varying degrees of (percent) evaporation, combinations of these evaporated liquids, and mixtures of neat liquids with the liquids at the varying degrees of evaporation. Diesel fuel samples were also collected in light of the fact that the diesel fuel product is purported to be identical to the home heating product without consideration of additives and the dyes used for identification. The samples were prepared with a deuterated internal standard containing benzene-d6, ethylbenzene-d10, and naphthalene-d8 and analyzed by GC/MS.

The data presented will demonstrate the utility of a quantitative approach and target compound ratio analysis methodology in differentiating complex mixtures of gasoline and No. 2 fuel oil from No. 2 fuel oil alone. The analytical methodology presented can be applied to further develop the criteria for the identification, classification, and discrimination of petroleum products and ignitable liquids.

Fire Debris, Ignitable Liquids, GC/MS

B78 Source Inference of Gasoline: The Contributions of Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Isotope Ratio Mass Spectroscopy (GC/IRMS) Analyses

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After attending this presentation, attendees will understand the possibilities and limitations of GC/MS and GC/IRMS results, used separately or in combination, for the comparison of gasoline samples from a source inference perspective.

This presentation will impact the forensic science community by providing new knowledge regarding the contribution of fire debris analysis in the association between ignitable liquid traces and possible ignitable liquids.

When gasoline is used to start and/or propagate arson, source inference of gasoline can establish a link between the fire and a potential source. This source inference is an interesting alternative to provide evidence in the event where physical traces (DNA, fingerprints, shoe prints, etc.) left by the perpetrator are rare, or more often than not, disregarded.

The main goal of this research was to develop a GC/IRMS method for the analysis of gasoline samples and to assess its potential to infer the source of gasoline traces compared to the GC/MS performances. An instrument that simultaneously analyzes samples by MS and IRMS was used in this research. An analytical method was developed, optimized, and validated for this instrument. Next, a large sampling of gasoline was collected at several time intervals, representing a regional area market and gasoline of different octanes. After collection, the samples were analyzed, either at that time or after several degrees of evaporation, by GC, then by MS and IRMS. In this research, isotope ratios were confined to measurements of $\delta^{13}C$. Finally, obtained data were processed and interpreted using chemometric methods.

The analyses revealed that the methodology, both for MS and for IRMS, allowed differentiation of unweathered gasoline samples from different gas stations. It also demonstrated that each new filling of gas station tanks generates an almost unique blend of gasoline. GC/MS achieved a better discrimination of samples from different stations, while GC/IRMS was more effective in distinguishing samples collected after each tank filling. Thus, these results indicate that the two components of the analytical strategy can be complementary to the analysis of unweathered gasoline samples.

The results also illustrated that the evaporation of gasoline samples does not compromise the possibility of grouping samples derived from the same source by GC/MS; however, it is necessary to make a selection of variables in order to eliminate those which are influenced by evaporation. Conversely, results demonstrate that the evaporation of gasoline samples has such a strong influence on the isotopic composition of the samples that it is not possible, even by performing a selection of variables, to properly group evaporated samples by GC/IRMS.

Arson, Gasoline, Source Inference

B79 Morphology and Microanalysis of Aluminum (Al) Powders From Amateur Improvised Explosive Device (IED) Methods

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After attending this presentation, attendees will better understand the forensic potential of combining elemental microanalysis and micromorphology to provide insight into the method of Al powder production.

This presentation will impact the forensic science community by discussing the differences in surface characteristics, elemental composition, and particle micromorphology of Al powders manufactured using various amateur methods and extracted from commercially available products. These results may provide valuable lead identification for forensic investigations.

Starting materials for an IED are readily obtainable from local commercial sources. Al powder, a common metallic fuel, has a wide variety of legitimate uses and is widely available without significant regulatory constraints.[1] Al powders can be obtained from industrial manufacturers or can be produced inexpensively using basic instructional manuals and videos. Due to the online sharing of instructional manuals and published books on how to construct IEDs, bomb-makers are now informed on the easily accessible household materials that can be used to make explosive chemical mixtures.[2]

For this project, Al powders from Al foil, spray paint, pyrotechnics, and Al cans were obtained and produced. Al powder was extracted from Al flake-containing spray paint and pyrotechnics, and was manufactured from Al foil and Al cans using ball-milling, grinding, and blending techniques. For Al flake-containing spray paints, acetone was added to solubilize the binders and additives, followed by centrifugation to form an Al powder pellet; the supernatant was removed to isolate the Al powder pellet, which was then dried by solvent evaporation. For the pyrotechnics, several separation techniques were used to remove the explosives and additives. Density separation techniques were used to separate the charcoal and elemental Silicon (Si) from the Al powder. A hand magnet was used to remove iron filings from "gold" sparklers. Lastly, a ball-milling technique, which used a small 6 lb. rotary dual drum ball mill and ball bearings, and a grinding technique (using a coffee grinder) were used to manufacture Al powder from Al foil and Al cans.

Preliminary results obtained from Scanning Electron Microscopy (SEM) micrographs demonstrate that Al powder manufactured by ball milling could be confidently differentiated from those extracted from an Al flake-containing spray paint. Furthermore, SEM with Energy-Dispersive X-ray Spectroscopy (EDS) analysis of the Al flake-containing spray paints provided additional information that could differentiate between brands and among products within brands. Four different manufacturers of Al flake-containing spray paints were studied: (1) Manufacture A's product contained Al and iron; (2) one of the products from Manufacturer B contained Al, potassium, copper, Si, sodium, and titanium (the other product contained only Al); (3) Manufacturer C's products contained Al and Si; and, (4) Manufacturer D's product contained only Al. All of these elements were present in the Al powder after an acetone wash, which is how amateur bomb-makers are extracting Al powder from spray paints, so these differences are also expected to be present in case samples.

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2. Larabee A. 2015. *The Wrong Hands: Popular Weapons Manuals and Their Historic Challenges to a Democratic Society*. Oxford University Press, New York, NY.

Aluminum Powder, SEM/EDS, Microanalysis

B80 The Effect of Time on the Quantity of Triacetone Triperoxide (TATP) on Different Surfaces

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After attending this presentation, attendees will understand the best practices for identifying the type of solvent that can be used with TATP swabbing, how long the TATP can survive before decomposition, and how the fingernail of a suspected person can be used to identify the explosives.

This presentation will impact the forensic science community by clarifying the best solvent that can be used to identify the explosives and by explaining how important the use of the suspect's fingernails is in proving that he/she touched the explosives.

In forensic science, the contribution of explosives trace detection in samples from skin, vehicles, hands, and clothes of the suspects has been essential in several forensic cases involving terrorists. Knowing the types of explosives used by terrorists can prevent future attacks by prohibiting these chemicals and controlling their precursors. The forensic analytical report could be used as evidence in judicial proceedings. Recently, the use of peroxide explosives, such as TATP and Hexamethylene Triperoxide Diamine (HMTD), has gradually increased by the terrorists around the world in the production of Improvised Explosives Devices (IEDs) due to their readily available precursors. Many studies have been published on sampling procedures for the collection of residues of firearms and explosives from suspects and/or clothing. The residues generally left inside baggage, bags, vehicle interiors, and garments are collected by a vacuum-lifting procedure and filters. Samples are usually collected by cotton swabs from smooth surfaces, such as skin, work surfaces, floors, leather, plastic, and post-blast debris. Solid Phase Extraction (SPE) is used for swabs and filters of explosives. For the efficient recovery of explosives residues, various materials are used for the sampling media; however, most forensic laboratories are using cotton-based material due to availability, ease of use, and low cost. A wide range of solvents has been reported to assist in the recovery of explosives residues in the sample media which were used.

Knowing the interval of time in which explosives may be active can play an important role in the investigation. Therefore, in this study, TATP was prepared, purified, and analyzed. A known quantity of TATP was applied to different service areas (such as hands, nails, tables, and car seats), then swabbed after different time intervals. The swabs were extracted by an organic solvent and analyzed by gas chromatography. The results disclose that the quantity of TATP left was dependent on the type of surface at a specific time.

Several solvents with different polarities were used. A known amount of TATP was applied to a clean surface of a lab bench. Johnson & Johnson® cotton pads were pre-wet with the selected solvent and directly used to swab the TATP. The pad was left in the ultrasonic for 30 minutes, the mixture was filtered and transferred into a 10mL volumetric flask, and topped with solvent in the presence of hexadecane. The results demonstrate that methanol was the best solvent, while acetonitrile was the least efficient. The excellent results with methanol can be attributed to the hydrogen bond formation with TATP rather than polarity.

A known quantity of TATP was applied to different parts of a vehicle interior when the outside temperature was 34 ºC-38ºC. The vehicle had only been used by the driver. After six hours, the TATP was swabbed and analyzed. Most of the TATP was lost from the surface areas of the car. The foot area under the passenger seat showed the best recovery as it had not been touched and did not face the sun like the dashboard.

A specific quantity of TATP was inserted under a fingernail. After a certain period of time, the fingernail was cut and the top of the finger was washed with methanol. The nail and the methanol were placed in a container and left in the ultrasonic for 30 minutes. When the fingernail was cut directly and immediately washed, the recovery of TATP was very high (97%); however, after only two hours, the TATP was found to be in the range of ca. 10%. After 48 hours, the concentration of TATP may remain under the fingernail, but in low concentrations (5%).

It can be concluded that TATP is easily decomposed; however, it can be detected from the suspect areas.

B81 Characterization of Pre- and Post-Burn Smokeless Powders by Direct Analysis in Real-Time Time-of-Flight/Mass Spectrometry (DART®-TOF/MS) vs. Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will understand how smokeless powders may be analyzed and classified pre- and post-burning using DART®-TOF/MS and how these results compare to the more traditional GC/MS results.

This presentation will impact the forensic science community by providing a rapid means of smokeless powder identification and differentiation by DART®-TOF/MS, which may be applied to Gunshot Residue (GSR) and explosive analysis, such as debris analysis following a bombing event.

Smokeless powders were analyzed by DART®-TOF/MS for the presence of several organic compounds of interest, including diphenylamine, nitroglycerin, ethyl centralite, dinitrotoluene, phthalates, and others. Positive and negative mode analysis was performed for each sample, with helium carrier gas at 200˚C and grid voltages set at 150 V. Unburned samples were analyzed as neat samples, as the smokeless kernel itself, and as extracts. Extracts were prepared by following a simple GC/MS extraction, the National Center for Forensic Science's procedures using dichloromethane. Extraction is not necessary for DART®-TOF/MS analysis of solid smokeless powder kernels; however, extractions were performed to determine if extraction increased the number of organic compounds observed and to allow for GC/MS testing. Each smokeless powder was subsequently burned and analyzed. Unburned or partially burned smokeless powder particles and burned smokeless powder residues may be recovered following an explosive event, such as discharge of a firearm or an Improvised Explosive Device (IED). Therefore, unburned and burned samples were analyzed to simulate possible real-world evidence. Burned and unburned samples were then compared and differences in the identifiable organic compounds were recorded to determine the effect of burning on the smokeless powder and residue composition. Sample extracts were later analyzed by GC/MS to confirm composition and compare results obtained by DART®-TOF/MS and GC/MS.

Compounds of interest were easily identified in smokeless powders analyzed via DART®-TOF/MS, and within seconds. Following characterization of the organic components of each smokeless powder, a classification scheme may be developed to further characterize smokeless powders. Classification of smokeless powders based on the organic components may aid investigators in determining the brand or origin of a suspect smokeless powder, burned or partially burned, recovered at a crime scene.

Sample preparation for DART®-TOF/MS is simple and requires minimal effort, because smokeless powder kernels can be analyzed directly by DART®-TOF/MS. Additionally, DART®-TOF/MS is a more rapid technique than GC-MS. DART®-TOF/MS samples may be analyzed in seconds, and mass spectra may be observed and evaluated almost instantly; GC-MS may take more than ten minutes to run one sample after sample analysis, and mass spectra may not be observed or evaluated until the sample run has been completed. DART®-TOF/MS is a viable method for rapid analysis of smokeless powders, which may be applied to explosive and GSR analysis.

Smokeless Powders, Explosives, Gunshot Residue

B82 Assessing the Quality and Reliability of Drug Identifications

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After attending this presentation, attendees will better understand how to evaluate laboratory data to obtain information on the quality of drug identifications.

This presentation will impact the forensic science community by providing an approach for estimating the quality and uncertainty of drug identifications in the forensic chemistry laboratory.

The Drug Enforcement Administration (DEA) laboratory system processes thousands of suspected drug exhibits every year, seized by federal, state, and local law enforcement officials during undercover operations and via interceptions of drug smuggling operations, among others. After laboratory analysis, results are summarized in official laboratory reports that are forwarded to the submitting investigative agency. Many of these laboratory reports are then used by government officials during court trials and sentencing procedures and, as such, should present accurate and scientifically supported results and conclusions, as incorrect decisions based on inaccurate reports could have significant legal and personal consequences. Laboratory reports and case documentation should also fulfill laboratory accreditation requirements and provide users in the judicial system, such as attorneys and jurors, with information regarding the quality of laboratory processes and resulting drug identifications.

The DEA laboratory drug identification process is separated in to three phases. Phase I includes evidence submission and Chain-Of-Custody (COC) procedures such as barcoding, secure vault storage, and safety protocols. Phase II forms the core of the laboratory identification process, as it incorporates the analytical scheme — the combination of sampling protocols and tests performed by expert analysts in order to achieve an unambiguous and scientifically supported identification. Phase III includes preparation of the final laboratory report by the analyst, and the technical and administrative reviews performed by laboratory managers. These peer-review steps ensure that analytical scheme requirements have been met, that identification results are accurately reported, and that the analytical case file contains all the documentation required to support the identification.

Even when best laboratory practices are in place and the appropriate analytical scheme is followed, the extensive series of laboratory procedures required to report a final identification may be susceptible to errors. Many of these errors are unforeseen or unanticipated and may occur during instrument operation, sample handling, and during the report-writing stages, among others. Consequently, the possibility of reporting a misidentification is always present. It is therefore crucial to assess the performance of a laboratory's identification procedure so that the quality of reported identifications can be described and communicated to the report recipients. This will undeniably result in a better understanding of the frequency of procedural errors and will help to evaluate and improve quality assurance measures throughout laboratories.

This presentation will include results from the evaluation of historical DEA laboratory system-wide Proficiency Testing Program (PTP) data obtained during the years 2005-2016. Analysis of this data provides estimates of the sensitivity (true positive rate) and specificity (true negative rate) of the DEA drug identification process, as well as estimates of the probabilities associated with Type I and Type II errors (false positive and false negative rates, respectively). This presentation will also demonstrate how Bayesian analysis can be used, in combination with the response rates, to evaluate the confidence and uncertainty associated with identification results. The PTP data and its evaluation and discussion will demonstrate that the DEA identification process is highly sensitive and specific, with rates of type I and type II errors below 1%.

Criminalistics, Seized Drugs, Identification

B83 2017 Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Update — A Discussion on Drug Analysis Techniques and A-B-C Categorization

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After attending this presentation, attendees will better understand the principles behind the categorization of analytical techniques used for drug analysis.

This presentation will impact the forensic science community by providing up-to-date information on the resources offered by SWGDRUG, in addition to other recent activities.

SWGDRUG was formed in 1997 in a joint effort between the United States Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). SWGDRUG works to improve the quality of the forensic examination of seized drugs and to respond to the needs of the forensic community by supporting the development of internationally accepted minimum standards, identifying best practices within the international community, and providing resources to help laboratories meet these standards. This presentation will comprise a discussion on SWGDRUG categories of analytical techniques and will also provide attendees with information on past and current SWGDRUG activities.

The development of new instrumental technologies and their introduction and use in seized-drug laboratories have brought up the need to clarify the classification of techniques into A, B, or C categories. In this presentation, the basis behind the three separate categories will be defined and discussed, from the point of view of structural characterization, discrimination ability, and the theoretically predictive value of each technique. Via example illustrations, this presentation will also discuss and emphasize the important factors to consider when establishing an appropriate laboratory analytical scheme, when the choice of techniques may depend on the particular analytical and judicial requirements of a laboratory.

During the summer of 2016, version 7.1 of the SWGDRUG Recommendations was approved and published (www.swgdrug.org). Also, Supplemental Document 6 (SD-6), titled "Examples of Measurement Uncertainty for Net Weight and Count Extrapolations," was finalized and posted. This document provides step-by-step examples for estimating uncertainty for scenarios in which the net weight of an exhibit is obtained via extrapolation or when the total count of a dosage unit exhibit needs to be extrapolated.

Core committee members are also working on revising Part IVB of the SWGDRUG Recommendations, pertaining to the validation of analytical methods. Revisions include clarifications on the performance characteristics to be evaluated during the validation of both qualitative and quantitative methods. Examples of method validation schemes for routinely used techniques, such as color test, Gas Chromatography/Mass Spectrometry (GC/MS) and Infrared (IR) spectroscopy, will also be provided in a separate supplemental document. This presentation will also summarize recent updates on SWGDRUG resources, such as the MS library, the IR library, and the Drug Monographs.

The SWGDRUG core committee includes representatives from federal, state, and local law enforcement agencies in the United States, Canada, Brazil, Great Britain, Germany, Austria, Switzerland, Australia, and Singapore. The following forensic organizations are represented: the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalistica y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). Core committee members also include forensic science educators and representatives from forensic science organizations across the United States, the American Society of Crime Laboratory Directors (ASCLD), the American Society for Testing and Materials (ASTM), and the National Institute of Standards and Technology (NIST).

Criminalistics, SWGDRUG, Drug Analysis

B84 No Lab, No Plea … Eliminating a Controlled Substances Backlog

James T. Miller, BS, Houston Forensic Science Center, 1301 Fannin Street, Ste 170, Houston, TX 77002*

After attending this presentation, attendees will better understand the testing guidelines implemented and the tools used to clear a backlog and maintain a turnaround time of 30 days or less. Attendees will also comprehend how to apply these tools in their own jurisdictions.

This presentation will impact the forensic science community by providing guidance on implementing managed processes, as well as testing guidelines that aid in the reduction of controlled substance backlogs. The Houston Forensic Science Center (HFSC) will share the lessons learned from its successful process in an effort to assist other jurisdictions in creatively managing their resources so they too can clear backlogs and maintain incoming caseloads.

Much attention has been paid to new legislation spreading across the United States regarding mandatory testing of sexual assault kits; however, it is often overlooked that in the controlled substance community, it has long been common practice for all seized drugs to undergo forensic testing, which creates a caseload of tens of thousands of cases. Due to limited resources, this often leads to large backlogs. Not only does this create strain within the lab, it also impacts the legal system. Also, drug convictions often move rapidly through the legal system as many defendants plead guilty rather than go through a lengthy trial. Recently, it has been discovered that some defendants have pleaded guilty to a drug charge prior to the seized substance being tested in the laboratory, only to find that subsequent testing showed no illegal substances were identified. This has led to approximately 200 drug case exonerations in Harris County, TX. The Houston-area legal system responded by instituting a "No Lab, No Plea" policy. While all involved agreed the policy was necessary, it did add pressure to clear the drug testing backlog and maintain a reduced turnaround time. Even though Federal funds are available for such upticks in work in the DNA area, the Controlled Substances Section had to find creative ways to increase capacity while reducing backlogs and turnaround times. By communicating with all stakeholders, HFSC's Controlled Substances Section established guidelines that reduced the amount of testing being requested, simultaneously prioritizing cases moving rapidly though the legal system. The section also developed managed processing plans through a mini Lean Six Sigma assessment that increased efficiency and reduced turnaround times to fewer than 30 days.

In this presentation, the Controlled Substances Section will share some of its process development results to illustrate how to communicate with stakeholders and use available resources to clear backlogs and maintain a 30-day turnaround time.

Backlog, Controlled Substances, Process Management

B85 The Implementation of Blind Proficiency Testing in Drug Analysis

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After attending this presentation, attendees will better understand the advantages and challenges of implementing a blind proficiency program in drug analysis.

This presentation will impact the forensic science community by providing a model for implementing a blind proficiency program in controlled substance testing. Laboratories, saddled with backlogs and high-priority cases, often do not have the time or resources needed to implement a blind proficiency program. After attending this presentation, attendees will recognize the benefits of blind testing despite the challenges and costs associated with the program.

Accrediting bodies require laboratories to participate in a minimum of one proficiency test annually in each area listed on the scope of accreditation. To fulfill this requirement, laboratories have used open proficiency testing provided by a commercial vendor as a tool to evaluate the performance and competency of staff, as well as to gauge the system's overall quality. The successful completion of these open proficiency tests demonstrates the quality of the test results and the staffs' competency. These commercially created tests attempt to mimic real casework, but often do not encompass the entire scope of testing. While the external commercial proficiency exams meet accreditation requirements and demonstrate competence, the laboratory is often limited to administering tests provided by the vendors. This means the tests may not always be representative of the majority of a laboratory's real casework. Blind proficiency testing has shown to be an advantageous quality control tool that can objectively test an entire system; however, implementing such a program can be challenging.

The Houston Forensic Science Center (HFSC) created its blind proficiency testing program in September 2015. The goal was to create and submit blind samples equal to 5% of the Center's average monthly caseload. Initially, tests had been created using old proficiency samples and non-controlled substances created in-house by the Quality Division staff. HFSC quickly learned of the challenges associated with obtaining samples of controlled substances that could mimic actual current casework. Another challenge involved packaging and describing evidence in a manner reflective of the laboratory's primary client. To further complicate matters, HFSC's Laboratory Information Management System (LIMS) is connected to the client's management systems. This allows for information exchange that could potentially expose the blind sample as a test.

As a result, HFSC had to work with the client to ensure tests remained disguised from receipt to reporting. To master evidence packaging, HFSC's quality team met with narcotic sergeants to learn their packaging and submission process. In addition, HFSC had to make a formal request to the Houston Police Department to receive approval for the use of narcotics' evidence submission forms and envelopes.

Obtaining controlled substances proved to be the greatest challenge due to state laws surrounding the forfeiture and destruction of seized drugs. Alternatively, negative samples that can be equally challenging to the system can be submitted, but it has proven to be difficult to create samples similar in appearance to true controlled substances. The key to overcoming these challenges has been a collaboration between the laboratory, the client, and the legal system.

Despite these challenges, the value gained from the program has been immeasurable. One researched benefit of blind testing programs is behavior change described as the "observer effect." Research has shown that when individuals believe they are being watched, their behavior changes. For this reason, a blind proficiency program can be a tool to combat perceived bias in forensic science. In addition, the clients and the analysts unanimously agree the program instills added confidence in testing results. The blind proficiency program is just one tool that HFSC uses to provide its clients with better service.

Blind Proficiency Test, Quality Control, Drug Analysis

B86 Quantifying Uncertainty in Estimations of the Total Weight of Drugs in Groups of Complex Matrices Using the Welch-Satterthwaite Equation

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After attending this presentation, attendees will better understand the use and robustness of a new statistical method to quantify estimations of total drug weight in groups of items, based on the Welch-Satterthwaite equation.

This presentation will impact the forensic science community by introducing a new method to quantify estimations of total drug weight in groups of complex matrices.

In a recent case at the drugs department of the Netherlands Forensic Institute (NFI), materials were received from the Schiphol national airport consisting of 43 T-shirts, boxer shorts, shorts and blouses; 12 socks; 8 vests and short trousers; 5 pieces of denim clothing; and 2 towels. Based on a color test, and verified by Gas Chromatography/Mass Spectrometry (GC/MS) analysis, the items were impregnated with cocaine. This type of casework is encountered often and the crucial question is: what is the total drug weight in the combined items? In order to answer this question, standard laboratory procedures have been developed. First, the total weight of each item is determined, then repeated concentration measurements are performed on some or all of the items (in this particular case, for example, not all items from groups 1 and 2 were sampled). The concentrations are measured using Gas Chromatography/Flame Ionization Detector (GC/FID) on samples taken from different locations on the items.

In the literature, guidelines exist on the expression of uncertainty in analytical measurements; additionally, the International Organization for Standardization (ISO) 17025 requires that forensic laboratories determine uncertainty on their measurements. The issue of how to sample from consignments of drugs, and how large samples should be in order to obtain required results, has been widely discussed. Frequentist and Bayesian methods have been described in cases in which no measurement uncertainty is attached; however, these studies do not address the question of how to deal with a situation in which considerable uncertainty is attached, which may differ for each item.

The question as to the total amount of drug weight, and the precision of its estimation, in the combined items may get complicated, and statistical methodology to handle this has been described.[1] The methodology is based on the following assumptions: (1) within each of the groups, the relative standard deviation on repeated measurements is the same; (2) the measurements are unbiased estimators of the real concentrations of drugs in the items, and repeated measurements on the same item (at different locations) are normally distributed; and, (3) when sampling takes place in one or more of the groups, the real concentrations of drugs in the items of the group are normally distributed.

It may happen that, for example, the assumption of a constant relative standard deviation is violated, or that normality of the measurements is untenable (e.g., for data with point masses at zero or if certain items in a group have a very high standard deviation). In cases such as these, it makes sense to use a model in which the relative standard deviation may be different for each item. A method is described for dealing with this type of situation based on the Welch-Satterthwaite equation, both for cases in which all items are sampled and cases in which this is not so. In this set-up, the assumption of constant relative standard deviations is not necessary.

In this presentation, case examples will be presented in which the method is applied, both for the situation in which all items are sampled and the situation in which this is not the case. Furthermore, results of simulation studies will be shown that study the effect if the data are not normally distributed but uniformly or if a substantial amount of them contain zeros (measurements are on locations containing no drugs at all). The simulation studies suggest that both the method described in this presentation and the methods described by Alberink et. al yield reliable results, including if data have point masses at zero or a large standard deviation.[1] Coverage of 95% intervals is always close to 95%. If the assumption of common relative standard deviations clearly does not apply, it is nonetheless advised that the method based on the Welch-Satterthwaite equation be used.

Reference(s):

1. Alberink I., Sprong A., Bolck A., Curran J.M. Quantifying Uncertainty in Estimations of the Total Weight of Drugs in Groups of Complex Matrices. *J. Forensic Sci.* 59 (6) (2014) 1614-21.

Drug Weight Estimation, Measurement Uncertainty, Welch-Satterthwaite Equation

B87 Estimations of Measurement Uncertainty for Weights of Controlled Substance Evidence and the Effect of Environmental Conditions

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WITHDRAWN

B88 An Analysis of Illicit Drugs in Wastewater: A Forensic Perspective

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After attending this presentation, attendees will understand the principles of wastewater analysis. Attendees will also be informed of the contribution this tool can bring in a forensic perspective, in particular deciphering the dynamic structure of illicit drug consumption in several types of environments.

This presentation will impact the forensic science community by emphasizing the value of the information that wastewater analysis can deliver and the added value of triangulating this data with other complementary sources of information.

In 1920, Edmond Locard formulated one of the core principles of forensic science, stating (in its epitomized form) that "every contact leaves a trace." By extension, this principle can also be applied to the consumption of a drug or, in particular, to an illicit drug. Actually, when one consumes such a substance, in order to assimilate and eliminate it, the product is transformed into its related metabolites. In other words, cocaine will be excreted via urine and feces in the form of its metabolite, benzoylecgonine, as well as the parent compound, cocaine itself.

These traces are then relics conveying objective information about the type, the relative quantity, and the pattern of consumption of a specific population. To obtain this information, the sampling strategy is essential. It is necessary to choose a place where these residues of consumption of the target population are combined; in this case, the entrance of the wastewater treatment plant of the area under investigation. Another application of this wastewater-based approach focuses on (semi-) closed environments, such as a prison or music festival. These configurations influence the sampling setting as well as the interpretation of the obtained data.

This study presents the application and limitations of this approach of wastewater analysis using results gathered over a three-year period, both for longitudinal and comparative studies. The main application of these results focuses on the determination of the consumption of illicit drugs in a population based on the amounts of the drug residue present in the target wastewater treatment plant, known as back-calculation. From these loads, it is also possible to infer the quantity of related illicit drugs or even the number of doses. While such findings are the most commented aspects of wastewater analysis, especially by the media, this study advises that there is more potential in these results, mainly by performing cross-analysis. A discussion on this interdisciplinary approach will be illustrated by presenting the added value of combining wastewater results with existing data related to illicit drugs, such as epidemiological or police data, as well as public health information.

Illicit Drugs, Wastewater, Triangulation

B89 Forensic Body Fluid Identification and Differentiation by Raman Spectroscopy

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The goal of this presentation is to educate attendees regarding how multivariate data analysis can be leveraged to extract information from Raman spectra and build predictive models.

This presentation will impact the forensic science community by offering a new approach for body fluid analysis, which, in many respects, is an improvement over the current methods.

The ability to identify body fluid traces at crime scenes and preserve any DNA present is critically important in forensic science. Identification can be difficult because many of the current techniques are specific to one body fluid, and typical biochemical methods are destructive, which prevents any further analysis. To develop a universal, confirmatory, non-destructive approach that can be used to differentiate and identify body fluids, the specificity of Raman spectroscopy was combined with the analytical power of statistical modeling.

Raman spectra were collected from 75 body fluid samples, including peripheral blood, saliva, semen, sweat, and vaginal fluid. After preprocessing the experimental spectra, the samples were split into calibration and validation datasets. Several chemometric analysis techniques were trained and tested to find the best model. These included Partial Least Squares Discriminant Analysis (PLSDA), Support Vector Machine Discriminant Analysis (SVMDA) modeling, and variable selection by interval PLSDA (iPLSDA) and Genetic Algorithm (GA). By exploring so many different combinations of classification algorithms and variable selection methods, this research was able to study patterns in the data, the effects of various modeling parameters, and to ultimately determine the most robust method for differentiation. All of the models were internally cross-validated during calibration and externally validated with a test dataset.

The first PLSDA model, which used the entire spectral range, misclassified 2.3% and 2.1% of the calibration and validation spectra, respectively. When an SVMDA model was trained by the same dataset, it misclassified 0.9% and 0.5% of the calibration and validation spectra, respectively. When iPLSDA was employed for variable selection, the rate of error in calibration predictions decreased; however, the error rate of validation predictions increased. Lastly, the dataset produced through variable selection by GA was used to train a final PLSDA and SVMDA model. The rate of misclassifications in the calibration dataset by the PLSDA model decreased to 2.2%, while the misclassifications in the validation dataset dropped to 1.7%, both slightly lower than the first PLSDA model. The final SVMDA model built on a dataset produced by GA performed the best. This model accurately predicted the identity of 99.9% of the spectra from the calibration dataset. More importantly, it correctly predicted the identity of 100% of the spectra in the external validation dataset.

All five body fluids were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is reliable, non-destructive, and is not specific to one body fluid, offering substantial advantages over the current techniques used to identify body fluids.

Forensics, Body Fluids, Chemometrics

B90 Accurately Estimating the Time Since Deposition (TSD) of Bloodstains Aged for More Than Two Years

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After attending this presentation, attendees will better understand how Raman spectroscopy, coupled with multivariate statistical analysis, can be used to analyze bloodstains aged for more than two years and subsequently: (1) confirm that the stains are blood and not another body fluid; as well as, (2) accurately predict the TSD.

This presentation will impact the forensic science community by demonstrating the ability to expand upon a unique non-destructive approach for the confirmatory identification of blood over time and the accurate estimation of bloodstain age, or TSD. Additionally, this presentation can significantly help with crime scene reconstruction by providing more clues about the time of a crime, or order of events, particularly when a body is not available for physical examination.

The identification of a body fluid stain is an important and necessary aspect of many forensic investigations. For blood in particular, knowing the TSD is highly desired in forensics, but it can be extremely complicated to accurately determine in practice. Although there have been numerous attempts to solve this problem using a variety of different techniques, currently no established well-accepted method exists. Since the amount of suspected blood evidence may be miniscule, it needs to be preserved and analyzed efficiently. Therefore, a non-destructive method to competently identify human blood and predict the TSD would be highly valuable. Raman spectroscopy is a technique that has the potential for both non-destructive confirmatory identification of blood and for detecting (bio) molecular changes over time that can be associated with the age of a bloodstain.

Raman spectroscopy has proven to be a versatile and effective analytical technique for numerous forensic applications, including the identification of drugs, explosives, gunshot residue, inks, and paints. Raman analysis often requires no sample preparation, is considered non-destructive, and has the ability to analyze microscopic amounts of sample. This technique is based on the detection of light that is inelastically scattered by a sample upon irradiation from a monochromatic light source. A Raman spectrum contains numerous distinctive bands that correspond to specific molecular vibrational modes. For blood in particular, Raman spectra provide rich detail and it has already been the subject of analysis in many forensic studies.

For this work, a previously developed Raman spectroscopic methodology for determining the TSD of bloodstains was tested.[1] Raman spectroscopy and chemometric modeling were used to analyze fresh bloodstains stored under ambient conditions for more than two years. A recently developed Support Vector Machines Discriminant Analysis (SVMDA) model was used for blood identification.[2] This model allowed for confirming a fluid's identity as blood through differentiation of blood from four other body fluids (i.e., saliva, semen, sweat, and vaginal fluid).

To provide quantitative predictions of the TSD, Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR) models were built on spectra collected at 14 time points. Both models were internally Cross-Validated (CV) and externally validated. The PLSR and PCR models had a CV Root Mean Squared Error (RMSE) of 0.17 and 0.18, respectively. They both showed a high degree of linearity with an R^2 of 0.98. Also, both models demonstrated similar external TSD prediction abilities with an RMSE of prediction of 0.29 and 0.31 for PLSR and PCR, respectively.

These results demonstrate that Raman spectroscopy can be used as a non-destructive analytical tool for confirming the identity of blood over time and discriminating between bloodstains on the scale of hours to days to years. This is very important for forensic science in helping to reconstruct a crime scene, as well as in establishing the relevant association of multiple bloodstains. This approach shows potential for practical use in the field to predict the TSD with a high degree of accuracy, especially since portable Raman spectrometers are now available. In the future, more work will be conducted regarding analyzing bloodstains left to age under different environmental conditions as well as on different substrates.

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Bloodstains, Raman Spectroscopy, Chemometrics

B91 Toward Surface-Enhanced Raman Spectroscopy (SERS) -Active Forensic Evidence Swabs for Human Bodily Fluids

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After attending this presentation, attendees will better understand how SERS could be used in the forensics workflow for the confirmatory analysis of human bodily fluids.

This presentation will impact the forensic science community by providing results of the fabrication of SERS-active evidence swabs, identification of human bodily fluids by spectroscopic analysis, and the further extraction, quantification, and Short Tandem Repeat (STR) typing of the samples collected on the swabs. SERS-active evidence swabs are not currently available to the forensic science community.

The collection and identification of human body fluids at a crime scene can be a crucial aspect of an investigation. In the crime laboratory, evidentiary swabs that may potentially contain human body fluids are screened, using both presumptive and confirmatory tests. These tests, such as the Acid Phosphatase (AP) test and the Christmas tree stain for semen and the Kastle-Meyer and Takayama tests for blood, are quite time consuming, expensive, only test for one body fluid, and are prone to both false positives and false negatives; however, recent literature reports have indicated that Raman spectroscopy may have a far lower limit of detection than traditional methods and may allow investigators to perform one type of measurement for all body fluids, potentially leading to higher efficiency, more definitive results, and higher accuracy. Another recent report utilized SERS as a means to decrease the limit of detection of certain forensic samples. SERS, in which an analyte is placed on or near a nanostructured metal surface, has the potential to increase the Raman cross-section of the analyte by many orders of magnitude.

This presentation reports on the development of SERS-active forensic evidence swabs by attaching silver nanoparticles grown via the hydrogen reduction method to the fibers of commercially available swabs. For this study, SERS-active swabs were used to collect and measure the Raman spectrum of semen. Different swab fabrication parameters, such as reaction time (30 minutes-360 minutes), reaction temperature (40°C-100°C), and swab pretreatment protocols were varied in an effort to maximize the Raman signal of the semen. Integrated SERS intensity of semen-specific Raman bands were compared to silver particle size, silver concentration and spacing between particles on the swab fibers. Minimum volumes of semen that could be detected using the SERS-active swabs were also found. DNA extraction and quantitation was performed on semen samples collected on pristine swabs and SERS-active swabs, both those that had been exposed to laser radiation for Raman analysis and those that had not. Although extraction yields were higher for pristine swabs due to some dissolution of silver on the SERS-active swabs, STR typing was found to be possible.

Raman, SERS, Evidence Swab

B92 A New Approach Combining *In Silico* and High-Resolution Melt (HRM) Analysis to Quickly Identify New Loci for Body Fluid Identification Using DNA Methylation Melt Analysis

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After attending this presentation, attendees will be informed of a new combined approach using bioinformatic tools and HRM analysis to quickly and inexpensively identify genome locations from an array dataset for DNA methylation analysis.

This presentation will impact the forensic science community by demonstrating that HRM is an excellent tool to identify epigenetic loci for body fluid identification at a research level and to be used as a body fluid discrimination method easily incorporated into the daily routine of forensic laboratories.

DNA methylation is a natural process in the mammalian genome involving the addition of a methyl group to the 5'carbon of cytosines in a dinucleotide Cytosine-Guanine (CpG) pair.[1] Tissue-specific Differentially Methylated Regions (tDMRs) present different methylation patterns depending on the cell type analyzed, making them interesting targets for body fluid discrimination.[2-7] To fully take advantage of this approach, it is necessary to identify new tDMRs that consistently show methylation differences in body fluids. Whole genome analysis of CpGs using a commercially available array followed by a confirmatory Polymerase Chain Reaction (PCR) -based assay can be costly and time consuming. Alternatively, a computer-based, *in silico* approach can save much effort through the use of bioinformatics analysis.

Bisulfite-modified PCR is a useful method to determine relative levels of DNA methylation. In this procedure, unmethylated cytosines are chemically converted to uracils, which then are converted to adenines during real-time PCR. The methylated cytosines are protected from bisulfite conversion; therefore, methylated DNA will have higher melt temperature (T_M) than unmethylated DNA.[8-10] Since HRM is cost- and time-effective, it constitutes an excellent experimental tool to perform screening for multiple tDMRs quickly. Due to its fast turnaround, HRM also has considerable potential to be used as a standard method to discriminate body fluids in forensic laboratories.

Blood, buccal swabs, vaginal, and semen samples were collected from volunteers according to the approved Institutional Review Board (IRB) 13-0555 from Florida International University. DNA was extracted using the EZ1® DNA Investigator kit and the BioRobot® EZ1 automated purification workstation according to the manufacturer's specifications. Fifty nanograms of DNA were bisulfite modified using the EpiTect® Fast DNA Bisulfite Kit according to manufacturer's instructions. Primers specific for the CpG of interest were designed using either the Methprimer or the BiSearch online tools.[11,12] Bioinformatic analysis was performed using R software to determine relevant CpGs to discriminate blood, saliva, and vaginal epithelia. Array data from the report by Park and colleagues was analyzed and the M-value calculated to perform statistical analysis.[13] CpGs identified as statistically significant were further filtered for relevance using the ratio of methylated versus unmethylated probes and comparing such ratio between body fluids. Real-time PCR reactions were performed using the Epitect® HRM kit on a Rotor Gene 6000 real-time machine.

Results illustrate that using bioinformatics tools to perform array data analysis reduces the cost to identify new genome locations for body fluid identification using DNA methylation. The most successful approach was to use the calculated M-values to statistically identify significant differences between blood, saliva, and vaginal epithelia from the array study.[13] The CpGs were further sorted by analyzing the relative light emission from the probes pertaining to one body fluid in comparison to the other two. To date, this approach provided 71% success in identifying new CpGs *in silico.* For example, from seven CpGs identified as potential blood markers, five proved to show a difference in their T_M for blood when compared to saliva, semen, and vaginal epithelia, using HRM.

Using the information gained from these studies, a protocol for a multiplex qPCR with melt analysis that includes semen-, blood-, and vaginal epithelia-specific primers capable of providing results in approximately two hours was successfully tested. In conclusion, the combination of new bioinformatics and HRM analysis provides a cost- and time-effective approach to identify epigenetic loci for body fluid discrimination using DNA methylation. Moreover, due to a high throughput of HRM, this method will allow forensic laboratories to determine differences in DNA methylation for body fluids without the need to invest in expensive DNA sequencers, reagents, or personnel training and time.[2,6,9]

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High-Resolution Melt, DNA Methylation, Body Fluid Identification

B93 The Development and Validation of a Dual-Genus Quantitative Polymerase Chain Reaction (qPCR) Assay for African and Asian Elephants for Forensic Purposes

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After attending this presentation, attendees will understand how a qPCR assay has been developed that will detect and quantitate African and Asian elephant DNA simultaneously. Attendees will also learn how to efficiently create qPCR assays for wildlife identification and quantitation.

This presentation will impact the forensic science community by providing a standardized qPCR assay that detects and quantitates elephant DNA for use in wildlife investigations. The development of this assay fills a research gap in wildlife forensic science.

Real-time PCR is most commonly used in the forensic community to quantify small amounts of human DNA in evidentiary samples. In the growing field of wildlife forensic genetics, real-time PCR is utilized primarily for identifying the species of origin from illegally traded animal byproducts. Although DNA sequencing of mitochondrial DNA is the most common approach for species identification, it can be costly. Utilization of a multiplex real-time PCR assay can be a fast, inexpensive, and robust approach to species identification that can aid law enforcement in prosecuting crimes against animals.

African (*Loxodonta africana)* and Asian (*Elephas maximus*) elephant populations are categorized under Appendix I and II of the Convention on the International Trade of Endangered Species (CITES), respectively. CITES is an agreement that regulates plant and animal species throughout the world to ensure that international trade of their products does not impact their survival. An Appendix I listing includes species that are threatened with extinction; thus, trade of these plants and animals is highly restricted. Species not facing extinction that require extra attention and regulations so they don't become exploited and over-utilized are listed in Appendix II. The primary reason for the decline of these two animals is the illegal trade of their ivory. Other reasons for the decline in the elephant population are deforestation and human conflict.

In wildlife crime laboratories, species of origin can often be determined by morphology. This method is limited by the expertise of the taxonomist and the condition of the animal product. Ivory is commonly carved into small figurines and trinkets. Elephant meat, hair, and hide are traded, which can make it difficult to identify the species. These limitations have led to the development of genetic tests to identify species of origin in wildlife investigations. The *cytochrome b* (*cyt b*) region of the mitochondrial genome is well established as a genetic marker for species identification. African and Asian elephants do have highly similar genomes; however, variation exists in portions of their *cyt b* gene.

In this study a dual-genus, real-time PCR assay to identify elephant DNA for forensic purposes was developed. By eliciting information from the variable areas of the *cyt b* gene in elephants, both genera of animals can be differentially identified and quantified in a robust and cost effective assay. Costs were decreased by scaling down reaction volumes and using one set of primers. Following the assay development, a rigorous developmental validation was conducted according to current community recommendations set forth by the Scientific Working Group for DNA Analysis and Methods (SWGDAM). The completion of this work provides an assay that can generate data of evidentiary quality for wildlife crime laboratories.

Elephant, qPCR, DNA

B94 The Individualization of Pubic Hair Bacterial Communities and the Effects of Storage Time and Temperature

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After attending this presentation, attendees will better understand a novel forensic technique using the pubic hair microbiome that has the potential to link individuals who have come into contact with each other. Additionally, attendees will understand the impact of storage time and storage temperature on the pubic hair microbiome.

This presentation will impact the forensic science community by introducing a novel forensic method with the potential to associate two individuals in a sexual assault. Attendees will also be informed regarding how to best store these types of samples for future analysis.

A potential application of microbial genetics in forensic science is the detection of transfer of the pubic hair microbiome between individuals during sexual intercourse using high-throughput sequencing. In addition to the primary need to show whether the pubic hair microbiome is individualizing, another aspect that must be addressed before using the microbiome in criminal casework is the impact of storage on the microbiome of samples recovered for forensic testing.

To test the effects of short-term storage, pubic hair samples were collected from volunteers and stored at room temperature (~20°C), refrigerated (4°C), and frozen (-20°C) for one week, two weeks, four weeks, and six weeks in addition to a baseline sample, followed by amplification and sequencing of the V3/V4 region of the 16S rRNA gene. Individual microbial profiles ($R^2 = 0.69$) and gender ($R^2 = 0.17$) were the greatest sources of variation between samples. Because of this variation, individual and gender could be predicted using Random Forests supervised classification in this sample set with an overall error rate of 2.7% $\pm$ 5.8% and 1.7% $\pm$ 5.2%, respectively. There was no statistically significant difference attributable to time of sampling or temperature of storage within individuals. Further work on larger sample sets will quantify the temporal consistency of individual profiles and define whether transfer between sexual partners can be detected. For short-term storage ($\leq$ six weeks), the microbiome recovered was not significantly affected by the storage time or temperature, suggesting that investigators and crime laboratories can use existing evidence storage methods.

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

Bacterial Forensics, Human Microbiome, Storage

B95 The Development and Validation of a New 13-Loci Short Tandem Repeat (STR) Multiplex for *Cannabis sativa* Genetic Identification

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After attending this presentation, attendees will understand the basic principles behind using an STR multiplex method for individualizing marijuana samples.

This presentation will impact the forensic science community by providing an STR panel that could not only assist law enforcement agencies in verifying legal marijuana products, but also aid in the linkage of illegal cases. This method could also serve as an additional tool to previously established marijuana profiling programs used in federal agencies such as the United States Customs and Border Protection (CBP) and the Drug Enforcement Administration (DEA).

Forensic DNA typing is typically performed on human DNA samples; however, the molecular analysis of plant DNA is increasingly being studied and considered for use in criminal justice systems around the world. Plant DNA can be used to link a suspect/victim to an area or, in the case of marijuana, can be used to aid in the investigation of drug cases. Marijuana (*Cannabis sativa* L.) is a plant cultivated and trafficked worldwide as a source of fiber (hemp), medicine, and intoxicant. The development and validation of a method using molecular techniques such as Short Tandem Repeats (STRs) could serve as an intelligence tool to link multiple cases by means of genetic individualization/association of *Cannabis* samples. In 2003, the first polymorphic STR markers were published for *Cannabis sativa*. Previous research has shown the utility of these markers in individualizing marijuana samples; however, the technique has been scarcely used in crime laboratories due to lack of standardization and validation.

For this purpose, a new 13-loci STR multiplex method was developed, optimized, and validated according to the International Society of Forensic Genetics (ISFG) and Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. The 13-loci multiplex mainly consisted of previously described tri- and tetra-nucleotides *Cannabis* STRs: ANUCS501, 9269, 4910, 5159, ANUCS305, 9043, B05, 1528, 3735, CS1, D02, C11, and H06. Validation studies were comprised of: (1) species specificity; (2) sensitivity; (3) Hardy-Weinberg and linkage equilibrium in a reference population; (4) heterozygous Peak Height Ratios (PHR); (5) inter-loci balance; (6) stutter ratios; and, (7) precision and accuracy. In addition, a sequenced allelic ladder consisting of 55 alleles was designed to accurately genotype 101 *C. sativa* samples from three seizures provided by a federal agency.

Using an optimal range of input DNA (0.125ng-0.5ng), validation studies revealed minimal artifacts and stutter (average stutter ratio of 0.021 across all loci), relatively balanced heterozygous peaks (average PHR of 0.83 across all loci), and a well-balanced electropherogram (inter-loci balance range: 0.500-1.296). The combined power of discrimination of this multi-locus system was 1 in 55 million with a sensitivity of 125pg of template DNA. The 13-STR panel was found to be specific for *C. sativa*; however, non-specific peaks were produced with *Humulus lupulus*.

In conclusion, the results of this research demonstrate the robustness and applicability of this 13-locus STR system in identification of *Cannabis* samples for intelligence purposes.

Forensic Botany, Cannabis Sativa, Short Tandem Repeats

B96 The Development and Testing of a Top-Down Proteomic Method for the Confirmatory Identification of Saliva

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After attending this presentation, attendees will understand some of the issues with current techniques for the identification of saliva, how protein mass spectrometry can be used to identify a panel of salivary protein biomarkers that are specific to saliva, and why protein mass spectrometry is a more sensitive and reliable method for confirmatory identification of saliva than methods currently in use.

This presentation will impact the forensic science community by providing a confirmatory method for identifying saliva in forensic casework, thereby potentially aiding in sexual assault investigations.

While DNA analysis is often considered the "gold standard" of forensic biology, being able to determine the source fluid(s) in addition to a DNA profile can provide critical context to a case. Immunochromatographic and enzyme-based tests are commonly employed for screening evidentiary material for saliva; however, neither of these assays provide true confirmatory results as false positive results with non-target fluids and false negative results with dilute and/or degraded samples are well known. Several emerging techniques for confirmatory body fluid identification seek to remedy this gap by developing more reliable analytical techniques based on protein mass spectrometry, messenger RNA (mRNA), micro RNA (miRNA), and epigenetic profiling of body fluids.

Several studies employing protein mass spectrometry have been successful in identifying saliva; however, serious throughput limitations exist, which can prohibit the adoption of these techniques for routine serological screening. For example, multi-day sample preparation workflows coupled with 60+ minute analytical runs are not uncommon.

The goal of the current research is to create a faster, laboratory-compatible workflow for saliva identification. This was achieved by direct enrichment for low molecular weight salivary proteins and analysis on an AB SCIEX™ TripleTOF® 5600 platform. Using this approach, sample preparation can be completed in less than two hours from collection to injection, therefore allowing same-day analysis of evidentiary samples. Initial studies have identified several saliva-specific candidates, such as basic salivary proline-rich protein 1/2, salivary acidic proline-rich proteins 1, 2, 3, and 4, and submaxillary gland androgen-regulated protein 3B. Further studies are underway to identify additional biomarkers and to identify the most reliable biomarkers for identifying saliva by characterizing the fluid in the population by analyzing 50 unique saliva samples.

In conclusion, this study illustrates that protein mass spectrometry is a sensitive and specific method for the confirmatory identification of saliva, providing a truly confirmatory method for the detection of saliva in sexual assault evidence.

Saliva, Confirmatory Identifiation, Proteomics

B97 A Rotationally Driven Microdevice (RDM) and Its Associated Low Footprint Instrument With the Potential of Fully Automated Integrated DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and Separation for Forensic DNA Analysis

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After attending this presentation, attendees will better understand the forensic application of RDMs and the recent advances toward a novel microfluidic device platform for forensic DNA analysis.

This presentation will impact the forensic science community by describing the development and proof-of-principle for an integrated "sample-to-answer" RDM platform for DNA analysis with the potential for fully automated genotyping.

Microdevices offer numerous advantages over the conventional methods used in forensic laboratories for rapid STR profiling, including: (1) smaller reagent and sample consumption; (2) the ability to integrate processes limiting the operator steps (hands free); (3) expediting sample-to-answer response; and, (4) portability. While several companies have successfully demonstrated the capability of Rapid DNA instrumentation, and while some are portable, they are still large and prohibitively expensive.[1-3] There is a need for the development of an inexpensive, rapid, and portable device for DNA extraction, Short-Tandem Repeat-Polymerase Chain Reaction (STR-PCR), and DNA fragment separation in forensic analysis. To meet these needs, this presentation reports the development of a unique, multi-layer, RDM and the associated centrifugal platform that can perform DNA extraction, amplification, and separation. This device is fabricated using a print-cut-laminate process that has been recently described using common office equipment, a laser cutter, and inexpensive substrates.[4] Unlike previously described systems, this does not require bulky instrumentation (external pumps, air compressor, and large actuators) as the integration of the processes and all fluid movement within the microchip rely on centrifugal force to drive fluidic movement. Therefore, this platform has the potential to be fully automated, compact, portable, and cost-effective human Identification (ID) system.

Initially, three separate rotationally driven microfluidic chips were designed (extraction, PCR, and electrophoresis), and their functionality demonstrated independently. The fabrication of RDMs from Polyester film (Pe), Pressure-Sensitive Adhesive (PSA), and other proprietary materials not typically used in microfluidics allowed for rapid, cost-effective, in-house prototyping. Non-solid phase extraction from buccal swabs using an enzyme-based reaction (ZyGEM® prepGeM) was successfully demonstrated on no fewer than 20 microdevices with different DNA donors and showed an average yield of 5.9ng/μL ± 2.4ng/μL, with all extractions leading to full STR profiles using a platform with spin-based capabilities. The same platform was used for microdevice PCR with rotation-driven fluid flow and reagent mixing. Successful PCR of ten STR markers in fewer than 20min was demonstrated with different substrates that were compatible with integration with the other steps. In this study, 100% of the profiles obtained were concordant with conventional typing methods, and presented satisfactory intra- and interlocus balance. Finally, electrophoresis on an RDM comprised of common materials, a Cyclic Olefin Copolymer (COC), and integrated gold electrodes was successfully demonstrated. The sieving matrix and sample were loaded centrifugally, and electrophoresis was complete in <8min with an unprecedented effective separation

length of only 4cm. This process was performed on a different platform with heating, spin control, and a high-voltage power supply for electrophoretic separation with optical detection capabilities.

Once the individual sub-assays were demonstrated, the architectural features needed for the integration of the three processes into a single hybrid RDM was designed and tested. A unified, integrated platform possessing all of the necessary hardware needed to perform sample-to-answer STR profiling was designed and manufactured in-house with off-the-shelf components, laser-cut materials, and 3D printed parts. While the forensic data quality of the integrated assay requires further optimization, proof-of-principle for integrated "liquid" extraction, STR amplification, and microchip electrophoresis is demonstrated on a cost-effective microdevice instrument whose small footprint is unprecedented. This system is a potential game changer for the criminal justice system where adoption of the Rapid DNA systems is limited by the cost of the instrument and the price of cartridges. In summary, this study presents the first rotationally driven, integrated, sample-to-result RDM for forensic DNA analysis operated by a device that is truly portable and can significantly reduce the price of Rapid DNA analysis.

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Centrifugal Device, Rapid DNA, Integrated Device

B98 A Novel, Extraction-Polymerase Chain Reaction (PCR) Microdevice on a Rotation-Driven Platform: Buccal Swab to Short Tandem Repeat (STR) Product in Less Than Two Hours

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After attending this presentation, attendees will better understand the applications and capabilities of forensic microdevices, as well as the challenges of transitioning conventional DNA workflow onto the microscale.

This presentation will impact the forensic science community by describing an integrated plastic microdevice for DNA extraction and STR amplification that is capable of producing Capillary Electrophoresis (CE) -ready STR product from a buccal swab in less than two hours.

Microdevices have numerous advantages over conventional methods, including smaller reagent volumes, smaller equipment footprints, reduced contamination risk, and faster sample-to-answer time. Three unique aspects of the device presented in this work allow for successful integration. First, microfluidic flow control is achieved by exploiting centrifugal force on a simple rotational platform. This is coupled with the use of simple "passive" valves specifically positioned to physically isolate the different pre-electrophoresis chemistries. Second, enzyme-mediated DNA liberation (non-solid phase extraction) is fast (<10 minutes total) and effective as it circumvents the need for wash steps, hazardous chemicals, and solid beads, thus simplifying its incorporation into the overall microdevice architecture. Third, non-contact heating is integral to both DNA liberation and PCR amplification of STR loci. An Infrared (IR) -mediated heating system consisting of a platform, lamp, and fan is used to directly heat and cool the device, avoiding the need to transfer liquid into microtubes as required by conventional thermocyclers. During DNA liberation, the enzyme (a thermostable protease) is heated by the infrared lamp, lysing cells and exposing the nuclear DNA. During Infrared-mediated PCR (IR-PCR), thermal cycling of the sample is facilitated by the lamp and fan. Direct heating of the sample (instead of indirect heating on a heat block), small sample volume, and the effect of IR radiation on water (exciting the stretch mode) combine to dramatically reduce ramp and hold times, allowing IR-PCR with 28 cycles and a 10-minute final extension to occur in 45 minutes.

All microdevices were fabricated using laser ablation and thermal bonding of Poly(Methyl-Methacrylate) (PMMA) layers. Using this microdevice, the enzyme-mediated DNA liberation module produced DNA yields similar to or higher than those produced using the traditional (tube-based) protocol. Initial microdevice IR-PCR experiments to test the amplification module and reaction (using Phusion® Flash/SpeedSTAR™) generated near-full profiles that suffered from inter-locus peak imbalance and poor adenylation (significant –A); however, subsequent attempts using KAPA2G and *Pfu* Ultra polymerases generated full STR profiles with improved inter-locus balance and the expected adenylated product. An integrated run designed to test microfluidic control successfully generated CE-ready STR amplicons in less than two hours (<1 hour of hands-on time). Using this approach, high-quality STR profiles were developed that were consistent with those produced using conventional DNA purification and STR amplification methods. This method is a smaller, more elegant solution than current microdevice methods and offers a cheaper, hands-free, closed-system alternative to traditional forensic methods.

Forensic Microdevice, Microfluidics, IR-PCR

B99 Ultrahigh-Speed Polymerase Chain Reaction (PCR): A Method for Obtaining Short Tandem Repeat (STR) -Based Genotypes in Eight Minutes or Less

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After attending this presentation, attendees will better appreciate new methods to accelerate the PCR reaction using a specially designed seven-loci STR multiplex that permits complete amplifications in eight minutes or less, thus aiding in the analysis of time-sensitive forensic casework.

This presentation will impact the forensic science community by demonstrating an approach toward optimizing rapid, multiplex PCR amplifications, which can be easily implemented in their own laboratories. This procedure uses small, high-speed thermal cyclers and fast polymerases with high processivity and inhibition resistance. The loci used in this multiplex are a small subset of the standard Combined DNA Index System (CODIS) set, making them readily compatible with current statistical approaches. This new procedure should be particularly useful for situations in which screening of suspects and crime scene samples is necessary.

It is often extremely important to rapidly screen suspect samples at border controls and police stations where the individuals in question can only be detained for short periods of time. Current DNA typing methods provide the best biometric information yielding identity, kinship, and geographical origin, but they are not sufficiently fast to permit identity of a suspect DNA in real time. Current rapid DNA systems take approximately 90 minutes, and full laboratory DNA analysis is even slower.[1,2]

Ultra-high speed PCR coupled with rapid polymerases can greatly reduce the processing time from sample to STR genotype.[3] Coupling these advances with new thermal cycler designs can make the analysis even faster. Using Peltier and flow-based thermal cyclers, PCR reactions have been optimized, resulting in the completion of singleplex reactions in four minutes and complete multiplex reactions in eight minutes. When coupled with microfluidic electrophoresis, complete genotypes can be produced in 15 minutes or less.[3]

To accomplish this, specially engineered enzymes and buffer systems have been tested to rapidly amplify autosomal and Y-chromosomal Short Tandem Repeat (Y-STR) multiplexes.[3,4] The designed multiplex includes D5S181, D13S317, D7S820, CSF1PO, D16S539, Penta D, and Amelogenin, which have sizes between 106bp and 454bp. A complementary rapid Y-STR multiplex has also been developed. By using off-the-shelf components and commercially available enzymes, it is possible to create a procedure that acts as a quick, highly informative sample screening process that also retains sufficient DNA for later manual processing using standard STR or Y-STR kits.

The first phase of this study further accelerated a previously reported amplification by increasing DNA concentration and reducing cycle number in order to produce a 7-locus multiplex amplification in eight minutes. Two different non-hotstart polymerases were compared in these studies. Additional work was performed on a novel flow-based thermal cycler in which the sample moves fluidically between two thermally isolated regions. This system was able to produce singleplex PCR reactions in four minutes. This work is currently being expanded to permit multiplex amplifications. In the current research, control DNA standards 9948 M DNA and K562 F DNA were used, as well as donated saliva samples from five adults. Samples were analyzed using gel, capillary, and microfluidic electrophoresis separation approaches.

The results of this study demonstrate the application of ultra-high speed PCR for the successful amplification of two different PCR multiplexes. With such a procedure in place, any crime laboratory can produce a nearly instantaneous genotype from buccal samples.

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Rapid PCR, Ultrafast Amplification, STR Genotypes

B100 Rapid DNA Analysis for Disaster Victim Identification

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After attending this presentation, attendees will understand the applicability of Rapid DNA analysis technology to Disaster Victim Identification (DVI) and family reunification. Attendees will also learn: (1) the impact of tissue degradation on the selection of samples for generations of Short Tandem Repeat (STR) profiles in mass casualty events; and, (2) the use of field-based kinship analysis software to assist in both victim identification and family reunification.

This presentation will impact the forensic science community by presenting processes that enable rapid identification of human remains and kinship identification in field-forward settings. The ability of first responders, primarily individuals without backgrounds in laboratory-based forensic DNA analysis and genetics, to perform STR analysis as well as sophisticated genetic analysis offers the potential to change the current paradigm in DVI. In particular, eliminating the interval between the disaster and receipt and analysis of samples at a laboratory will minimize deterioration of sample quality and time-to-profile generation and, most importantly, will accelerate familial reunification and bring closure to grieving family members.

The fully integrated Rapid DNA Analysis™ and kinship determination system is based on 27-locus FlexPlex™ chemistry. This six-color assay is modeled after Promega's® Fusion 6C chemistry with two additional Y-chromosomal Short Tandem Repeat (Y-STR) loci (DYS570 and DYS576) and the substitution of Penta D with D6S1043. The assay contains all expanded Combined DNA Index System (CODIS), United Kingdom, INTERPOL, European Standard, German and Australian core loci, and D6S1043, an important STR marker broadly used in China. Accuracy, concordance, precision, resolution, Personal Health Record (PHR), sensitivity, species specificity, and all other relevant measures meet or exceed required metrics. Developmental validation of the system is in progress.

Data demonstrating the functionality of the Accelerated Nuclear DNA Equipment (ANDE) Rapid DNA system for fully integrated, fully automated processing of a number of DVI sample types will be presented, with a focus on bone, muscle, and liver. Data on Rapid DNA processing of degraded tissues will also be presented based on mock samples exposed to excessive heat, explosive materials, microorganisms, and other conditions associated with mass casualty events. Data supporting the use of automated kinship analysis software and database creation will also be presented.

In conclusion, this study demonstrates the broad applicability of Rapid DNA analysis in mass disasters with an easy-to-use mobile system that can generate STR profiles from a broad range of sample types and perform kinship analysis.

Rapid DNA Analysis, Disaster Victim Identification, Kinship Analysis

B101 The Recovery of Mitochondrial and Nuclear Touch DNA From Spent Cartridge Casings

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After attending this presentation, attendees will understand the advantages and disadvantages of Short Tandem Repeat (STR) analysis and mitochondrial DNA (mtDNA) sequencing of touch DNA from spent cartridge casings for the identification of the loader of a firearm.

This presentation will impact the forensic science community by detailing how the recovery of touch DNA from spent cartridge casings is influenced by casing caliber and how the type of testing employed, either mitochondrial or nuclear, influences DNA typing success and the potential identification of the loader of a firearm.

Crimes involving firearms are common, yet the weapon itself is seldom recovered at a crime scene. In contrast, cartridge cases that are ejected during a shooting are regularly left behind by the shooter and are commonly submitted as forensic evidence in an attempt to identify the loader or shooter of the gun. Fingerprints may be deposited on cartridge cases by the loader; however, they are seldom recovered following a shooting. Due to the lack of fingerprints, DNA profiling may be attempted. This has had limited success from spent cartridge cases, most likely because the DNA is in trace amounts or highly degraded, possibly due to the intense heat generated or other aspects of the shooting process. Thus, mtDNA, which is present in hundreds of copies per cell encased inside the mitochondria, may be more advantageous for DNA testing from spent cases.

In the research presented, all testing had Michigan State University (MSU) Institutional Review Board (IRB) approval. Volunteers loaded magazines of firearms with 0.22 and 0.45 caliber cartridges. The volunteers also provided a buccal swab for reference. Firearm examiners at the Michigan State Police Lansing Forensic Laboratory subsequently fired the weapons. The ejected cartridge cases were collected and taken to the MSU Forensic Biology Laboratory for DNA analysis. DNA was isolated using an optimized double swabbing technique and organically extracted, followed by purification using pre-treated Amicon® filters. DNA was quantified via an Alu-assay using real-time Polymerase Chain Reaction (PCR). STR analysis was then performed using a PowerPlex® Fusion kit, and results were assessed based on the number of alleles consistent and inconsistent with the loader. MtDNA sequencing was also performed, and results were categorized as consistent or inconsistent with the loader or the presence of a mixture. The two analysis methods were then compared.

MtDNA haplotypes and STR profiles were typically in agreement with regard to the mtDNA classification and number of alleles consistent with the loader. Mitochondrial haplotypes consistent with the loader had a median of 11 loader alleles in STR profiles, while haplotypes inconsistent with the loader had a median of 7.5 loader alleles. Haplotypes displaying a mixture had a median of nine; however, the methods differed in terms of amplification success and identification of the loader. MtDNA sequencing proved to be more robust than STR analysis, as mtDNA was successfully amplified from all extracts, whereas complete STR profiles were rare. Alternatively, STR profiles that had numerous loci amplifications were more individualizing than mtDNA in that some volunteers shared haplotypes, while STR results were unique. Cartridge caliber also played a substantial role in DNA results. Significantly more DNA was recovered from 0.45 caliber cases than 0.22 caliber cases ($p = 0.0023$), with 0.45 caliber cases also producing a significantly higher median number of loader alleles ($p = 0.0062$) even though both calibers were loaded into magazines in the same manner. On the other hand, classification of mtDNA haplotypes did not differ significantly between case caliber ($p = 0.321$). Given these results, it is important to recognize that STR analysis and mtDNA sequencing generate different outcomes, each having distinct advantages and disadvantages. The results also demonstrate the value of the optimized DNA isolation methods, which were successful in obtaining DNA from spent cases, which is vital if DNA profiling of cases is to widely undertaken.

Cartridge Casings, Touch DNA, Mitochondrial and Nuclear DNA

B102 Deliberations on Y-Chromosomal Short Tandem Repeat (Y-STR) Interpretation, Present and Future

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After attending this presentation, attendees will better understand the proper analysis of Y-STR profiles, with particular consideration paid to rapidly mutating loci and mixed haplotypes, and the current and future approaches for statistical analysis of Y-STR matches.

This presentation will impact the forensic science community by informing attendees of issues related to the application and interpretation of male-specific STR testing.

Y-STR typing methods were developed shortly after autosomal STR typing became adopted in forensic laboratories. It was quickly recognized that a male-specific typing test could be valuable in cases in which male DNA would otherwise be swamped by the amount of female DNA in certain samples. This opened up investigative opportunities for sexual assault cases that may have previously been passed over, particularly cases in which no spermatozoa were detected, either because the incident was not immediately reported or no ejaculation occurred.

Despite its advantage over autosomal STR typing in these cases, Y-STR typing was not implemented by forensic laboratories as readily. While the methodology required to obtain Y-STR profiles is nearly identical to that used in obtaining traditional STR profiles, a completely new approach to interpretation and statistical analysis had to be developed. What does the Y-STR profile mean? First, it is a true haplotype rather than a DNA profile of independent loci. All of the loci are linked and theoretically inherited as a group. Second, a match to an individual male is also likely to be a match to any patrilineal relative and to an unknown number of unrelated males. Third, the strength of the match is limited by the size of the database used for comparison and by the number of loci tested. Fourth, samples containing haplotypes from more than one male proved very difficult to reliably interpret.

New kits that include more Y-STR loci are now commercially available; however, only a small percentage of the samples in the commonly used Y-STR population databases contain haplotypes with these additional loci. Some of the new kits also have rapidly mutating loci that can assist in differentiating close male relatives. What impacts do the additional loci and the rapidly mutating loci have on conclusions and statistics, particularly for mixed haplotypes?

A two-hour block of time has been scheduled for deliberations on the present and future approaches to Y-STR interpretation. The panel convened for this session consists of analysts, researchers, and statisticians with extensive experience in the development of Y-STR interpretation approaches and in the application of Y-STR analysis to forensic casework. The discussion will include topics such as the cost-benefit of searching more haplotypes or more loci, comparing the counting method with the likelihood ratio methods, familial/pedigree studies, and the impact of future typing technologies.

Y-STR, Interpretation, Statistics

B103 Evaluating the Efficiency of the Use of the Qiagen® QIAsymphony® With High-Throughput Y-Screening Utilizing Phosphate-Buffered Saline as an Alternative to Conventional Serology

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After attending this presentation, attendees will better understand research that is taking a new approach to screening sexual assault evidence — a male DNA (Y) -screen using a phosphate-buffered saline cell extraction on substrates.

This presentation will impact the forensic science community by providing a conventional serological alternative for screening sexual assault kits that is scalable in such a way that it is affordable to all laboratories, regardless of laboratory size or throughput.

Sexual assault kit backlogs have been a common topic in news stories in recent years throughout the United States. According to the Rape, Abuse and Incest National Network (RAINN), there are 237,868 victims of rape/sexual assault per year. This project developed a high throughput screening method for the presence of male DNA. The goal of this project was to increase laboratory efficiency by performing a Y-screen utilizing the Qiagen® QIAsymphony® on a Phosphate-Buffered Saline (PBS) extract instead of completing conventional serology.

Historically, serology screening has been the method for processing sexual assault kits. Drawbacks of this method include: false positives and negatives, difficulty in automation, subjective interpretation, and lengthy time commitments to a microscope. Y-screening enables the process to be automated, using a 96-well format allowing simultaneous screening on a Polymerase Chain Reaction (PCR) -based method. This method brings objectivity to the screening process and provides a downstream correlation similar to the current DNA analysis techniques. Y-screening has been employed for a number of years.

This proposed process is unique in that this approach took a uniform cutting from the evidence and cells were removed from the substrate with PBS. The PBS extract was screened during the Y-screen. The extraction was performed on the Qiagen® QIAsymphony®, which lysed all cells in the PBS extract. Quantitative PCR with simultaneous male and human DNA quantitation setup was then conducted on the Qiagen® QIAgility® robotic liquid handler with the use of the Quantifiler® Trio DNA quantification kit by ThermoFisher Scientific or Qiagen® Investigator® Quantiplex HYres kit. Quantitation was performed on an Applied Biosystems® 7500 Real-Time® PCR system. The screening of the samples was based on the presence or absence of male DNA. During the Y-screen, the samples with the presence of male DNA proceeded to full DNA analysis. In order to do so, the remaining portion of the PBS extract was taken through the traditional DNA analysis procedures. Unlike conventional serology, this resulted in a quantitation that will directly correlate to downstream DNA analysis. In addition, it minimized the number of times that an analyst needed to go back to the evidence.

This project compared and evaluated laboratory efficiency of the Y-screen model versus conventional serology through various studies. Variables such as temperature, incubation time, PBS volume, and agitation method were optimized for the Y-screen. In addition, the amount of sample extract added to the quantitation reaction for the Y-screen was also studied and optimized. During the sensitivity study, the Y-screen had increased sensitivity when compared to semen serology. Although the supplies needed for the Y-screen procedure are costlier than conventional serology, the Y-screen approach is a more cost-effective method when analyst time is factored. It was concluded that the Y-screen is a valuable method for use on sexual assault kits for the Marshall University Forensic Science Center DNA Laboratory. It is not only cost-effective but also an objective method with thoroughness and specificity.

DNA, Y-Screen, Serology

B104 Why Perform Y-Screening? Validation of a Novel Sperm Lysis Protocol to Improve Sexual Assault Triaging

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After attending this presentation, attendees will better understand the process of screening sexual assault evidence using a novel sperm lysis protocol and commercially available quantitative Polymerase Chain Reaction (qPCR) kits.

This presentation will impact the forensic science community by demonstrating a method for triaging sexual assault kits by rapidly lysing sperm cells, allowing for the detection of male DNA with Y-specific qPCR.

Many laboratories in the United States are making efforts to reduce backlogged sexual assault cases and/or reduce the processing time for incoming cases. These laboratories often screen large amounts of evidence, looking for the best samples to test in order to get a perpetrator's DNA profile. This often involves presumptive tests for semen, amylase, or blood. Some laboratories have begun to use qPCR as a screening tool for male DNA since most sexual assault cases have a male suspect. Consequently, there is demand for a quick sperm lysis protocol, which would allow for rapid high-throughput screening of potential sexual assault evidence.

Typically, DNA is released from sperm using a combination of detergents and reducing agents, which can be inhibitory to downstream assays, such as PCR and real-time PCR. Most sperm lysis procedures require additional steps to remove these potentially inhibitory reagents from the DNA extract. These clean-up procedures add more transfer steps, which lengthen the process and can add cost. In addition to the time and cost considerations, extra transfer steps can lead to sample switching, contamination, or loss of DNA.

This presentation will describe the validation of the ZyGEM® PDQeX for DNA extraction, including sperm lysis. This instrument uses a novel protocol and reagents for sperm lysis. The end result is DNA ready for qPCR or for Short Tandem Repeat (STR) profiling without additional clean-up steps. Data from the developmental validation of the manufacturer, as well as data from the internal validation of this system by the New York City Office of Chief Medical Examiner, will be presented.

The validation data includes sensitivity, precision and accuracy, mixture, contamination, and known and non-probative studies. This data has shown that the system can lyse sperm mixed with overwhelming quantities of female epithelial cells. Sensitivity studies have illustrated that male/female mixtures containing as few as one sperm per microliter can be lysed and then detected and quantified using qPCR. Mock casework and stability studies have shown that the system can extract DNA from realistic and challenging mock casework samples, such as mock vaginal swabs and sperm deposited on denim and other fabrics. The qPCR data was also used to accurately estimate the amount of template DNA for STR amplification with commercially available kits. The quantification data was found to be a good estimate for the purposes of STR amplification, indicating that the DNA extracts were not inhibiting qPCR or STR amplification.

This data will demonstrate that this system can extract DNA from typical sexual assault evidence that is ready for commercially available qPCR and STR kits. This process enables laboratories to use Y-specific qPCR to rapidly screen sexual assault kits for useful evidence. The screening method is an efficient way for laboratories to triage samples with sufficient male DNA to pursue STR analysis.

Y-Specific qPCR, Sperm Lysis, Male Screening

B105 Proximity Ligation Real-Time Polymerase Chain Reaction (PLiRT-PCR): A Protein-Based Confirmatory Method for Processing Sexual Assault Kit Samples for Semen and Sperm Cells

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After attending this presentation, attendees will possess valuable insight on the research efforts performed to integrate a potential confirmatory detection method of semen and spermatozoa to sexual assault casework workflow.

This presentation will impact the forensic science community by demonstrating that PLiRT-PCR can be easily integrated into forensic laboratories, as it requires only a thermocycler and a real-time PCR system as well as utilizing a small fraction of the total reaction.

Sexual violence affects millions of Americans. According to the United States Department of Justice's National Crime Victimization Survey (NCVS), each year there is an average of 288,820 victims of rape and sexual assault ages 12 or older. Every two minutes, a victim is being sexually assaulted. Therefore, processing sexual assault evidence is time sensitive. Rapidly confirming semen, and generating the assailant's DNA profile, is crucial for solving crimes and bringing closure to the victims and their families.

A large number of sexual assault kits submitted to forensic laboratories have yet to be analyzed. The current procedures of sexual assault workflow are presumptive serological testing, which include microscopic sperm slide searches, followed by differential extraction methods. This workflow is labor intensive, time consuming, and significantly affects case backlog.

This study has developed an alternative detection method with the potential for replacing microscopic sperm confirmation and enabling the processing of multiple samples at a time on a 96-well plate. In as few as three hours, the assay confirms whether a sample contains semen or sperm cells simultaneously and assists analysts in deciding whether to proceed to differential extraction, therefore helping to reduce backlogged cases.

The PLiRT-PCR assay begins with a small cutting from the evidence (e.g., a fifth of a swab) that is placed in lysis buffer to break open the sperm cells, thus saving the rest of the evidence for the downstream analysis pipeline: differential extraction, quantification, and Short Tandem Repeat (STR) analysis. The lysis step is followed by a binding reaction of specific antibodies against proteins of interest. The antibodies are coupled to short DNA strands, forming so-called pairs of proximity probes. Upon simultaneous detection and proximal binding of the probes to their respective target proteins, the two attached DNA strands are brought in close proximity and are hybridized to a connector oligonucleotide complementary to the oligonucleotide ends of the assay probe pair. Then, the DNA strands undergo an enzymatic ligation reaction, forming a new amplifiable DNA product that can be amplified and measured in a highly quantitative manner by quantitative Polymerase Chain Reaction (qPCR).

This presentation discusses the application of PLiRT-PCR for the identification of semen and sperm proteins from blind samples provided from Bode Cellmark by using only 2µL of the sample. The assay supports both swab samples and other substrates that have different dilution amounts of body fluids spotted on them. Once semen and/or sperm was determined, differential extraction, quantification, and STR typing using Applied Biosystems® Quantifiler® Trio, GlobalFiler™, and Yfiler® Plus kits were performed. Samples that tested positive with the PLiRT-PCR assays displayed a positive male quantification result with the Y-screen assay, Quantifiler® Trio, further validating the sensitivity of this assay.

This study demonstrates that PLiRT-PCR can be a powerful, robust, and quantitative confirmatory technique the DNA analyst can use to assess whether evidence from sexual assault kits contain semen or sperm cells. Without adding additional resources, the assay utilizes common instruments in the laboratory. This method is more sensitive than the currently used protein-based detection techniques and assists in deciding which sample to process with the differential extraction methods.

PLiRT-PCR, Sexual Assault, Spermatozoa

B106 Improving Seminal Fluid Detection Sensitivity in Extended Postcoital Intervals by Triple Quadrupole (QQQ) Mass Spectrometry

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After attending this presentation, attendees will better understand the use of protein mass spectrometry for the detection and quantitation of human seminal fluid during an extended postcoital interval.

This presentation will impact the forensic science community by illustrating how protein mass spectrometry can be used to enhance the potential for the successful detection and confirmatory identification of seminal fluid in an extended postcoital interval. The results of this study can provide the forensic science community with a powerful tool to aid in obtaining potentially probative evidence in sexual assault investigations.

This work adapted a fully validated multiplex QQQ Multiple Reaction Monitoring (MRM) method for multiple body fluid identification for the focused identification of seminal fluid protein biomarkers. The modified assay provides enhanced sensitivity for seminal constituents that surpasses the sensitivity currently achievable with commonplace serological screening techniques. This makes it possible to confidently detect the presence of seminal fluid in extended postcoital intervals.

Previous research has resulted in the development of a qualitative mass spectrometry-based assay for the confirmatory identification of human seminal fluid. The current study has demonstrated that Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) can be utilized to both detect and quantitate seminal fluid four days postcoitus in authentic samples. Initial limits of detection and quantitation were determined using heavy isotope-labeled peptide standards complimentary to the targeted peptide fragments of interest. By comparison, paired analysis using the ABAcard® p30 and RSID™-Semen immunochromatographic assays failed to produce a positive test result one and three days postcoitus, respectively.

The goal of the current research was to facilitate the analysis of challenging sexual assault evidence by optimizing the quantitative tandem mass spectrometry method to increase the detection and quantitation of seminal fluid protein biomarkers. Using an Agilent® 6495 QQQ mass spectrometer coupled with a 1290 UPLC system, seminal fluid-specific proteins (selected based on previously acquired time-of-flight data) were analyzed. The specificity of the protein biomarkers as well as the potential for matrix interference was evaluated through analyses of two-, three-, and four-component mixtures of seminal fluid in combination with commonly analyzed body fluids (i.e., peripheral blood, vaginal and menstrual fluids, saliva, and urine). The sensitivity achieved by the MRM seminal fluid assay was established using a serial dilution of semen spotted onto vaginal swabs. It was found that a 1:131,072 dilution of seminal fluid in a vaginal fluid matrix could be reliably detected by the assay. This translates into a doubling of the detection interval for authentic postcoital samples — enabling detection up to more than eight days postcoitus.

Additionally, intact synthetic proteins (prostate-specific antigen and semenogelin-1) were used to create a "standard detection curve." These data provide insight into the lower limits of detection and quantitation of natural seminal fluid proteins. The ability to quantify seminal fluid protein biomarkers can be used to establish background interferences (semenogelin-1) and baseline levels (prostate-specific antigen) of the targeted proteins.

In conclusion, this study provides comprehensive data detailing the sensitivity and specificity of a novel serological assay approach utilizing protein mass spectrometry for the detection and quantitation of human seminal fluid.

Proteomics, Postcoital Interval, Sexual Assault

B107 Evaluating the Utility of Highly Sensitive Male Quantitative Polymerase Chain Reaction (qPCR) Targets for Simple, Low-Cost, and Rapid Screening of Sexual Assault Samples

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After attending this presentation, attendees will understand the determination of the optimal genomic targets for the detection of male DNA in sexual assault cases.

This presentation will impact the forensic science community by evaluating the comparative utility of several male qPCR targets as a potentially valuable tool to improve a forensic laboratory's capacity to screen a high volume of sexual assault case samples.

The reduction of Sexual Assault Kit (SAK) backlogs has become of top concern and priority for many jurisdictions across the country. In 2011, the National Institute of Justice (NIJ) published a special report in response to recent discoveries of thousands of untested SAKs in police evidence rooms nationwide. In this report, one of the priorities identified for crime laboratories is to "create a plan to handle work if large numbers of previously untested SAKs are suddenly sent to the crime lab." This plan must include a more streamlined process for triaging SAKs to identify the presence of testable male DNA in these samples. Historically, serological screening using color immunological tests, chemical tests, or microscopic analyses have been the laboratory method of choice for initial processing of SAKs; however, these conventional methods have several drawbacks, including false positives and negatives, difficulty implementing automation, subjective interpretation, and the length of time the tests take in the laboratory. In the past decade, advancements in forensic quantitation systems based on real-time PCR have greatly improved the sensitivity and reliability of sample detection, allowing for higher throughput screening of sexual assault samples. Screening for male DNA (Y-screen) enables an objective, quantitative analysis of the presence of male DNA in a sexual assault sample. The process is easily automatable, using a 96-well format allowing for the screening of a high number of samples simultaneously using a real-time PCR-based method. These methods provide a downstream correlation with the profiling results obtained with commonly employed Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) genotyping systems.

This study evaluates the utility of two different qPCR targets for detection and screening of male DNA in sexual assault and other casework samples. The methods studied include the use of a quick and simple lysis step to non-differentially lyse the cells in conjunction with two high-copy targets located on the Y chromosome. Studies performed include sensitivity, precision/reproducibility, male:female mixtures, and correlation with downstream STR and Y-STR results of mock sexual assault samples.

Results indicate that both tested targets are adequate for use in the accurate detection of male DNA, with the high copy target enabling the most sensitive detection of male DNA in sexual assault samples. Sensitivity studies using a dilution series conducted in triplicate with the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2372 Component A indicated the ability to consistently detect male DNA at quantities below one picogram. Mixture studies demonstrated the ability to consistently detect low quantities of male DNA (10pg) in all male:female mixtures tested, including 1:8000 male:female mixtures. Additionally, a quantitation threshold value that may reliably be used as a stopping point was determined for STR and Y-STR analysis, although this value may require adjustment based upon the sensitivity of the genotyping systems and interpretation criteria employed in each forensic laboratory. This study demonstrates the utility of real-time PCR male-specific targets in high throughput and fast screening of sexual assault cases. These methods will undoubtedly improve a laboratory's capacity to screen a high volume of sexual assault samples.

SAKs, Male DNA, QPCR

B108 Microchip-Based Antibody-Mediated Differential Lysis of Sperm Cells

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After attending this presentation, attendees will better understand the forensic applications of modular microfluidics, as well as the benefits of a separation module for the differential extraction of sperm-containing evidence samples from sexual assaults.

This presentation will impact the forensic science community by describing a separation module for the cell sorting and differential extraction of forensically relevant sexual assault samples on a rotationally driven microdevice.

These modular systems allow for the incorporation of advanced microfluidics technologies in forensic laboratories without the wait for development of Micro Total Analysis (μTAS) systems for casework samples. Modular devices would require a small footprint, would replace only limited steps of the workflow, and could potentially decrease the amount of time needed for sample processing, particularly in regard to the differential extraction and amplification steps.

Due to the increasing sexual assault kit backlog and the increased legislation proposed to reduce this backlog, there is a significant driving force to develop methods that decrease the sample processing time for these samples. Manual differential lysis separates only non-sperm from sperm cells and includes many analytical steps and significant user intervention, leading to an increased potential for contamination, increased processing time, and imperfect separation of male/female fractions. The proposed microchip has the potential to decrease the manual intervention currently required for differential extraction while minimizing the need for difficult mixture interpretations. This sexual assault microdevice incorporates an antibody-binding chamber, which utilizes polystyrene beads coated with an antibody specific to an antigen on the acrosomal cap of sperm cells. Previous studies identified a sperm-specific antibody (SPAG-8) and showed that, when coated onto the bead surface, it was capable of improving sperm cell DNA yield when processed in microcentrifuge tubes. Furthermore, studies using a modified ZyGEM® prepGEM™ differential method confirmed that this DNA liberation method was able to improve efficiency of sperm cell lysis when compared to traditional differential lysis/extraction methods. In these studies, the SPAG-8-coated bead mechanism was tested again, but testing was in the plastic microchip environment. Semen dilutions were processed on a simple chip that included an inlet reservoir, an antibody-bead binding chamber, and an outlet reservoir; following binding, samples were removed from the chip for modified ZyGEM® prepGEM™ differential extraction and quantitation of human DNA. Runs that included the SPAG-8-coated beads had 39% more sperm cell DNA captured versus on-chip incubation of the same samples *without* beads or antibody. In addition, these data demonstrated a 20% and 38% increase in the amount of sperm cell DNA captured on the microchip using SPAG-8-coated beads versus what was obtained using manual differential methods (modified ZyGEM® prepGEM™, and differential using the QIAamp® DNA Blood Mini Kit), respectively.

In broadening the search for a sperm-specific antibody, Male-Enhanced Antigen 1 (MEA-1) was also evaluated for its ability to separate cells associated with sexual assault samples. MEA-1 is encoded by the male-histocompatibility gene (HY) and is purported to be present on the cell membrane of *all* male epithelial cells; however, the literature data is contradictory. If expression is truly male-cell specific, the addition of MEA-1 to SPAG-8 in the microchip antibody-binding chamber may lead to male and female fractions with enhanced purity. Thus, similar to previous work, the binding performance of MEA-1 was evaluated via flow cytometry. Initially, MEA-1 displayed an average binding affinity of 10.7% to male buccal epithelial cells versus 9.8% to sperm cells, indicating that the antigen is indeed expressed evenly on all male cells, regardless of tissue origin. A follow-on study was performed to determine the difference in expression between male cells (buccal and sperm) and female cells (buccal and vaginal). MEA-1 antibody binding was ~6.5-fold higher in male buccal cells and sperm cells than female buccal and vaginal epithelial cells, respectively. Although flow optimization using these antibodies (and their respective fluorophores and isotype controls) is needed to combat the overall low average binding affinity

in male cells, these data clearly indicate a much higher MEA-1 expression rate in males; thus, these results are promising. Optimization is critical for future work. Adding a male-specific antibody could bring an added value to this microdevice upon commercialization. Future work on this project will include integration and testing of this module into a microdevice that also includes downstream DNA liberation and Infrared-mediated Polymerase Chain Reaction (IR-PCR) -based Short Tandem Repeat (STR) amplification.

Microdevice, Sexual Assault, Antibody

B109 We Did It! Houston's Sexual Assault Kit Backlog Elimination Story

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After attending this presentation, attendees will have a better grasp of the gravity of the nationwide Sexual Assault Kit (SAK) backlog. This presentation will share the challenges and successes associated with eliminating a backlog of SAKs in Houston, TX, the fourth-largest city in the United States, and the achievement of a sustainable, 30-day turnaround time on sexual assault cases.

This presentation will impact the forensic science community by introducing workflow and personnel management strategies that helped Houston eliminate its SAK backlog and achieve a sustainable, 30-day turnaround time.

In 2013, the Houston Police Department (HPD) had 6,663 untested SAKs on property room shelves, the oldest dating back to the 1980s. At the time, the HPD Crime Laboratory was tasked with outsourcing the untested kits. Each case would have to be internally reviewed following the outsource testing in order to upload relevant information into the Combined DNA Index System (CODIS), the national DNA database. To complete this task, the laboratory had to quickly develop a plan to locate the evidence, prepare the documentation, and ship the items for testing. Internal training began as the laboratory prepared for the large amount of case reviews that would occur when the completed cases returned. Meanwhile, in April 2014, HPD's Crime Laboratory ceased to exist. The Houston Forensic Science Center, an independent local government organization, took over management and oversight of Houston's forensic operations, including DNA testing. By February 2015, the outsourcing project had been completed and analysts had successfully reviewed close to 10,000 cases. More than 4,200 profiles were uploaded into CODIS, resulting in more than 1,800 hits. The project also helped with the identification of a serial rapist and confirmed that no one had been wrongfully accused.

With efforts focused on reviewing the outsourced cases and their accompanying reports, the laboratory was cognizant that it could end up with a backlog of current cases. It developed a plan to maintain a workflow for SAK processing in order to prevent a backlog from reoccurring. Unfortunately, the outsourcing project ended before the laboratory had a fully functioning workflow for SAK processing. Each month, the number of backlogged SAKs grew. By October 2015, the backlog had risen to 716 kits. The laboratory began operating under the new workflow with the help of 14 people whose job it was to whittle away at the backlog. On April 6, 2016, Sylvester Turner, Houston's newly elected mayor, instructed the laboratory to eliminate the backlog and reach a sustainable 30-day turnaround time by July 1, 2016. On that day, the backlog (counting both those that were untested and those already in progress) was 575 SAKs. The laboratory succeeded by utilizing different resources to achieve the goal. The laboratory's staff had to be reorganized to focus on SAK processing, additional instruments had to be quickly purchased, and 99 kits had to be outsourced. By combining into one report male quantification for screening and the DNA results, the SAK workflow was streamlined to nine days from extraction to report completion. The laboratory completed 702 SAK cases, reviewed data from the 99 outsourced kits, and achieved a 30-day turnaround time in only 90 days.

Sexual Assault Kit, Backlog, Workflow

B110 The Development of a Dissolvable Swab for Increased Biospecimen Recovery

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After attending this presentation, attendees will understand existing forensic swab techniques and processes and the potential of a high-surface-area dissolvable swab to enhance biospecimen acquisition and subsequent recovery for forensic analysis.

This presentation will impact the forensic science community by discussing the increases in forensic competence as advances in material technologies are applied to biospecimen acquisition and processing for improving forensic analyses. Swab fiber technology with increased acquisition properties and complete specimen release through total swab dissolution allows for the collection of small amounts of cellular evidence while still producing robust Short Tandem Repeat (STR) profiling using standard methods.

Swabs are routinely used by crime scene investigators and forensic scientists for the collection of a wide range of evidence types for analysis. The most commonly used swab device for collection of biological specimens is the sterile cotton swab due to its ease of use, low cost, and ability to collect on multiple substrates such as wood, fabric, and metal. Although cotton swabs readily adsorb biological material, they exhibit low efficiency of DNA sample release. While material and manufacturing advances have resulted in a diversity of new forensic swab types and materials, sampling and extraction efficiency from these swabs varies significantly by substrate and sample type. The loss of biological material for downstream processing can be 40%-80% from merely the swab extraction process.

The primary objective of this study was to evaluate the effectiveness of high-surface-area, dissolvable, electrospun nanofibers on biospecimen capture and DNA recovery. Using prototype swabs made from sheets of electrospun biopolymeric material, experiments were conducted that evaluated adsorption of high and low volumes of biological material (wet and dried cells) from glass slides, and subsequent DNA extraction using commercially available forensic analysis kits. Both manual and automated DNA recovery was investigated. DNA extraction was conducted using the QIAGEN® QIAamp® DNA Mini Kit or the QIAGEN® EZ1 Robot. The Quantifiler® Human DNA Quantification Kit, in conjunction with an Applied Biosystems® 7500 Real-Time Polymerase Chain Reaction (PCR) instrument, was used to estimate the quantity of human DNA present in each sample. Following DNA quantification, STR typing was performed on all samples of DNA recovered from the dissolvable swabs using the GlobalFiler™ PCR Amplification Kit and analyzed using the GeneMapper™ ID-X software.

Dissolvable nanofiber swabs were shown to have excellent performance in cellular adsorption (99%) and in DNA extractions (82%). All DNA samples recovered from dissolvable electrospun swabs were also capable of producing full, high-quality STR profiles. The results of these experiments demonstrate the potential usefulness of high-surface-area, dissolvable, nanofiber swabs for both enhanced biospecimen capture and increased DNA recovery of biological evidence.

Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the United States Army Small Business Innovation Research (SBIR) Program Office or the Army Research Office.

Swab, Dissolvable, DNA

B111 A Comparison of Methods of Analysis for the Extraction and Identification of Drugs Using Microfluidic Mass Spectrometry (MS) From Different Substrates

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After attending this presentation, attendees will better understand the utilization of a microfluidic extraction device and how, in conjunction with High Performance Liquid Chromatography (HPLC) and high- and low-resolution MS, it allows for accelerated analysis of multiple drugs of abuse in a single sample run.

This presentation will impact the forensic science community by introducing multiple methods for accelerated analysis of numerous drugs (up to 70) at the same time, which can aid in determining the efficiency of the system depending on the Microfluidic Device (MFD) MS instrumental set up.

This specific MFD was originally developed for the extraction of dyes from fibers; however, it is now being fully tested for other areas of forensic science that use extraction, such as inks on questioned documents or analgesic tablet analysis. Benefits of this device include: (1) smaller amounts of sample needed, allowing for less damage to the evidence; (2) less contact between the examiner and the evidence, allowing the integrity of the evidence to be kept intact; and, (3) speed of extraction, which is generally less than five minutes per sample run.[1]

The primary focus of this presentation is to compare the different methods of MFD-MS to illustrate the versatility of the device and to describe how high-resolution MS can be utilized for facilitated identification of the extracted drugs. Increasing the efficiency of drug analysis is essential in the forensic community simply because it allows for faster analysis of data, meaning rapid identification of the drugs used by perpetrators. Also important is ensuring that the majority of the evidence is kept accessible if later testing is needed, which this extraction device enables.

In these experiments, the MFD was used to extract a standard solution containing 70+ drugs from multiple substrates including Quantisal™ saliva collection devices, cotton swabs, paper, and more. Multiple instrumental set ups of the MFD-MS were tested: (1) directly connecting the MFD to the Shimadzu® LCMS-8060 series Triple quadrupole (QqQ) MS and directly injecting the sample into the MS; (2) extracting the sample using the MFD and introducing the extraction to HPLC for separation, then analysis via the QqQ MS; (3) extracting the sample using the MFD, adding 2mL of Methanol (MeOH) to enhance solubility and ion signal, and analysis through HPLC/MS; and, (4) connecting the MFD to the HPLC system using a T-valve for direct connection into the HPLC/MS system. These methods were also compared using the MFD attached to a different MS instrument, specifically, an Agilent Technologies® 6520 series quadrupole Time-Of-Flight (qTOF) MS. Comparison of analysis via these MS instruments demonstrates the diversity of compatibility this MFD possesses. The efficiency of each set up was compared by determining the number of drugs identified from a known standard solution of 70+ drugs. Sample preparation for all substrate types can be generalized in the following steps. First, a sample of the standard drug solution containing the 70+ drugs was administered onto the substrate (~2mL-3mL). Second, using either scissors or a Harris® micro-punch tool, a small portion (~0.5mm-2mm) of the substrate was removed. Third, the removed sample was placed in the sample cavity of the MFD sample chip and inserted directly into the MFD. From there, a computer program determining the number of flushes of solvent into the cavity for extraction was selected, and the process began.

In conclusion, the experiments conducted reveal the versatility and diversity of this MFD for extraction of multiple drugs on multiple different substrates, and also exhibits its compatibility with different MS instruments, which is beneficial as many crime laboratories have different instruments available. This MFD-MS and MFD-HPLC/MS set up will allow accelerated analysis of multiple drugs, increased integrity of evidence, and less contact between the evidence and the examiner, which are all essential for forensic analysis today.

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Drug Analysis, Mass Spectrometry, Microfluidics

B112 Ionization Efficiency of Drugs Using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

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After attending this presentation, attendees will better understand the ionization efficiencies of controlled substances utilizing DART®-MS. Specifically, this presentation will demonstrate that chemical properties of controlled drugs play a critical role in ionization and directly relate to DART®-MS sensitivity.

This presentation will impact the forensic science community by explaining the ionization efficiencies of drugs using DART®-MS and how this can be an effective technique for the identification of seized drugs.

DART®-MS is a fast ionization technique for mass spectral analysis of compounds. DART®-MS allows for ambient ionization of small molecules without sample preparation. The samples are directly introduced into the ion-source using capillary glass tubes and are desorbed from the sample surface by a flow of heated nitrogen gas while being ionized. DART®-MS, in combination with a High-Resolution Accurate Mass (HRAM) spectrometer, can deliver quick results with accurate mass determinations and highly specific mass-to-charge spectral data.

Ionization efficiency can be affected by a number of factors, such as MS interface design, inlet temperature, matrix composition, nitrogen or helium gas, or an analyte's chemical properties. Much of the research conducted to ionization efficiencies to date has been performed using Liquid Chromatography/Electrospray Ionization/Mass Spectrometry (LC/ESI/MS). Ionization efficiency studies using DART®-MS have been limited to instrument conditions, such as inlet temperature, capillary temperature, type of ionizing gas, and grid voltages. Presently, there is little information concerning ionization efficiencies for seized drugs of abuse.

The purpose of this work was to study ionization efficiencies for cocaine, heroin, oxycodone, fentanyl, acetylfentanyl, caffeine, and levamisole utilizing a previously developed 24-second DART®-MS screening method. Equimolar concentrations (400nM) of each analyte were prepared in methanol using an internal reference standard, Diethyltoluamide (DEET, 10ppm). Mass spectral acquisition of data was performed in positive mode utilizing a DART®-Simplified Voltage and Pressure (SVP) ion source with an Exactive™ Plus Mass Spectrometer. Five microliters of each sample solution were directly introduced into the ion source via glass capillary tubes, utilizing the Dip-It linear rail system connected to the DART®-SVP ion source. Data acquisition included fragmentation patterns utilizing Source-Induced Dissociation (SID), generated at 30eV, 60eV, and 100eV.

Preliminary results revealed cocaine had the greatest ionization efficiency, followed by levamisole and acetylfentanyl. These results explain why trace submissions of cocaine exhibits naturally demonstrated greater signal response on DART®-MS. The results also indicated that ionization efficiencies for other basic drugs are different and are significantly dependent upon the chemical property of each analyte. It was disclosed that for compounds that ionized more efficiently, the sensitivity response on DART®-MS was much greater. Likewise, compounds with weak ionization efficiencies revealed diminished sensitivity. In either case, confirmation of drugs was always obtained by an orthogonal technique, such as an LC/ESI/MS or GC/MS. Overall, this approach appeared to be an effective way to study ionization efficiencies of commonly encountered drugs in the laboratory.

DART®-MS, Drugs, Ionization

B113 Rapid Screening of Emerging Novel Psychoactive Substances (NPS) Using a Portable, Ambient Sampling Mass Spectrometer (MS)

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After attending this presentation, attendees will better understand how the merging of ambient ionization techniques and portable mass spectrometric technologies can be utilized to rapidly characterize new psychoactive substances onsite and offer high throughput detection via automated library searching methods of evidence ranging from trace residues surfaces to paraphernalia types, such as blotter paper.

This presentation will impact the forensic science community by introducing attendees to recent advances in portable, ambient sampling MS technology and the application of such a system for the analysis of new psychoactive substances.

The creation and use of NPS continues to increase throughout the world. In Europe alone, it has been reported that from the period of 2005-2014, over 400 NPS were reported to the European Union Early Warning System. In 2014, it was estimated that two NPS appeared on the market every week.[1] NPS are unregulated recreational drugs that have been synthetically modified to mimic traditional drugs of abuse while avoiding scheduling as an illicit substance. NPS pose a serious challenge for forensic identification in comparison to traditional drugs of abuse as structural characteristics can be variable and unknown.[2] The frequency and complexity of NPS evidence point to the need for robust screening methods, particularly portable technologies that could expedite legal investigations and reduce the burden on the crime laboratory system. In this study, a portable MS coupled with conventional and ambient ionization methods, such as Desorption Electrospray Ionization (DESI) and Paper Spray Ionization (PSI), was investigated as a flexible asset to combat the emerging threat of NPS.[3,4]

Substituted phenethylamines, particularly the 2C-series and prominent analogues featuring the addition of *N*-benzylmethoxy derivatives (i.e., NBOMes or "N-bombs") were the focus of this study. Analytical standards and mock forensic evidence were investigated via ESI, DESI, and PSI on a FLIR® Systems AI-MS 1.2 portable Cylindrical Ion Trap (CIT) MS.[5,6] The FLIR® Systems AI-MS 1.2, ruggedized for fieldwork, features an atmospheric capillary inlet with on-board high voltage and syringe pumping for coupling to conventional and ambient ionization methods. Both MS and Tandem Mass Spectrometry (MS/MS) data were collected for all analytes of interest in this study, incorporated into a simplified user interface that allows automated identification of threats via data dependent scanning and spectral database matching.[7] Automated spectral interpretation and simplified ionization methods, in turn, allow the collection of reliable and accurate data by non-technical users and first response personnel.

Data collected in this study suggest the use of interchangeable ionization sources outfitted on the AI-MS 1.2 system can provide a high throughput fieldable solution for NPS identification. Trace analysis, in the form of surface residues, was demonstrated by determining Limits of Detection (LODs) via surface swabbing PSI/MS from a variety of surfaces of forensic relevance. LODs ranged from low to high nanograms of deposited analyte from the relatively smooth surfaces (i.e., glass, aluminum, plastic identification and electronic entry cards, and plastic storage bags) and textured and geometrically complex substrates (i.e., brass key, ceramic floor tile, and textured laminate countertop). Furthermore, application to realistic paraphernalia types, such as blotter paper, was shown, allowing accurate identification of illicit species even when present in complex mixtures.

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Field Analysis, Ambient Mass Spectrometry, NPS Detection

B114 Optimal Headspace Extraction for the Detection of Volatile Organic Compounds (VOCs) Released From Synthetic Cathinones Using Solid-Phase Microextraction (SPME) for Field Application

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After attending this presentation, attendees will better understand the headspace analysis of synthetic cathinones. This presentation will provide detailed information regarding the volatile organic compounds associated with these drugs.

This presentation will impact the forensic science community by presenting a detailed method to sample synthetic cathinones in a non-destructive manner and by introducing a precise, safe, volatile compound in the form of a Controlled Odor Mimic Permeation System (COMPS), which will equip canine teams with the ability to detect synthetic cathinones (bath salts) currently being smuggled across United States borders. Because of an inadequate knowledge of the volatiles of these bath salts, narcotic detection canines have been unable to detect these illicit substances.

Methylone, ethylone, Methylenedioxypyrovalerone (MDPV), and α-Pyrrolidinopentiophenone (α-PVP, also known as Flakka), collectively referred to as bath salts, are a new trend of illicit substances known as synthetic cathinones. Designed by chemically modifying the core structure of the compound cathinone (the natural amphetamine of the khat plant), synthetic cathinones became prevalent within the United States in the mid-2000s. As an inexpensive and less-controlled alternative to the traditional Methylenedioxymethamphetamine (MDMA, Ecstasy), it has become heavily abused, prompting emergency scheduling by federal regulators. As a result of the drastic increase in popularity and abuse of synthetic cathinones, research studies have been conducted to gain a deeper understanding of its toxicological effects; however, the analytical components (sampling and analysis) required to understand and characterize bath salts are still vastly underrepresented.

This study will present results for an optimal extraction method that has been developed, which can be applied to the indirect analysis of synthetic cathinones. Method development was conducted using a headspace SPME technique on an actual confiscated bath salt sample known as Methylone (Molly). After performing extraction optimization, it was determined that a Polydimethylsiloxane Divinylbenzene (PDMS/DVB) -coated fiber, in addition to complimentary ionization techniques, resulted in the most beneficial set for the extraction and analysis of synthetic cathinone volatile components. This method allows for cathinone derivative samples to be rapidly sampled by non-invasive means, followed by analysis utilizing traditional gas chromatography/mass spectrometry. Determination of the VOCs comprised in these drugs can be beneficial in the creation of an effective canine training aid utilizing the Controlled Odor Mimic Permeation System (COMPS). COMPS involves a deployable source housing a target odor in a permeable polymer, which releases the odor at a known and controlled rate. This controlled system will aid canine teams throughout the country in the detection of synthetic cathinones, a task that a large number of canine teams are currently unable to achieve.

Synthetic Cathinone, SPME, COMPS

B115 Improving Qualitative Synthetic Cathinone Identification by Gas Chromatography/ Mass Spectrometry (GC/MS) Using Cold Electron Ionization (Cold-EI)

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After attending this presentation, attendees will understand the merits of Cold-EI in comparison to those of classical Electron Ionization (EI) in the analysis of synthetic cathinones. Attendees will be shown that better identification of synthetic cathinones is possible with Cold EI due to the presence of molecular ion in the mass spectra compared to no molecular ion in the EI spectra.

This presentation will impact the forensic science community by suggesting an alternative avenue for improved identification of forensic drugs using MS. Examples include analyses of emerging drugs, such as synthetic cathinones that yield inconclusive mass spectra due to the lack of molecular ions under classical EI.

Synthetic cathinones are emerging drugs with molecular structures similar to that of cathinone, the active ingredient in the khat plant. They are specifically designed to avoid legal prosecution of the manufacturers and dealers. Synthetic cathinones, which are beta keto-amphetamines, produce amphetamine-like effects when abused. Many synthetic cathinones are scheduled in the United States to protect the public interest. They became known as bath salts, mainly because they are typically misbranded when sold.

Synthetic cathinones are notoriously labile compounds that undergo extensive fragmentation when analyzed using classical EI. As a result, the resulting mass spectra contain little to no molecular ion peak, making the identification uncertain. The interpretation of the spectra is further complicated because synthetic cathinones produce relatively few fragments. Cold-EI relies on vibrational cooling of the analytes prior to ionization, thus reducing thermally induced fragmentation. This technique has been shown to increase the relative abundance of the molecular ion for thermally labile compounds and maintain the integrity of the fragments obtained by classical EI, thus improving the confidence in their identification. The confidence can be further improved by performing Tandem Mass Spectrometry (MS/MS) analyses.

This study subjected a total of 35 controlled synthetic cathinones and positional isomers to analyses by two GC/MS instruments; one equipped with a classical EI ion source and a quadrupole analyzer and another equipped with a cold-EI ion source and a quadropole Time-Of-Flight (qTOF) analyzer. Both instruments used 30m x 0.25mm, 0.25µm Elite-5MS columns for the GC separation.

As expected, all the classical EI mass spectra of synthetic cathinones contained molecular ion with relative intensity of less than 1%. In most cases, more of the molecular ions survived when the analysis was conducted by cold-EI ionization, with some analytes reaching molecular ion relative intensities of more than 25%. MS/MS analyses were performed on analytes that provided molecular ion with more than 5% relative intensity.

To reveal the additional discrimination that the presence of the molecular ion in the mass spectra brings to the spectra, the MS data obtained by the two methods was subjected to Principal Component Analysis (PCA).

Cold Electron Ionization, Synthetic Cathinones, Molecular Ion

B116 Using Coupled Columns With Ultra High-Performance Supercritical Fluid Chromatography (UHPSFC) for the Analysis of Synthetic Cathinones

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After attending this presentation, attendees will understand the uses and principles of UHPSFC with coupled columns for the separation of synthetic cathinones.

This presentation will impact the forensic science community by demonstrating the benefits of coupled column UHPSFC in drug analysis. Screening or confirmation of bath salts, including their positional isomers, using coupled columns is accomplished with significantly greater reliability compared to the use of a single column. Identification can be accomplished by retention time, Ultraviolet (UV) spectrum, and molecular ion and major fragment ions.

UHPSFC has been used to separate bath salts, including their positional isomers. The current method uses a Torus™ DIOL column with a 10mM ammonium formate in methanol, run isocratically with 3% methanol and 97% carbon dioxide as the mobile phase. This method separates 10 out of 15 controlled bath salts and 28 out of 34 isomers with a resolution of 1 or greater. In order to achieve better separation, coupled columns using different stationary phases were investigated. The two coupled columns were connected through a short length of narrow bore tubing and isocratic conditions were employed to the combined columns. The retention times from each column were approximately additive, so an estimation of the retention times of the coupled columns could be achieved prior to testing.[1] In order to investigate if greater separation of bath salts and their positional isomers could be achieved by using a coupled column approach, eight columns were tested. The columns include seven 1.7µm 3.0x100mm achiral columns (Torus™ DIOL, CSH Fluoro-Phenyl, HSS C18 SB 1.8 µm, BEH 2EP, 1-AA, Torus™ DEA, Torus™ 2PIC) and one 2.5µm 3.0x150mm chiral column (Trefoil CEL 1).

A combination of Torus™ DIOL and Torus™ 2PIC run with 3% 10mM ammonium formate in methanol has demonstrated significantly better resolution, in which 11 out of 15 controlled bath salts and, more importantly, 32 out of 34 positional isomers were separated in less than 15 minutes. In contrast to the use of a single column, six positional isomers of pentedrone and 4-methylethcathinone are now fully resolved. Percent methanol, temperature, pressure, and flow rate can also be adjusted in order to increase the resolution.

The effects of using coupled columns compared to single columns on the retention factor, selectivity factor, and efficiency were also investigated. During coupling, both the connecting tubing and temperature gradients caused by the increased pressure can contribute to band broadening.

This project was supported by the National Institute of Justice, Office of Justice Programs, and the United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

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Synthetic Cathinone, Supercritical Fluid, Coupled Column

B117 The Stability of Select Synthetic Cathinones in Non-Alcoholic Beverages

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After attending this presentation, attendees will better understand the role of synthetic cathinones in today's drug-facilitated sexual assaults and how various matrices and storage temperatures can affect the stability of specific synthetic cathinones.

This presentation will impact the forensic science community by offering information on the stability of select synthetic cathinones in beverages, an area of cathinones that has been investigated insufficiently. Through presenting this stability research, samples from suspected spiked drinks can be accurately analyzed and properly stored to aid in the investigation of drug-facilitated sexual assault cases.

Cathinone is a beta-ketone amphetamine found in the leaves of the khat plant (*Catha edulis*). While cathinone is a Schedule I drug, many of its derivatives continue to be unscheduled and referred to as new psychoactive substances. These cathinones can cause euphoria, tachycardia, paranoia, increased sex drive, and lowered inhibitions. It is for these reasons that synthetic cathinones can be used as aids in Drug-Facilitated Sexual Assault (DFSA). When combined with alcohol, synthetic cathinones can lead to an ideal situation for someone to be taken advantage of. These drugs have been seen in recent studies of sexual assault, and their prevalence in the United States has increased over the past ten years. Since synthetic cathinones are becoming a more regular part of forensic casework, exploring the stability of these drugs in different matrices is crucial. Knowing the stability of synthetic cathinones can assist in choosing appropriate storage after seizure to ensure accuracy at the time of testing. This research investigates the stability of naphyrone, alpha-Pyrrolidinopentiophenone (a-PVP), and Methylenedioxypyrovalerone (MDPV) in tap water and Coca-Cola® under different storage conditions throughout a one-month period.

The stability of these three drugs was evaluated based on the matrix, the storage temperature, and the extraction day. Each beverage was spiked at 1mg/L and stored in 1mL individual aliquots at room temperature, 4°C (refrigerator), and -20°C (freezer), all in the absence of light. Aliquots from each storage temperature and matrix were extracted using Clean Screen® DAU cartridges on days 0, 3, 7, 14, and 30 to evaluate how stable each drug was in the matrices at each temperature. Analysis of these extractions was conducted via Gas Chromatography/Mass Spectrometry (GC/MS) in full scan mode.

Naphyrone appears to be stable in both matrices at all temperatures, with no significant drug loss during the 30 days; however, a-PVP was not stable in water at room temperature. By day 14, most of the drug had degraded; however, in the refrigerator and freezer, a-PVP remained mostly stable. Coca-Cola® spiked with a-PVP and MDPV in both water and Coca-Cola® all followed the same trend of room temperature being the least-stable storage condition.

A study comparing the efficiency and recovery of synthetic cathinone extractions using solid phase extraction, Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), and Filter And Shoot (FASt) extractions was also completed, but by using Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS). Comparable results were observed for all drugs.

Synthetic Cathinones, Stability, DFSA

B118 Ultraviolet (UV) Spectra of Synthetic Cathinones: Resurrecting an Old Technique

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After attending this presentation, attendees will understand the value of UV spectroscopy for distinguishing positional isomers of the synthetic cathinones. Attendees will also better understand how ortho, meta, and para alkyl substitution on the aromatic moiety in the synthetic cathinones produces predicable shifts in the major UV absorption bands. Observation of differences in the near UV spectra of positional isomers of synthetic cathinones can permit the differentiation of these isomers when the electron impact mass spectra of the isomers are uninformative, as is often the case.

This presentation will impact the forensic science community by demonstrating the utility of UV spectroscopy as a detection mode for liquid and supercritical fluid chromatography. A scheme for the prediction of the position of major UV absorption bands for synthetic cathinones having multiple substituted aromatic moieties will be presented.

UV spectroscopy was at one time a widely used instrumental method for the analysis of controlled substances. Analysts would record the near UV spectra of the suspected controlled substance in an appropriate solvent and then consult tables of λ_{max} values to make an identification; however, the latest Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines now relegate UV spectroscopy to Category C, the lowest category of analytical technique for the analysis of controlled substances. Nevertheless, for certain types of analytical problems confronting forensic drug chemists, UV spectroscopy may be very useful, if not indispensable. The synthetic cathinones are a case in point: their electron impact mass spectra (a Category A technique) typically show weak molecular ion peaks and uninformative fragmentation patterns. Their UV spectra often provide information about the substitution of the aromatic moiety in these compounds. The basic UV chromophore of the synthetic cathinones can be viewed as acetophenone. Acetophenone has four electronic transitions in the near UV: in n-hexane these bands are found at 238.7nm (ε = 12,500), 276.5nm (ε = 900), 285.2nm (ε = 880), and 321.0nm (ε = 44). The very weak 321.0nm transition is the n$\rightarrow\pi^*$ transition of the carbonyl; the remaining bands are $\pi\rightarrow\pi^*$ transitions involving the benzene ring. The strongest band is designated $^1B_{1u}\leftarrow{}^1A_{1g}$. The synthetic cathinones α-PVP, α-PBP, pentedrone, buphedrone, ethcathinone N,N'-dimethylmethcathinone, and methcathinone have an acetophenone-type structure with substitution on the acetophenone methyl group. When these compounds are analyzed by Ultra High-Performance Supercritical Fluid Chromatography (UHPSFC) with a near UV detector, spectra of these compounds were dominated by a strong peak at 238nm-239nm. Analysis of other synthetic cathinones under the same conditions demonstrated that ortho alkyl substitution increases the wavelength of the $^1B_{1u}\leftarrow{}^1A_{1g}$ transition by 0.9nm, meta alkyl substitution increases the wavelength of the band by 4.4nm, and para alkyl substitution increases the wavelength of the band by 10.9nm. For multiple substituted benzene rings, the effects of alkyl substitution are generally additive. Using the increment values for ortho, meta, and para alkyl substitution of 0.9nm, 4.4nm, and 10.9nm, respectively, yields the following predicted λ_{max} values for the $^1B_{1u}\leftarrow{}^1A_{1g}$ transitions of di-alkyl substituted cathinones: 2,3-dimethylmethcathinone 243.9nm (observed 243nm), 2,4-dimethylmethcathinone 250.4nm (observed 250nm), and 3,4-dimethylmethcathinone 253.9 nm (observed 254 nm). These results illustrate that near UV spectra can differentiate alkyl-substituted synthetic cathinones and that rules can be formulated for the prediction of the locations of UV transitions of new alkyl-substituted synthetic cathinones.

Cathinones, Ultraviolet Spectroscopy, Aromatic Substitution

B119 The Evolution of Forensic Household Dust Analysis Over the Past Century

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After attending this presentation, attendees will recognize how forensic household dust specimens are formed and how the fibrous components form a cage-like structure that acts as a snare, which in turn entraps an array of human and animal hairs, natural and synthetic fibers, and an assortment of particulate materials. Attendees will learn about the development of techniques used to study household dust specimens in the forensic laboratory over the past century and the probative value of household dust specimens.

This presentation will impact the forensic science community by demonstrating the probative value of an underused form of physical evidence — forensic household dust specimens.

In the late 19th century, Hans Gross, a German magistrate, speculated in his writing that dust is a representation of a person's environment in miniature. Gross further proposed that by recognizing the constituents composing a particular dust sample, one could estimate the surroundings from which the dust originated, and that this information could be used to help solve crimes. At approximately the same time, the importance of trace evidential material in solving crimes was being popularized by Sir Arthur Conan Doyle through his fictional character, Sherlock Holmes, who solved many mysteries by reconstructing the events of the crime from dust traces left at or taken away from the crime scene. Gross's and Conan Doyle's writings are believed to have inspired many European and American scientific detectives to look for valuable dust clues while investigating crimes.

In the early 20th century, Edmond Locard established a police laboratory in Lyon, France, and soon his ability to solve crimes by analyzing the dust found on a suspect became known throughout the world. Locard's successful implementation of scientific methodology in criminal investigations and his belief that dust analysis could link every criminal to his or her crime are probably responsible for the use of trace evidence in contemporary times. Moreover, his work served to spur the development of forensic laboratories throughout the world.

Between 1910 and 1970, forensic laboratories were developed in France, England, Sweden, Germany, Switzerland, and Austria as well as the United States. In the early 1930s, a national crime laboratory was maintained by the Federal Bureau of Investigation (FBI) in Washington, DC.

During this era, forward-thinking scientists and scientific investigators such as Edmond Locard and many others throughout the world advanced the use of microscopic trace evidence and the analysis of dust traces to solve crimes and reconstruct events.

As postulated by Edmond Locard, the ultimate goal of trace forensic evidence in a criminal investigation is to identify the people, places, and things involved in the commission of the crime. A commonly encountered but often ignored form of physical evidence, traces of household dust, has the potential to achieve these goals. Dust bunnies, as they are often called, should be a unique entangled conglomeration of fibers containing a variety of inorganic and organic particulates from the immediate environment that are formed over time. Thus, in principle, if one or more dust bunnies are found associated with a crime, it should be possible to positively identify the location from which it originated and maybe the people involved in the event.

In the past few years, several papers have been presented which demonstrate that household dust specimens may in fact be unique to a given location. These preliminary studies have focused on developing a rapid microscopic method for tabulating the materials, such as animal and human hair, natural and synthetic fibers, and the particulates commonly found in household dust specimens.

The data collected in prior studies and for newly acquired specimens was tabulated on a revised dust tabulation data sheet specifically designed for this study. The newly acquired and previously acquired data study was combined and subjected to rigorous statistical analysis. A number of interesting trends were found, extensively studied, and reported.

A subsequent study postulated that the probative value of household dust specimens would be enhanced not only if the room could be identified by its material makeup but also by the habitual occupier(s) of the room. Thus, the latest study focused on both the common materials present in the dust and the materials from the occupant(s), which is trapped inside the dust bunny (likely originating from the habitual occupier of the room). Promising results were statistically evaluated and reported.

It is believed that the combination of these different approaches will greatly enhance the discriminating power, as well as the probative value, of household dust by enabling one to not only identify a location, but also to identify its habitual occupant(s).

Edmond Locard, Household Dust, Trace Evidence

B120 The Probative Value of Very Small Particles (VSPs) Adhering to Common Items of Physical Evidence

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After attending this presentation, attendees will appreciate the potential probative value of VPSs, present as dust on or within common items of physical evidence.

This presentation will impact the forensic science community by increasing awareness of the potential value of such particle combinations on common items of physical evidence, as well increasing the awareness of the computational methods that can be applied to recognize associations and measure the strength of associations based on these combinations.

Particle combination analysis uses co-occurring particles to test alternative attribution hypotheses. One application of particle combination analysis is the exploitation of the thousands of VSPs that are found in and on items of evidence, using these particles to test associations and enhance probative value. The combinations of VSPs are so complex that, until recently, there was no practical method to identify and interpret these combinations.

This presentation will cover the application of statistical methods of particle combination analysis to Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) analytical results for very small particles recovered from the surface of common items of physical evidence, such as handguns, cell phones, ski masks, and drug packaging.

VSPs were collected from actual items of evidence from cases in one jurisdiction where detectives had determined that the items were no longer of value and had approved them for disposal. Particles were harvested from plastic drug packaging by directly applying commercially prepared SEM stubs analogous to those commonly used in protocols for the recovery of possible Gunshot Residue (GSR) from a subject's hands. Clean room swabs, slightly moistened with pre-filtered distilled water, were used to recover VSPs from handguns, cell phones, and ski masks. Two separate specimens were recovered from each evidence item.

The SEM stub specimens from drug packaging were suitable for SEM/EDS processing without further preparation. The swab specimens were prepared for SEM by extraction of particles into an aqueous suspension, followed by low-vacuum filtration onto 0.4 micrometer pore size 13mm polycarbonate filters and mounting on SEM/EDS stubs.

For each specimen, up to 10,000 VSPs were individually characterized by semi-automated SEM/EDS analysis, binning the analytical response for each particle into 18 X-ray energy bins corresponding to a set of 18 elements. The data sets were filtered to reduce noise represented by: (1) particles having no dominant elemental composition detected under the analysis conditions; and, (2) elements present in low quantities for any given particle.

Sets of Target Particle Types (TPTs) were defined based on normal mixture modeling using a training set composed of random sampling from all sources. Multinomial distributions were defined for each source based on the numbers of particles corresponding to each of the TPTs. For comparison of TPT profiles, the probability density of the observed count in a test specimen was assigned in each of the N multinomial densities (corresponding to each of potential sources). This probability was used as the measure of correspondence to each of the reference sources. The probabilities can be used for classification or for ranking of candidate sources as in a library search.

Measurements of probative value were defined using a Bayesian classifier applied to the multinomial probability densities, assuming an equal prior among all N classes. This results in posterior probabilities obtained using the classifier for all N sources. A corresponding likelihood ratio was calculated as a measure of evidential weight, based on assumptions of the representativeness of the N sources.

This project was supported in part by awards from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the author and do not necessarily reflect those of the Department of Justice.

Particle Combination Analysis, Trace Evidence, Evidential Value

B121 Using Kernel-Based Methods for Inferring the Source of Very Small Particles (VSPs) From Recovered Forensic Materials

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After attending this presentation, attendees will better understand a method that objectively models pairwise scores that are often obtained in the analysis of complex and high-dimensional forensic data. This method can be used to estimate the weight of evidence for a particular object of forensic interest.

This presentation will impact the forensic science community by: (1) providing a method to infer the source of forensic material by using the VSPs found on the surface of the material; and, (2) providing a method that models the dependency structure of pairwise scores and provides a numerical weight of evidence.

The objective comparison of complex signals in chemistry and, more particularly in forensic chemistry, with the view of inferring the source of a particular "trace" object is an ongoing issue. These issues show up either in how measurements are taken (dimensionality and/or compositional data) or the production/mass manufacturing of the trace material (e.g., paint, metals), leading to difficulty in the identification of identical materials.

Instead of looking at the trace material itself, the VSPs found in contact with the trace material are considered. VSPs are picked up in the environment(s) in which the trace material has been. These VSPs offer information regarding the geographic origins of the material despite non-uniqueness of the trace material due to manufacturing processes and allow a measure of relation between VSPs on trace material and known material, assisting in source identification of trace material.

Initial efforts classified sets of VSPs using a multinomial classifier. In order to reduce the complexity of the parameter space for this model, the types of the VSPs were categorized as determined by their dominant compounds.[1] Each source was characterized by a vector of relative proportions of these Target-Particle Types (TPTs), which could be used as parameter estimates in a multinomial model. The definition of TPTs involved the use of unsupervised clustering techniques with their inherent drawbacks (e.g., arbitrary choice of a limited number of TPTs to keep the dimension of the parameter space reasonable).

In this project, a method is proposed that circumvents these problems and enables the assignment of a probability distribution to control material from any given source based on its chemical signal. Instead of estimating parameters for TPTs, the raw data is directly worked with by leveraging the dimension reduction and discriminative powers of kernels, then extending the work of Gantz and Saunders for pairwise-score parametric models.[2] The subsequent model only requires the estimation of three parameters (once a kernel is chosen) and is subsequently used to infer the source of a trace object using a simple Bayes classifier.

The application of this method to the inference of the source of trace objects based on VSPs is illustrated. A dataset of VSPs recovered from carpet fibers throughout the United States is used and this method is applied method to: (1) reduce the complexity of compositional data obtained by SEM/EDS; and, (2) infer the source of the trace material. Initial results of the method have a slightly improved classification rate of the trace fibers than that of the multinomial classifier, while significantly reducing the complexity of the parameter space.

This method can be extended to VSPs found on other types of recovered forensic materials such as weapons, drugs, or Improvised Explosive Devices (IEDs), and to other types of complex chemical signals.

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High-Dimensional, Weight of Evidence, Forensic Material

B122 Forensic Analysis of Trash Bags: Part I — Microscopical Discrimination of Trash Bags

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After attending this presentation, attendees will understand that trash bags have differing physical features: color, type of closure, surface textures, and heat seals. The bags may also contain different crystalline additives with varying concentrations. Attendees will also understand the role of light microscopy (both reflected light and Polarized Light Microscopy (PLM)) in the forensic analysis of trash bags.

This presentation will impact the forensic science community by informing attendees of a database that was created and includes different brands of trash bags with their descriptions, dimensions, thicknesses, and distinguishing features. This database includes more than 80 different brands of trash bags. Such a database will be useful to forensic examiners as it includes trash bags sold in the United States.

Trash bags can appear as evidence at crime scenes as they can be used to package everything from controlled substances to dead bodies. A frequently asked question is whether a trash bag found at the crime scene can be associated with a roll or box of trash bags found in a suspect's possession. The physical measurements (such as length, width and thickness), optical properties and the additives of each trash bag would be compared. Physical matches of tears, perforations or manufacturing imperfections have proven useful for identifying sequentially manufactured bags.

Eighty-eight trash bag samples were collected, most from the Washington, DC, area and a few were purchased in the Midwest. Boxes or rolls of trash bags were purchased at supermarkets, department stores, and dollar stores. Special emphasis was placed on "store brands." Roughly equal numbers of white "kitchen" trash bags and the large, dark trash and lawn and leaf bags were collected for examination. A majority of trash bag samples were found to have been manufactured in the United States, while a very few were manufactured in China and Canada.

Photomicrographs of the side-edge and bottom-edge heat seals and surface textures were obtained for each bag using a low magnification reflected light stereomicroscope equipped with a 14-megapixel eyepiece digital camera. For transmitted light microscopy, pieces of each trash bag were cut from the side edge. The side seal was retained so that the extrusion direction of the sample could be determined. The pieces of trash bags were cleaned with methanol, left to dry overnight, then permanently mounted on a standard microscope slide with a cover slip for further microscopic examination (brightfield and Polarized Light Microscopy (PLM)). Photomicrographs of the trash bags were obtained under each of the viewing conditions.

The thicknesses of the trash bags varied from 10.1µm to 1,100µm, with majority of the trash bags having a thickness of 22.9µm. The dimensions of the trash bags varied from 55.8cm x 60.9cm to 98.4cm x 114cm, with majority of the trash bags having dimensions of 60.9cm x 68.5cm. The dimensions and thicknesses differentiated the trash bags into 31 and 23 different groups, respectively. PLM provided little differentiation of the trash bags. While crystalline additives in the bags were visible due to their birefringence, the particles were too small to be identified *in situ* by optical crystallography. At most, bags with significant concentrations of additives could be distinguished from those with low concentrations of additives. It was found that the side and bottom heat seals were very useful for distinguishing different brands of trash bags. The side-edge and bottom-edge heat seals differentiated the trash bags into 37 and 31 different groups, respectively. Such differences presumably reflect differences in the processing of the side and bottom heat seals (e.g., the width of the heated metal strips used to create the seals). The side and bottom heat seals provide better brand discrimination than Fourier transform infrared spectroscopy or X-ray diffractometry.

Trash Bags, Reflected Light Microscopy, Polarized Light Micrscopy

B123 Forensic Analysis of Trash Bags: Part II — Fourier Transform Infrared (FTIR) Spectroscopy and X-Ray Diffraction (XRD)

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After attending this presentation, attendees will understand the use of FTIR and XRD to discriminate between various brands of trash bags. Trash bags are common in forensic investigations because they can be used to conceal victims, dispose of evidence, or transport controlled substances. Attendees will understand the potential for using FTIR and XRD to discriminate trash bags of different brands by identifying and quantifying crystalline additives.

This presentation will impact the forensic science community by providing forensic examiners with instrumental methods of analysis for the discrimination of trash bags from different sources. Additionally, this study provides data on trash bags used in the United States and Canada, while other publications on the forensic examination of plastic bags have examined samples used in other countries.

Trash bags are typically manufactured from virgin polyethylene pellets, pigments, and other particulate additives melted together to form a molten plastic. The melted plastic is extruded through a ring-shaped die and mandrel, which impresses tool marks into the bag surface, creating identifiable patterns. Such patterns can be useful in identifying sequentially manufactured bags, but would have less utility for the identification of the brand of trash bag. On the other hand, FTIR and XRD can provide information on the brand of trash bag and permit the comparison of trash bags when the questioned and known trash bags were not sequentially manufactured. These instrumental methods would also be of value when only fragments of bags are recovered at a crime scene.

This research investigated the discriminatory power of FTIR and XRD for the forensic examination of trash bags. Eighty-eight different samples representing 28 different brands were examined. Most of the samples were purchased in the Washington, DC, area; a few bags were purchased in the Midwest. While samples of national brands were collected, special emphasis was placed on "store brands." Features of the bags, such as color, odor, type of closure, dimension, thickness, and place of manufacture were recorded for each sample to create a database.

Square pieces of each trash bag were cut from the side-edge, approximately 5cm x 5cm in size. These pieces were cleaned with methanol, in order to eliminate possible interference of coatings on the surfaces of the bags. Two strips were then cut from each square, one for FTIR analysis and the other for XRD. The samples analyzed by FTIR were scanned in transmission mode from 525cm^{-1} to 4,500cm^{-1}, 128 scans per sample. The samples used for XRD analysis were glued to a standard microscope slide. The samples were flattened on the slide using a rubber roller and left to dry before testing. The diffraction angle (2q) was scanned from 3° to 40° at 2° per minute.

The FTIR and XRD analyses of the trash bags provided basic information on their compositions. Most samples were composed of Low-Density Polyethylene (LDPE); a small number of samples were comprised of Linear Low-Density Polyethylene (LLDPE). Some bags had no additives; some contained either calcite or talc but not both; and some contained both calcite and talc. XRD revealed the presence of a low concentration of an additional additive, montmorillonite, which was not detected by FTIR. Based on their FTIR spectra, the bags could be placed into 14 groups based on the presence or absence of additive peaks and their relative intensities. Based on their XRD patterns, the bags could be placed into 18 groups based on reflections present in the diffraction patterns. The discrimination of the trash bag samples in this research demonstrates the value of XRD for polymeric material containing a number of additives. Based on the results of this project, XRD should be preferred to FTIR for the analysis of trash bags.

Trash Bags, FTIR, X-Ray Diffraction

B124 An Assessment of the F-108 Polymer and Capillary Electrophoresis Single-Strand Conformational Polymorphism (CE-SSCP) to Screen Human DNA Mixtures

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After attending this presentation, attendees will understand the principles of CE-SSCP analysis, why the F-108 polymer is used in contrast to the Performance Optimized Polymers (POP), and how the two are utilized to identify Single Nucleotide Polymorphisms (SNPs) in forensic samples that have previously been identified as human DNA mixtures.

This presentation will impact the forensic science community by demonstrating the application of CE-SSCP to quickly identify SNPs in or around regions within the commonly used Short Tandem Repeat (STR) loci. This will allow scientists to compare a mixture sample with overlapping alleles to a reference sample, thus allowing scientists to screen and determine which samples may need to be further analyzed via next generation sequencing.

Current technology in forensic science uses instrumentation such as a thermal cycler to amplify the DNA by the Polymerase Chain Reaction (PCR) and a CE instrument to produce DNA profiles. These profiles are a series of peaks of varying heights that represent alleles from loci that were amplified in the PCR. At each locus in a single source sample, there should be a maximum of two visible alleles. But often there are more, indicating the presence of multiple contributors to the sample, commonly referred to as a mixture. Due to technological advances, the process of DNA typing is becoming extremely sensitive, allowing for the testing of DNA collected from touched objects. As a result, the amount of forensic mixtures obtained for analysis has increased; however, the actual interpretation of these types of samples remains an issue. Current methodologies only address allelic variation by fragment length and not the actual nucleotide sequence within the amplican. As a result, there may be alleles of the same size yet different sequence among the contributors in a forensic mixture sample, which further hinders the interpretation of useful results.

In traditional forensic CE, DNA is separated by a denaturing polymer (e.g., POP-7) that serves as an entanglement matrix that allows smaller fragments of DNA to move faster than larger fragments. A linear relationship is observed between fragment size and migration time. The denaturant keeps the single-stranded DNA from changing its conformation as it migrates through in the capillary. Another matrix, Pluronic F-108, is unlike the POP matrix because it is non-denaturing. F-108 is a Poly(Ethyleneoxide)-Poly(Propyleneoxide)-Poly(Ethyleneoxide) (PEO-PPO-PEO) triblock copolymer. The non-denaturing aspect of this polymer makes it ideal for the detection of SNPs in the DNA sequence using a phenomenon known as SSCP. The principle of SSCP is based on the fact that single-stranded DNA has a defined conformation (secondary structure). A change in this conformation in a non-denaturing matrix because of the presence of a SNP causes the single-stranded DNA to partially reanneal and migrate differently through the matrix even if only one nucleotide has changed out of several hundred bases.

DNA was extracted from three different Fast Technology for Analysis (FTA) cards containing blood samples from different contributors, and the extracted DNA was processed for downstream analysis. The locus D16S539 was amplified. The resulting PCR products were quantified and prepared for Sanger sequencing. The sequences were separated using the POP-7 polymer to identify SNPs within the amplicons. Several SNPs were subsequently identified in each sample. Additional DNA was extracted from those FTA cards and amplified for STR analysis where each sample produced a single peak for each allele on POP-7. That same PCR product was analyzed using the CE-SSCP method with the F-108 polymer. This approach was able to distinguish the presence of polymorphisms in sequence, even when the length of the amplicon was the same.

In conclusion, results from this study determine that the CE-SSCP technique can aid in resolving forensic mixtures. This method uses the same STR product and the same CE instrument but with the non-denaturing F-108 polymer to identify areas of SSCP, which suggests the presence of SNPs. Thus, incorporating this polymer could make the process of screening samples quicker and more efficient for subsequent next generation sequencing analyses.

Mixtures, CE-SSCP, SNPs

B125 An Improved Capillary Electrophoresis (CE) System for Human Identification

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After attending this presentation, attendees will better understand the challenges to implementing new forensic technologies such as Next Generation Sequencing (NGS) and rapid DNA, will understand why CE will remain the method of choice for forensic DNA laboratories, and will learn about the advantages of using eight-color Short Tandem Repeat (STR) multiplexes and a CE system in the forensics workflow, such as the use of more mini-STRs to provide improved results with degraded samples, more usable data from inhibited samples, and improved overall efficiencies.

This presentation will impact the forensic science community by suggesting improvements that eight-color STR multiplexes will offer to the forensic DNA workflow, including more usable data from inhibited samples, enhanced results when degradation is present, and improved overall efficiencies.

Rapid DNA and NGS hold great promise for the forensic community to extend the reach and depth of DNA typing. While both of these approaches are powerful complements to traditional CE STR typing, neither approach is likely to replace CE analysis for the majority of forensic samples in the near future. CE will very likely remain the "workhorse" of forensic DNA typing. As such, improving upon CE technology is critical for the advancement of forensic DNA typing.

The Spectrum CE System offers increased spectral capacity by allowing the continued analysis of existing four-, five- and six-color multiplexes, as well as a new family of eight-color multiplex STR systems. With the inclusion of additional colors, more loci can be designed in the smaller size ranges. This will increase a laboratory's chance of success with degraded samples, a situation that forensic laboratories often encounter with their casework samples. Additionally, improved multiplex configurations will provide more complete and informative results with inhibited casework samples, which are quite commonly processed in forensic casework laboratories. The narrower range of product amplicon sizes will enable more consistent results with variable "direct amplification" samples. This CE system also offers increased workflow flexibility with four continuously accessible plate positions, and allows laboratories to process more samples at one time than is currently possible with existing CE instrumentation. This design improves laboratory efficiency by reducing scheduling conflicts, increasing overnight/weekend throughput, and reducing the number of instruments needed in the laboratory. Lastly, the system's analysis software, which has been specifically intended for human identification and forensics applications, offers several benefits, including time savings, ease of use**,** accuracy, and additional integrated post-genotyping applications.

Capillary Electrophoresis, DNA Typing, Eight-Color STR Multiplex

B126 A Comparison of Differex™ and Organic Differential DNA Extractions for the Acquisition of a Male Profile From Samples That Exhibit Mold Growth

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The goal of this presentation is to provide attendees with knowledge on which, if either, of the two DNA extractions explored is most suitable for mixed male and female DNA samples contaminated with mold growth.

This presentation will impact the forensic science community by potentially aiding forensic scientists in making a decision regarding how to process a DNA sample contaminated with mold growth. Finding an optimal method to process mold-contaminated vaginal swabs in sexual assault cases could allow for the analysis of samples previously deemed untestable.

Backlogged cases in crime laboratories have increased dramatically since 2005 due to lack of funding and personnel. The statute of limitations has also been extended in some states, allowing more sexual assault evidence kits to qualify for testing. These backlogs can range from months to years since collected and stored. Recently, there has been a push to allot funds for the processing of sexual assault evidence kits in an attempt to reduce these backlogs. The most commonly tested piece of evidence in sexual assault evidence kits is a vaginal swab. If not collected and stored properly, vaginal swabs can develop mold over time. This can be a result of varying environmental conditions or exposure to humidity; however, it has become common practice to dispose of samples with excess mold growth because it is believed that no accurate human DNA profile can be obtained. Some agencies choose not to process these samples due to the expense involved and the limited chance of obtaining successful results. While this practice may seem rash, it has been determined that the mold slowly degrades the DNA, causing it to become more fragmented. Additionally, mold acts as an inhibitor for Polymerase Chain Reaction (PCR), a crucial step in current forensic DNA analysis. PCR inhibition can cause loss of signal, peak imbalance, and/or allelic dropout.

This research attempts to compare the effectiveness of the standard organic differential extraction protocol to the Differex™ System on vaginal swab samples that exhibit varying degrees of mold growth. Optimal packaging procedures currently exist for sexual assault evidence to prevent the development of mold. These procedures reduce the incidence of mold growth, but they are not as effective if the sample has not been dried properly prior to packaging or if the sample is exposed to moisture during storage. There is little research and information on what to do with samples previously collected that may contain mold growth. Traditionally, a differential DNA extraction procedure is performed on these samples. This extraction involves wash steps, which theoretically get rid of any contaminants. While the organic differential extraction technique is regarded as the gold standard, the Differex™ System uses the same principle and is less time consuming. The Differex™ System employs the use of DNA IQ™ magnetic resin to bind the DNA, effectively reducing the sperm loss that is common to other differential techniques. This could allow for a partial or complete male profile to be obtained from samples contaminated by mold growth.

Mock sexual assault samples were prepared by obtaining sterile cotton swabs containing female epithelial cells and vaginal fluids. Four swabs were submerged in each of the following seminal dilutions: 1:2; 1:8; 1:32; and 1:128. The swabs were then stored in improper conditions to promote mold growth for 0, 1, 2, 3, 6, and 14 months. Photographs were taken of each swab to record mold growth. One-half of each swab was subjected to a standard organic differential extraction, while the other half was processed using Differex™. DNA quantitation values and profile quality were compared between the two extraction methods.

Examining alternative means of dealing with mold-contaminated samples is essential not only to further advance testing of backlogged cases, but also to avoid disposing of essential incriminating evidence that could potentially lead to the incarceration of a rapist. Comparing current DNA extraction methods could result in the discovery of an optimal technique for samples of this nature. An optimal method could limit the severity of inhibition and allow more accurate results in data analysis.

Differex™, Organic Differential, Mold

B127 Effects of Swab Collection and Storage Methods on DNA and Messenger RNA (mRNA) Profiling of Forensic Stains

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The goal of this presentation is to evaluate the effects of different swabbing methods and the subsequent storage conditions of collection swabs on combined DNA and mRNA profiling of trace evidence.

This presentation will impact the forensic science community by providing crime scene investigators with directions on optimal procedures for the collection and storage of biological stains that are expected to undergo DNA/RNA co-analysis.

The increasing sensitivity of multiplex Polymerase Chain Reaction (PCR) assays for the simultaneous amplification of Short Tandem Repeat (STR) loci now "trace DNA"). Determination of tissue origin of trace DNA is often crucial for the reconstruction of crime dynamics. Recently, several robust protocols for DNA/RNA co-extraction from stains have been described and validated for forensic purposes, thus enabling the single pipeline analysis of both STRs and mRNA profiles to identify body fluids.[1] So far, limited research has been dedicated toward identifying an optimal solution for the retrieval and preservation of RNA from forensic stains. Trace samples are usually collected by means of wet swabs moistened with water; however, it has been suggested that this may not be the most suitable method for RNA because of its high susceptibility to hydrolytic breakdown.[2] Storage conditions of swabs after collection may also affect the quantity and quality of retrievable nucleic acids. It has been shown, for example, that freezing rather than drying swabs prior to extraction results in an improvement of DNA recovery.[3]

The goal of this study was to evaluate the impact on DNA/RNA retrieval rates and integrity for downstream applications of: (1) different moistening agents applied to collection swabs; and, (2) different storage conditions prior to DNA/RNA extraction of the swabs used for stain collection.

Mock stains were created by applying the following to glass slides: whole blood; 1:10 diluted blood treated with luminol; saliva; semen; and skin (by rubbing one thumb/index fingertip over the designated area for approximately 10s). Moistening agents applied to collection swabs were: water; ethanol; and RNAlater™. Swabs were either air-dried or frozen for one to seven days prior to DNA/RNA extraction. DNA and RNA were co-isolated and quantified with the Plexor® HY System and 2100 Bioanalyzer, respectively.[2] DNA was genotyped using the AmpF*l*STR® Identifiler® Plus PCR amplification kit and 3500 Genetic analyzer and mRNA profiling was performed.[4]

Concentration of total RNA isolated from mock stains was significantly higher for swabs treated with RNAlater™ compared with water and ethanol, with consequent effects on mRNA profiling. Interestingly, the impact of moistening media on DNA recovery rates varied among tissues. In skin samples, both ethanol and RNAlater™ significantly outperformed water, also generating more complete STR profiles. On the contrary, DNA retrieval from whole blood and semen stains was significantly lower with ethanol; however, because of the higher DNA yields obtained, on average, from whole blood and semen when compared to skin, moistening agents had no effective impact on STR typing in this case. Finally, no significant differences in DNA/RNA recovery were observed depending on storage conditions and storage time of collection swabs.

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Trace Collection, Swab Storage, MRNA Profiling

B128 A Method for Optimizing Pressure-Based Extraction and Direct Polymerase Chain Reaction (PCR) of Simulated Sexual Assault Samples

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After attending this presentation, attendees will better understand a novel method for differentiating, extracting, and amplifying epithelial and sperm mixture samples.

This presentation will impact the forensic science community by providing results for a method that can decrease the analysis time for sexual assault samples. Attendees will see results of a new method to aid in direct PCR for amplification of simulated sexual assault samples. These results will add to previous work performed using pressure cycling by demonstrating a method to eliminate the long cell lysis and purification process as well as coupling these methods with direct PCR.

In this study, pressure cycling is used to prepare differentiated lysates of epithelial and sperm mixtures for rapid analysis. It is widely known that many Sexual Assault Kits (SAKs) currently await processing. For example, in January of 2016, more than 13,400 SAKs had been reported as not being analyzed in the state of Florida.[1] This study presents a method for faster analysis of these types of samples. Epithelial and sperm mixtures often require large amounts of time and effort to differentiate the fractions. The goal is to develop a rapid STR-based screening that eliminates the cell lysis, purification, and quantitation steps for a faster analysis.

This study involved the use of alkaline lysis and direct PCR to extract and amplify epithelial and sperm DNA from simulated SAK samples, with various concentrations of epithelial and sperm cells. The mixtures that were tested ranged from 1:1 to 20:1, epithelial: sperm. The first part of the project consisted of the optimization of pressure cycling and alkaline lysis for simulated sexual assault samples. Concentrations of Sodium Hydroxide (NaOH) and temperature were tested, as well as pre-pressure steps. Concentrations of 0.4N, 0.3N, 0.2N, and 0.1N NaOH were used to determine an increase in epithelial recovery. Immunomagnetic cell capture prior to the Pressure Cycling Technology (PCT) step was used to increase the differential recovery of epithelial and sperm fractions. This technique utilizes the EasySep™ Human EpCam Positive Selection Kit to capture epithelial cells with antibodies that target epithelial cell receptors. These are then attached to dextran-coated magnetic particles added to the sample. Once the sample tube was inserted into a magnet, excess epithelial cells were trapped on the sides of the tube. In the second part of the project, the simulated sexual assault samples were processed with pressure cycling and a direct PCR protocol was developed. Cotton swabs with epithelial and sperm cells were treated at room temperature with 0.2N NaOH in a PULSE™ tube. In the same tube, pressure cycling was performed with the optimized protocol using the Barocycler® NEP 2320; this step was repeated and the epithelial lysate was neutralized. Then the swab was treated again with alkali and heated to 95°C to lyse the sperm cells. These were also neutralized and the cotton swab removed. Both lysates were amplified with direct PCR using a 7-locus primer set on the Applied Biosystems GeneAmp® PCR System 9700 and/or amplified with rapid/direct PCR on a Streck Philisa® Thermal Cycler. Amplification conditions and speed were optimized by examining the effect of primer, polymerase, buffer, and dNTPs. The STR multiplex was then analyzed on an Applied Biosystems® ABI PRISM® 310 Genetic Analyzer to assess the quality of the mixture separation.

The results demonstrate the potential of alkaline lysis and pressure cycling to greatly expedite the analysis of differential extraction. In regard to optimization of the pressure cycling, the use of 0.2N NaOH resulted in improved recovery and removal of epithelial cell DNA from the substrate, 115% compared to the unmodified protocol, according to data obtained from quantification with Plexor® HY. Genetic profiles from the ABI PRISM® 310 Genetic Analyzer revealed a slight majority male profile for the sperm fraction at a 20:1 female epithelial cell to sperm cell ratio. The addition of the immunomagnetic cell capture treatment prior to the PCT step produced a clear majority male profile with female allelic dropout. The addition of faster amplification to this protocol can greatly increase the speed of the results obtained.

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Direct PCR, Pressure Cycling Technology, Simulated SAK Samples

B129 The Use of Direct Polymerase Chain Reaction (PCR) on Semen and Spermatozoa and the Development of a Differential Isolation Protocol

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After attending this presentation, attendees will recognize the potential of direct PCR use in cases of alleged sexual assault to provide a higher level of sensitivity. Attendees will also learn a novel differential isolation protocol for use with direct PCR to obtain both female and male profiles.

This presentation will impact the forensic science community by demonstrating a more rapid method to determine the DNA profiles within sexual assault samples that also provides greater sensitivity. A differential isolation protocol for use with direct PCR will be presented to separate the male and female fractions of a simulated sexual assault mixture.

Sexual assault samples can be some of the most common samples encountered in forensic analysis. These samples can require a significant time investment due in part to differential extraction processes. This presentation reports on the first recorded use of direct PCR to successfully amplify semen for STR analysis. Amplification without prior DNA extraction, known as direct PCR, has gained increased interest in forensic science due to the reduced time to DNA profile and increased sensitivity of the technique. Benefits of direct PCR include reduced time and expense compared to standard DNA extraction prior to amplification. In this study, the potential for direct amplification of spermatozoa and seminal fluid is investigated in order to determine the donor. Stains containing seminal fluid (pure and mixtures) were subjected to the technique of direct PCR and a differential isolation method was investigated prior to direct amplification.

Neat seminal fluid, dilutions ranging from 1:5 to 1:160, and German DNA Profiling (GEDNAP) samples were successfully amplified using a direct method. All samples amplified full profiles to the 1:160 dilution in which approximately 50% of a full profile were obtained. All GEDNAP stains produced low-level but complete and reproducible profiles containing the same alleles as when analyzed using a traditional differential extraction technique. GEDNAP samples represent simulated case samples that are used for proficiency testing relating to presumptive testing and Short Tandem Repeat (STR) analysis. A mild differential isolation technique to enrich spermatozoa was developed and successfully implemented to separate and directly amplify a mixture of semen and female epithelial cells. Profiles resulting from the differentially isolated samples show high levels of amplification with good balance across all loci. Global balance ranged from 0.78 to 0.87 for liquid mixtures and 0.78 to 0.9 for dried mixtures. Aliquots of samples subjected to the differential isolation protocol were stained with hematoxylin and eosin for sperm scoring. To verify that the spermatozoa were being lysed during the direct PCR, samples stained after PCR showed a complete lack of intact spermatozoa. While the differential isolation protocol adds a small amount of time to the direct PCR process, it resulted in strongly enhanced male profiles. Even in samples with an excess of female epithelial cells, direct PCR can produce male profiles with surprisingly strong peak heights if combined with the differential isolation protocol. Direct PCR can offer increased sensitivity with reduced time and cost for sexual assault samples compared with traditional differential extraction methods.

Direct PCR, Differential Isolation, Spermatozoa

B130 Botanical DNA Evidence in a Case of Illegal Drug Trafficking: The Use of High-Resolution Melting (HRM) With the Internal Transcribed Spacer (ITS) Approach in the Identification of Psychedelic Fungus (*Psilocybe sp.*)

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After attending this presentation, attendees will better understand how molecular biology techniques such as real-time Polymerase Chain Reaction (PCR) strategies, analysis of HRM curves, and DNA barcoding can help identify psychedelic fungus in illegal crops.

This presentation will impact the forensic science community by demonstrating how the results of coupling real-time PCR analysis with a DNA barcoding approach provided elements that were used in a drug trafficking court case in Chile.

Currently, as supporting evidence, DNA analysis is practically the only element that can be used as a reliable identification tool, due to the high variability of DNA nature across all species. One way to identify a distinctive DNA fragment for a species is the study of PCR products analyzed via real-time PCR. One of the most popular sequences of forensic interest at the generic and intra-generic levels in plants is the ITS. ITS refers to the spacer DNA situated between the small-subunit ribosomal RNA (rRNA) and the large-subunit rRNA genes in the chromosome or to the corresponding transcribed region in the polycistronic rRNA precursor transcript. ITS1 is located between the 18S and 5.8S rRNA genes, while ITS2 is between the 5.8S and 25S rRNA genes in plants.

Real-time PCR has many advantages over other molecular techniques since it does not require electrophoretic analysis. With real-time PCR, it is possible to distinguish PCR products using their melting Temperature (Tm) curves via differential analysis. In nature, there are more than 200 species of fungi with hallucinogenic properties. These fungi are classified as *Psilocybe*, *Gymnopilus*, and *Paneolus*. They contain active principles with hallucinogenic properties, such as ibotenic acid, psilocybin, psilocin, or baeocystin.

In Chile, fungi seizures are mainly composed of mature specimens or spores; however, it was found that clandestine laboratories processed fungus samples at the stage of mycelium. In this transient stage of growth (mycelium), traditional taxonomic identification is not feasible, making it necessary to develop a new method of study.

The case described in this presentation refers to the genetic analysis of mycelia of psychedelic fungi collected from a clandestine laboratory. The identity of fungus species was achieved using an ITS and HRM analysis approach. A genetic match was confirmed between the HRM curves obtained from the mycelia (evidence) and biological tissue extracted from the fungus' cap (the *Psilocybe sp.* mushroom, which served as a control). Therefore, mycelia recovered from the evidence and the fungus control were genetically indistinguishable. This HRM strategy enabled the molecular traceability of the psychedelic fungus and proved the usefulness of this approach for the identification of closely related species. The suspect was convicted of drug trafficking.

Forensic Botany, Psychedelic Fungus, High-Resolution Melting

B131 DNA Recovery From Waterlogged Bone: A Test of Three Methods

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After attending this presentation, attendees will better understand the effect of water on the quality and quantity of DNA retrievable from skeletal remains, as well as which extraction method, organic phenol-chloroform or solid-phase, and bone type is best for the isolation of DNA from skeletal remains found in water.

This presentation will impact the forensic science community by providing information concerning a neglected area of research: the quantity and quality of DNA retrievable from waterlogged skeletal remains, and the most successful type of bone (compact or spongy) for DNA extraction.

Following water-related accidents and mass disasters occurring over or in water (e.g. ferry disasters and commercial plane crashes), soft tissue on victims' remains could be entirely absent after a temperature-dependent period of time. Due to the physical and chemical components of bones that protect them from environmental deterioration and biological attack, the ability to identify the victims may rely on the examination of skeletal remains.[1,2] Ordinarily, DNA recovery from skeletal remains is difficult and results in a low yield of DNA due to degradation and Polymerase Chain Reaction (PCR) inhibitors.[3] Few case studies discuss remains found submerged in water. For example, a Short Tandem Repeat (STR) profile was successfully extracted from a 67-year-old bone found in a fresh water lake by creating and using a unique extraction protocol; however, a later study using the same protocol on remains found in seawater was unsuccessful.[4,5]

In this experiment, samples were derived from 12 pig (*Sus scrofa*) humeri and 12 pig ribs, divided into a total of 24 humerus and 24 rib samples. Cut bones and waterproof dataloggers were submersed in water and left outdoors. Water temperature was recorded hourly. Data from the loggers were monitored. Approximately every 500 Accumulated Degree Days (ADD), three rib and three humerus samples were collected and stored in a -20°C freezer. After collection, the DNA was extracted in triplicate using these different methods: organic phenol-chloroform, DNeasy Blood and Tissue Kit, and ChargeSwitch® gDNA Plant Kit. The quality and quantity of the DNA was determined using the NanoDrop™ Spectrophotometer, Qubit® 2.0 Fluorometer, and real-time quantitative PCR (qPCR), respectively.

In assessing the quality of DNA extracted from samples across 3,000 ADD, Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) analyses indicated a significant difference among methods (F=9.83, p=0.0004); the ChargeSwitch® gDNA Plant Kit had a decreased mean 260/280 ratio compared to DNeasy Blood and Tissue Kit. Qubit® quantitation values were highest using the ChargeSwitch® gDNA method and rib bone type. ANOVA analysis did not indicate a significant difference between bone type (F=200, p=0.1666) or among methods (F=0.87, p=0.4278). ANOVA analysis of Cycle threshold (C_t) from qPCR indicated a significant difference among the methods (F=5.36, p=0.0096). The ChargeSwitch® gDNA Plant Kit had the lowest mean C_t value (28.88) compared to DNeasy Blood and Tissue Kit (34.34) and organic phenol-chloroform (33.48). Analysis also indicated a significant difference between bone types with respect to DNA yield. The mean C_t value for humeri (33.81) was greater than the C_t value for ribs (30.83), indicating that the ChargeSwitch® gDNA Plant Kit method and rib bone type produced higher quantities of DNA.

Overall, this study suggests that magnetic bead technology is the most beneficial method for recovery of DNA from waterlogged bone, and ribs may be better suited for recovery of host DNA, contrary to common practice and belief.

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Waterlogged, DNA Analysis, Skeletal Remains

B132 Protein Profiling of Decedent Scalp Hairs to Investigate the Potential Mechanisms for the Formation of Postmortem Root Bands

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After attending this presentation, attendees will better understand the proteomes from different segments of the pre-keratinized region in anagen hairs exhibiting and not exhibiting a Postmortem Root Band (PMRB). Manifestation of a PMRB is one form of decompositional change that is presented as an ellipsoidal-shaped banded area in anagen and early catagen-phase hairs derived from cadavers.[1-5] It has been demonstrated that these banded areas are gas pockets, as they appear dark in transmitted light microscopy and bright in reflected light microscopy.[4] Although the microscopic characteristics of PMRBs have previously been well investigated, the mechanism for their formation requires further research.[1-5]

This presentation will impact the forensic science community by contributing to the understanding of the possible biochemical mechanism(s) of the formation of a PMRB. The knowledge gained from this research will be useful to forensic science by providing support when determining whether the hair evidence was shed postmortem.

Anagen head hairs were collected at the Federal Bureau of Investigation Laboratory from human volunteers and at the University of Tennessee Knoxville Anthropology Research Facility from deceased donors of known postmortem interval. Hairs were trimmed to approximately 1cm from the proximal end to facilitate handling. A visual qualitative assessment of decomposition was performed for each hair using transmitted light microscopy. For each hair exhibiting a PMRB, three areas of the root were segmented for protein extraction: below (proximal), at (medial), and above (distal) the band. Hair segments (~300µm in length) of homologous locations were also obtained from postmortem and antemortem hairs that did not exhibit PMRBs. In total, at least 72 hair segments were processed for protein extraction and enzymatic digestion using trypsin.[6]

The peptide/protein composition of banded and non-banded hairs was characterized using nano-liquid chromatography/tandem mass spectrometry using labeled synthetic peptides and angiotensin added as internal standards. Chromatograms of the segments (i.e., proximal, medial, and distal) as well as the proteomes of these segments for both banded and non-banded hairs were compared. Preliminary qualitative and semi-quantitative analysis of protein profiles reveals subtle differences between the different hair segments. It was anticipated that the number, quantity, or modification of proteins in the non-PMRB hair segments may be different than in the PMRB hair segments because microscopic analysis of the banded segment showed significant decomposition. Results from this comparative proteomics analysis of the hair segments will be presented.

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Postmortem Hair Root Banding, Hair Decomposition, Liquid Chromatography

B133 The Effect of Using an Extra Polymerase Chain Reaction (PCR) Cycle With GlobalFiler® When Amplifying Skeletal Samples

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After attending this presentation, attendees will understand the advantages and disadvantages of altering the suggested number of PCR cycles as a means to increase Short Tandem Repeat (STR) success for low template and/or degraded bone samples.

This presentation will impact the forensic science community by providing insight into the benefits and cautions when increasing the PCR cycle number using a common STR amplification kit in an attempt to improve the DNA typing results from low-template and/or degraded skeletal samples for human identification.

Using STRs to identify human remains has its challenges. By their nature, bone samples often contain low amounts of DNA template. Depending on the length of time and types of conditions they were exposed, bones are also often highly degraded and/or inhibited. When DNA degrades, it breaks into increasingly smaller fragments which may lead to amplification failure of the longer STR markers (>250bp). In addition, the low amount of DNA template (<100pg) available for amplification also often negatively affects STR results due to stochastic effects, such as allele and/or locus dropout or drop-in, allele imbalance, and increased stutter. A common approach to improving STR results from low-template samples (low copy number typing) is to increase the number of PCR cycles, but this method may also result in more complicated STR profiles.

In this study, various bone and tooth samples (N=24) were harvested from cremated, embalmed, buried, and decomposed human remains to compare the effects of increasing the number of PCR amplifications from 29 to 30 using the GlobalFiler® DNA Amplification Kit. Each bone was sanded, chipped, and cleaned with a wash series of 15% bleach, water, and ethanol before being powdered in a 6700 SPEX® freezer mill. DNA was extracted from 100mg of bone powder by following the recommended bone protocols using the QIAamp® DNA Investigator Kit or the PrepFiler® BTA Forensic DNA Extraction Kit (2x 50mg powder per sample – pooled elutes). The quantity and quality of each sample was determined using the Quantifiler® Trio DNA Quantification Kit, amplified using the GlobalFiler® PCR Amplification Kit (29 or 30 cycles), and detected using the 3500 genetic analyzer.

The results of this study demonstrated that when the number of PCR cycles was increased from 29 to 30, the number of alleles detected and the peak heights increased significantly (p <0.05). Although improvement in STR results was observed in almost all samples, the most notable improvement was observed in samples with the lesser DNA template (<0.05ng); however, no notable improvement in average heterozygote peak height balance was observed. While adding another PCR cycle increased the number of alleles detected and the average peak height, an increase in PCR artifacts was also observed in STR profiles generated using 30 cycles; however, this increase was minimal as only seven drop-in alleles, six off-ladder peaks, and one event of pull-up were observed in a total of 48 amplifications.

Overall, this research has shown that regardless of the DNA extraction method used in this project, consistently more alleles were recovered from bone and tooth samples with the addition of an extra PCR cycle using the GlobalFiler® PCR Amplification Kit with minimal adverse STR artifacts.

Skeletal Samples, Short Tandem Repeats, Low Copy Number

B134 Comparative Analysis of Quantity and Overall Quality of Trace DNA Evidence Collected From Substrates Found at Crime Scenes

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After attending this presentation, attendees will be able to compare various surfaces from which DNA is recovered based on the quantity and quality of the samples.

This presentation will impact the forensic science community by providing insight for crime scene analysts regarding information of the benefits and drawbacks of the substrates from which DNA samples are retrieved at a given crime scene.

The ability to recover high-quality trace DNA samples from a potential crime scene is an important aspect of forensic science and depends on a wide range of factors, such as the characteristics of the contributor, the different types of surfaces, the environment, and the time of recovery.[1] In this study, the number of variables was limited to the types of surfaces and the time of recovery. In the present investigation, samples were prepared by depositing a trace amount of saliva on various surfaces (21 locations), followed by periodic swabbing of the surfaces using the double-swab method at timed intervals for three months.[2] Insight into differences in recoverable DNA concentration due to surface characteristics (e.g., wood flooring, ceramic tile, plastic, granite, glass, and brick) was achieved through quantification of DNA. Data from a real-time quantitative PCR (qPCR) multiplex assay determined that the ratio of the shorter CSF locus (67bp) to the longer TH01 locus (170bp-190bp) target increased over time, indicating degradation of the samples.[3] The qPCR degradation ratio varied from 3.6 to 4.7 and correlated with the percent degradation determined by the relative DNA absorbance measured at 260nm. The percent degradation of DNA was 57% (±2.6%) from samples obtained from brick (most degraded) as compared to 20% (±2.4%) from samples obtained from the plastic surface (least degraded). To assess the quality of the recovered DNA, the extracted DNA was amplified using AmpFℓSTR® Identifiler® primers, followed by capillary electrophoresis. Among all the materials tested, only the DNA obtained from the glass sample provided a complete profile after three months. These results indicate that the quality and quantity of DNA obtained from deposited saliva samples depends on the type of surface from which the sample is obtained. Porous surfaces (e.g., brick) provide lower amounts and lower quality of DNA compared to smooth surfaces (e.g., plastic, glass). The results of this study will aid crime scene analysts by providing information regarding the most desirable location to retrieve sufficient quality DNA samples at a given crime scene.

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Forensic Science, DNA, Crime Scene Investigation

B135 An Analysis of the Complex Biological Components of Touch DNA

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After attending this presentation, attendees will better understand the complex biological components of touch DNA and the ways in which this important forensic evidence can be collected and analyzed.

This presentation will impact the forensic science community by providing a description of the relationship between the basic and applied scientific aspects of touch DNA. A systematic evaluation of the components of fingerprints, as well as the optimization of their collection and analysis, could help advance touch DNA technology and ultimately provide the forensic scientist with expanded capabilities.

Touch DNA is a trace-level sample left by human physical contact with surfaces, such as countertops, weapons, or clothing, that results in the transfer of epithelial cells. With advances in knowledge and technology over the past decade, forensic scientists have been increasingly more successful in profiling the nuclear DNA found in these touch samples, providing valuable evidence in many cases. Fingerprints are a prime example of the touch sample, but they also contain other biological components, such as microbial signatures and cell-free DNA, that could play a role in individualization.

The experimental approach described here was two-pronged, as it is rooted in both the basic and applied sciences: (1) basic science — analysis of the biological components of a fingerprint using massively parallel sequencing technology and evaluation of the differences between individuals; and, (2) applied science — optimization of the collection and analysis techniques of DNA in fingerprints deposited on various substrates and development of a set of fingerprint-positive controls for co-analysis with questioned samples to increase confidence in both positive and negative results.

To attain the basic scientific goals of the project, massively parallel sequencing technology was used to analyze the cell-free DNA and the microbiome of fingerprints. Cell-free DNA was collected as the portion of nucleic acid contained in a sample supernatant. Various extraction techniques were evaluated, including protocols involving carrier DNA and sample concentration. Metagenomic analysis of the microorganisms in fingerprints was accomplished after careful optimization of DNA extraction techniques designed to minimize bias introduced in the sample by factors such as incomplete homogenization of the sample matrix or insufficient and incomplete cell lysis. Bioinformatic analysis of sequence data provided an estimate of variance between samples and identified sequences that may be common between individuals. Further work will confirm the results.

To meet the applied science objectives of the project, a "fingerprint solution" was developed by the proportional combination of the major chemical components of an eccrine fingerprint. Buccal epithelial cells were collected in suspension, treated to reduce clumping, and counted using a hemocytometer. This number was equated to the DNA content of a certain volume of the suspension; therefore, a known quantity of DNA could be added to the fingerprint solution. The mixture was deposited on a surface and collected in parallel with true fingerprints, thus acting as a known positive control with the chemical characteristics of the fingerprint. The procedure has been tested and optimized for a number of surfaces and collection devices. The results of both the basic and applied experiments will be presented and discussed in the context of operational forensic science.

Touch DNA, Microbiome, Cell-Free DNA

B136 The Evaluation of a Hand-Held Raman Spectrometer for Field Identification of Controlled Substances

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After attending this presentation, attendees will understand the advantages of using a hand-held Raman spectrometer over colorimetric field tests for suspected controlled substances. Attendees will also be aware of the types of samples that are suitable for Raman spectroscopy identification.

This presentation will impact the forensic science community by presenting an evaluation of an alternative method of preliminary identification of unknown substances. This method offers a safe, non-contact method of field analysis for law enforcement officers.

The PGR-1064™ Raman Spectrometer was evaluated for its suitability as a field test for suspected controlled substances. Its suitability was determined using controlled substance identification data as well as user observations about the functionality of the spectrometer. There are many advantages of Raman spectroscopy over commonly used colorimetric field tests. With Raman, there are no harsh chemicals or solvents, there is no need to open the packaging, and the interpretation of the results is not subjective.

Two identical PGR-1064™ Raman Spectrometers were used in this study. Blind triplicate scans of 135 samples suspected to contain controlled substances were taken with each Raman spectrometer and searched against two of its library databases. Each sample was then analyzed using Fourier Transform Infrared (FTIR) with an Attenuated Total Reflectance (ATR) attachment; IR spectroscopy is complementary to Raman spectroscopy. Once each sample was preliminarily identified using both Raman and FTIR, the results were compared to the true identity of the substance from Gas Chromatography/Mass Spectrometry (GC/MS) analysis.

Twenty-one samples had at least one compound that was not in the current library of the Raman spectrometer. Analysis of the 14 samples of plant material was not successful; the material was too dark and charred when it came into contact with the laser. Excluding the new compounds and plant material, the Raman library correctly identified the sample or part of the mixture in at least one of the scans 79% of the time. Separating results by compound, cocaine was identified 74% of the time (203/274 scans) and methamphetamine was identified 83% of the time (121/146 scans).

The Raman spectrometers evaluated in this study are suitable for field use for preliminary identification of suspected controlled substances. This technology would be most effective at identifying suspected cocaine and methamphetamine.

Field Test, Hand-Held Raman Spectroscopy, Controlled Substances

B137 The Evaluation of Portable Hand-Held Raman Systems for the Presumptive Identification of Narcotics

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After attending this presentation, attendees will understand the advantages and limitations of portable Raman spectroscopy and its potential as a supplemental approach for the field testing of narcotics.

This presentation will impact the forensic science community by demonstrating the potential applicability of using portable hand-held Raman spectroscopy systems to detect illegal substances quickly and accurately using a single test, either within a laboratory system or as a field test by law enforcement.

Historically, presumptive testing for narcotics has involved colorimetric tests, otherwise known as spot tests. These tests are fast, sensitive, and can categorize a suspected illegal substance to a particular class of drugs; however, the interpretation of the color change is subjective and false positives and negatives are possible. Hand-held Raman devices have been developed for forensic application to eliminate the need for colorimetric testing. These user-friendly systems offer a non-destructive means to detect potential controlled substances, precursors, and cutting agents quickly and accurately either within a laboratory system or as a field test by law enforcement.

The goal of this research was to evaluate two portable hand-held Raman systems to determine their ability to accurately analyze narcotic samples. The Thermo Scientific TruNarc® and Chemring Detection Systems PGR-1064® were used to test more than 100 case samples by colorimetric and Gas Chromatography/Mass Spectrometry (GC/MS) analysis in the Palm Beach County Sheriff's Office Chemistry Unit. Case samples, which included opiates, stimulants, hallucinogens, and pharmaceutical tablets, were scanned in triplicate on three consecutive days in order to determine reproducibility. Results of the Raman scans were compared to the laboratory results. The TruNarc® successfully detected the target drug in 77% of the case samples and generated reproducible results in 84% of the case samples when the results were compared to the rescans on days two and three. An added benefit to the TruNarc® system is the Type H kit, which utilizes Surface Enhanced Raman Spectroscopy (SERS) to increase Raman scattering and fluorescence quenching, allowing drugs in low concentration or those with high fluorescence to be detected successfully. The PGR-1064® successfully detected the target drug in 36% of the case samples and generated reproducible results in 60% of the case samples when the results were compared to the rescans on days two and three.

These Raman detection systems exhibited the potential to provide accurate and reproducible results for single-component samples through certified reference standards; however, there are intrinsic challenges to the technology of Raman spectroscopy when dealing with mixtures. Case sample's homogeneity was unpredictable when adulterants, diluents, and other components were found within the samples. As a result, the laser was likely not always focused on the target drug within a sample. Additionally, limited sample quantities resulted in inconclusive or unidentified results. The ability to detect forensic narcotic samples likely depends on sample purity, amount, and where on the sample the laser is focused.

The data presented suggest that hand-held Raman systems have the potential to detect substances of abuse depending on the specific sample, although further evaluation is necessary for implementation within a laboratory and as a field test.

The opinions, findings, conclusions, and recommendations stated in this presentation are those of the authors and do not necessarily reflect the vendors or the Palm Beach County Sheriff's Office.

Presumptive Testing, TruNarc®, PGR-1064

B138 Seized-Drug Mass Spectral Libraries: Data Quality Control Measures

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After attending this presentation, attendees will better understand spectrum self-consistency as well as consensus comparison among several spectral library entries as a means for mass spectral library quality control.

This presentation will impact the forensic science community by providing greater understanding, and thus greater confidence, as to how curated mass spectral libraries are created and maintained.

Spectrum quality control measures suitable for forensic drug analysis have been developed. The mass spectral library maintained by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) currently includes close to 2,300 spectra (version 04-01-2015). The National Institute of Standards and Technology (NIST) Mass Spectrometry Data Center has reviewed each SWGDRUG spectrum and brought the level of curation up to the standards of the NIST library. Having high-quality, controlled-substance libraries is critical to the criminal justice system.

Each spectrum in the SWGDRUG library was compared via standard library search methods to spectra of the same compound found in any of five mass spectral libraries. These libraries were: the NIST/Environmental Protection Agency (EPA)/National Institutes of Health (NIH) library (240,000 compounds), the Wiley Designer Drug® library (16,343 compounds), the Wiley Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites® (8,650 compounds), the Cayman Chemical Company library (748 compounds), and the Adams Essential Oil Components library (2,200 compounds). Confirmation of a good-quality SWGDRUG spectrum was contingent primarily on matching with a match factor of 750 or better (out of 999) any and all spectra in the five reference libraries. Outlier spectra in any library were flagged for further scrutiny using NIST's Mass Spectral (MS) Interpreter program.

Although the number of SWGDRUG spectra that were found to contain errors is small (on the order of fewer than 1%), the variety of errors found was wide. In essence, each error found was unique to that entry. Systematic, library-wide errors were not found.

Spectral errors are generally of three types: (1) missing peaks; (2) spurious peaks not attributable to the compound structure; and, (3) incorrect mass assignments (often from incorrect rounding of mass values). In addition, there are a variety of compound identification errors. These include: (1) incorrect chemical structure; (2) incorrect chemical name; (3) incorrect Chemical Abstracts Service (CAS) number or other identifier; and, (4) incorrect isomer identification. In a few cases, a compound exists only in the SWGDRUG library, so comparison was not possible. These were flagged for measurement by NIST for addition to the NIST library.

The use of NIST's MS Interpreter program was instrumental for adjudicating cases in which only a few measurements were available. MS Interpreter assigns peaks to plausible molecular substructures based on a set of rules for estimation of fragmentation energetics using a chemical structure (connection table) type analysis. MS Interpreter is freely available at chemdata.nist.gov/mass-spc/interpreter/.

Of the 2283 SWGDRUG spectra examined, 605 were not in the NIST library, 89 had only one measurement on record in any library, 10 had only poor quality spectra available, and 12 had incompatible spectra in two or more libraries. Additionally, 173 NIST/EPA/NIH entries required CAS number updates, 14 had incorrect chemical structures, and 4 had incorrect compound names.

The compounds not in the NIST library or of poor quality will be measured and added, if certified samples can be found. This should be completed in time for next year's library release, NIST17, available Spring 2017.

Mass Spectral Database, Consensus Comparison, Quality Control

B139 Evaluation of a New Technology for the Collection and Analysis of Breath Components for Marijuana Detection Using Capillary Microextraction of Volatiles (CMV)

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After attending this presentation, attendees will be more familiar with the new CMV technology.

This presentation will impact the forensic science community by providing insight into a hopeful new technology for the non-invasive collection and detection of drugs (i.e., marijuana) in the breath of potentially intoxicated motorists.

Increased legalization of medical and recreational marijuana usage in the United States has led to an urgent need for forensic field tests for the detection and confirmation of drugged driving. Marijuana use has been found to have a detrimental effect on an individual's driving abilities, causing an increase in public concerns for road safety. The non-invasive approach of breath collection has made exhaled breath an attractive sample to collect for field detection of drugs. Breath analysis for marijuana detection, although potentially advantageous, has been limited by the available breath collection devices and analysis techniques. The endogenous compounds of breath, which naturally form in the body, make up the complex background of normal breath, which may interfere with analyses developed for the determination of compounds in the breath associated with the smoking of marijuana. Previous studies characterizing the breath of individuals who have smoked marijuana used filters as sample collection devices resulting in low collection efficiencies.

This study proposes the evaluation of a new technology, CMV, for its suitability for the collection of breath aerosols and volatiles in exhaled breath analysis. The CMV preconcentrates breath components using a mini capillary tube filled with Polydimethylsiloxane (PDMS) -coated glass filter strips. The CMV offers dynamic sampling of Volatile Organic Compounds (VOCs) with a simple coupling to the inlet of a Gas Chromatograph (GC) for analysis, avoiding expensive thermal desorption instrumentation needed for bulk sorbent-type collection devices. CMV offers a 5,000-fold increase in surface area and an improved collection capacity over the static, single Solid-Phase Microextraction (SPME) fiber based on the same preconcentration fundamentals. The collection efficiency and analysis of commonly known volatiles associated with normal breath and marijuana smoking were studied using a simulation of synthetic breath composed of vapors generated by permeation into a flow of humidified nitrogen. After collection of the synthetic breath onto the CMV, two extraction methods were tested for efficacy in releasing analytes for analysis. The comparison of the direct thermal desorption and online supercritical Carbon dioxide (CO_2) extraction of a CMV into a GC/Mass Spectrometer (GC/MS) inlet was used to determine the recovery profiles of the two recovery approaches. The chemical characterization of these breath components would provide a foundation and better understanding of breath collection with CMV. This understanding permits the differentiation between normal breath constituents and the exogenous compounds resulting from the smoking of marijuana. Reliable demonstration of the CMV for breath collection would serve as a proof of concept for future applications of the CMV for detection of marijuana smokers' breath for drug-impaired driver management. The portability and sensitivity of the CMV could aid law enforcement agencies in the future during traffic patrols of drug-impaired drivers.

Breath Analysis, Breath Collection Device, Drug Detection

B140 High-Throughput Analysis of Controlled Substances: Combining Multiple Injections in a Single Experimental Run (MISER) and Liquid Chromatography/Mass Spectrometry (LC/MS)

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After attending this presentation, attendees will understand how the application of MISER to LC/MS can provide an efficient, high-throughput method of analysis for seized drug submissions.

This presentation will impact the forensic science community by demonstrating the efficiency, simplicity, and versatility of MISER LC/MS analysis of controlled substances, especially when applied to multiple-unit and/or multiple-component submissions.

Forensic drug laboratory personnel are frequently faced with the challenge of efficiently managing cases and evidence backlogs while also performing high-quality analyses that will fulfill jurisdictional and other requirements. Conclusions of analysis must be scientifically supported by the use of reliable and robust analytical techniques. Furthermore, analytical results need to be generated in an efficient and timely manner. The ability to perform high-throughput analysis is therefore a crucial need in today's forensic laboratories. This is especially true for state, local, and federal laboratories that routinely receive multiple-unit exhibits, as jurisdictional requirements often involve the testing of a large number of individual units in order to make an inference on the entire seizure, or on a high proportion of it.

MISER is an ideal technique for the rapid analysis of multiple-unit drug exhibits. It is a variation of flow-injection analysis in which samples are analyzed by direct injection into an eluent flow provided by a liquid chromatograph. In combination with an autosampler, samples can be continuously injected, allowing the rapid evaluation of their contents. The collection of results from multiple samples within a single chromatogram (misergram) also allows simple evaluation of data. Contrary to routine Gas Chromatography (GC) or LC applications, the proportion of strong solvent employed during MISER analysis should be sufficiently high to ensure only minimal interaction with the stationary phase, thus accelerating the passing of the analyte(s) through the column. Although sample components are not separated on the basis of retention time, this apparent limitation in selectivity is easily remedied by the use of an MS detector.

Included in this presentation are the results of several experiments in which the MISER technique was utilized in combination with LC/MS in the analysis of controlled substances. In one experiment, the analysis of 28 randomly selected units from a large cocaine submission was completed in approximately 36 minutes, demonstrating the time-saving advantages MISER offers. Isocratic solvent flow conditions were optimized for both rapid elution and minimal separation of the primary constituents present in the samples, and the samples were able to be injected every (approximately) 1.2 minutes.

Another drug submission consisting of oxycodone tablets was analyzed using similar solvent flow conditions, but with the mass analyzer programmed to perform three types of experiments on each sample: (1) full mass analysis (m/z 50-500); (2) then Tandem Mass Spectrometry (MS/MS) on the most intense ion detected during the full mass analysis scan; and, (3) followed by Triple Mass Spectrometry (MS/MS/MS) analysis on the most intense fragment ion detected during the MS/MS. This experiment sequence provided additional fragmentation information for the oxycodone present in the tablets, since the MS/MS analysis resulted in too few ions for unambiguous characterization.

In addition to multi-unit drug submissions, MISER LC/MS is well-suited for multiple-component mixtures, as demonstrated by the analyses of opium samples. Solvent conditions in one example were shown to produce slight separation of the major constituents while maintaining rapid elution; however, a much simpler misergram was produced when the eluent flow contained a higher proportion of organic solvent such that all sample components co-eluted. Furthermore, co-elution of multiple components did not preclude the ability of the mass analyzer to provide structural information on each individual substance present in the samples.

This presentation will benefit the forensic science community by offering a versatile and highly efficient screening method for multiple-unit and multiple-component seized drug submissions.

Controlled Substances, MISER, LC/MS

B141 Using Solid Phase Extraction (SPE) to Reduce Interference in Gas Chromatography/ Mass Spectrometry (GC/MS) Analysis of Fire Debris Samples

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After attending this presentation, attendees will better understand how SPE with GC/MS increases the chances of determining whether a hydrocarbon accelerant is present, then correctly identifying the accelerant used, even in the presence of matrix interferences.

This presentation will impact the forensic science community by describing another way to improve the identification of fire debris samples that otherwise would have been unidentifiable due to matrix interferences.

Interferences can be broken down into three types: (1) substrate products; (2) pyrolysis products; and, (3) combustion products.[1] Their presence makes identification of the accelerant components challenging, thus hindering the interpretation of arson samples. To reduce the interference and make it easier for identification, a simple method of extraction with SPE cartridges can be used to purify the samples. Silica and amino propyl cartridges were evaluated as they remove compounds with which hydrogen bonds, but they do not have π bond interactions, such as aromatics. Accelerants containing hydrocarbons were used in this study.

There were two phases to the research. The first phase was to document the degree to which SPE affects the components of ten neat accelerants of different classes. The neat accelerant samples were collected using passive headspace with activated charcoal strips to adsorb the compounds.[2] The cans were heated overnight in an oven. Approximately 1mL of Carbon Disulfide (CS_2) was used to extract the compounds from the strips in amber vials. Three activated charcoal strips were used per sample during the heating stage of research. One strip went through a Silicon Dioxide (SiO_2) cartridge, another through an amino (NH_2) cartridge, and the third strip remained as is. Each accelerant was sampled in triplicate. All extracts were analyzed by GC/MS.

The chromatograms from the neat, silica cartridge and amino propyl cartridge were compared for each accelerant by first isolating the six most prominent peaks. The compounds were then identified using the reference library from the GC/MS and verified from the National Center for Forensic Science (NCFS).[3] The areas for these six prominent peaks were normalized for each sample, then the mean normalized area was calculated and standard deviation was calculated for the triplicate samples. Finally, the upper and lower confidence limits were calculated for the differences in the mean normalized areas of the neat versus the mean normalized areas for each of the sample sets run on the different SPE media. In all of the tested accelerants, there were no statistical differences in the normalized means of the six most prominent peaks.

Phase two of the research focused on testing the effectiveness against interferences. Various interfering matrices were placed in the cans, then burned until a smoldering smoke was observed. At this point, the cans were spiked with an accelerant mix consisting of a 1:1:2 volume/volume (v/v) gasoline, kerosene, and diesel and sealed with three charcoal strips inside. The strips were extracted as before and one strip was analyzed with no clean-up, while the other strips were cleaned via SPE, one strip using the silica cartridge and the other using the amino-propyl cartridge. After the extracts were analyzed by GC/MS, the prominent interference peaks in the samples were normalized against the largest accelerant peak and decrease in the normalized intensity was documented.

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Arson, GC/MS, Solid Phase Extraction

B142 Developing Quantitative Measurements and Test Materials for Fingerprint Development Reagents Using Inkjet Printing

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After attending this presentation, attendees will: (1) understand the motivation and potential benefits of using standardized and quantifiable test materials to evaluate the stability and efficacy of their fingerprint development reagents; (2) understand what has taken place to create these materials and evaluate their stability and lifetime; and, (3) possess insight into potential issues that can arise with long-term storage of their reagents.

This presentation will impact the forensic science community by presenting a method through which examiners can reliably and reproducibly monitor the efficacy of their fingerprint developers using standard test materials, thus minimizing issues arising from using one's own fingerprint.

While the use of fingerprint development reagents is universal throughout forensic science laboratories, the methods for ensuring the reagents are working properly are not. Numerous methods exist, though the most common is to develop one's own fingerprint to see if the reagents are working properly. Due to the inherent variability in fingerprint composition, this practice does not provide a means for reproducible evaluation of the reagents over a period of time. In order to truly understand the efficacy, quality, stability, and lifetime trends of the developing reagents, a quantitative method of evaluation needs to be developed.

The work presented here highlights the development of quantifiable fingerprint reagent test materials created using high precision inkjet printing. Using this technology, it is possible to create highly reproducible (~1% Relative Standard Deviation (RSD)) deposits of chemicals to test the reagents. The test materials contain an array of arrays with increasing surface concentrations of an analyte(s) of interest. By providing a range of surface concentrations, it is possible to obtain a better understanding of the efficacy of the reagent beyond a single-point red light/green light approach. The original work began with the creation of amino acid-containing test materials to quantifiably evaluate reagents such as ninhydrin and 1,2-indanedione on porous surfaces. With the high precision of inkjet printing, it is not only possible to evaluate the efficacy of these reagents but also to establish limits of detection for the reaction to be observed.

This presentation will focus on the development of these test materials, which includes establishing appropriate test levels, evaluating the lifetime of the materials under different environmental conditions, identifying necessary storage considerations, and obtaining feedback from forensic laboratories throughout the country. This presentation will also discuss methods that have been developed to quantitatively evaluate the initial amount of material deposited to create the materials, the reactions, and potential solvent bleed of various developer reagents. In addition to the creation of amino acid standards, standards for the reagents which react with sebaceous components, cyanoacrylate, and non-porous surfaces will also be discussed. Advantages of this type of test material and potential issues with the use of one's own fingerprint as a check method will also be presented.

Latent Fingerprints, Developer Reagents, Inkjet Printing

B143 Investigating the Utility of Automated Flash Chromatography in Forensic Drug Analysis

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After attending this presentation, attendees will understand how automated flash chromatography systems can be utilized in forensic drug analysis. Such systems are capable of isolating and purifying components of a liquid mixture, much like a modern High-Performance Liquid Chromatograph (HPLC); however, unlike HPLC, flash chromatography systems are capable of collecting the isolated components for further analysis.

This presentation will impact the forensic science community by demonstrating a means to isolate and collect components of a liquid mixture that require additional analysis in order to attain an identification. This becomes especially important when encountering emerging synthetic drugs before reference material is available.

Gas chromatography/mass spectrometry is the gold standard for analyte identification in most forensic drug analysis laboratories; however, when new synthetic drugs are detected, more information may be needed to make an identification. Ideally, Nuclear Magnetic Resonance (NMR) spectroscopy would be used to gain insight into the molecular structure of the unknown compound. Unfortunately, NMR analysis requires fairly pure samples to avoid complicating the spectra with signals from multiple compounds. Therefore, the compound of interest must be isolated and purified before it can be analyzed via NMR spectroscopy. Several isolation and purification techniques, such as liquid-liquid extraction, could be attempted to meet this requirement; however, automated flash chromatography was explored as it provides a means to isolate compounds of interest when little-to-no chemical information is known.

Because synthetic drug standards are expensive and are only sold in small quantities, known mixtures of common controlled and non-controlled substances were used for this study as a proof of concept. Various method parameters, including different columns and mobile phases, were tested. Polar silica columns and non-polar C18 columns were both tested. Water, methanol, acetonitrile, hexane, ethyl acetate, and isopropanol were tested to determine the optimal mobile phase for the separation. Utilizing automated flash chromatography equipped with an Ultraviolet (UV) detector, cocaine was successfully isolated and collected from a mixture containing equal parts lidocaine and benzocaine. For this specific mixture, a silica column with a methanol mobile phase was found to provide the best separation when the cocaine was in freebase form. To successfully isolate the cocaine, a liquid-liquid extraction containing a 1:1 ratio of sodium bicarbonate and chloroform was first performed to convert any salts to freebase. The sample mixture was then run on the automated flash chromatography system. Results showed clear separation of cocaine from lidocaine and benzocaine. Further analysis of the purified cocaine was successfully performed via NMR spectroscopy.

The same process used to develop the method used for this study could be employed for unknown compounds, such as synthetic cannabinoids. Because so many parameters can be altered which affect the separation, it is suggested that quick tests using Thin-Layer Chromatography (TLC) be used prior to attempting a full-scale separation on an automated system. Once optimal method parameters are determined, automated flash chromatography systems provide a simple technique for the successful isolation and collection of analytes requiring further analysis for identification, making it a useful tool in forensic drug analysis.

Drug Analysis, Flash Chromatography, Synthetic Drugs

B144 The Use of Near-Infrared Spectroscopy (NIRS) and Chemometrics in Evaluating Growth Periods of Cannabis Seeds Seized and Cultivated in a Greenhouse

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After attending this presentation, attendees will be able to assess the classification of cannabis seeds seized and cultivated in controlled conditions during their different growth stages.

This presentation will impact the forensic science community by providing a new and effective classification of cannabis plants by age and, consequently, by providing a fast and reliable solution to gathering information about indoor cultivation time, establishing a connection between the cultivation site, the trafficked seeds, and the route of cannabis trafficking.

In recent years, cannabis drug trafficking has been changing in Brazil. An exponential increase in the seizure of cannabis seeds sent by mail has been observed by the Brazilian Federal Police (BFP). This change in the cannabis trafficking scenario may be the result of BFP efforts to eradicate the cultivation of the plant in large-scale farms and the change of the outdoor setting for indoor cultivation. This same tendency is also observed in Europe.

Even though a simple chemical analysis of the seized drug is sufficient to identify cannabis as a drug for legal purposes, classification by age may be an important tool in forensic intelligence (e.g., to gather information regarding indoor cultivation time, establish a connection between the cultivation site and the trafficked seeds and route trafficking); however, the high intrinsic variability present in cannabis samples (brands, varieties, chemotypes and gender) makes it difficult to obtain a classification standard. Therefore, this study sought to develop a method for classifying cannabis germinated in a homemade greenhouse according to the growth period, using Near-Infrared Spectroscopy (NIRS) and chemometrics.

Twenty-nine seeds seized by BFP were cultivated in a homemade greenhouse, in three different growth periods and controlled conditions. The harvest was performed by removing the entire plant from the soil; the loss on drying was estimated by gravimetry until the sixth day post-harvest. The leaves, stems and inflorescence were ground in an agate mortar and directly analyzed by NIRS. The influence of the sample homogeneity on the classification was evaluated using triplicates, and at random.

The NIR spectra were obtained using a PerkinElmer® 400 IR spectrometer equipped with integrating sphere and Indium Gallium-Arsenic (InGaAs) detector. The spectra were measured with resolution of 4cm^{-1} in the range between 10,000 and 4,000 cm^{-1}. A total of 32 scans were performed for each sample.

Principal Component Analysis by intervals (*i*PCA) was performed to assess the spectral region. This provided the best separation between the groups. The PCA, Hierarchical Cluster Analysis (HCA), Partial Least Squares Discriminant Analysis (PLSDA) and Support Vector Machines Discriminant Analysis (SVMDA) were performed and the samples (n=87) were classified by age (5.5 weeks, 7.5 weeks, and 10 weeks). The software MATLAB with PLS_toolbox and iToolbox (http://www.models.kvl.dk) were used.

An absorption band above 8,000 cm^{-1} was observed, with only the absorption band associated with the third overtone of CH_3, CH_2 and CH being approximately 8,500 cm^{-1}. The absorption bands associated with the second and first overtone of CH_3, CH_2, and CH were observed in the area of 6,900 and 5,800 cm^{-1}. In the spectral region between 5,000 and 4,000 cm^{-1}, there was information associated with the combinations of CH_3, CH_2, CH, C-C, and CHO, among others.

The *i*PCA in the raw spectra demonstrated the best separation between the samples by age, identified in the spectral region 4,000-4,375 cm^{-1}, associated with CH_3, CH_2, CH, C-C, and CHO combinations. Both discrimination analysis algorithms were carried out in this interval. The PCA and HCA exhibited good separation between the three groups of cannabis with different growth periods. The PLSDA and SVMDA classified the samples with good

results in terms of sensitivity and specificity. The sensitivity and specificity for SVMDA classification were equal to unity.

These results revealed that, in the early stages of the cultivation of indoor cannabis, the age of the plant can be predicted by NIRS and chemometric tools; however, a larger study may be needed to confirm this observation due to the low number of samples obtained.

Cannabis, Near-Infrared Spectroscopy, Chemometrics

B145 Forensic Intelligence in Illicit Markets: The Contribution of Chemical Analysis on Counterfeit Watches

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After attending this presentation, attendees will understand the mechanism of intelligence gathering through the chemical analysis of counterfeit goods. The added value that such information has on understanding the functioning of illicit markets will also be demonstrated.

This presentation will impact the forensic science community by highlighting the scope of the contribution that forensic science can provide from a security perspective, beyond the general emphasis that is placed on forensic science's role in criminal courts.

The practice of forensic science relies fundamentally on the study of trace evidence as remnants of a criminal activity and on the extraction and contextualization of the information this conveys. Counterfeit watches, as products of an illicit activity, hold trace details of their production and/or distribution that can provide information about the structure of illegal trafficking. Fundamentally, by the ability to reveal links between specimens or cases, trace evidence provides pertinent information to investigative activities. When combined with other types of intelligence gathered through spatiotemporal analysis of seizures or monitoring of internet sites selling counterfeit goods, trace evidence offers a powerful tool to decipher the structure of illicit markets.

The contribution of forensic intelligence in gathering information regarding illicit markets through the example of chemical analysis performed on seized counterfeit watches will be illustrated. By considering three types of chemical analyses — the profiling of perfumed plastic straps, the composition of leather and plastic straps, and the metal composition of watchcases — the challenges that characterize the extraction of intelligence from counterfeit watches will be illustrated.

The first challenge, data production, pertains to establishing an analytical strategy that provides representative and informative data on the composition of the different watch parts. Headspace/Solid-Phase Microextraction followed by Gas Chromatography/Mass Spectrometry (HS/SPME-GC/MS) was used for the extraction of volatile compounds from the watch straps, while X-ray spectroscopy and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) after acid dissolution were used to obtain the elementary composition of watchcases.

The second challenge, data treatment and organization, consists of creating reliable and informative profiles from the analytical results. Subsequently, the profiles for each of the counterfeit watches were compared and grouped by means of similarity indexes, providing intelligence about possible links between specimens.

The integration of this information with other types of intelligence, known as contextualization, is the third challenge. Contextualization revealed forensic science's contribution to the information regarding the illicit counterfeit watches market. Information gained through chemical analysis corroborated existing information from other sources and also revealed new links between seizures and specimens.

This presentation exemplifies a more general approach of forensic intelligence for concealed markets. The extraction and exploitation of information from an illicitly produced item to gain information about its production is a generic mechanism that is already widely used. For instance, the chemical profiling of illicit drugs has already been extended by analogy to other types or counterfeit goods (medicines, cigarettes, etc.) or illegal activities (forged documents, doping agents, etc.).

Illicit Markets, Chemical Analysis, Counterfeit Watches

B146 An Analysis of Ignitable Liquids Using Portable Ion-Trap Gas Chromatography/ Mass Spectrometry (GC/MS) With Solid-Phase Microextraction (SPME) Sampling

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After attending this presentation, attendees will better understand the impact samples, sampling time, and sampling conditions have on data that was collected at fire scenes and analyzed using portable GC/MS with SPME sample introduction.

This presentation will impact the forensic science community by informing attendees how portable GC/MS may be applied to fire investigations, as well as by demonstrating the advantages and limitations of performing this method.

The analysis of Ignitable Liquid Residues (ILRs) from a fire scene is performed to provide investigative leads, as well as to determine whether or not the crime of arson was committed. Typically, samples that potentially contain ILRs at a fire scene are identified, collected, packaged and transported to the laboratory for GC/MS analysis. Determination of the presence of an ILR is achieved by the evaluation of GC patterns and by the conclusive identification of individual components present in the sample using both GC and MS data. There are many challenges to the successful performance of this type of analysis. Proper sample identification, collection, packaging, and transport are critical. If these tasks are not performed correctly, results of analytical testing may be meaningless or even misleading. In addition, laboratory backlog may delay analytical turnaround time. As a result, data may not be representative of the sample collected. This is because the longer these volatile and semi-volatile samples are stored, the more likely they are to change their chemical composition and, therefore, their chromatographic profile. This can significantly complicate the interpretation of the analytical data from the collected sample.

For these reasons, it may be desirable to take the analytical laboratory to the field and perform GC/MS during the fire scene investigation as the quality of the sample is at its greatest at the scene. Results are characteristic of the sample collected before storage and time have had a chance to alter its chemical composition. In addition, when analysis is performed at the scene, investigative leads are available in near-real time. Also, the use of portable GC/MS will prevent the submission of poorly collected samples to the laboratory for analysis. Portable GC/MS can be used to establish the presence of an ILR at the scene, preventing the submission of negative samples to the laboratory.

There are a number of different portable GC/MS systems available to perform field analysis. These systems vary based on the type of GC column and mass analyzer used, as well as how the sample is collected and introduced to the system. These factors can play a critical role in the chromatogram collected and, therefore, impact data interpretation. It is important that factors such as sampling conditions, as well as degree of sample weathering, impact the chromatogram generated before a method is applied to this type of evidence.

For this research, a portable ion-trap GC/MS with an SPME sampling accessory was used to analyze ILs representative of a range of different classes of these compounds. The portable GC-MS system used weighs less than 15kg and is designed for use in the field. Sampling was performed using SPME. Ignitable liquids tested included lighter ILs such as gasoline and paint thinner, as well as heavier ILs such as diesel and kerosene. Testing was performed to determine the impact sampling time and degree of sampling weathering had upon the resulting chromatogram. This is critically important because an understanding of these effects is necessary if proper interpretation of GC/MS data is to be achieved.

Results revealed that the chromatograms recovered from an IL are dependent upon many factors, including degree of aging of the sample, the type of IL analyzed, and other sampling conditions. A summary of the data will be presented so an understanding of how these factors impact the data may be understood. A discussion of the analytical advantages and disadvantages to the method will also be included. Attendees will understand the variability they should expect to see in the data should this method be applied in the field to samples collected during a fire scene investigation.

B147 The Utilization of Controlled Odor Mimic Devices to Improve Law Enforcement Canine Training

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After attending this presentation, attendees will understand the advantages and shortcomings of odor detection canine training, why the canine's generalization-discrimination balance must be enhanced, and how odor recognition validation tests are conducted during canine trials.

This presentation will impact the forensic science community by demonstrating the canine's ability to become proficient in discriminating between target odors vs. similar odors and detecting mixtures of odorants. This will minimize both false positives and false negatives.

Canines are well known for their ability to detect and distinguish between large varieties of odors. Despite their discriminating power, previous studies have revealed some canine's deficiencies in proficiently detecting mixtures and generalizing molecularly similar compounds with varying vapor pressures and volatilities. In collaboration with the Naval Research Laboratory (NRL) and Naval Surface Warfare Center (NSWC), Florida International University (FIU) explored the capability of improving canine performance in detecting these odors using an odor delivery device. Field trials were then designed in order to demonstrate that the canines are capable of being trained to effectively discriminate between similar compounds.

In order to accomplish these tasks, it was necessary to first be able to deliver the target odors to canines. Known odor concentrations were released by utilizing Controlled Odor Mimic Permeation Systems (COMPS), a patent-pending device developed by FIU scientists. Utilizing COMPS ensured consistent and measurable delivery of a wide variety of target odor compounds, allowing for reduction in detection bias that may otherwise result from differences in odor vapor pressure and volatility that are characteristic of various odor compounds. The concentrations of the target odors were measured using gravimetric and headspace analysis. A variety of polymer bag densities and pore sizes were used to control dissipation rates of these compounds in the COMPS devices. The effect of exposure to temperatures and humidity on COMPS was also examined and the data gathered was utilized to build a variety of COMPS devices that were then used during canine training.

By combining COMPS with traditional canine field trials such as double-blind Odor Recognition Tests (ORTs), canines were able to be graded on their ability to recognize and distinguish between molecularly similar compounds in a controlled setting. The positive and negative canine alerts were statistically compared using the Positive Predictive Value (PPV). The results of using these COMPS will be discussed, as well as their ability to provide in-depth training of law enforcement canines to an ever-wider variety of compounds.

Advances in the use of odor detection canines are necessary for the enhancement of this field's scientific validity. The versatility, reproducibility, and low cost of this technology have the potential to revolutionize canine training and minimize its deficiencies. In conclusion, results from this study validate that utilizing COMPS is a safe and effective training tool for law enforcement canines.

Canine Training, COMPS, PPV

B148 An Investigation of Factors Affecting the Formation of 3D Fabric Imprint Patterns in Automotive Finishes

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After attending this presentation, attendees will better understand the variables involved in the formation and quality of fabric imprint patterns in automotive finishes during vehicle-pedestrian impacts.

This presentation will impact the forensic science community by providing the means to establish an alternate evidentiary link between a vehicle and pedestrian in the absence of or in conjunction with tissue or paint transfer. This evidence may also provide additional information for the purpose of reconstructing vehicle-pedestrian impacts.

In vehicle-pedestrian impacts with sufficient force, imprint patterns from clothing fabric may be formed on vehicle bumpers and in automotive finishes. Even at low speeds, hit-and-run vehicle-pedestrian impacts are far too common.[1-4] A crime laboratory's focus in the event of a hit-and-run is currently on the individualization of paint left behind by the vehicle, either on the pedestrian or in the surrounding area.[5-6] In the absence of paint evidence, or of hair and tissue on the vehicle, it is often difficult to prove an evidentiary link between the vehicle and the victim.

The goal of this study is the elucidation of pattern production mechanics — type of fabric, surface coating, angle of impact, and impact force of the vehicle—and the eventual individualization of imprint patterns to specific fabrics. Rather than crashing full-sized vehicles, this preliminary study currently utilizes a standard six-foot pendulum. A dome-shaped weld cap is mounted to the front of the pendulum arm and covered with a layer of foam, then the fabric of choice, simulating a human kneecap. The pendulum impacts a section of either a door or fender, cut to an approximate 23cm x 23cm square and mounted in a custom-built frame using C-clamps. To vary the impact force, the pendulum arm is raised or lowered by five-inch increments.

In keeping both surface coating and fabric type consistent, and performing impacts normal to the car surfaces, preliminary results do show the consistent, repeatable formation of fabric imprint patterns, provided a narrow range of pendulum heights is reached or exceeded. Precise impact force can be calculated through the analysis of high-speed video, which is currently being assembled and optimized and will be utilized in further tests repeating the above procedure. This will allow for the determination of impact force and the study of pattern variability with changing fabric type, surface coating, or angle of impact.

Initial examinations of imprint patterns have been performed with Digital Single-Lens Reflex (DSLR) photography and optical microscopy with oblique lighting. In the future, micro-level terrain mapping may be able to provide individualizing characteristics. As little research in this area has been published, this study will be supported by a survey of industry professionals. This survey is currently undergoing Institutional Review Board (IRB) evaluation prior to distribution.

Once these variables — impact force, fabric type, surface coating, and angle of impact — are fully examined on a small scale, a continuation of this study will seek to analyze a more true-to-life impact, using mannequins, an 80-foot pendulum, and full-sized vehicles.

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Forensic Science, Fabric Impressions, Hit and Run

B149 The Characterization and Rapid Detection of Synthetic Cannabimimetic Materials Using Ion Mobility Spectrometry (IMS) for In-Field Forensic Investigations

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After attending this presentation, attendees will be able to evaluate the efficiency of a rapid and portable technique, IMS, for the identification of individual compounds and mixtures of Synthetic Cannabinoids (SCBs) for use in forensic field investigations.

This presentation will impact the forensic science community by providing detection parameters necessary to create and program a spectral library of SCBs into a commercially available IMS. As a result, this research may facilitate law enforcement's efforts to analyze suspected SCBs in the field and reduce the backlog of screening examinations of drug submissions in the laboratory.

The abuse of synthetic cannabimimetic or SCBs, which mimic the psychoactive effects of Δ^9-Tetrahydrocannabinol (THC) found in marijuana, has increased since their initial discovery. The marijuana-like effects of these designer drugs and marketing as "legal highs" have influenced their rising popularity in recent years. Although legislation has been passed to outlaw the manufacture, distribution, and use of a large number of SCBs, law enforcement officials are constantly challenged with the daunting task of rapidly detecting new chemical modifications of SCBs used by manufacturers to circumnavigate local and federal legislation. Routinely used screening methods for SCBs, such as color tests and microcrystalline tests, have been shown to be subjective and possibly lead to false positives. Although confirmatory identification of these SCBs has been successful using laboratory confined instrumentation, such as Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS), to date, there are no reliable and rapid analytical techniques available for detection and characterization of SCBs to support law enforcement efforts in the field. The concerning lack of objective analyses for SCBs may be addressed by investigating the reliability of IMS, which is an analytical tool that is sensitive to trace amounts of compounds.

In this study, the detection parameters (i.e., drift times (ms) and reduced mobility (K_o) values) of 21 widely abused SCBs were determined using IMS, and sensitivity, reproducibility, and selectivity of the instrument were evaluated.

This study will increase knowledge of the structures and detection methods for an ever-evolving class of materials (SCBs), which have greatly challenged forensic practitioners and law enforcement officials in clandestine field operations. The development of new rapid and portable analytical methodologies will assist with the identification of suspected SCBs for criminal justice purposes.

Twenty-one target compounds, which are widely abused and represent a variety of SCBs from different sub-classes with varying structures, have been analyzed. Samples included 21 commercially available certified reference SCBs at various concentrations (0.1mg/mL-1.0mg/mL). A certified THC standard was used as a positive narcotic control. Positive and negative verification standards were also used as positive instrumental controls. Detection parameters of the test samples were obtained using a Smiths Detection IONSCAN 400B IMS with an atmospheric pressure chemical radioactive 63Ni ionization source in positive (narcotics) mode. Each sample was analyzed in triplicate using approximately 2µL-5µL of the chemical standard solutions. Confirmatory analysis of test samples was performed using GC/MS. To determine variation of instrument detection, time studies were also conducted. The IMS detection parameters (ms and K_o) for each SCB were recorded and evaluated for reproducibility using statistical software.

Presumptive testing using IMS, limit of detection studies, and mixture analysis was conducted on selected SCBs to determine the efficacy of IMS as a quick and reliable technique for in-field use for law enforcement. Using IMS, all 21 SCBs were detected, and selected SCBs could be observed in trace concentrations as small as 0.09µg/mL. Analysis of mixtures containing multiple SCBs revealed that at least one compound can accurately be

identified when compared to a standard. Limit of detection experiments were also performed on six selected SCBs.

In this study, 21 SCBs were analyzed using IMS and were shown to exhibit high reproducibility and stability over the course of three years with similar K_o values as provided by the United Nations Office on Drugs and Crime (UNODC). Due to the similarities in the detection parameters obtained in this study and those reported for the selected SCBs in the literature, IMS (positive mode) is a suggested tool for the presumptive identification of SCBs in forensic field investigations.

Synthetic Cannabimimetics, Ion Mobility Spectrometry, In-Field

B150 The Identification of Synthetic Cathinones Commonly Found in Designer Drugs Utilizing Liquid Chromatography/Electron Spray Ionization/Quadrupole Time-of-Flight/Mass Spectrometry (LC/ESI/q-TOF/MS) and Direct Analysis in Real-Time-Mass Spectrometry (DART®-MS)

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LC/ESI/q-TOF/MS and DART®-MS methods were developed for the identification and confirmation of several commonly encountered synthetic cathinones. The goal of this presentation is to demonstrate two fast, efficient techniques for the identification of these emerging compounds.

This presentation will impact the forensic science community by demonstrating how effective both the LC/ESI/q-TOF/MS and DART®-MS methods are when identifying and confirming the presence of synthetic cathinone derivatives.

Analytical techniques such as Gas Chromatography/Mass Spectrometry (GC/MS), Gas Chromatography/Flame Ionization Detector (GC/FID), Fourier Transform Infrared (FTIR), and Liquid Chromatography/Mass Spectrometry (LC/MS) are routinely employed at crime laboratories to identify the components found in designer drugs. Due to the structural similarities, many synthetic cathinones have similar Electron Ionization (EI) fragmentation patterns and retention times. The use of additional analytical techniques will assist in the positive identification of these compounds. The purpose of this study was to develop two independent qualitative methods for the identification and confirmation for several synthetic cathinones utilizing LC/ESI/q-TOF/MS and DART®-MS techniques.

The use of LC/ESI/q-TOF/MS for the confirmation of synthetic drugs has gained popularity over the past few years. It has been widely used for the identification of unknowns and targeted searches for compounds in complex matrices. This analytical technique has an advantage over traditional methodologies as it can deliver accurate mass and provide highly specific mass-to-charge spectral data with accuracies in the milli-Dalton (mDa) range.

DART®-MS is a rapid and efficient analytical technique that requires no sample preparation. The sample is directly introduced into the ion source utilizing a capillary glass tube and is desorbed from the sample surface by the flow of heated nitrogen while being ionized. DART®-MS coupled with High Resolution Accurate Mass Spectrometer (HRAMS) can produce rapid results with accurate mass determinations and very specific mass-to-charge spectral data.

Methylone, ethylone, butylone, pentylone, dimethylone, and dibutylone were the cathinones chosen for this study. In addition, several 3,4-methylenedioxyphenethylamines, such as 3,4-MDMA, 3,4-MDEA, 3,4-MDDMA (3,4-methylenedioxydimethylamphetamine), and 3,4-MBDB (3,4-methylenedioxy-phenyl-2-methylbutanamine), were analyzed for comparison. All analytes were prepared at a concentration of 10ng/µl in LC/MS grade methanol for both methods.

An Ultra High-Performance Liquid Chromatography (UHPLC) equipped with a binary pump and degasser interfaced with Agilent's® 6520 Q-TOF MSD was used. A 6.5-minute method employed an ESI source operating in positive mode with fragmentor voltage 150V; skimmer voltage 65V; octopole 1RF voltage at 750V; and Collision-Induced Dissociation (CID) analysis performed at 15V.

A 24-second method was developed applying DART®-MS in positive mode using Source-Induced Dissociation (SID) generated at 1V, 30V, and 60V utilizing a Simplified Voltage and Pressure (SVP) ion source with Thermo Fisher's Exactive™ Plus Mass Spectrometer Data acquisition.

Both methods have demonstrated successful identification, ion separation, and excellent mass accuracy for the ten compounds studied.

LC/ESI/q-TOF showed peak separation and mass spectral data with distinguishable fragmentation patterns for all compounds analyzed. In addition, this study demonstrated that isobaric compounds can be identified using LC/ESI/q-TOF/MS by both retention time and CID experiments.

DART®-MS coupled with SID experiments demonstrated ion separation and mass spectral data with distinguishable fragmentation patterns for most of the compounds when analyzed independently. The analysis of the ten component mixture posed a challenge due to the structural similarities and the combination of a significant

number of fragment ions. Many other product ion peaks overlapped and could not be attributed to a single cathinone in the mixture.

Both methods are very beneficial to the forensic community and each analytical technique has the potential to dramatically streamline sample analysis, minimize the number of sample preparation steps, and enable rapid characterization of emerging structural analogs.

DART®-MS, LC/ESI/q-TOF/MS, Synthetic Cathinones

B151 The Development of a Unified Gas Chromatography/Mass Spectrometry/Flame Ionization Detector (GC/MS/FID) Method to Determine Various Classes of Synthetic Drugs Using Retention Indices

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After attending this presentation, attendees will understand the method used to separate, identify, and quantify different classes of synthetic drugs using GC/MS/FID. The benefits of identifying these drugs using a library of retention indices rather than simply relying on a mass spectral library will be demonstrated. This retention index library can then be used by the forensic science community to identify synthetic drugs in street samples.

This presentation will impact the forensic science community by providing a more systematic and efficient method for the identification and quantification of various classes of synthetic drugs, which will allow for faster scheduling of these drugs and will allow crime laboratories to stay up-to-date on this growing problem.

The purpose of this research was to develop a single method using GC/MS/FID that would allow various classes of synthetic drugs, including synthetic cannabinoids, synthetic cathinones, piperazines, 2Cs, synthetic opiates, and benzodiazepines to be identified and quantified. This is important due to the growing abuse of emerging synthetic drugs in this country as an alternative to more historic illegal forms. To prevent this abuse, many states have passed laws banning these drugs, and the Drug Enforcement Agency (DEA) has begun scheduling and/or temporarily scheduling these drugs; however, as soon as one synthetic drug is scheduled, its structure is modified so that the new compound does not fall under DEA regulations. Due to the growing number of synthetic drugs being produced and the lack of research being conducted, crime and clinical laboratories are having difficulty identifying and analyzing these drugs efficiently. Therefore, developing a single analytical method to identify and quantify different classes of synthetic drugs using common instrumentation is needed so crime laboratories can analyze these drugs quickly and increase their productivity.

GC/MS was used to identify the synthetic drugs and a library of retention indices was created from reference standards as a better form of identification rather than relying on mass spectral libraries alone. GC/FID was used to quantify the drugs due to the documented reactivity of some of the synthetic drugs when using MS.[1] To separate these drugs, an Rtx-5 Amine column along with a base-deactivated split inlet liner with base-deactivated wool was used as it demonstrated the best inertness for this wide range of compounds. The method successfully separated six classes of synthetic drugs and was used to identify and quantify synthetic drugs in street samples; however, it was determined that derivatization of the 2Cs was needed for optimal separation performance due to the reactivity of the compounds. To date, only two of the synthetic cathinones posed a problem of co-elution, and thus could only be semi-quantified. For determination of the retention indices of the drug compounds, the Massachusetts (MA) Extractable Petroleum Hydrocarbon (EPH) Aliphatic Hydrocarbon Standard mix was used. Method precision (as percent of Relative Standard Deviation (%RSD)) was relatively low for all the compounds, with the synthetic cannabinoids showing the best precision and the synthetic cathinone, methylone, showing the poorest. Retention indices were calculated for all compounds, but extracted ion chromatograms were needed to calculate 2C-N's retention index.

With this method, six classes of synthetic compounds and street samples were able to be separated, identified using retention indices, and quantified. Utilizing retention indices has been demonstrated to be a clearly better method for identification than reliance on mass spectral data alone, especially for compounds that are isomers and have similar mass spectra. This is because retention indices are more specific to the target compound than retention times and mass spectra. The retention index library can then be expanded to include more synthetic drugs, and additions will need to be made as new synthetic drugs are uncovered in recreational use. This will provide a more systematic and efficient method for the identification and quantification of synthetic drugs, allowing for faster scheduling of these drugs and allowing crime laboratories to keep up with this growing problem. Also, once these compounds are identified, more toxicological research can be conducted on the drug effects at certain doses.

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Synthetic Drugs, Retention Indices, Gas Chromatography

B152 The Analysis of the Fatty Acid Content of Fingerprint Residues Using Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will understand the within-person chemical variability of fingerprint residues of individuals.

This presentation will impact the forensic science community by providing attendees with an additional means of latent print analysis through the determination of the chemical composition of fingerprint residues. Often, smudged fingerprints deposited at crime scenes are of little value to latent print examiners because smudged prints lack minutiae that are necessary for comparisons. In cases in which DNA or fingerprint minutiae in the fingerprints are not able to identify a suspect or victim, the chemical composition of fingerprint residues could provide a useful investigative lead, if the residues enable the classification of individuals into groups according to biometric traits. For this approach to be possible, one must first establish that the chemicals found on a person's fingers are endogenous and not exogenous, then determine that the within-person variance is smaller than the between-person variance.

One of the recurring issues in forensic science is human subjectivity, especially within the field of fingerprint examination. Smudged fingerprints at crime scenes that contain little to no detail often cause problems to examiners who, in turn, are unable to make an identification or exclusion. Touch DNA is one solution to this problem, but mixed DNA and stochastic dropout are major barriers to interpretation. In recent years, researchers have begun to explore the chemical composition of fingerprint residues to provide an alternative means for including or excluding potential donors.

Research into the chemical composition of fingerprints has shown that it may be possible to determine sex, age, and race from residues that are left behind when a fingerprint is deposited on a surface; however, some issues have been encountered. Lipids, one of the major components of these residues, are also found in personal care products, such as moisturizers and cosmetics. It has been difficult for researchers to distinguish fingerprint residues deposited by an individual from the residues left behind by these products. The purpose of this study is to determine the variability of fingerprint residues within an individual over the course of several months, and to assess the relative proportion of endogenous and exogenous sources of lipids.

This study involved the collection of fingerprint residues of six individuals (three males and three females) over the course of three months. Natural, eccrine, and sebaceous secretions were collected from each participant multiple times per week. Saponification and derivatization were performed on each sample to convert the various types of lipids/glycerides to Fatty Acid Methyl Esters (FAMES). Analysis was performed using a standard GC/MS system with an HP5 column. Data analysis consisted of the identification of peaks and extraction of peak areas. One-way Analysis of Variance (ANOVA) was used to assess within-group variance to between-group variance at a 95% confidence interval to determine the variability of fingerprint residues deposited by individuals over a period of time. Results showed that within-person variability is smaller than between-person variability.

Fingerprint, GC/MS, Fatty Acid

B153 An Elemental Characterization of Firearms Discharge Residue Using Complexing Agents and Triple Quadrupole Mass Spectrometry (QQQ MS)

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After attending this presentation, attendees will better understand a new analytical approach to detecting metal cations from firearms discharge residue using an instrument found in many forensic toxicology laboratories

This presentation will impact the forensic science community by offering attendees a new method to screen for and identify elements associated with Gunshot Residue (GSR).

Several methods have been published reporting on the analysis of organics found in Firearms Discharge Residue (FDR) using Liquid Chromatography/Mass Spectrometry (LC/MS). The mass spectrometers used include Time-Of-Flight (TOF), quadrupole Time-Of-Flight (qTOF), and Triple quadrupole (QqQ) designs. For LC/MS analysis, a substrate such as a hand swab is typically extracted, concentrated, and introduced into the chromatographic system and analytes such as Diphenylamine (DPA), ethyl and methyl centralites, dinitrotoluenes, and other diphenylamines are detected. This capability has the potential to increase the utility of FDR analysis in that current methods using Scanning Electron Microscopy/Energy-Dispersive X-Ray Spectroscopy (SEM/EDS) target the inorganic particulate residues from the primer. Even better would be a procedure that allows for detection of the inorganic and organic constituents of FDR from a single sample. The goal of this project was to demonstrate a novel method of detecting the key elemental constituents of FDR using complexing agents and direct infusion sample introduction using a QqQ instrument. This successful demonstration opens the doors to further development of method in which the organic and inorganic constituents of FDR can be characterized from a single sample.

Crown ethers are macrocyclic complexing agents that bind with +1 and +2 cations. For this project, 15:5 crown ether was chosen because its cavity size is amenable for binding known GSR metals (Pb, Ba, Sb, Cu). These crown ether metal complexes were created independently in methanolic solution and were analyzed using Electrospray Ionization (ESI) -QqQ via direct infusion, positive mode. The parent ions in all cases were in the form of M-L-NO3 (cation-ligand (15-crown-5)) and nitrate ion. Detection limits were established (ppm range in solution corresponding to ~ µg total solid) and Multiple Reaction Monitoring (MRM) transitions optimized such that the parent ion yielded the corresponded element as the transition product. This allows for detection of isotopes of the elements that occur at a natural abundance of ~ 5% or more via M-L-NO3 à M. For example, the metal complexes in which ^{208}Pb, ^{207}Pb, ^{206}Pb, and ^{204}Pb were all observed with MRM analysis, detecting all but ^{204}Pb, which has a natural abundance of <5%. Semi-quantitative results were obtained using an internal cesium spike. This element is monoisotopic and not expected to be detected in typical GSR samples. Antimony (Sb) proved to be the most challenging to detect, either due to low concentrations in sampled residues, poor initial dissolution, poor binding efficiency, or some combination of factors. This presentation will detail experimental methods, figures of merit, and the results from authentic shooting sampling events in which lead, barium, and copper were easily and routinely detected.

Firearms Discharge Residue, Complexing Reagents, Mass Spectrometry

B154 The Analysis of Gunshot Residue and Plastic Deposits From 3D Printed Polymer Firearms

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After attending this presentation, attendees will better understand the residues that result from the discharge of an Acrylonitrile Butadiene Styrene (ABS) polymer firearm.

This presentation will impact the forensic science community by providing information that may be applied to casework in determining indications of polymer firearm use in crimes.

In recent years, there has been a significant increase in the availability of 3D polymer printers for use by individuals. These personal 3D printers are relatively sophisticated, while their affordability and ease of use make them ideal for home hobbyists. At the same time, designs and instructions for producing firearms by 3D printer are readily accessible on the internet. The production of these types of guns raises many concerns to law enforcement and has an unknown impact on the forensic science laboratory analysis of evidence collected during the investigation of shooting events.

Samples were collected during the discharge of five different 3D printed polymer firearms including both .22 Long Rifle (LR) and .380 Automatic Colt® Pistol (ACP) calibers. Scanning electron microscope stubs with carbon impregnated adhesive tabs were secured near the guns during discharge. Samples were analyzed for primer Gunshot Residue (pGSR) using a Scanning Electron Microscope with Energy Dispersive Spectrometer (SEM/EDS) and automated GSR analysis software. The instrument was operated using parameters typically used in casework at the Colorado Bureau of Investigation Forensic Services Laboratory. Each sample analyzed was positive for the presence of particles characteristic of Gunshot Residue (particles with lead, barium, and antimony combined in discreet particles with distinctive morphologies of pGSR). The number of characteristic GSR particles detected varied among the guns. This study attempted to determine if residues originating from a polymer gun have distinctive features that differ from the typical pGSR particles that are recognizable by the SEM/EDS automated particle analysis technique. Some of the detected pGSR particles have unusual shapes. This indicates a fusion of residues containing both polymer and typical primer components.

ABS plastic residues were present in samples, both on the SEM stubs and in the residues collected from the muzzle discharges. The detection of ABS residues presents analytical challenges for routine forensic polymer analysis. Infrared (IR) microspectroscopy was employed to characterize the polymer residues.

This presentation will report the results of the analysis of samples collected from the discharge of 3D printed polymer guns.

Gunshot Residue, 3D Printed Firearm, Polymer

B155 A Study on the Persistence of Gunshot Primer Residue on Clothing Subjected to Handling

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After attending this presentation, attendees will better understand the persistence of gunshot primer residue on clothing.

This presentation will impact the forensic science community by providing attendees with an insight into the dynamics of gunshot primer residue particle transfer on clothing. This presentation describes the methods used to artificially create the transfer of gunshot primer residue from one item of clothing to another and provides the results of this study.

Gunshot primer residue is produced by a firearm when it is discharged. The primer for centerfire cartridges is composed of lead styphnate, barium nitrate, and antimony sulfide. The residue from the primer explosion escapes from openings in the gun and can be deposited on a person's hands and clothing. These particles can be collected and analyzed using automated scanning electron microscopy energy dispersive X-ray. Characteristic gunshot residue primer particles have a molten appearance and are composed of barium, antimony, and lead. During a crime scene investigation, evidence technicians may package an item of clothing that has gunshot primer residue on it in the same evidence container as other items of clothing from the crime scene. While studies have been conducted as to how long gunshot primer residue persists on a living person's hand, studies have not been conducted about how long it will persist on clothing that is subjected to handling, such as packaging as evidence. Additionally, it has also not been determined whether gunshot primer residue easily transfers from one item of clothing to another item of clothing with which it may come into contact.

This presentation will detail the results of a study using a controlled mechanism for the transfer of gunshot primer residue from one item of clothing to another.

Gunshot primer residue was placed on clean cloth swatches of different types of clothing materials to create positive gunshot primer residue samples. Clean cloth swatches were then packaged with the positive gunshot primer residue cloth swatches in the same manner that samples are received in the laboratory. The cloth swatches were then subjected to different types of handling to mimic real-life conditions. The surfaces of the clean cloth swatches were then sampled using standard gunshot primer residue collection stubs. Those stubs were analyzed using scanning electron microscopy energy dispersing X-ray spectroscopy instrumentation using standard laboratory procedures for the analysis of gunshot primer residue.

This research takes a novel approach by investigating the likelihood of gunshot primer residue transfer between articles of clothing.

Gunshot Residue, Evidence, Scanning Electron Microscopy

B156 Clearing a Firearms Backlog Using Process Management and Available Resources

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WITHDRAWN

B157 Microscopical and Ultrastructural Investigation Into Possible Chemical and/or Mechanical Degradation Mechanisms of Hair Roots Containing Induced Postmortem Root Band (PMRB) -Like Features

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After attending this presentation, attendees will understand the ability of various ionic and pH-specific buffers to elicit PMRB-like ultrastructural features in antemortem hairs as observed by light and scanning electron microscopy.

This presentation will impact the forensic science community by presenting possible mechanisms of hair root degradation for the formation of PMRBs with the goal of ultimately better understanding the complex processes that result in true PMRB formation *in situ*.

A PMRB is a microscopic feature that results from degradation of the pre-keratinized region of the roots of anagen and early catagen hairs obtained from cadavers.[1,2] A PMRB is located *in situ* approximately 0.5mm from the root tip and is surrounded by root sheath issue. A PMRB forms due to degradation to the Intermacrofibrillar Matrix (IMM) (or cell membrane structures) in the cortex, resulting in elongated, gas-filled void spaces. Scanning Electron Microscope (SEM) analysis has shown that the degradation is confined to the cortex and does not extend to the hair cuticle.[3] The morphology of the IMM degradation is highly complex; in some instances, the elongated void spaces are lined with jagged "saw-teeth" that are interpreted to be remnant IMM.[3] These structures may be due to chemical degradation or perhaps represent "pull apart" structures resulting from the build-up of decomposition gas. Resolution of this premise may provide valuable insight into the mechanism of PMRB formation.

Prior work has shown that exposing human scalp hairs to slightly alkaline (pH 7-8) aqueous ammonium salt solutions results in degradation that is microscopically similar to that observed in known PMRBs.[4] In addition, similar PMRB-like decomposition could also be achieved by immersing antemortem anagen head hair in various pH-specific (pH 6-8) aqueous buffers. The mechanism and manner for this decomposition is not known.

One hypothesized mechanism for *in vitro* band formation is the presence of the ammonium ions or ammonia gas that may chemically attack the IMM. Another hypothesis is that the change in hair pH, due to immersion in various buffers, may lead to IMM degradation/ collapse. Alternatively, decomposition gases may build up and subsequently become trapped inside the cortex, thus mechanically disrupting the integrity of the pre-keratinized region of the root. This process is hypothesized to be possible due to the presence of the root sheath or cuticle trapping the gas, preventing it from escaping from the hair into the surrounding tissue.

This work investigates whether the damage to the IMM is of chemical or mechanical origin and if the presence of the root sheath plays a role in the formation of PMRBs. To address this question, antemortem anagen hairs were obtained from living human donors. Hairs with root sheaths were included to better mimic *in situ* hair follicle conditions; hairs without root sheaths were included to investigate whether the root sheath is necessary for the development of PMRB-type degradation. Hairs were either left intact (controls) or embedded and sectioned by ultramicrotome. Hairs were then incubated in the following test solutions: 100mM ammonium acetate (pH 7.8); a proportional mixture of sodium phosphate solutions (pH 7.8); or ultra-pure water. After three to ten days, the hairs were examined via light and electron microscopy.

Preliminary data suggest intact hairs with and without root sheaths exposed to the ammonium acetate and pH 7.8 solutions developed dark PMRB-like degradation similar in appearance to true PMRBs; these results agree with prior work.[4] The hair samples immersed in ultra-pure water did not produce PMRB-like degradation. Next, in an attempt to investigate the potential effects of containment on the occurrence of IMM degradation, thin-sections of hair were immersed in the same solutions. If gas were produced while immersed in the various buffers it would not be able to build up in the hair shaft. These sections were compared to similarly prepared healthy anagen head hairs. Preliminary data suggest that the untreated and water-exposed head hairs exhibit little degradation to the cortex of the pre-keratinized region; however, hair sections immersed in ammonium acetate solutions, both with and without root sheath, do exhibit significant ellipsoidal voids similar to features seen in known PMRBs in the

pre-keratinized region. Sectioned hairs exposed to the pH-matched buffer exhibit some voids, but these are less prominent than those observed in hairs exposed to ammonium acetate solutions. Taken together, these results suggest that ammonium may play a role in the chemical attack of the IMM and that mechanical damage due to gas buildup is a less likely mechanism for PMRB formation.

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Postmortem Hair Root Banding, Hair Microscopy, Trace Evidence

B158 Multivariate Classification Model Transfer of Ultraviolet (UV) /Visible Spectral Data From Acrylic Fibers Without Standards

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The goal of this presentation is to illustrate the value of transfer of multivariate classification models for spectra of trace evidence fibers between laboratories. Such efforts could save time and resources in forensic analyses and contribute to an understanding of differences in spectra from multiple laboratories due to variations in sample preparation, environmental conditions, and instrumental signal response.

This presentation will impact the forensic science community by exploring the ability to transfer multivariate classification models between laboratories, which could save time and resources in forensic analyses. Successful sharing of such information between forensic laboratories may be valuable for confirming fiber identification and assessing the reliability of comparisons.

UV/visible microspectrophotometry is commonly employed for discriminating metameric fibers in forensic casework. Recent studies have demonstrated that multivariate classification techniques are an effective tool for characterizing such fibers. The ability to transfer multivariate classification models between laboratories could save time and resources in forensic analyses; however, issues transferring models of this type from one laboratory to another can arise as a result of differences in sample preparation, environmental conditions, and instrumental signal response. Spectra of 12 blue acrylic fibers were examined at five separate locations, including three academic research laboratories and two forensic laboratories. The data received from these facilities were analytically assessed in three manners. Multivariate classification models were initially constructed on each individual laboratory's dataset to evaluate intra-laboratory variability between samples. In a subset of the study, discriminant analysis was performed after merging all data collected in the study. Lastly, the transferability of classification models was assessed by predicting class membership of samples analyzed at a single laboratory using models built from the spectra collected at the four remaining locations.

Principal Component Analysis (PCA) followed by Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), or Support Vector Machine Discriminant Analysis (SVMDA) was used to evaluate the agreement of results among the laboratories. An average classification accuracy of 93.2% was found after training discriminant analysis models using data collected at the four laboratories and using the information collected at the fifth laboratory as an external test set. For comparison, intra-laboratory studies that were conducted produced an average classification accuracy of 96.3%. The reduction in the discriminative abilities of the transferred models was likely due to the differences in spectral noise and peak intensities experienced between laboratories. On the whole, the errors generated by QDA were lower than those resulting from LDA and SVMDA. In conclusion, this inter-laboratory study confirms the ability to preprocess spectra to explore the agreement of spectral comparisons among forensic laboratories.

Inter-Laboratory Comparison, Classification Model Transfer, Inter-Laboratory Reliability

B159 Photobleaching in Cotton Fibers Dyed Using Red, Yellow, and Blue Direct Dyes During Examination With Microspectrophotometry (MSP)

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After attending this presentation, attendees will understand the susceptibility of cotton fibers to loss of color, or photobleaching, when being analyzed using MSP. Attendees will also understand how spectra obtained by performing Ultraviolet/Visible (UV/Vis) spectroscopy measurements on dye solutions compare to the spectra obtained from MSP of fibers dyed with the same dye.

This presentation will impact the forensic science community by increasing forensic examiners' awareness of the tendency of dyed cotton samples to undergo photobleaching while performing MSP analysis, which may affect procedures for examining dyed cotton fibers, and will also demonstrate the usefulness of MSP on fibers by comparing spectra of the dye solutions with the dyed fibers.

MSP is a promising technique for the analysis of trace evidence such as fibers and paint. Color can be a powerful tool for comparison of many types of trace evidence, but previously large samples or elaborate procedures were required for analysis of color in paint or fiber. This rapid, non-destructive technique combining microscopy and UV/Vis spectroscopy can eliminate the need for time-consuming extraction and analysis of dyes from textile fibers when performing analysis of colored fibers and can directly analyze color from multiple layers of paint, all while performing microscopic analysis of these materials.

This study will present an experiment in which bleached cotton fabric was dyed with direct dyes in blue, yellow, and red colors at different concentrations. The dyed cotton fibers were then examined using MSP. Photobleaching was investigated by measuring a specific spot on the fiber periodically over the course of one-half hour, which was considered the longest time a forensic examiner might ever leave a sample in the MSP. Visible color loss and a reduction in absorbance was observed for all three colors, but was most pronounced for the fibers dyed with red dye. Since forensic examiners expect MSP to be a non-destructive method, it is important to be aware of situations in which their selected analysis might unexpectedly be destructive. While some MSP vendors are educating examiners during training about the possibility of photobleaching of dyed cotton fibers, an informal poll of forensic trace evidence examiners indicated that this was not common knowledge among the trace evidence community. It is recommended that fiber trace evidence examiners become aware of the possibility of photobleaching when analyzing cotton fibers using MSP and that they understand how spectra collected using MSP from a fiber compare with UV/Vis analysis of dye solutions in the liquid state. After attending this presentation, attendees should better understand both concepts.

Microspectrophotometry, Fiber, Photobleaching

B160 Probing the Effects of Inherent Variability in Forensic Fiber Analysis

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After attending this presentation, attendees will better understand the various sources of variability inherent in forensic fiber analysis, which stem from both the sample itself and the methodology used.

This presentation will impact the forensic science community by increasing awareness of this variability and providing some suggestions as to how analysts might reduce this variability in their measurements. An eventual impact of this study will be the creation of a reference database that can be used by fiber examiners to compare their results and techniques to a standard set of known data.

In this experiment, a reference collection of synthetic fibers was used to investigate the effects of polymer type, starting diameter, shape, and compression on the resulting Fourier Transform Infrared (FTIR) spectra. Using a randomized experimental design, reference fibers were embedded in polyethylene and hand-sectioned to determine cross-sectional shapes and diameters via Scanning Electron Microscopy (SEM). A separate randomized experimental design was created to test two different known torques (5N·m and 7 N·m) to flatten these fibers in a diamond compression cell. A torque wrench with a 3D printed adapter was used to help standardize compression, and the resulting thickness of the fiber films was measured for uniformity with an optical profilometer. The starting versus ending thickness information was compiled and compared with the polymer material and shape to define any trends, note inconsistencies, and identify places for improvement. A similar action plan was taken to measure FTIR spectra before and after compression, and the spectral features were compared to see if any optimization in the process could be obtained.

SEM analysis verified that even along a single fiber there existed measurable variation in starting diameter, and the profilometer measurements showed that cross-sectional shape and different torque strengths influenced the fiber film thickness. Large relative standard deviations in the measurements resulted from different areas of the film being measured; for example, trilobal fibers, such as nylon, produced widely different thicknesses after compression, depending on the orientation of the fiber when it was placed in the diamond cell.

This study takes the first steps in understanding how differences in fiber type, fiber shape, and methodology affect the outcome of fiber analysis, with concerted effort placed on characterizing that variability. Primarily, this research seeks to raise awareness of the variability that accompanies the analysis process by evaluating and refining analytical techniques commonly used by forensic examiners. The end goal is to establish a database based on the Microtrace fiber reference collection of chemical spectra that combines microscopic measurements of fiber cross-section and diameter with FTIR spectra, and eventually add Microspectrophotometry (MSP) and Raman, where appropriate.

Fibers, Reference Database, Trace Analysis

B161 Applications of Infrared (IR) Imaging and Chemometrics to Facilitate the Forensic Examination of Automotive Paint

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WITHDRAWN

B162 Evaluating the Application of Micro X-Ray Fluorescence (XRF) and Micro Raman Spectroscopy to the Analysis of Duct Tapes: Intra-Roll and Inter-Product Correlations

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After attending this presentation, attendees will better understand XRF and Raman microscopy in applications in the analysis of duct tapes; this analysis is an important element in forensic identification of these materials.

This presentation will impact the forensic science community by serving as key aspect of duct tapes analyses and as an example of a practical application of XRF and Raman spectroscopy in duct tape identifications.

XRF and Raman spectroscopies are useful tools for identification substances and confirming their identity with little or no sample preparation. XRF provides information about elemental composition of the material, whereas Raman spectroscopy supplies molecular information. Both techniques are able to record not only spectra of small particles, but also hyper-spectral images, as well as collect average spectra over certain areas. Multivariate Analysis (MVA) can produce chemical distributions of elements and/or material classification based on Principal Component Analysis (PCA), Partial Least Square Discriminative Analysis (PLSDA), in particular, with association between elements that can aid in the identification of bonded phases. The analysis of micro XRF and Raman data of duct tapes can be used to identify the source of a duct tape or the vendor of the product.

XRF and Raman analytical microscopes were used in this study. XRF spectra of the materials were collected using 30keV acceleration voltage and with an X-ray spot size of 1.2mm. Two excitation wavelengths (532nm and 785nm) were used to collect Raman spectra.

The spectra of duct tapes from different sources were collected and analyzed by micro XRF and Raman spectroscopy. XRF analysis was performed in the range of 1.00keV-40.96keV. There are no spectral features in the energy range above 15keV; spectra were truncated and analysis was performed in spectral range of 1.00keV-15keV. Some tapes contain elements Ti, Ca, S, and Al in a fiber substrate, which may be used for duct tapes differentiation. Classification of duct tapes-based PCA of the spectra will be shown. Small pieces of glue from the tapes were collected and Raman spectra of this material were measured in the range of $100cm^{-1}$-$3,500cm^{-1}$. MVA was applied to these spectra to extract differences in connection with different source of the tapes. The data demonstrates that MVA allows differentiation of the samples, for example, duct tapes #1020 and #1110 or #1230. PCA of duct tapes exhibits significant separation between duct tapes of different vendors and brands. Raman spectra of the materials note many common features with some differences, which may originate from the filler. Data fusion technology was applied to the set of XRF and Raman data to create PCA and PLSDA models. Misclassification in PLSDA models was studied using randomly selected samples from available data. Results of standard and data-fused analysis are compared and discussed. In conclusion, this study provides methods that allow one to differentiate duct tapes based on spectra analysis of micro XRF and micro Raman data.

Duct Tape, XRF, Raman Spectroscopy

B163 Discrimination of Soil Organic Matter Via Nuclear Magnetic Resonance (NMR) Spectroscopy Combined With Interval Extended Canonical Variate Analysis (iECVA)

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After attending this presentation, attendees will be able to apply the techniques presented. Attendees will be able to extract the organic matter from soil samples, prepare samples for NMR analysis, and understand how iECVA can be combined with NMR to pinpoint the exact location of a soil sample.

This presentation will impact the forensic science community by disseminating new methods for soil analysis. The new methods developed will provide both guidance to practitioners for soil sample collection and arguments to establish convincing corroborative evidence in court between a suspect and a crime scene, between a victim and a crime scene, or between a victim and a suspect when soil evidence is found.

A new method for the analysis of Soil Organic Matter (SOM) is described. Modern detection and identification methods for soil evidence at crime scenes suffer from some drawbacks. In particular, most techniques of identification are destructive and rely primarily on the inorganic fraction of soil. Forensic scientists have not given much consideration to the analysis of SOM. Forensic geoscientists also face the following problem: if a soil sample is found on a potential suspect, what is the probability that this soil sample comes from one particular area versus another? (i.e., is there a way to assess how common the observed points of similarity or difference between soils are?). Another issue is the small amount of published guidance regarding the small-scale spatial variability of the soil considered. Assessing this variability is important in determining where samples should be collected in order to adequately represent an area of forensic interest. In view of these current shortcomings, the work presented here seeks to: (1) identify the SOM at nine different locations in New York City by Nuclear Magnetic Resonance (NMR) spectroscopy; (2) combine iECVA with NMR to pinpoint the exact location of a soil sample; and, (3) produce a method (i.e., empirical base match probability estimate) to determine the probability of finding similar soil samples from a particular park in the wider environment.

Soil samples from New York City Central Park were collected in nine different locations. The organic matter of the soil samples was analyzed by liquid state ^{1}H NMR and solid state ^{13}C NMR using spectrometers JEOL 300 MHz instrument for liquid NMR experiments and AVIII 400 MHz instrument for solid NMR experiments. The iECVA was combined with NMR results using computers to discriminate the nine different locations of interest. The ability to correctly assign the origin of the soils was assessed. It is expected that the method developed in this presentation (i.e., NMR spectroscopy combined with iECVA) will produce superior results (i.e., better ability to correctly assign the origin of the soil sample) than the traditional techniques. Finally, statistical analyses (iECVA and Wilks' lambda statistic) were used to assess the degree of small spatial variability in the soil properties observed by NMR.

Soil, NMR, Statistics

B164 Using Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) to Detect Volatile Compounds Remaining From the Storage of Dead Mice

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After attending this presentation, attendees will understand the principles of how Volatile Organic Compounds (VOCs) from decomposition can be used as evidence and how the volatile profile is affected once the decomposed body is removed.

This presentation will impact the forensic science community by demonstrating that the VOCs released by decomposition can be collected, analyzed, and differentiated, allowing for detection of a decomposing body, even after removal. This is important as it can aid investigators in the discovery of the body or in determining that a body was present, but moved to a secondary crime scene.

One of the main objectives in forensic science is to analyze evidence so a linkage can be made. The linkage can be between a number of different elements, such as the victim(s), suspect(s) and crime scene(s). These linkages help law enforcement officers reconstruct the events that took place during a crime, which aids their investigation. One possibility is linking a body to a certain area based on the VOCs detected in the surrounding area (soil, air, etc.). Body odors are emitted from both living and decaying bodies. Each occur due to different taphonomic processes; therefore, the VOCs released from the body could be different. There are five stages of decomposition: (1) fresh; (2) bloated; (3) active decay; (4) advanced decay; and, (5) dry remains. The two main processes that are involved in the stages of decomposition are autolysis and putrefaction. As these processes occur, different VOCs are released from the body.

In this study, four mice were placed into four separate quart-sized arson cans. Three of the cans were used for data collection, while the fourth can was utilized for photographing the decomposition process. The headspace above the decaying mice was analyzed by utilizing three different SPME) fibers (one fiber per can): (1) Polydimethylsiloxane (PDMS); (2) Polydimethylsiloxane/Divinylbenzene (PDMS/DVB); and, (3) Polydimethylsiloxane/Divinylbenzene/Carboxen (PDMS/DVB/CAR). The VOCs collected using these fibers were analyzed and compared using a splitless method for GC/MS. Cleaning and storage conditions in addition to instrument parameters were assessed to determine the optimal procedure. This allows for a fast and simple sample collection methodology without any sample preparation prior to analysis. The run time for each sample was 25.67 minutes. A diluted standard mixture, of similar compounds to those observed in previous literature, was prepared and run using a split method to confirm that the method was working properly.

VOCs associated with decomposition stages of the mice were collected once a week for each can using a different fiber. The most abundant VOCs present within the first week of decomposition consisted of dimethyldisulfide, dimethyl trisulfide, dimethyl tetrasulfide, phenol, and indole for all three cans and all fibers. These results support previous studies that indicate sulfide compounds are among the first to be released during decomposition. By day 13 of decomposition, the PDMS fiber only detected five compounds (abundances of $2x10^5$ or greater). By day 16 of decomposition, the PDMS/DVB fiber detected more than ten compounds and the PDMS/DVB/CAR fiber detected more than 13 compounds (both with abundances of $2x10^6$ or greater). I n addition to the compounds listed above, additional compounds included: hydrocarbons, esters, amines, alcohols, ketones, and furans. Based on these results, the PDMS/DVB/CAR fiber was chosen as the optimal fiber. This fiber was then utilized to determine the VOC signature that remained after the mice were removed from the three cans.

Decomposition, Volatile Organic Compounds, SPME-GC/MS

B165 Black Box and White Box Forensic Examiner Evaluations — Understanding the Details

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The goal of this presentation is to assist attendees in understanding the issues involved in using the black box approach to assess the accuracy and reliability of forensic examiners.

This presentation will impact the forensic science community by describing an approach for assessing potential areas of strength and weakness in forensic science, as well as by offering several objective measures to support admissibility requirements.

Recently, there has been a great deal of interest in using "black box and "white box" techniques to evaluate decisions made in a variety of forensic disciplines. This presentation will help dissect the details of conducting such evaluations, which are not as deceptively simple as they seem.

Black box evaluations are conducted by assessing the examiner's decision without regard to how those decisions are made. Black box evaluations can provide a useful overall understanding of the accuracy, reproducibility, and repeatability of the decisions made in response to a given task. Such evaluations do not attempt to assess how a specific examiner performs on specific data — but black box evaluations are a necessary first step toward such detailed tests. Black box evaluations provide a means of quantifying forensic examinations for which quantitative models do not (yet) exist and, therefore, provide both an interim solution while such models are under development, as well as a means of validating such models.

Conversely, white box evaluations are conducted to gain an understanding of how and why examiners make decisions. White box evaluations are detailed assessments of the bases of examiners' decisions, focused not just on the end decisions but on the features and attributes used by the examiners in rendering conclusions. While analyses of black box results deal with the inter-examiner variability of decisions, white box analyses also deal with inter-examiner variability of the detection of features and other attributes.

This presentation will discuss topics that should be considered in the design of black and white box evaluations, including: (1) representativeness of data (dealing with heteroscedastic data, avoiding biased data selection); (2) assessing accuracy vs. reproducibility and repeatability (methods of measurement, data selection implications); (3) test size (precision of measurement, measuring rare events); (4) the Hawthorne effect (dealing with the differences between behavior in tests vs. operations, minimizing differences between test and operational procedures); (5) measuring rates of errors and non-consensus decisions; and, (6) dimensions of examiner skill (accuracy and effectiveness).

Evaluation, Error Rates, Examiner Accuracy

B166 The Implications of Latent Print Quality, Black Box, and White Box Studies

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The goal of this presentation is to help attendees understand the implications of this series of studies on the latent print examination discipline.

This presentation will impact the forensic science community by describing a portfolio of research that was conducted to evaluate the latent print examination process.

In response to a fingerprint misidentification in the 2004 Madrid bombing case, a proactive, internal Federal Bureau of Investigation (FBI) Laboratory review committee evaluated the scientific basis of friction ridge examination and recommended a portfolio of research.[1] To address those recommendations, since 2007 the FBI Laboratory and Noblis® have engaged in a research program evaluating the latent print examination process, which has resulted in eight journal publications to date.[2-9] This portfolio of work has provided a systematic series of analyses of key aspects of the latent print examination process.

The Latent Quality Study involved: (1) conducting a detailed survey of how quality and clarity are assessed within the latent fingerprint community; (2) developing guidelines and metrics for describing the clarity of friction ridge impressions; and, (3) developing software tools to provide objective, reproducible methods for assessment of friction ridge impression clarity.

The Black Box Study was a large-scale study of the accuracy and reproducibility of latent print examiners' determinations. The follow-on Black Box Repeatability Study retested examiners to evaluate the repeatability of their determinations.

The Sufficiency for Value Study evaluated how image clarity and feature content are associated with the assessment of latent value by latent print examiners.

The White Box Study investigated the relationship between examiners' annotations and their determinations. This included: (1) analyses of how examiners assess the sufficiency of information for individualizations; (2) analyses of how examiners revise their analysis of a latent after comparison with an exemplar; (3) analyses of inter-examiner variation of minutia markup; and, (4) analyses of the factors associated with exclusion decisions.

These studies have been critically important with respect to fingerprint examiner testimony in the legal system and are frequently introduced in court in admissibility hearings; in response to these results, some agencies have changed their policies on how examiners testify in court. These studies have also had an impact on laboratory standard operating procedures, examiner training, certification and competency testing, and quality assurance.

This presentation will provide summaries and a synthesis of these studies to date and will discuss their implications and recommendations on how the results of these studies and their implications may be used to enhance the field of latent print examination.

This research has a variety of implications or recommendations on latent print business processes, including not only error rates, but consensus rates, standardized markup for detailed casework documentation, verification (and blind verification), proficiency testing, conflict resolution, and the effects of human factors.

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Latent Prints, Error Rates, Examiner Accuracy

B167 Toward Reform: Implementing Quantitative Methods Into Practice for Latent Print Examination

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After attending this presentation, attendees will have a greater understanding of how friction ridge comparisons may be quantified, the policies and procedures guiding the use of such methods in practice, and the next steps currently under way to ensure these methods are accessible to the broader forensic community.

This presentation will impact the forensic science community by: (1) introducing a method to evaluate and express the significance of latent print examination results in quantitative terms; (2) discussing the progress of implementing this method into practice at the United States Army Criminal Investigation Laboratory; and, (3) exploring strategies to facilitate the transition of this method to other federal, state, and local forensic service providers.

Over the past several years, there has been significant emphasis on implementing reform in the pattern evidence domains by identifying, developing, and integrating relevant quantitative methods into laboratory procedures for the evaluation and interpretation of impression evidence. This emphasis has resulted in promoting awareness of the need for such methods, stimulated conversations and debates on how to effectively achieve such endeavors, and encouraged practitioners, statisticians, scientists and other relevant academia to partner to achieve a common objective. Over the past few years, the United States Army Criminal Investigation Laboratory has taken incremental steps forward to facilitate the transition from solely subjective, experience-based practices to integrating more robust, scientifically demonstrable, and data-driven practices for latent print examination. As part of this process, prototype software has been developed to quantify the correspondence between friction ridge impressions based on the geospatial arrangement of friction skin features and estimate the related likelihoods to assist analysts in their interpretation of source associations. Following robust evaluations of the method against ground truth data sets and pseudo operational trials using casework datasets, the method is being implemented into routine practice in a stepwise fashion. This presentation will provide a general explanation of the statistical methods employed, discuss policies and procedures governing its use in casework, and discuss possible strategies to transition the method to other federal, state, and local forensic service providers in an effort to assist the broader friction ridge community in its incremental progression toward reform.

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

Fingerprint, Probability, Statistics

B168 A Question of Context Within Fingerprint Examinations: Decision-Making Processes, Contextual Information, and Examiners' Motivation

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After attending this presentation, attendees will have a different perspective regarding the accessibility of contextual information for fingerprint examiners. The goal of this presentation is to provide attendees with new suggestions for the practice of forensics, especially for fingerprint examinations; however, these guidelines will be provided from the examiners' point of view and examiners' performances from different countries. Taking this into consideration, attendees will receive input that will be of value for new implementations of guidelines and for a better understanding regarding the effects of contextual information.

This presentation will impact the forensic science community in three ways. First, the research conducted used only fingerprint examiners; although the sample is not extensive, no lay people were participants. This means the results were closer to the community of forensic professionals. Second, in addition to the computer-based experiment, this presentation provides qualitative input from examiners in different countries. There are some common points within participants' ideas, which need to be further acknowledged by the forensic community. Third, the mix methodology between quantitative and qualitative research is a very important point as the results will provide not only a functional view (the task), but also addresses a very important issue — the examiners' motivations.

Criminal investigation is an area of interest in which there is a need to systematically retain links with forensic science disciplines. Among those, the forensic community found various disciplines that have been the target of research within cognitive bias, namely fingerprints and DNA. Until 2004, after an erroneous decision by fingerprint examiners from the Federal Bureau of Investigation (FBI), this field received enormous focus on research relative to examiners' performances.

From all of the suggestions previous research proposed, the Analysis, Comparison, Evaluation (ACE) methodology has been given an independent phase (verification), changing the methodology into what is currently known as Analysis, Comparison, Evaluation-Verification (ACE-V). On the other hand, the vision on contextual information acquired great dimensions, making this type of variable appear negative for examiners' performances.

This presentation has a different perspective. Examiners from 12 countries (England, Scotland, the United States, Brazil, China, Belgium, Germany, the Netherlands, Portugal, Switzerland, Australia, and New Zealand) were asked to perform a computational experiment in their respective laboratories. Participants (n=65) with varying experience (from trainees to senior examiners) completed tasks similar to fingerprint examinations. Within the task, participants had different types of contextual information in four blocks (control/no context; type of crime; suspect's criminal record; and a previous conclusion from another examiner].

Examiners were also individually interviewed (n=50) on topics related to the ACE-V methodology, the current guidelines within their laboratories, contextual information, and their motivation to perform their work.

Quantitative results suggest that there are types of contextual information that affect examiners' performances in a more negative way, whereas some types of contextual information do not affect performance as much. These results contradict other research that suggested examiners will make misguided decisions in their casework when they have certain types of contextual information, such as the type of crime.

Qualitative data from interviews provided a view that has not been acknowledged to a great extent. Examiners have claimed that there are some guidelines that may not be useful or even possible to accomplish. Regarding their motivation, there are two distinct types of motivation, one of these being a motivation toward performing their work being linked with having some type of contextual information or instant feedback from other departments.

This presentation opens a dialogue on the accessibility of contextual information by examiners, on their motivation (which seems to be something that has been forgotten by research), and on future guidelines in a worldwide perspective. The latter item is included here as global crime is increasingly becoming an issue, and international partnerships have been part of the communication between forensic communities (e.g., the Prüm Treaty).

B169 Resolving Latent Conflict: What Happens When Latent Print Examiners Enter the Cage?

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After attending this presentation, attendees will be better informed regarding consultation and conflict resolution methods used during the verification phase of the Analysis, Comparison, Evaluation-Verification (ACE-V) process at the Houston Forensic Science Center (HFSC). Attendees will also be advised of trends observed when this process is utilized via the study of approximately 2,000 completed latent print examination cases. The goal of this presentation is to use these results to inform policies regarding conflict resolution and case processing for other forensic disciplines.

This presentation will impact the forensic science community by fostering better understanding of consultation and conflict between examiners of different experience, training, and employment backgrounds. This study focuses on data gathered from two years and approximately 2,000 latent fingerprint examination cases completed at HFSC. The results will shed light on assessing the types of conflicts that arise and will more predictably and accurately resolve those disagreements.

Verification, which provides scientific validity and scrutiny to a reported conclusion, is the final stage of the latent print analytical process. Despite standard operating procedures and quality controls designed, in part, to mitigate differences, latent print processing and analysis inherently lends itself to conflict. This conflict arises when two latent print examiners, analyzing and comparing the same friction ridge impression, do not use or interpret the same points of data when formulating their conclusions.

In order to preemptively moderate potential conflict, HFSC's Latent Print Section has implemented a standard indicating the number of minutia required before a latent impression is deemed suitable for comparison. Despite this standard, differing levels of experience and training remain and the ability to identify minutia presence and type signals from competing factors, such as pressure and distortion effects, background interference, and processing technique, may create differences of opinion regarding the comparison conclusions. As a result, latent print examiners may consult with each other regarding the impression and the corresponding area(s) of an exemplar, noting both similarities and/or differences used to formulate their respective conclusions. From this point, a consensus agreement regarding the conclusion of the comparison may be reached; however, if the two examiners cannot agree amongst themselves, the conflict is escalated until either a supervisor or the collective decision of the examiners in the section determines the reported conclusion.

While studies have scrutinized latent fingerprint comparisons, past research has not thoroughly examined the verification stage of the process in a casework setting. This study presents an analysis of approximately 2,000 cases completed by HFSC latent print examiners. This analysis focuses on overall occurrence of consultation and conflict resolution over a two-year period with an emphasis on rate and trends of occurrence and outcomes in relation to examiner demographics, such as experience level. Inferences for policies to reduce trends in intra- and inter-experience-level conflict occurrences will be made.

Latents, Verification, Consultation

B170 The Evolution of Latent Print Testimony

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After attending this presentation, attendees will possess a long-range perspective of where latent print testimonial practices have been, where they are headed, and why they are headed there. Attendees will understand the hallmarks of the "dogma" versus the "transparent" expert and will be aware of the main arguments and literature supporting change in the way reports and testimony are presented. Attendees will also understand some potential barriers to this change and the research that is needed to reduce or remove those barriers.

This presentation will impact the forensic science community by embracing the American Academy of Forensic Sciences (AAFS) meeting theme, *Our Future Reflects Our Past: The Evolution of Forensic Science*, to examine both the past and the future of latent print testimony and reflect on how we are currently working to bridge that gap while still testifying every day during a time of change. This presentation will provide a context to the several philosophies of latent print reporting and testimony that are currently being used by different laboratories and practitioners.

Friction ridge comparison testimony in the United States has long been characterized by speaking in absolutes: fingerprints are unique, the Analysis, Comparison, Evaluation-Verification (ACE-V) methodology has a zero error rate, and the testimony presented by the expert should be regarded as an incontrovertible fact. Once the National Research Council released their watershed Report in 2009, questioning and criticizing these clear overstatements of the strength of the evidence, many commentators and professional organizations recommended that the friction ridge community rethink the way their evidence was presented in reports and in court. Yet change has been slow to come. While some agencies have begun a shift in the way they present their findings, many others still testify the same way, or nearly the same way, they always have. Differing schools of thought have evolved regarding how latent print conclusions ought to be presented, and these are causing a philosophical rift in the community.

This presentation offers the historical context of where American friction ridge testimony has been, lays out the arguments for why it needs to change, describes some recent efforts to improve, and highlights some likely directions for the future of friction ridge reporting and testimony in the United States. This presentation will include examples of some new modes of reporting and testimony, along with discussion of challenges that may accompany those new styles, such as concerns about juror comprehension and examiners' discomfort with leaving absolute source attribution behind without a quantitative model to support a probabilistic conclusion, and will also examine some of the recent literature that surrounds the issue of new styles of testimony.

Latent Print, Reporting, Testimony

B171 Reconstruction of Decompositional Events by the Use of an Arthropod Community on a Large Number of Partially Burnt Human Mummies in the Catacombe dei Cappuccini in Palermo, Italy

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After attending this presentation, attendees will understand not only how to perform a successful collection of biological stains on human corpses hundreds of years postmortem in a difficult environment that was rearranged and burnt, but also that it is possible to do so.

This presentation will impact the forensic science community by determining that entomological stains can not only be collected hundreds of years postmortem but can also be analyzed to reconstruct both decompositional events and the storage of corpses, even though the environment was burnt.

In July 2012, 622 mummies were examined in the basement of the Capuchin monastery in Palermo, Italy. Corpses were dated to between the 17th and 20th centuries. Their decompositional end state was termed "mummification" by the local population even though skeletonization and differential decomposition were much more abundant. In fact, the mummies must have passed through different, mixed stages of (often active) decomposition.

There were no statistical differences in the type of decay between mummies of males, females, monks, or regular priests nor between the mummies of persons with other occupations (lawyers, etc.). Some of the mummies were stuffed with straw (i.e., they did not contain relevant amounts of body tissue). Inside the skulls, insect remains could be collected.

Only 260 of the 622 mummies showed signs that may have been caused by insect activity (i.e., skin lesions in cases in which skin was intact). The insect remains found on those 260 partially burnt mummies were unusual. Very few blowfly remains (Calliphoridae) were seen even though this group of flies is usually very common in early decomposition. Instead, other arthropods were found (e.g., *Hydrotaea ignava* (Diptera: Muscidae), *Fannia scalaris* (Diptera: Fanniidae), *Conicera tibialis* (Diptera: Phoridae), *Leptocera* sp. (Diptera: Sphaeroceridae), *Necrobia rufipes* (Coleoptera: Cleridae), *Gibbium psylloides* (Coleoptera: Ptinidae), *Oryzaephilus surinamensis* (Coleoptera: Silvanidae), Alysiinae (Hymenoptera: Braconidae), *Tinea pellionella* (Lepidoptera: Tineidae), and a number of pseudoscorpions (Pseudoscorpionida, Arachnida)).

Certain coffins that could be opened also contained fragments of Clerid, Dermestid, and Staphylinid beetles. The insect findings were compared to arthropod recoveries from human corpses in similar climatical environments.

Comparing the mummies of males, females, virgins, monks, priests, and members of other professions, only priests and virgins showed a significant difference in insect colonization patterns (via the Wilkoxon test). This may be due to very different methods of mummification rather than a reflection of the life of the persons before death; however, the state of the teeth revealed significant differences between the health status of the groups (e.g., mostly missing teeth; also flat, filed-off teeth due to sand in baked goods — an indicator of age at death).

During further searches, a "colatoio" (preparation) room was found in which the fresh corpses had been stored, then dried and reassembled. The method of mummification was often not elaborate, and methods have changed several times over the centuries, as could be deduced from the insect remains.

Mummies, Arthropods, Decomposition

B172 DNA Recovered From a Victim's Pockets Solves a Cold Case: A Case Study

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After attending this presentation, attendees will understand the importance of utilizing techniques currently used in the field today to help aid in the re-examination of old evidence in unsolved cold cases.

This presentation will impact the forensic science community by encouraging the re-examination of the victim's pockets of clothing worn at the time of death in crimes in which robbery could be a possible motive. Evidence that is suitable for further testing should then be swabbed for the presence of touch DNA left behind by the perpetrator.

A case study will be presented that highlights this method of swabbing the victim's pockets for touch DNA left behind by the perpetrator, which ultimately aided in solving the case after 22 years. The perpetrator can deposit skin cells when reaching into the victim's pockets in the process of robbery. This touch DNA can then be collected and analyzed in order to develop a DNA profile.

On the night of November 6, 1993, a witness stated he saw a car driving down the street and then stopping. Minutes later, he heard gunshots and saw an individual exit the passenger side of the car and flee. A 17-year-old boy was found in the driver's side area of the car shot multiple times in the head. The victim's left front pants pocket was turned inside out and there was change lying on the ground next to the driver's door.

In 2015, a request was made by the prosecutor's office cold case unit to re-examine the victim's clothing worn at the time of death. The pants were examined and the pockets were swabbed on the inside for the presence of touch DNA. The swabs were extracted using organic extraction methods. The extracted DNA was quantified using the Quantifiler® Duo quantification kit using a Real-Time Polymerase Chain Reaction (RT-PCR) instrument. The DNA was amplified by using the PowerPlex® Fusion amplification kit. The amplified DNA was loaded onto the genetic analyzer for the detection of DNA fragments. Fragment analysis was performed using GeneMapper® ID software.

A Short Tandem Repeat (STR) DNA mixture profile was developed from the victim's left front pants pocket. The DNA mixture consisted of the victim and an unknown major contributor. This unknown profile was entered into the Combined DNA Index System (CODIS). The unknown profile hit to a convicted offender. After releasing the name to the agency, it was learned that this individual had been interviewed in the initial investigation and was a person of interest.

In conclusion, touch DNA in the victim's pockets that was left behind by the perpetrator over 20 years ago was still able to be collected and a DNA profile developed, which ultimately aided in solving the case.

Touch DNA, Cold Case, Criminalistics

B173 The Identification of Silk Forgeries

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After attending this presentation, attendees will better understand how to use Capillary Electrophoresis/Mass Spectrometry (CE/MS) to identify naturally aged vs. artificially aged silk.

This presentation will impact the forensic science community by introducing proteomics techniques and explaining how they could be used in forgeries.

Forgery in art and cultural heritage is almost as old as the objects themselves; however, cultural heritage forgery is seldom investigated in forensic settings. The identification of the forgery is similar to the forensic analysis of documents, inks, and paints and similar to age identification of proteinaceous specimens, such as hair or teeth. In this presentation, one of the most famous cases of silk forgery in the United States will be discussed as will the analytical techniques developed and used to identify the real and forged specimens. Forgery identification includes the identification of the materials, dyes, and age of the specimens.

In 1924-1925, a number of medieval Islamic silks were excavated from tombs at a site called Bibi Shahrbanu near Rayy, Iran. Although these silks were not necessarily all from the Buyid dynasty, as a collective they are referred to as the "Buyid" silks as they are thought to be from that approximate time period. Additional pieces surfaced in the 1940s, but doubts arose regarding the authenticity of some of the fragments, which led to discussions among curators, art historians and dealers. Accurate dating of many Buyid fragments has yet to be ascertained.

Recently, several new techniques for dating proteinaceous historical artifacts have been developed using modern separation/mass spectrometry and proteomics techniques.[1] By using these techniques, several biomarkers of natural aging in proteinaceous cultural heritage objects were identified. The dating study is based on racemization and deamidation of amino acids in proteinaceous specimens. CE/MS was used to measure D-amino acid/L-amino acid (D/L) of Aspartic Acid (Asp) and a proteomics technique was employed to measure the deamidation rate of asparagine in silk proteins. To test the authenticity of the Bibi Shahrbanu silk, 12 samples from the Textile Museum in Washington, DC, were analyzed and the change in racemization and deamidation in both authentic and unknown silk samples was compared. These results were also compared to the rate of racemization and deamidation of the artificially aged silk proteins. Modern silk samples were aged using pH, heat, and radiation. Thirteen silk fragments were HCl digested and analyzed by chiral CE/MS and the D/L Asp was measured. For the deamidation study, these samples were enzymatically digested and analyzed by proteomics techniques. Two of the fragments that were originally purchased by the museum were among these samples. One of these fragments was carbon dated and used as the control. The results were consistent with the skepticism regarding the authenticity of nine fragments and pointed to the possibility that they were artificially aged to look older. The results of this investigation will be discussed.

Reference(s):

1. Moini M., Rollman C., France C. Dating Human Bone; Is Racemization Dating Species Specific? *Anal Chem.* 2013, 85, 11211-5.

Silk Forgery, Proteomics, Racemization Dating

B174 Lessons From Inside the Barrel: A Forensic Case Report of a Double Gunshot Suicide

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After attending this presentation, attendees will understand the complexity and importance of the interpretation of biological traces in challenging forensic gunshot cases.

This presentation will impact the forensic science community by highlighting the value of precise and cross-sectional analyses of biological traces involving molecular methods in forensic settings and examinations.

A 45-year-old man was found dead, seated in the driver's seat of a vehicle parked next to a remote cemetery in the countryside. The right hand of the corpse was lying on its lap, closed around a .38 special Smith & Wesson® revolver. Behind the driver's seat, balanced between the front-seat pocket and the back seat row was a 9mm Glock® handgun, the barrel pointing toward the front. The corpse presented with a gunshot entry wound on the right temple with a corresponding gunshot exit wound above the left ear. Interestingly, a second gunshot entry wound was found at the back of the neck with an exit wound located on the chin, slightly to the left of the facial midline.

A thorough external examination of the corpse by forensic experts was performed onsite, during which backspatter was identified and collected on the right hand of the corpse, but not on the left. To complete the forensic investigations, an autopsy and post-mortem imaging (Computed Tomography (CT) and Magnetic Resonance Imaging (MRI)) were performed. In addition, both firearms seized onsite were subjected to endoscopy of the barrels, visualizing the biological stains inside and enabling their systematic and precise collection by DNA-free cotton swabs moistened with sterile, desalted water. Analysis of the collected biological material was performed in the context of a research project funded by the Swiss National Foundation which investigates the phenomenon of biological stains inside gun barrels after close-contact gunshots.

Interdisciplinary work involving forensic and criminal investigation experts allowed a reliable and comprehensive reconstruction of the incident, revealing that the two firearms had been fired simultaneously with close contact of both muzzles to the skin, confirming the act to be suicidal. Analyses of organ-specific regulatory RNA, more specifically microRNA (miRNA) in the biological traces collected from the right hand of the corpse and from the barrel of the .38 special Smith & Wesson® revolver, which had inflicted the shot to the right temple, revealed both blood-specific and brain-specific miRNA. Interestingly, on the swabs collected from the barrel of the Glock® handgun, which had equally been used with close contact to the skin at the back of the neck, causing extensive injury to the spinal cord, no organ-specific miRNA traces could be detected.

The presented case demonstrates the complexity of backspatter composition and distribution as a function of different gunshot wound locations, firearms, and munitions. Importantly, this case substantiates the reconstructive value of cross-sectional molecular analysis methods and points toward their increasing importance in forensic examinations in the future.

Firearms, Contact Shot, Backspatter

B175 Gunshot Residue (GSR) Identification Errors and Sample Significance

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The goal of this presentation is to describe analyst errors made in the interpretation of spectra by Energy Dispersive X-ray Spectroscopy (EDS) of GSR samples in the Scanning Electron Microscope (SEM), as well as population statistics as they relate to GSR sampling.

This presentation will impact the forensic science community by describing errors in the interpretation of EDS spectra in GSR particle analysis made in case work and in determining sampler significance for GSR.

SEM/EDS is being used by crime laboratories worldwide for the analysis of GSR samples in shooting cases. Unfortunately, the operators of these instruments often have inadequate training for the interpretation of EDS spectra, usually relying solely on software identification of peaks. Newbury analyzed this problem, but did not focus on mistakes made in GSR analysis.[1] Element peaks are misidentified, missed, or fabricated.

Case 1: Arsenic with the major sulfur peak confused for lead.

Case 2: Mistaken assignment of trace antimony in particles with major barium and trace calcium.

Case 3: Misreporting spectra due to software misplacement of element symbols on spectra.

Case 4: Missing element peaks that are obscured by peaks of other elements.

Case 5: Claim of trace element presence (antimony or lead) using presumptive (thumbnail) spectra listings without confirmation spectra.

Case 6: Assignments of particle spectra to consistent, "highly specific" (= characteristic), "unique" (= characteristic), and characteristic GSR.

Case 7: Ignoring a major contribution of iron (+ trace copper) in antimony/barium particle spectra (= friction-brake particles) and calling these particles GSR.[2]

Case 8: Failure to identify the source of trace elements represented in a GSR spectrum. Barium sulfate particles are ubiquitous in the urban environment and often well-populate a sampler. Thus, nearby barium-containing particle(s) may contaminate a particle's spectrum. This can occur for the consistent GSR particles composed of lead and antimony which produces an erroneous assignment of characteristic GSR.

For many crime lab analysts, there is a failure to consider statistical significance of a GSR particle burden of sampler. The SEM/EDS analysis results of the (usually) two samplers from a suspect (left and right hands) require statistical criteria for acceptance of these samplers as representative of a GSR population that existed on the suspect's hands prior to sampling. A single-characteristic GSR particle without supporting consistent GSR on a sampler cannot be separated from a rare contamination event or outlier.[3] This is especially true due to possible GSR contamination in the police environment from officers, who occasionally qualify their firearms, or GSR-contaminated suspects who have been in the police environment previous to the suspect. The criterion for a GSR sampler to have a significant burden of characteristic (Lead, Antimony, and Barium (PbSbBa)) GSR particles is three (except perhaps in military cases); however, the criterion for assigning significance to a sample when it has one characteristic GSR particle with two or more consistent GSR particles has not been established.[4]

There is no reason not to combine the two samplers from a suspect's hands for the determination of sampler significance.

Reference(s):

1. Newbury D. Mistakes encountered during automatic peak identification of minor and trace constituents in electron-excited energy dispersive x-ray microanalysis. 2009. *Scanning*. 31:1-11.
2. Torre C., Mattutino G., Vasino V., Robino C. Brake linings: A source of non-GSR particles containing lead, barium and antimony. 2002. *Journal Forensic Sci*. 47(3):494-504.
3. Bernet V., Lewis T. *Outliers in statistical data.* 3rd edition 1994. John Wiley and Sons, New York.
4. Wright D.M, Trimpe M.A. Summary of the FBI Laboratory's gunshot residue symposium. 2006. *Forensic Sci. Communications*. 8(3):1-17.

B176 A Post-Incident Investigation of Ammonia Contamination of Food Products in a Cold Storage Facility

Kelly L. Wouters, PhD, Armstrong Forensic Lab, Inc, 330 Loch'n Green Trail, Arlington, TX 76012*

After attending this presentation, attendees will understand a protocol used to conduct a large-scale investigation regarding the sampling and analysis of numerous food products located in a cold storage facility, following an ammonia refrigerant leak. The protocol includes the development of statistically based sampling plans, sample collection procedures, and analytical methodologies.

This presentation will impact the forensic science community by providing examples and suggestions for dealing with similar incidents in the future. No published guidelines are available for this type of investigation. This presentation will illustrate the decisions and flexibility required to overcome the specific challenges associated with interfacing between numerous parties, including the business representatives, insurance companies, attorneys, and both state and federal governmental agencies.

This presentation will discuss an extended investigation following an ammonia refrigerant leak at a large-scale cold storage facility. Numerous types of food products were stored in the warehouse, including baked goods, meats, and seafood. Due to the variations in product compositions, packing techniques, exposure potentials, and requirements imposed by regulatory bodies, several different processes were utilized for sample collection and analysis.

The sampling plan for most of the products evaluated was based on a hypergeometric distribution, a statistically based sampling method commonly utilized by forensic scientists in other applications. The sampling plans were modified, as needed, to suit the needs and requests of the specific product owners. In total, more than 3,000 samples were collected for evaluation and analysis.

The primary instrumental analytical technique utilized for the investigation was Ion Chromatography (IC). Residual ammonia, when dissolved in the moisture contained in the product and/or extracted under acidic conditions, will be in the form of the ammonium cation. The IC technique is a sensitive method for detecting and identifying ionic species, including ammonium. For some products, organoleptic testing was employed at the request of regulating authorities. The organoleptic testing involved both taste and odor evaluations via a panel of analysts. All testing was based on published methodologies, adapted for use in this unusual application.

A summary of the analytical data and observations will be presented. Strategies for data interpretation will be discussed. Overall, the results of the investigation established the ammonia release had little to no impact on the food products stored at the facility. No health hazards were indicated, although ultimately, the decision regarding whether the products were suitable for public consumption was not made by the laboratory. The final decisions regarding the acceptability of the affected products varied greatly, depending on many factors. Over the course of this extensive investigation, numerous unexpected twists and challenges were encountered and overcome, and the narrative of the project should be both engaging and informative to other forensic investigators.

Ammonia, Food Products, Ion Chromatography

B177 The Indirect Detection of Bleach (Sodium Hypochlorite) in Bleach-Tainted Infant Eye Drops

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After attending this presentation, attendees will better understand the methodology that can be used to test a matrix for the presence of bleach (sodium hypochlorite), even if all of the active ingredient in the bleach has broken down, will learn how suspect eye drops were determined to be positive for bleach, and how this information was used in court to convict a mother of assaulting her daughter.

This presentation will impact the forensic science community by shedding light on bleach detection in difficult matrices and by illustrating the possibility of identifying bleach in a matrix even when all of the active ingredient in bleach has broken down.

An eye drop sample that reportedly left a pharmacist ill after inhaling its fumes, left a detective with a chemical burn on her skin after spilling it, and left a toddler blind after receiving it as treatment for a month from her mother was received for analysis at the Forensic Chemistry Center (FCC). The suspect eye drops tested positive for an oxidizer, had a pH of 6.1 when received, contained chloride and chlorate, but tested negative for bleach.

Sodium hypochlorite (NaOCl) is a strong oxidizer that is used as a bleaching agent, a sanitizer, a clothing whitener, and a deodorizer. It is readily available in most households. It is caustic, causes damage to tissues when it comes in prolonged contact with the human body, has chloride and chlorate as breakdown products, and rapidly degrades in matrices that can be oxidized. Methodology to characterize the stability of sodium hypochlorite in beverages has been developed and published by the FCC.

Twenty-three beverages were spiked at three levels with sodium hypochlorite and were monitored for sodium hypochlorite stability, pH, chloride and chlorate content, and visual and organoleptic characteristics over a 13-day period. This study revealed that sodium hypochlorite adulteration can be determined in a suspect matrix even when all of the active hypochlorite has broken down. This is accomplished using spot tests for oxidizing agents and Ion Chromatographic (IC) anion analysis for bleach degradation products. Based on the excess chloride found in the suspect eye drops, the amount of equivalent bleach was estimated and spiked into control eye drops to be used for comparison to the suspect eye drops. Comparisons were made using IC, Gas Chromatography/Mass Spectrometry (GC/MS), Stereoscopic Light Microscopy (SLM), Fourier Transform Infrared (FTIR) spectroscopic imaging, and Liquid Chromatography/Charged Aerosol Detection (LC/CAD). It was determined that the laboratory-fortified control eye drops were very similar to the suspect eye drops using the above-listed techniques. These results were presented in court and led to the conviction of the toddler's mother, who was sentenced to 40 years in prison for the assault.

Sodium Hypochlorite, Bleach, Eye Drops

B178 What Happened to Flight MH17?

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After attending this presentation, attendees will better understand how the origin of an airplane explosion may be determined and, more specifically, what brought down Flight MH17 and how Disaster Victim Identification (DVI) processes can benefit from the inclusion of new technologies.

This presentation will impact the forensic science community by familiarizing attendees with some of the results of the initial investigation into the crash of Flight MH17. Non-accidental airplane crashes are relatively rare events. This presentation will discuss how to obtain and examine relevant materials from one of these events. This presentation will also explain the benefits of designing DVI processes to include forensic examinations; in particular, the novel inclusion of mobile Computed Tomography (CT) scanners, dual-beam X-ray scanners, and hand-held X-ray scanners.

At approximately 3:15 p.m. on July 17, 2014, Malaysia Airlines Flight MH17 from Amsterdam to Kuala Lumpur crashed in the vicinity of Hrabove in the eastern Ukraine province of Donetsk. All 298 passengers and crew were killed. Initial reports hinted of a non-accidental cause of the crash. At the time of the crash, an armed conflict between Ukrainian military and armed separatist forces was taking place in the crash area, which precluded the possibility of examinations at the crash site.

One week later, on July 23, 2014, the first human remains arrived in the Netherlands. At the military base in Hilversum, a disaster mortuary had been established at the Corporal Van Oudheusdenbarracks. The DVI process took place at this location.

Prior to start of the DVI process, plans were made to include forensic examinations within the DVI process, as this might be the only opportunity to obtain forensic evidence to determine what happened to Flight MH17; however, the DVI process took top priority as it could not be impeded as a result of the forensic examinations.

The multidisciplinary approach used at the Netherlands Forensic Institute (NFI) to examine invasive traumas led to a plan that included the use of a mobile Computed Tomography (CT) scanner, a dual-beam X-ray scanner, and a portable X-ray scanner to screen the human remains for foreign (metal) fragments.[1-3] It was thought that once recovered fragments had been identified as being non-airplane in nature, this information could be combined with a passenger seating map to determine the origin of the explosion.

The recovered fragments were sent to the NFI, where they were tested for explosive residues. After the fragments were cleaned, they were visually examined. Those of unknown origin were further analyzed with Scanning Electron Microscopy in combination with Energy Dispersive X-ray (SEM/EDX) analysis. This analysis confirmed that multiple fragments were unalloyed steel on which molten and resolidified layers were present. With the use of a Focused Ion Beam (FIB), local cross-sections were made and it became clear there were aluminum and glass layers present on the particles. The elemental composition of the glass layers revealed zirconium was present in the glass. A subsequent query of the NFI glass database disclosed only a single hit of glass containing zirconium: a cockpit window.

In December 2014, parts of the wreckage arrived at the Gilze-Rijen airbase in the Netherlands. From the wreckage, more fragments were recovered and analysis determined the same results as those obtained from the human remains. Reference samples from various glass sources in the airplane verified that only the outer and inner layers of the cockpit windows were made of zirconium-containing glass. This means that the steel fragments originated outside of the airplane and that probably a missile brought down Flight MH17.

Reference(s):

1. Vermeij et al. Analysis of microtraces in invasive traumas using SEM/EDS. *Forensic Sci. Int.* 214(1) pp. 96-104.

2. Zoon et al. Microanalysis of Invasive Traumas - an integrated multidisciplinary approach into the manner of death. Chin. *J. Forensic. Sci.* (2012), 4, pp. 54-61.
3. Vermeij et al. Microscopic Residues of Bone from Dissolving Human Remains in Acids. *J. Forensic Sci.* 60(3) 770-776.

MH17, Explosion, DVI

B179 DNA Testimony in the Past, Present, and Future

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After attending this presentation, attendees will be aware of various perspectives on the evolution of forensic DNA-related testimony, will realize what approaches are more effective for judges and juries, and will benefit from the opportunity of asking questions of the invited panel.

This presentation will impact the forensic science community by encouraging a dialogue between DNA experts and members of the legal system.

DNA fingerprinting, as it used to be known, was first applied to criminal cases in the late 1980s. Most notably, Dr. Alec Jeffreys used his technique in 1986 to exclude a 17-year-old boy as the perpetrator of two sexual assaults in the Leicestershire area of England and to later identify the true perpetrator, Colin Pitchfork. The first person convicted by DNA evidence in the United States was Tommy Lee Andrews, who was found guilty in 1987 in Orange County, FL. At the end of 1988, the Technical Working Group on DNA Analysis Methods (TWGDAM) held its first meeting to discuss the best approaches to implementing DNA analysis in crime laboratories, including quality control measures. This ultimately led to the Guidelines for a Quality Assurance Program for DNA Analysis in 1991 (updated in 1995). These guidelines have since evolved into the Quality Assurance Standards (QAS) required by the Federal Bureau of Investigation (FBI) for all forensic DNA laboratories that participate in the national DNA database system, Combined DNA Index System (CODIS). Due to the solid foundation provided by the QAS, DNA analytical techniques have successfully endured admissibility hearings and have been accepted in courts throughout the country over the last 30 years.

In those early days, most DNA testing was restricted to blood and semen. These types of stains yielded relatively straightforward Restriction Fragment Length Polymorphism (RFLP), and later Short Tandem Repeat (STR) Polymerase Chain Reaction (PCR), profiles. Mechanisms were established and promoted by TWGDAM and later the QAS to allow the objective comparison of evidence results to known references. If a match was determined, the statistical significance of the match was reported. In this way, DNA testimony differed from the more traditional police sciences of fingerprint analysis and firearms comparisons in which there were generally no qualifications made on putative identifications other than the expert's opinion.

As technology improved, the nature of biological evidence expanded beyond the rich sources of DNA to extremely trace level samples. This increased complexity of the resulting DNA profiles, their interpretation, and the statistical analysis applied to inclusions. Consequently, presenting DNA analysis to juries has shifted focus from describing the nature of DNA and its capacity to identify individuals to explaining how DNA can be transferred between objects and the intricacies of dropout and mixture ratios. Future technologies promise to provide additional levels of information from biological material beyond today's STR profile or Single Nucleotide Polymorphism (SNP) haplotype, such as the type of cell, the age of the stain, the physical description, and even the age of the donor. How will this impact future testimony?

A two-hour block of time has been devoted to explore the past, present, and future of DNA testimony. The panel convened for this session will provide the perspective of the judge, the prosecutor, the defense attorney, the DNA expert, and the independent consultant on effective testimony techniques and will discuss the pitfalls to avoid in light of the emerging changes in DNA interpretation and statistics.

DNA, Testimony, Statistics

B180 Ashes to Ashes: An Analysis of Enhanced Methods for Genetic Identification of Human Cremated Remains

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After attending this presentation, attendees will better understand the potential and limitations of genetic investigations of cremated human remains.

This presentation will impact the forensic science community by providing a way to gain maximum information from cremated human remains, which have historically been very difficult to identify.

Human cremation is a common funeral practice in many cultures throughout the world. In the United States, the preference for cremation continues to increase, with the rate of cremation soon expected to surpass that of burials. After cremation, there may be a need to confirm the identity of the remains for reasons including civil or criminal cases, paternity or kinship analysis, or identification of missing or deceased individuals. This has traditionally fallen to anthropologists, who can employ various metric analyses to assess whether the contents of an urn are in fact human cremated remains and can assist in determining a biological profile including sex, stature and weight.

Historically, genetic examination of cremated remains has been limited due to lack of remaining DNA and concerns regarding contamination. The cremation process involves burning at temperatures up to 1,000°C, reducing the corpse to skeletal remains or bone fragments, which are then often further ground to a sand-like consistency; however, depending on the method used to grind the remains, sizeable bone fragments may still be found in the urn returned to family members. Previous investigations into DNA analysis from cremated remains have shown limited success. Studies testing the sand-like portion of the remains found that small amounts of nuclear and mitochondrial DNA were recoverable, but the potential for contamination was too great to ensure reliable results, while examination of remaining bones or teeth resulted in little to no DNA recovery; however, advances in DNA extraction and detection methodologies may present an opportunity to re-examine the process of genetic identification from the remaining bone fragments.[1,2]

This work examines adjustments or enhancements along the entire DNA analysis workflow to improve identification methods from human cremated remains. DNA was extracted from bone fragments using either a commercial silica-based method or an enhanced extraction method that modifies the commercial kit to determine which process resulted in maximum DNA recovery. Multiple extracts from a single bone fragment were pooled, then concentrated using an Eppendorf Vacufuge™. All extracts were quantified using real-time Polymerase Chain Reaction (PCR) to assess nuclear or mitochondrial DNA (mtDNA) recovery. Concentrated extracts were then amplified using either the GlobalFiler® PCR Amplification Kit with an increased PCR cycle protocol and analyzed using capillary electrophoresis or an in-house-developed whole mtDNA genome multiplex for sequencing on the Illumina® MiSeq®. Multiple samples from individual sets of remains were examined to assess consistency across results.

Results indicate that low levels of nuclear DNA can be recovered from cremated bone, with partial Short Tandem Repeat (STR) profiles obtained from pooled and concentrated extracts; however, exaggerated stochastic effects such as increased stutter, allele drop-out, and peak-height imbalance were observed in some profiles due to the low amount of starting template and use of an increased-cycle PCR, thereby complicating the profile interpretation. Results also revealed that sufficient mtDNA can be recovered from cremated remains for whole mtDNA genome sequencing using a multiplex amplification approach. Massively parallel sequencing of amplified mtDNA yielded an average coverage of 10,500 reads across the genome, with 97% of the genome covered by at least 100 reads. Preliminary variant analysis suggests the remains are likely to be from a single source.

Reference(s):

1. von Wurmb-Schwark N., Simeoni E., Ringleb A., Oehmichen M. Genetic investigation of modern burnt corpses. *Int Congr Ser.* 2004:1261:50-52.

2. Tsuchimochi T., Iwasa M., Maneno Y., Koyama H., Inoue H., Isobe I., Matoba R., Yokoi M., Nagao M. Chelating resin-based extracting of DNA from dental pulp and sex determination from incinerated teeth with Y-chromosomal alphoid repeat and short tandem repeats. *Am J Forensic Med Pathol.* 2002:23(3):268-271.

Cremated Remains, STR Profiling, MtDNA Sequencing

B181 The Short-Term Effects of Surface and Subsurface Burial on DNA From Human Skeletal Remains

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After attending this presentation, attendees will understand how mitochondrial and nuclear DNA quantities vary within the human femur and how they are affected by short-term soil surface exposure and burial.

This presentation will impact the forensic science community by providing insight into both the variable levels of DNA throughout human femora and the differential degradation after short-term surface and subsurface burial.

Forensic biologists utilize human skeletal remains as a source of DNA for identification purposes. In particular, the femur and other weight-bearing long bones are commonly sampled at the midshaft diaphysis for DNA analysis in a forensic setting; however, Mundorff and Davoren demonstrated that DNA quantities vary throughout the human skeleton, with cancellous bones such as the patella and tarsals producing greater profiling success than the midshaft of weight-bearing long bones, although only one location on each bone was tested.[1,2] Previous research conducted at Michigan State University using bovine and porcine models demonstrated that DNA quantity and quality vary widely within a femur, with higher DNA yields at the epiphyses than at the diaphyses.[3] In contrast to these animal models, DNA variation within human bones has yet to be rigorously examined.

Following death, skeletal DNA begins to degrade, which can be exacerbated by unfavorable environmental factors such as temperature, moisture, pH, and burial conditions. Over time, this results in reduced DNA yields, potentially to a point where DNA is no longer retrievable; however, exactly when and to what extent DNA degradation occurs during the taphonomic process is unresolved. The goal of this study was to establish the short-term effects of surface and subsurface burial on human femoral DNA throughout the length of the bone.

Three unpreserved human bilateral femur pairs were obtained. The majority of soft tissue was removed manually, and the bones were subsequently macerated in a 0.5% Terg-a-zyme® solution. A Dremel® tool with a cobalt drill bit was bleached and Ultraviolet (UV) -irradiated and used to drill five locations on each femur (midshaft, proximal and distal diaphysis, femoral neck, and patellar groove). Bone powder was collected and its mass recorded. One femur was buried in approximately 30cm of soil, while its counterpart was placed on top of the soil, exposed to the elements. A large dog crate with the bottom removed was staked to the ground in order to protect the remains from scavengers. Femora were retrieved from the site after seven days and resampled. The powder was digested in a demineralization buffer and a 1:1 phenol-chloroform extraction was performed, followed by Amicon® column filtration. DNA extract volumes were measured prior to storage at -20°C.[4]

Mitochondrial DNA quantities at the five femoral locations were examined using an in-house, human-specific, TaqMan® assay. An internal positive control template was included to assess any Polymerase Chain Reaction (PCR) inhibition. A Quantifiler® Human DNA Quantification Kit was used to assess nuclear DNA quantity. For both mitochondrial and nuclear assays, samples that demonstrated signs of inhibition were diluted tenfold and re-tested.

Results demonstrate that prior to surface exposure or subsurface burial, mitochondrial DNA quantity was highest in the more distal femoral regions (distal diaphysis and patellar groove), whereas nuclear DNA quantity was greater at the proximal end (neck and proximal diaphysis). Both surface and subsurface burial usually resulted in sharp decreases in DNA yields, sometimes by as much as 90%, even after only seven days. Bones that were placed on the soil surface had overall higher mitochondrial and nuclear DNA yields throughout the bone than did their buried counterparts. Finally, the proximal diaphysis maintained higher mitochondrial and nuclear DNA quantities after surface and subsurface burials than did the more peripheral regions.

In conclusion, short-term burial can have a strong and extremely rapid degrading effect on DNA from human bone, the extent of which varies across the length of the femur. Contrary to conventional thinking, the midshaft diaphysis generally does not have higher DNA yields before or after burial. Owing to this, the midshaft diaphysis should not be viewed as the optimal location for DNA recovery from human femora.

Reference(s):

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2. Mundorff A.Z., Davoren J.M. Examination of DNA Yield Rates for Different Skeletal Elements at Increasing Post Mortem Intervals. *Forensic Science International: Genetics.* 8:55-63.
3. Antinick T., Foran D.R. 2015 Intra-Bone Variation of Recoverable Nuclear and Mitochondrial DNA in Femora. *Proceedings of the American Academy of Forensic Sciences*, 67th Annual Scientific Meeting, Orlando, FL. 2014.
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DNA Quantity in Bone, Skeletal DNA and Burial, DNA Degradation

B182 A Specialized Workflow for Charred and Degraded Human Remains That Leads to Identifications

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After attending this presentation, attendees will learn how to obtain probative results from samples that have been degraded after prolonged exposure to fire. Attendees will see the effect of sample degradation and sample inhibition on forensic DNA cases and will learn about the tools that are available to address theses issues. Two cases, one from a recent transport disaster event and one from a missing persons' identification case from a 20th-century military conflict, will be presented to illustrate the typical condition of samples obtained from crime scenes and evidence exposed to fire.

This presentation will impact the forensic science community by identifying and sharing best practice methods that have enabled results to be generated from highly compromised samples that previously would have been unlikely to produce probative results. Attendees will learn bone and tissue preparation methods that can improve downstream success and, in these cases, led to positive identifications.

As a result of both the improved recovery of DNA from crime scene samples and increases in the sensitivity of forensic DNA testing systems, there is increased understanding of the robustness of DNA testing from degraded samples. Forensic analysts, therefore, are more frequently encountering challenging samples that require a specialized workflow to obtain probative data. These samples are often highly degraded and have been exposed to multiple Polymerase Chain Reaction (PCR) inhibitors from various environmental conditions. Degradation becomes one of the greatest challenges when processing evidence that has been subjected to fire. The length of exposure, the temperature of the fire, and preservation of evidence after collection affects the ability to recover a DNA profile with traditional Short Tandem Repeat (STR) testing systems.[1]

To improve the ability to maximize recovery from these complex tissue and bone samples, a specialized workflow has been developed. It involves combining efficient extraction, recovery, and subsequent analysis using commercially available technologies, including next generation autosomal STR marker systems, a highly sensitive quantification system, and an *Alu*-based maker system designed for highly degraded samples. This presentation will detail the proposed workflow, including tissue and bone preparation, analysis, and interpretation.

Two cases will be presented, one recent and one from a 20th-century military conflict. In both cases, samples were exposed to prolonged high-temperature fires. These cases will demonstrate the process that was used to achieve probative results from highly compromised samples exposed to and consumed by fire. In both cases, sufficient STR data was obtained to make a positive identification based on a family reference sample.

Reference(s):

1. K Ph.D, Kadunc R., Mann G., McLaughlin S. Comparison Of Quantity And Quality Of DNA Recovered From Burn Samples In Which Burn Temperatures And Conditions Were Varied. *The Internet Journal of Forensic Science. 2009 Volume 4 Number 2.*

Degraded, Bone, Human Remains

B183 DNA-Protein Crosslink Reversal and Mitochondrial DNA Amplification From Formaldehyde-Treated Unknowns From the Korean War

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After attending this presentation, attendees will understand the concept of formaldehyde-induced DNA-protein crosslinks and their effect on Polymerase Chain Reaction (PCR). Attendees will further learn how to use heat denaturation to reverse DNA-protein crosslinks for successful DNA amplification. The downstream ramifications of heat denaturation will be explained, and protocol modifications to accommodate single-stranded DNA (ssDNA) will be discussed.

This presentation will impact the forensic science community by offering guidance on how to successfully reverse formaldehyde-induced DNA-protein crosslinks from embalmed human remains.

Of the more than 800 Korean War Unknowns that were embalmed in formaldehyde and buried in the National Memorial Cemetery of the Pacific in Hawaii, only a few dozen have been identified to date. The formaldehyde treatment of the remains inflicted DNA-Protein Crosslinks (DPCs) that inhibit standard PCR amplification. Since the 1990s, attempts to conduct DNA testing of these Unknowns have been largely unsuccessful until Next Generation Sequencing (NGS) technologies became available. In 2015, the Armed Forces DNA Identification Laboratory (AFDIL) validated a hybridization capture and Illumina® sequencing protocol to obtain mitochondrial genome data from these and other degraded DNA samples. While this method was shown to be nearly 100% successful when tested with traditional degraded samples, it proved to be half as robust for the formaldehyde-treated Korean War Unknowns (with a success rate of 47%). Moreover, the DNA that could be sequenced from these Unknowns was shown to be very short in length, approximately 65 base pairs (bp) on average. The observations made over the course of the NGS validation indicated that most of the endogenous DNA was unamplifiable due to the presence of inhibitory DPCs.

To bolster the success rate of DNA testing for this set of Korean War Unknowns, a novel DPC reversal and DNA extraction procedure was recently developed at the AFDIL. The procedure involves standard bone powder demineralization, DPC isolation and denaturation, followed by proteinase K digestion, and DNA extraction and purification. For this, 29 samples and 6 reagent blanks were extracted with varying DPC denaturation temperatures (65°C, 72°C, and 95°C) and DNA purification methods (QIAGEN® EZ1® DNA Investigator, Microcon®, and Amicon® Ultra-4). After DPC reversal extraction, DNA was quantified using the Qubit® fluorometric assay as well as the Plexor® HY System. Amplification was attempted using a modified AmpFℓSTR® Y Filer® technique as well as mitochondrial control region primers. The results indicate that higher temperature (95°C) improves DPC denaturation and enables amplification of 100bp-250bp mitochondrial DNA fragments. Furthermore, this study demonstrated that DPC reversal at high temperature renders DNA single stranded, which has downstream ramifications. Specifically, ssDNA can lead to DNA loss when silica-based and filter-based purification methods are employed. In addition, the validated NGS library preparation protocol at the AFDIL requires double-stranded DNA substrate. Consequently, DPC reversal requires DNA extraction techniques that are amenable to ssDNA, such as phenol chloroform extraction, as well as a complementary-strand DNA synthesis step prior to NGS library preparation. The results of the DPC reversal extraction and DNA amplification will be presented and future goals for protocol development will be discussed.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense, its branches, the Defense Health Agency, the Armed Forces Medical Examiner System, or the United States government.

Crosslink, DNA Extraction, Heat Denaturation

B184 The Success of DNA Testing of Skeletonized Human Remains and the Comparison of Organic vs. Inorganic Extraction Protocols

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After attending this presentation, attendees will understand not only the success of skeletal elements for DNA testing as a whole, but will also increase their knowledge regarding different extraction techniques.

This presentation will impact the forensic science community by providing information on different extraction techniques for DNA testing of skeletonized human remains. This presentation will also encourage attendees to be flexible in protocols and procedures to allow for greater success.

Since 1992, the Armed Forces DNA Identification Laboratory (AFDIL) has processed more than 16,000 skeletal elements received from the Defense POW/MIA Accounting Agency (DPAA) for multiple DNA testing platforms. These skeletal elements have typically been exposed to a variety of environmental insults postmortem and range in time of death from 40 years to slightly more than 80 years. While the initial testing strategy focuses on Sanger sequencing of the mitochondrial DNA (mtDNA) Control Region (rCRS), in the past ten years, autosomal Short Tandem Repeat (STR) testing has become an increasingly prevalent testing option. Currently, more than one-third of the casework reported each month is some form of STR analysis. These include Low-Copy Y-chromosomal Short Tandem Repeat (Y-STR) Analysis (LCN-Y) and a variety of STR testing kits.

With the shift in focus to STR analysis, in addition to a desire to use less dangerous chemicals, AFDIL implemented an inorganic DNA extraction procedure in 2013. Validation studies indicated inorganic extractions provided a cleaner DNA extract, which is more suitable for STR analysis; however, there was some concern that the extract was too clean for the existing mtDNA protocols which were adapted specifically for extracts that included low-quality DNA as well as some inhibitory materials.

Over the subsequent 3.5 years, sample types have been tracked to determine the efficacy of inorganic extractions. While the general trend in STR analysis has seen a marked improvement, from ~20% success to a current 44% success, mtDNA success has slipped, from 92% to 85%. Initial evaluation of the samples indicates that skeletal materials exposed to inhibitory materials, such as formalin or fuel, were less successful overall when extracted using an inorganic protocol. In such instances, samples were re-extracted using a Phenol-Chloroform-Isoamyl Alcohol (PCIA) -based organic extraction and generated successful mtDNA results. An organic extraction protocol has been re-incorporated into day-to-day testing for severely compromised skeletal remains.

Frequently, the forensic community is eager to adopt a single strategy for processing of casework samples. A workflow with minimal decision points is simpler, especially when faced with a large number of samples or complex cases. This is seen to be of one of the great appeals in the move toward automation; however, this evaluation of extraction protocols used for skeletonized remains indicates that a careful evaluation of the sample upon receipt and a selection of a workflow based on downstream needs may be valuable. It is also indicative of a need to maintain multiple protocols within a laboratory, rather than to suffer loss of success or capabilities by eliminating "outdated" protocols.

DNA, Extraction Techniques, Skeletonized Remains

B185 A Comparison of the Deconvolution and Likelihood Ratios (LRs) Produced Using a Continuous Probabilistic Software From Low-Level Samples When Amplifying the Entire Extract or Splitting the Extract

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After attending this presentation, attendees will understand the potential benefits and differences of information gained from either a single analysis of a low-template sample versus splitting and combining replicate testing of the samples using a continuous probabilistic genotyping software.

This presentation will impact the forensic science community by demonstrating the range of LRs generated analyzing a single DNA profile versus splitting, then jointly analyzing, replicates of the DNA template.

Touch DNA samples typically contain much less than 1ng of total DNA. Strategies to maximize the genetic information from low-level samples is to: (1) concentrate the entire extract down to 10uL, then amplify the entire extract volume; or, (2) split the extract into multiple amplification reactions, then develop a consensus profile for interpretation.[1,2] Some laboratories may be hesitant to implement enhanced detection methods to increase the sensitivity of the amplification or analysis (e.g., increased cycle number, increased injection time/voltage, post-amplification de-salting, etc.) due to the potential for an increased detection of allele drop-in. Therefore, more information may be gained through replicate amplifications using standard protocols. Probabilistic genotyping software using continuous models of interpretation has the ability of analyzing replicates of the same DNA sample to produce a combined deconvolution and LR. This option poses the question, which analysis will produce a more informative result — amplifying and analyzing the entire DNA extract or splitting the extract and conducting a joint analysis? By splitting the DNA extract, the total DNA template used for the amplification is halved, but the replicate analysis may provide additional information for the statistical analysis.

In this study, DNA profiles were generated from a range of single source and two- and three-person mixed DNA samples in which the template DNA was amplified: (1) once with a determined quantity of template DNA; and, (2) in duplicate using one-half of the original quantity of template DNA in each amplification. All samples were amplified using the standard Polymerase Chain Reaction (PCR) amplification conditions and analyzed using capillary electrophoresis. The DNA profiles were then processed using a continuous probabilistic genotyping software. The duplicate DNA profiles generated from the split DNA extract were analyzed together, resulting in a single combined deconvolution and LR result. The information from deconvolved genotypes and the range of LR values for a single analysis compared to a joint analysis using replicate profiles will be presented.

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1. Gill P., Whitaker J., Flaxman C., Brown N., Buckleton J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci Int.* 112(1): 17-40.
2. Grisedale K.S., van Daal A. (2012) Comparison of STR profiling from low template DNA extracts with and without the consensus profiling method. *Investig Genet.* 3(1): 14.

Low-Template DNA, Likelihood Ratio, Probabilistic Genotyping

B186 The Application of Short Tandem Repeat (STR) Sequence Variation for the Selection of Novel STR Markers to Enhance DNA Mixture Deconvolution: What Do We Know and Where Are We Headed?

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After attending this presentation, attendees will better understand Massively Parallel Sequencing (MPS) of forensically related STRs, the value of capturing entire amplicon sequence information (primer-to-primer sequence data), and the potential impact increased allelic data will have on applications in forensic DNA human identification.

This presentation will impact the forensic science community by providing information on data that supports implementation of MPS and STRs into forensic laboratories, discussing best-practice analysis methods, and exploring the next steps in novel forensic STR determination for maximal DNA mixture deconvolution efforts.

DNA mixture deconvolution of crime scene samples remains one of the biggest challenges faced by forensic laboratories today. When DNA mixtures are encountered in a sample, the analyst would like to identify individual components of the mixture and characterize each component of the sample using mixture interpretation guidelines and statistical calculations. Once the different components of the mixture have been resolved, the analyst then is able to make comparisons of the mixture components to reference samples. Currently, the basis of forensic interpretations relies on the amplification of STR markers using the Polymerase Chain Reaction (PCR), allele sizes determined using Capillary Electrophoresis (CE), and the results used to query reference samples or existing profiles stored in the Federal Bureau of Investigation (FBI) Combined DNA Index System. DNA mixtures often complicate STR genotyping of samples, and complex mixtures of three or more contributors are being encountered routinely in forensic casework.

MPS is a novel yet robust technology for capturing large amounts of DNA data from minimal input DNA, which is useful for processing forensic evidence that is frequently limited in quantity and quality. Using MPS, multiplexing a large number of STR markers simultaneously is possible and will provide greater genetic information for STR genotyping of multiple contributors in mixture DNA samples. MPS also provides the opportunity to identify intra-allelic sequence variation within STRs not possible using conventional CE protocols. Although this technology offers promise, current panels that capture the core STR loci may not contain sequence variants that have sufficient variability to individualize components in a mixture sample. Additionally, the ability to capture flanking region sequence variation to exploit the full power of forensically relevant STR loci is needed. The application of such data will increase knowledge and understanding of each locus, increase the power of discrimination of a locus, and potentially aid in mixture deconvolution efforts. The underlying genetic variation needs to be described through studies on various population groups.

An in-depth examination of sequence variation in 58 STRs was performed on 780 individuals using the MiSeq® FGx™ Forensic Genomics System, STRait Razor, and in-house Excel® workbooks. A total of 747 autosomal, 228 X chromosome, and 324 Y chromosome STR alleles were identified by sequence compared to 357 autosomal, 107 X chromosome, and 188 Y STR alleles that were identified by length. Within the observed sequence variation, more than 500 novel alleles were identified and described. These population data illustrate the genetic variation that exists in the commonly used STR markers and suggest that a great deal of genetic diversity has yet to be uncovered in STR markers.

Continued efforts are focused on identifying substantially polymorphic STR loci for the creation of a novel STR panel. The characterization of novel STR markers containing high-resolution intra-allelic sequence variants will allow the forensic scientist to overcome certain challenges of interpretation of some complex mixture samples, increase the number of resolved profiles being compared to reference and suspect profiles, and expand the DNA database by increasing the number of forensic samples uploaded. The benefit from this revolutionary application will be an increase in the number of investigative leads and the overall resolution of more crimes.

Massively Parallel Sequencing, STR Sequence Variation, DNA Mixture Deconvolution

B187 Validation of Probabilistic Genotyping Software for Use in Forensic DNA Casework

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After attending this presentation, attendees will better understand the scientific foundation of software validation. Attendees will receive both theoretical and practical guidance on implementing validation of probabilistic genotyping software in a forensic DNA laboratory.

This presentation will impact the forensic science community by assisting forensic DNA laboratories in validating probabilistic genotyping software, which fills a current and critical need.

Complex profiles may encompass a multitude of confounding factors resulting from DNA profiling of a low quantity and/or low quality biological sample. The resulting profile may contain multiple contributors, may lack information from the true contributors (allelic drop-out), may include extraneous information unrelated to the crime-sample information (allelic drop-in), and may suffer from degradation or inhibition. It is now accepted throughout the worldwide forensic DNA community that a likelihood ratio approach is required to reliably interpret these types of profiles.

Accordingly, recent years have seen a proliferation of probabilistic models, implemented via software, offered to the community as solutions to this problem. Acceptance of such software in the user community, and subsequent acceptance by the court, relies heavily on their validation. Although these probabilistic models rely on different assumptions and make use of different types of information, they all enable the evaluation of evidence within a likelihood framework. While these software programs have proven generally useful to facilitate the interpretation of complex DNA profiles, few guidelines exist that describe the appropriate and sufficient validation of such software used in forensic DNA casework.[1,2] The validation of probabilistic models for use in forensic casework is not straightforward because the true weight of the DNA evidence cannot be determined; there is no "right" answer. Indeed, the generated likelihood ratio always depends on the model's assumptions. No "gold standard" exists in the form of a true likelihood ratio that can serve as a comparison.

Forensic science is not the first discipline to face the challenges of model and software validation. Consequently, the field can draw on the collective wisdom of other disciplines to guide its inquiry. This presentation will discuss general principles of software validation and how they could be applied to the interpretation software now being introduced into the forensic community. This presentation will first introduce some general definitions of model and software validation taken from existing fields. Importantly, the relationship between a statistical model and its implementation via software will be clarified. This presentation will then illustrate how these ideas can be translated into use for forensic casework. A set of considerations that seek to provide guidance in validating probabilistic genotyping software for casework use will be offered.

Finally, this presentation will make the case for transparency in software. Concerns about the reliability and reproducibility of software used in scientific computing have grown over the past few years. There is a strong movement for researchers to make the source code used for analyses freely available to the community at the time of publication. Easily accessible source code implementing a statistical method will allow scientists to perform all aspects of software validation. Finally, such transparency will promote standardization and will facilitate improvements and extensions to existing software, which will be a further benefit to the community.

Reference(s):

1. Scientific Working Group on DNA Analysis Methods. *Guidelines for the Validation of Probabilistic Genotyping System.* www.swgdam.org (2015).
2. Haned H., Gill P., Lohmueller K., Inman K., Rudin, N. Validation of probabilistic genotyping software for use in forensic DNA casework: Definitions and illustrations. *Science and Justice.* 56 (2016) 104–108

Probabilistic Genotyping, Software, Forensic DNA

B188 Assigning the Number of Mixture Contributors: A Fool's Errand

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After attending this presentation, attendees will understand that the number of contributors to assume for a mixture calculation cannot be anticipated in advance, and frequently isn't even the same for competing hypotheses. Overlooking this possibility, as nearly all currently available advanced software does, entails a substantial risk of very misleading results.

This presentation will impact the forensic science community by illustrating the need to be decidedly more cautious about pushing the envelope with even moderately complicated mixtures.

Some standard advice about DNA mixture analysis is to begin by deciding the number of contributors. This advice is not only bad, it's impossible. The advice was memorialized in a careful and influential 1998 paper.[1] In the context of the times and the paper, practically speaking, the advice was merely: decide if the mixture is two-person, in which case the paper described how to perform calculations. The paper's authors didn't seriously consider more complicated mixtures. Presently, complicated mixtures are considered seriously. This is a good time to review and, without disrespect, to reject the old advice.

As a working definition, "complicated" means that the number of contributors isn't obvious. Sometimes the number doesn't even exist in any practical sense. Imagine a mixture of many contributors with contribution amount ranging from major by gradations down to undetectable. Clearly, an undetectable contributor isn't a contributor in a practical sense. How about barely detectable? A small contribution may or may not count as significant, depending on context.

A clear and concrete example will be presented explaining how the differing contexts of two competing (i.e., prosecution and defense) hypotheses may require the use of different contributor numbers. In the example, the only reasonable analysis for the prosecution hypothesis is as a four-person mixture, but the only fair analysis for the defense hypothesis is as a three-person mixture. The resultant Likelihood Ratio (LR), for four-person versus three, is close to one — neutral evidence. Calculating instead as a four-person mixture throughout manufactures the illusion of strong evidence; it frames the suspect. Conversely, calculating uniformly as three-person mixture per the defense generates the illusion that the suspect is virtually excluded. Correctly employing the unconventional calculation is vital; it makes a huge difference.

Examples are fairly common in practice, and do not follow a single pattern. It is not good to say, after studying the one example, "I see where the problem comes from; I'll just look out for that situation and I'll be okay." A counterexample doesn't simply mean, "Here's an exception." A counterexample to a rule means the rule is wrong.

What is to be done? Since there is no ground-truth number of contributors, it is impossible to begin by "deciding the number of contributors." What can and must be done instead is to calculate likelihoods for the range of possible contributor numbers, each for the prosecution and defense separately. Generally, there will be one standout number for each side — maybe the same number, maybe not — which can be used.

Reference(s):

1. Clayton et al. Analysis and interpretation of mixed forensic stains using DNA STR profiling. *Forensic Science International.* 91 (1998) 55–70.

DNA Mixture, Number of Contributors, Bias

B189 The Use of Microhaplotypes in the Analysis and Deconvolution of Mixed DNA Samples

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After attending this presentation, attendees will understand some of the advantages of using Massive Parallel Sequencing (MPS) of regions containing multiple Single Nucleotide Polymorphisms (SNPs), known as "Microhaplotypes (MHs)," to analyze and deconvolute complex forensic samples that are composed of DNA from multiple contributors.

This presentation will impact the forensic science community by providing further understanding of additional methods that can be used in the analysis of forensic DNA samples.

Short Tandem Repeats (STRs) have been the standard DNA markers used in human identification due to the ease of amplification and their highly polymorphic nature, which leads to a high power of discrimination. Additionally, several software packages have been developed that allow the analyst to easily identify and interpret the results. While STRs have been vital in forensic DNA analysis, several characteristics have led to limitations in their use. STR fragments are amplified and labeled using fluorescent probes; the number of loci that can be analyzed in one assay is determined by the size of the fragments and number of dyes. Current Capillary Electrophoresis (CE) methods allow determining the size of the fragment but not the sequence; however, STR loci have complex repeats that vary in sequence, making alleles indistinguishable by size discernable only by sequence. Lastly, during amplification of the repeats, stutter effects can occur for some loci over 20% of the time, leading to the production of n-4 (also n-8/+4) fragments indistinguishable from true alleles of the same size.

MHs are loci of two or more SNPs within a short distance of each other (<250 nucleotides (i.e., micro)) with three or more allelic combinations (haplotypes). Conventional Sanger sequencing does not allow determination of the *cis/trans* relationship between individual SNP alleles (i.e., the haplotype). In contrast, MPS methods, when SNPs are in the same amplicon, allow sequencing of individual strands and, therefore, the detection of haplotypes at a locus. As MHs are sequence variations, stutter effects are of no concern, making MHs a potential resource for the analysis and deconvolution of imbalanced mixtures. Other characteristics, such as small amplicon size and lower mutation rate, make these markers potentially effective on degraded samples and in familial cases, respectively. Numerous MHs have been identified that are promising forensic markers; these MHs are currently being further characterized for their use in mixture deconvolution.

In this study, synthetic mixtures and forensic type samples were genotyped using both MPS (Thermo Fisher HIDS Early Access Ion AmpliSeq™ GlobalFiler™ Mixture ID Panel) as well as CE size analysis (GlobalFiler™) and the results were compared. Results from samples first analyzed by CE suggested they were potentially composed of multiple genetic contributors, though the interpretation using CE alone was questionable as potential minor contributors fell below analytical or stochastic thresholds and into stutter ranges. MPS of STRs of these samples showed sequence variations that provided more support for the presence of a mixture; however, MH genotyping conclusively indicated the presence of multiple contributors in mixtures of two, three, and four persons at 40:1, 10:1:1, and 10:1:1:1 ratios, respectively. Additionally, several forensic-type samples were found to contain DNA from more than one contributor, with the minor contributor(s) more easily identified using MHs. Using preliminary MH allele frequencies, work is being conducted to determine random match probabilities for minor contributors found in mixed forensic samples.

These results illustrate the capabilities of MHs in the detection of mixtures and suggest the ability to genotype multiple contributors in complex forensic samples.

DNA, Sequencing, Microhaplotypes

B190 An Error in the Likelihood Ratio: The False Match Probability (FMP)

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After attending this presentation, attendees will understand why the FMP is important in forensic science and how the FMP can be accurately and rapidly calculated.

This presentation will impact the forensic science community by describing how to quantify an error in forensic analysis and DNA mixture interpretation.

In identification science, there may many possible types. Observables that exist in the physical world can be conceptualized as random samples drawn from a type space. Probability and information statements made about observed types refer to the full sample space of all possible types.

A prior probability distribution describes the chance of observing a type before examining data. A posterior distribution over type space updates these probabilities based on examined data. The Bayes factor of a type in the sample space is a ratio of posterior to prior probabilities.

When stating a factor for a type exemplar relative to evidence, there is a chance of false match error. With a DNA mixture, this error is the probability of misidentifying a non-contributor type as a contributor, when the type coincidentally has a factor value at least as large as a given match statistic.

The probability of falsely matching a wrong exemplar by chance is the FMP. The error can be quantified by calculating the subset size of misidentified types having spuriously large factors.

The FMP can be costly to calculate exactly on a large type space; however, when a type is formed from independent subtypes, the factor is a numerical product of the subtype factor values. Independence helps with rapid and accurate calculation of the joint factor distribution. Evaluating the joint distribution's tail probability beyond a fixed factor value measures the type subset showing false matches.

A trier of fact does not want to make a mistake by wrongly convicting an innocent person. Most jurors do not know Bayes theorem or statistical independence. Few have studied mathematical probability, and fewer still have learned conditional probability. They rarely know about likelihood (the probability of data given a hypothesis), much less the Likelihood Ratio (LR) factor that contrasts two competing hypotheses. But they do understand the chance of error, and they want to avoid making a mistake.

Considering all the people in the world, what is the chance that a reported match statistic identifies the wrong person? DNA mathematics can randomly embed the 7.5 billion (10^{10}) people in the world in a dense space of a trillion trillion (10^{24}) possible genotypes. Population genetics can estimate prior genotype probability, while Bayesian update on evidence data can produce a posterior genotype distribution. Prior and posterior combine to provide a factor function over all genotype space.

The factor function's inverse connects extreme match values to an error subset of types. The one-dimensional tail probability of extreme match values is the multidimensional measure of non-contributor types. Actual objects in the physical world have types that are samples from the full type space. To determine an FMP relative to all the people in the world, it is computationally effective to reduce the function to a single variable and its tail probability.

The LR summarizes the probative value of evidence in forensic identification. The FMP puts an error rate to that LR value, customized to the evidence in a particular case. Both numbers are important to a trier of fact — the LR's strength of match, and the FMP's chance of error. While 1/LR is always an upper bound on LR error, calculating the FMP can provide an exact estimate of misidentification frequency. The FMP provides additional error information about an LR match statistic, simply expressed as the chance of making a mistake.

This presentation will introduce the FMP method and demonstrate its use in analyzing DNA in a sexual assault case and searching a DNA database. Understanding match statistic error will help an analyst better quantify the chance of making a mistake.

Likelihood Ratio, False Match, Error Rate

B191 Out of the "*Frye*" Pan and Into the Fire: KISS Your Judge and Defend Your Probabilistic Genotyping (PG) Software in Court

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After attending this presentation, attendees will be better prepared to present evidence concerning the general acceptance and reliability of various PG software programs.

This presentation will impact the forensic science community by providing laboratory managers and lawyers with the skills necessary to successfully defend the use of PG programs, which have been increasingly adopted by accredited forensic laboratories to improve reliability of the statistics used in DNA testing.

When a laboratory manager introduces probabilistic genotyping software to generate statistics in casework, he/she can expect *Frye* or *Daubert* challenges in court. These are claims, generally by the defense, that the results should be precluded because the new techniques are not generally accepted as reliable in the relevant scientific community (*Frye*) or that the techniques are not based on sufficient facts or data and that the proposed testimony is not the product of reliable principles and methods (*Daubert*). These attacks are made even though PG software has been used to generate statistics that sometimes exonerate suspects, in both pre-trial and post-conviction settings.

Laboratories and prosecutors must partner up when faced with these challenges to scientific evidence. Together, they must create an educational package with one student in mind — the judge, who will decide whether the jury will hear results from the software on which the laboratory has spent enormous resources of time and money. The presentation to the judge will have to be comprehensive, yet simple enough for a non-scientist to understand, following the Keep It Simple Stupid (KISS) rule of thumb. Sometimes this challenge can be met on paper, and sometimes it will have to be battled out on the witness stand.

Based on successful experience defending the Office of the Chief Medical Examiner's (OCME's) Forensic Statistical Tool (FST) in New York City, suggestions for the best ways to prepare and present complex scientific evidence will be offered, including: peer-reviewed articles, conference presentations and workshops; validations; DNA and mixture interpretation fundamentals; recommendations from the Scientific Working Group on DNA Analysis Methods (SGWDAM), National Institute of Standards and Technology (NIST), and international bodies; other agencies and laboratories using them; and problems PG software will avoid.

An important aspect of these scientific admissibility hearings focuses on what to expect from experts. Because this is an adversarial system, lawyers on both sides of a *Frye* hearing can be expected to research expert witnesses and vigorously challenge them on the basis of expertise, bias (financial or otherwise), and whether they advocate the use of alternative software that can be manipulated by varying thresholds on a case-by-case basis instead of using one set of validated thresholds uniformly across the laboratory.

PG Software, *Daubert*, *Frye*

B192 United States Population Sequence Data for 27 Autosomal Short Tandem Repeat (STR) Loci, 24 Y-Chromosomal Short Tandem Repeat (Y-STR) Loci, and 7 X-Chromosomal Short Tandem Repeat (X-STR) Loci

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After attending this presentation, attendees will better understand sequence-based STR allele frequencies, how this new data type can be implemented into statistical analysis, and which STR loci exhibit significant allelic gains by sequence in a large population dataset representative of four common United States populations.

This presentation and the availability of this data will impact the forensic science community by facilitating sequence-based statistical calculations when STR sequencing applications are utilized. Recent publications have been limited to smaller sample sizes, less loci, or populations less representative of the United States.[1-3]

STR loci allows for determination of repeat motif variations within the STR (or entire PCR amplicon) that cannot be ascertained by size-based Polymerase Chain reaction (PCR) fragment analysis. Sanger sequencing has been used in research laboratories to further characterize STR loci, but it is currently impractical for routine forensic use due to the laborious nature of the procedure in general and additional steps required to separate heterozygous alleles. Recent advances and cost reductions in Next Generation Sequencing (NGS), also known as massively parallel sequencing, have opened the door to routinely obtaining STR sequence data in the forensic laboratory, which may be particularly valuable in challenging cases of mixture interpretation or complex kinship. Thus, several commercial manufacturers have developed assays for sequencing various combinations of STR loci (autosomal, X, and Y), with some also containing Single Nucleotide Polymorphisms (SNPs) and mitochondrial DNA (mtDNA).

This sequence-based population study includes >1,000 DNA samples, divided among Caucasian, African American, Hispanic, and East Asian individuals, at 27 autosomal STR loci, 24 Y-STR loci, and 7 X-STR loci using an assay designed for NGS. Data analysis for genotyping was performed using the manufacturer's software and an alternate in-house pipeline based on freeware.[4,5] Allele calls for Capillary Electrophoresis (CE) and sequencing methods were compared for concordance at every locus. In the case of a discordant allele call, the cause was determined.

The resulting population data reveals general gains in discriminatory power per locus, as well as population-specific gains for some loci. Categorizing the 58 STR loci as simple or compound/complex based on the motif present in GRCh38 Human Genome Reference Sequence, the 22 compound/complex loci exhibit, on average, more than double the number of alleles by sequence compared to alleles by length, with some loci containing more than four or five times the alleles. The 36 simple repeat loci demonstrate a more modest increase in alleles by sequence compared to alleles by length, as expected. For the autosomal STR loci, the resulting sequence-based frequency data is applied to generate Random Match Probabilities (RMP) for each individual per locus and across the profile, and comparison to length-based RMP values reveals expected gains in statistics. Y-STR and X-STR loci are evaluated for gains in haplotype diversity.

This data is fundamental to implementation and support of sequencing technology, and the demonstrated gains in alleles and RMP values will assist laboratories in weighing costs/benefits.

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STR Sequence, United States Population, Allele Frequency

B193 An Evaluation of the Discriminatory Power, Ancestry Prediction, and Practical Considerations of the ForenSeq™ DNA Signature Prep Kit Against Traditional Short Tandem Repeat-Capillary Electrophoresis (STR-CE) Methods

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After attending this presentation, attendees will better understand the differences in the discriminatory power, ancestry prediction, and practical considerations (i.e., processing and analysis time and cost) of the ForenSeq™ DNA Signature Prep Kit and Universal Analysis Software (UAS) versus traditional STR-CE methods.

This presentation will impact the forensic science community by illustrating how, as this community begins to embrace next generation sequencing, it is crucial to fully understand the differences in discriminatory power, ancestry prediction, and practical considerations between this new technology and the methods currently in use.

To this end, the ForenSeq™ DNA Signature Prep kit was evaluated for its usefulness with a set of 120 self-identified samples from African American, United States Caucasian, and United States Hispanic and Colorado population groups. The ForenSeq™ kit contains 27 autosomal STRs, 24 Y-chromosomal STRs (Y-STRs), 7 X-chromosomal STRs (X-STRs), 95 identity-informative Single Nucleotide Polymorphisms (iiSNPs), 56 ancestry-informative SNPs (aiSNPs), and 22 phenotypic-informative SNPs (piSNPs). The samples, which were previously analyzed for the mitochondrial control region, were processed according to the manufacturer's recommended procedure using Primer Mix B with minor modifications at the library purification step. Sample libraries were sequenced with an Illumina® MiSeq® FGx™, and sequence data were analyzed using the UAS. Concordance data were generated by typing the samples with an AmpFℓSTR® Y Filer® Polymerase Chain Reaction (PCR) Amplification kit and a PowerPlex® Fusion 5C System using an ABI® 3500xL and performing analysis with GeneMapper® ID-X software. This allowed for the comparison of sequenced STRs from the ForenSeq™ kit with autosomal STRs and Y-STRs typed on a traditional CE platform. Additionally, ancestry prediction from the UAS was compared with the self-identified ancestry, maternal ancestry determined from the previously obtained mitochondrial region haplogroups, and paternal ancestry determined from Y-haplogroups predicted from both the Y Filer® loci and the ForenSeq™ Y-STR loci.

These high-quality samples resulted in full autosomal profiles for all samples and full Y Filer® profiles for all male samples in both ForenSeq™ and CE kits. The data between PowerPlex® Fusion and ForenSeq™ were 99.46% concordant across overlapping loci. The ForenSeq™ data showed the presence of isoalleles, which are length-based homozygotes with different sequences due to SNPs that further distinguish same-size alleles. Both kits exhibited imbalance at D22S1045, although the imbalance was more pronounced in the data generated by the ForenSeq™ kit. Allelic calls for Y-STR markers were 97.77% concordant across overlapping loci for ForenSeq™ and Y-Filer®. Regarding the ancestry prediction from the ForenSeq™ data set, the majority of samples matched the self-identified ancestry. Discrepancies between the two measures of ancestry were observed with 6 out of 90 samples. The additional comparison of the ForenSeq™ ancestry prediction with the mtDNA and Y-STR ancestries allowed for improved characterization of the admixed samples.

Comparing the cost of both workflows at the reaction level, the ForenSeq™ method was approximately the same cost as the traditional CE kits used in this comparison. Additionally, start-up costs, such as the cost of instrumentation and a laboratory's particular throughput, need to be considered. Furthermore, the time needed to process the ForenSeq™ workflow, starting with prepared extracts, was approximately 36 hours, compared to approximately 8 hours for the traditional methods. In regard to analysis, the evaluation of the ForenSeq™ data with the UAS was particularly time consuming for two main reasons: (1) it required navigating to individual calls; and, (2) population statistics and phenotypic/ancestry prediction had to be performed for each sample individually with no report generated by the analysis software. Analysis of the CE data with GeneMapper® ID-X was generally straightforward with appropriate reports for the analyzed data and more rapid compared to analysis of STRs within

the UAS.

ForenSeq™ offers a higher discriminatory power than traditional STR CE kits and additionally provides ancestry and phenotypic prediction that can be used for investigative leads; however, the practical considerations of cost, time, and low-throughput analysis of ForenSeq™ may limit the implementation of this protocol into forensic crime laboratories since traditional STR CE kits still provide sufficient data for DNA identification in a rapid and affordable manner.

The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command, or the Armed Forces Medical Examiner System.

Next Generation Sequencing, ForenSeq™, STRs

B194 An Evaluation of the ForenSeq™ System for Sequence-Based Y-Chromosome and Autosomal Short Tandem Repeat (STR) Typing

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After attending this presentation, attendees will better understand the performance of the Illumina® ForenSeq™ Next Generation Sequencing (NGS) Assay and its associated Universal Analysis Software (UAS).

This presentation will impact the forensic science community by describing the current practical utility and usability of the ForenSeq™ NGS Assay and its associated UAS.

The recent commercial availability of massively parallel sequencing components and systems designed specifically for forensic use has improved the feasibility of sequence-based typing of nuclear DNA markers commonly examined for forensic applications. One such assay, the ForenSeq™ DNA Signature Prep kit (compatible with the MiSeq® ForenSeq™ FGx™ instrument), simultaneously targets 58 Short Tandem Repeat (STR) loci and up to 172 single nucleotide polymorphisms. The associated ForenSeq™ UAS performs all secondary and tertiary analyses and presents the resulting STR genotypes in both repeat and sequence-based formats.

The potential utility of the ForenSeq™ assay and software system for Y-chromosome and autosomal STR typing was evaluated based on the examination of high-quality DNA samples amplified at the target DNA input. The performance of the assay/software combination was considered with respect to marker recovery metrics. Genotype concordance was assessed both across sample or lineage replicates and with standard Capillary Electrophoresis (CE) -based repeat number data. To test both the ForenSeq™ autosomal STR (auSTR) and Y-STR recovery rates and the UAS performance with respect to the detection of poor quality or inconclusive data, UAS-determined genotyping results were assessed prior to and after analyst review of the ForenSeq data. ForenSeq™ UAS-determined genotypes were compared directly to the CE-based allele calls developed from PowerPlex® Fusion System and the Y Filer® Plus PCR Amplification Kit. A total of 4,111 auSTR and 1,296 Y-STR loci were targeted for the samples examined in this study. Five runs of the MiSeq® FGx™ system were performed, with each run varying in terms of the number of samples multiplexed. Apart from the single run (Run #2) with the largest number of multiplexed samples (61 samples total), greater than 99% of the auSTR loci and more than 97% of the Y-STR loci were recovered. The lower recovery rates in Run #2 clearly reflected lower overall read quantities per sample.

Among the 4,167 total loci ultimately compared between the ForenSeq™ and CE data (3,212 auSTR and 955 Y-STR), only two UAS allele calls were found to be inconsistent with the CE-based data and, additionally, did not trigger quality control indicators in the software. The first of these was a sample that also typed differently between the GlobalFiler® and Fusion™ CE assays. The second of these instances represented a null allele in the ForenSeq™ assay. Overall, the auSTR and Y-STR ForenSeq™ results indicated high concordance with CE data developed using commercially available assays and were similar to concordance rates reported for other CE kit-to-kit comparisons. In this presentation, results from the concordance assessments will be presented along with additional information on the general performance of the ForenSeq™ chemistry and software.

Next Generation Sequencing, Illumina® ForenSeq™, NGS Concordance

B195 Forensic Analysis of the Entire Mitochondrial Genome on Ion Torrent™ Massively Parallel Sequencing (MPS) Platforms

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After attending this presentation, attendees will possess a basic understanding of the mitochondrial genome sequencing process on an MPS platform. Attendees will also better understand the benefits of sequencing the entire mitochondrial genome in order for forensic laboratories to be able to generate the maximum amount of discrimination power and to improve mixture detection and resolution.

This presentation will impact the forensic science community by providing information on the progress toward validating and implementing MPS technologies into forensic laboratories.

The mitochondrial genome (mtGenome) has been well-established as a useful genetic marker for the analysis of challenged or degraded forensic samples; however, sequencing of the mtGenome in forensic laboratories has been limited to HVI and HVII of the sequence variation-rich control region. This is largely due to limitations with Sanger-Type Sequencing (STS) methodologies, the current gold standard of forensic DNA typing. MPS technologies offer an alternative to these STS methodologies. More specifically, the Ion PGM™ and Ion S5™ Systems are promising MPS platforms for forensic analyses. A large multiplex, short-amplicon system was developed for sequencing the mtGenome on these Ion Torrent™ MPS platforms. The Applied Biosystems™ Precision ID mtDNA Whole Genome Panel is comprised of two multiplexes each with 81 primer pairs (plus degenerate primers) that generate amplicons ≤175bps in length, which facilitates the analysis of challenged and degraded samples. When used with the Ion Chef™ System, an efficient and largely automated workflow is generated worthy of consideration for forensic casework. Sequence data for the entire mitochondrial genome can increase discrimination power when generating mitochondrial haplotypes, and the increased resolution afforded by MPS technologies allows for detection of heteroplasmy levels at each nucleotide and provides avenues for mixture interpretation. Samples were sequenced on the Ion PGM™ and Ion S5™ Systems to evaluate the quality and efficiency of the Precision ID mtDNA Whole Genome MPS workflow. Metrics such as concordance, amplicon success, coverage, strand balance, and noise were analyzed to evaluate the quality and reliability of the data produced.

MtGenome sequence data were generated for 120 reference samples, and these genomes displayed few instances of amplicon dropout. Haplotype calls for these samples were concordant with mtGenome data generated by long Polymerase Chain Reaction (PCR) on both the Ion PGM™ and Illumina® MiSeq® platforms. Read depths for these samples ranged from 259X to 8,579X, and strand balance calculations demonstrated that reads were generated from both strands of the DNA. Any reads not attributed to nominal nucleotide calls were termed noise and ranged from 0.002% to 9.03% of the total read depth across the genome. A dilution series ranging from 1ng to 1pg of input genomic DNA illustrated the sensitivity of detection for this multiplex. Successful analysis of challenged samples (including bones, aged buccal swabs, and hair shafts) and mixture samples demonstrated the multiplex's success with forensically relevant samples. When analyzing the mixtures, the major contributor's haplotype was successfully identified with nuclear DNA ratios of 1:1, 1:5, and 1:10. Overall, results indicated robust and accurate data were generated, which supports the need for full validation studies with this MPS workflow in order to move this multiplex and MPS technology closer to implementation into forensic laboratories for routine mtDNA analyses.

Massively Parallel Sequencing, Forensic DNA Typing, Mitochondrial DNA

B196 An Analysis of Highly Degraded DNA From Bone Samples Using Probe Capture Enrichment of the Entire Mitochondrial Genome and Next Generation Sequencing

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After attending this presentation, attendees will better understand the efficacy of using probe capture enrichment of the entire mitochondrial genome coupled with next generation sequencing to analyze highly degraded DNA from bone samples.

This presentation will impact the forensic science community by evaluating a method that can be used to successfully analyze highly degraded DNA samples from crime scenes and to identify victims of mass disaster situations.

Forensic DNA samples are often highly degraded, making them unsuitable for traditional methods of DNA analysis such as Short Tandem Repeat (STR) analysis because target sequences and primer binding sites are not intact. Mitochondrial DNA (mtDNA) is useful for analyzing degraded DNA because of its high copy number. Traditional methods of mtDNA analysis, including Sanger sequencing, are commonly used to only target only the HVI/HVII regions of the mtDNA genome, limiting the discriminatory power of mtDNA analysis. Probe capture is a novel technique that uses mtDNA-specific probes to enrich and capture the entire mitochondrial genome. Analyzing the entire mitochondrial genome would allow detection of discriminating information outside of the HVI/HVII regions and increase the discriminatory power of mtDNA. Next generation sequencing, a massively parallel, clonal, and high-throughput technique, is an excellent tool for analyzing the entire mitochondrial genome of degraded DNA and allows for mixture detection and resolution. The purpose of this project was to determine whether probe capture and next generation sequencing on the Illumina® platform could be used to successfully analyze highly degraded mtDNA from bone samples dating to approximately 100, 2,000, and 4,000 years ago.

This optimized probe capture method for enrichment of the entire mtDNA genome was tested on forensically relevant bone samples dating to 19-20 BC, 10-8 BC, and 4000-1000 BC. Seven bone samples recovered from a comingled tomb in Rijeka, Croatia, and dating to approximately 100 years ago were successfully sequenced with coverage of the mitochondrial genome ranging from 52%-100%. The average read length for these samples ranged from 73bp-79bp, demonstrating that the DNA is highly degraded. Six prehistoric bone samples dating to approximately 2,000 years ago were successfully sequenced with coverage of the mitochondrial genome ranging from 26%-100%. The last set of samples was recovered from a necropolis on the island of Korčula, Croatia, and dates to approximately 4,000 years ago. These six samples were successfully sequenced with coverage of the mitochondrial genome ranging from 75%-100%. In this presentation, the results of this study and the historical information gathered from these results will be discussed in further detail.

Degraded DNA, Mitochondrial DNA, Probe Capture

B197 The Evaluation of a Nanopore Sequencing Platform for Concordance and Reliability in Short Tandem Repeat (STR) Analyses

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After attending this presentation, attendees will better understand current nanopore sequencing technology and its potential utility in the forensic science community, particularly with forensic STR analysis.

This presentation will impact the forensic science community by attempting to evaluate and determine the potential utility of a revolutionary small-scale nanopore sequencing platform for obtaining High-Throughput Sequencing (HTS) data.

The question in the forensic science community is not whether HTS will be implemented, but how and when. It is crucial to evaluate all potential avenues of HTS that can bring the forensic science community up to speed in the most cost- and time-efficient manner possible. This becomes increasingly imperative with the expansion of both the number of samples and the number of biomarkers per sample being employed in forensic laboratories today. This research works to evaluate and determine the potential utility of a revolutionary small-scale nanopore sequencing platform for obtaining HTS data for the forensic science community.

Research was conducted using the only nanopore platform currently commercially available: Oxford Nanopore's MinION™ nanopore system. Samples were extracted and amplified using STR amplification techniques currently used in forensic science laboratories. By evaluating the nanopore platform with commonly used, commercially available STR primers for amplification, implementation would potentially require fewer validations, implementation into the workflow would be more easily achieved, and the Combined DNA Index System (CODIS) data entry could be streamlined. In the MinION™ platform, DNA passes through a nanopore protein channel for sequencing. The unique amount of current blocked as each nucleotide moves through the channel is translated into a base call. It was posited that the 5' fluorophores incorporated during STR amplification would hinder this process and possibly block ligation of the necessary adapters for sequencing during the library preparation. Thus, STR amplicons were pretreated with ExoSAP-IT® reagent in an effort to remove the fluorophores for improved ligation efficiency and higher quality sequencing.

Products were analyzed by Capillary Electrophoresis (CE) after ExoSAP-IT® treatment to determine if the fluorophores were removed by the treatment. Data revealed incomplete cleavage of the 5' fluorophore mobility complex from all amplicons. One specific locus, D8S1179, had STR alleles detected on the CE, indicating the continued presence of the fluorophore; however, sequence data was still obtained upon analysis on the MinION™ nanopore sequencing device. These results demonstrated that the removal of the fluorophore is not necessary, and STR sequencing data is obtainable with fluorophores still attached. Alternatively, it is possible that the majority of the sequence data is originating from the non-tagged strand.

Overall, sequencing with the nanopore device yielded a total of 3,053 sequence reads less than 500bp in length, with the following loci identified: CSF1PO, FGA, TH01, TPOX, D5S818, D7S820, and D13S317. Additional sequence reads were identified for CSF1PO, TH01, and D7S820 but did not pass Quality Assurance (QA) thresholds. Only six of the identified loci were concordant with reference profiles obtained by CE analysis. Thus, an accuracy rate of less than 50% was observed. While these results are promising, they also illustrate that some additional work is needed to fully evaluate this HTS method. Continuing work will include replicate runs on an updated MinION™ platform, which includes a redesigned nanopore protein flowcell and improved bioinformatics software to address these exact error and accuracy issues. Also, additional concordance and accuracy data will be analyzed and compared to reference data obtained from CE, which may serve to further streamline and improve MinION™ data analysis.

This research works to provide information to the forensic science community about a small, portable HTS

platform that is potentially both time- and cost-efficient. The preliminary data determines that, with further improvements in error and accuracy rates, the potential uses of nanopore sequencing devices in forensics are tremendous.

Nanopore, Short Tandem Repeat, High-Throughput Sequencing

B198 The Development of a Nuclear Single Nucleotide Polymorphism (SNP) Probe Capture Assay for Massively Parallel Sequencing (MPS) of Degraded and Mixed DNA Samples

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After attending this presentation, attendees will better understand a novel method of enrichment called probe capture that can be used to enrich targeted SNP regions in degraded DNA samples as well as DNA mixtures for MPS.

This presentation will impact the forensic science community by demonstrating that extremely fragmented DNA can be captured and sequenced by an SNP probe capture assay targeting a large number of SNP regions. Further, the SNP probe capture assay designed in this project introduces the combined usage of tri-allelic SNPs, tetra-allelic SNPs, lineage SNPs, and micro-haplotypes, thereby improving the capability of detecting mixtures even if the minor contributor percent is extremely small.

Mini Short Tandem Repeat (miniSTR) primer kits are used for STR analysis when DNA samples are degraded; however, in some cases such as mass disasters and missing persons, the DNA is extremely degraded and miniSTR primer binding sites may not be intact. In such cases, mitochondrial DNA (mtDNA) can be analyzed, but since mtDNA is maternally inherited and the "product rule" is not applicable, it is not as discriminatory as nuclear DNA analysis. SNPs can be viable markers for degraded DNA analysis since the region of interest is only a single base; however, primer extension assays used in conventional SNP typing methods have limited multiplexing capabilities and require intact primer binding sites. Recently, MPS methods have been shown to sequence up to 172 SNPs across multiple samples simultaneously with high read depth per sequence. Also, the method of targeted DNA library preparation using probe capture enrichment has proved successful in enriching extremely degraded mtDNA for MPS without the need of intact primer binding sites. Therefore, the goal of this project was to design and test a probe capture assay targeting forensically relevant nuclear DNA SNPs for MPS of degraded DNA samples and mixed DNA samples.

A total of 451 SNPs were selected in the design and development of this forensic SNP probe capture assay. These SNPs include 136 Identity Informative SNPs, 41 Ancestry Informative SNPs, 24 Phenotypically Informative SNPs, 25 X chromosome SNPs, 81 Y chromosome SNPs, 31 Tri-allelic SNPs, 39 Tetra-allelic SNPs, and 36 Micro-haplotypes. The custom probe panel was developed and tested for read depth, sensitivity, capturing size-selected and degraded DNA, and detecting two-person mixtures at different ratios. The results of the coverage test consisting of 16 samples at 25ng indicated that 448 SNPs out of 451 showed coverage ≥10X in each sample. Three SNPs dropped out consistently and were excluded in later studies. The sensitivity of this capture system was tested by determining the number of SNPs with a read depth of at least 10X for sample DNA amounts ranging from 50ng to 50pg. For amounts of 5ng and greater, the percent of SNPs covered (measure of sensitivity) was 100% for all SNPs, and the correct SNP genotype assignment was at least 99.78%. The percent of SNPs covered and percent of SNPs with correct genotype assignment decreased as the sample amounts reduced from 1ng to 50pg. Next, the performance of the system was tested with size-selected DNA samples and mock-degraded DNA samples at varying sample amounts. With 0.5ng, size-selected sample DNA ≤75bp obtained coverage for 96.65% of all SNPs, and 95.09% of all SNPs obtained the correct genotype assignment. Mock-degraded samples at 10ng, 1ng, and 0.5ng with an average size of 150bp were sequenced. The coverage results for all SNPs were as follows: 100% for 10ng, 94.87% for 1ng, and 75.67% for 0.5ng. The mixture test contained samples at ratios ranging from 60:40 to 97.5:2.5. The X SNPs, Y SNPs, Tri-allelic SNPs, Tetra-allelic SNPs, and Micro-haplotypes detected 90% of the minor contributor SNPs for ratios between 60:40 and 90:10, and approximately 85% of minor contributor SNPs for ratios 95:5 and 97.5:2.5.

Based on these results, highly fragmented DNA samples can be successfully captured and sequenced for DNA samples fragmented to ≤75bp. Also, minor contributor ratios as low as 2.5% can be detected in mixtures. Therefore,

it is expected that this system can be successfully applied to analyze highly degraded DNA obtained from mass disasters and missing person cases as well as mixed DNA samples.

Single Nucleotide Polymorphism, Probe Capture, Massively Parallel Sequencing

B199 Toward a Vision of a National Forensic Science Academy Specializing in Leadership and Management

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After attending this presentation, attendees will better understand how the concept of a leadership academy developed, the steps being undertaken to develop a curriculum, and future efforts to make the project a reality.

This presentation will impact the forensic science community by providing a process for supervisors and managers to gain skills in leadership and management to assist them in becoming better leaders.

Forensic scientists are typically promoted into higher-level positions in a forensic science laboratory because the individual is good at casework and may volunteer for special projects; this becomes the basis of the promotion. Once scientists become supervisors, managers, or crime laboratory directors, they discover their new position requires a different set of skills, which they have not had the opportunity to acquire. "Flying by the seat of one's pants" all too often becomes the solution to the problem.

Unlike police ranks, which may often have leadership and management training programs, mentorship programs, and colleagues in other command positions within the agency to provide guidance, forensic scientists placed into management roles may have little training or support.

One of the more sought-after programs for leadership/management training within policing is the Federal Bureau of Investigation (FBI) National Academy (NA). The NA began July 29, 1935, and was created in response to a 1930 study by the Wickersham Commission that recommended the standardization and professionalization of law enforcement departments across the United States through centralized training. With strong support from the International Association of Chiefs of Police and with the authority of Congress and the Department of Justice, the "FBI Police Training School" was born.

A similar study for forensic science that recommends elements of standardization and professionalism is the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward,* which stated, "First-line supervisors need training in quality assurance, case file review, and basic supervision skills; and managers need training in fiscal management, quality systems management, leadership, project management, human resource management, and customer service."

The concept for a National Forensic Science Academy (NFSA) was proposed by Research Triangle Institute (RTI) International and the American Society of Crime Laboratory Directors (ASCLD) to The Laura and John Arnold Foundation (LJAF), who agreed that a need existed and funded a start-up effort to develop the curriculum for the program.

There exists a number of leadership and management programs directed toward crime laboratory managers: ASCLD and West Virginia University are two of many. RTI decided that rather than start from scratch, they would work with existing programs with the intention of integrating the different efforts into an interconnected, national program of blended learning and certification with minimum standards and flexibility for individual student needs.

This panel is made up of persons with broad expertise in the forensic science community and management/leadership training program who will provide a context to the development and implementation of the NFSA.

Leadership, Management, Continuing Education

B200 Data From the Federal Bureau of Investigation (FBI) Review of Microscopic Hair Comparison Laboratory Reports and Testimonies

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After attending this presentation, attendees will be better informed concerning current results from the review of the FBI laboratory reports and testimony in the discipline of Microscopic Hair Comparison Analyses (MHCA).

This presentation will impact the forensic science community by providing data from the FBI's MHCA review with details on statements that were marked as "inappropriate" in the review.

In 2012, the FBI, in conjunction with the Department of Justice, the Innocence Project, and the National Association of Criminal Defense Lawyers, engaged in a review of laboratory reports and testimony provided by FBI Laboratory examiners in cases involving MHCA. To the extent possible, the review has covered the period from the early 1970s through the end of 1999, when mitochondrial DNA testing of hair was fully utilized within the FBI Laboratory. The process this study used was to identify statements made by hair examiners that, when viewed alone, the FBI interpreted as not meeting accepted scientific standards for the discipline of MHCA. In April of 2015, the FBI issued a joint press release acknowledging that more than 90% of testimonies and 50% of laboratory reports in the MHCA cases reviewed had been identified as containing at least one scientific error. While these statistics are accurate, the press release did not contain the underlying data regarding these errors, nor how the FBI conducted its review. For example, when conducting the review of individual statements, language which has more than one interpretation was often conservatively marked as an error. The impact of single statements in the larger context of the examiner's complete testimony or report was not considered. The inclusion of language that properly described the boundaries of the science elsewhere in the testimony or report was not considered in deciding if a statement exceeded the limits of the science.

This presentation will provide the definitions of scientific errors used by the FBI and examples of statements that were marked as inappropriate in the FBI's review. The data will reveal that all 26 examiners that provided testimony on MHCA had at least one testimony statement flagged as inappropriate. Further, 24 of 34 examiners who issued laboratory reports on MHCA had at least one report with a statement marked as erroneous; however, it is noted that 26% of the total testimonies reviewed that contain inappropriate statements, as well as 26% of the total laboratory reports reviewed that contain inappropriate statements, were from a single examiner. It is also noted that two-thirds of testimonies with inappropriate statements were offered from the mid-1980s through the early 1990s.

More than 63% of statements marked as errors in testimony reviews and 99% in laboratory report reviews fell within the "Type 2" category in which it was deemed that the examiner offered or inferred a probability or a likelihood that a questioned hair originated from a particular source. Of these, nearly all of the Type 2 errors in laboratory reports and nearly one-half of the Type 2 errors in testimony were marked because the examiner indicated that a questioned hair was "consistent" with coming from an individual. Additionally, 20% of Type 2 errors marked in testimony were when the examiner used terms such as "rare," "unusual," or "highly unlikely" in describing the likelihood that two different people may have the same microscopic hair characteristics.

Twenty-five percent of statements marked as errors in testimonies were in the "Type 1" category. Type 1 errors involved a statement implying "the evidentiary hair could be associated with a specific individual to the exclusion of all others." Use of the words "match," "associate," or "unique" were the phrases most-often flagged for this category.

Error Type 3 required the examiner to "cite their experience of the number of cases or hair analyses worked in the laboratory and the number of samples from different individuals that could not be distinguished from one another as a predictive value to bolster the conclusion that a hair belongs to a specific individual." Approximately 12% of the statements marked as testimonial errors fell within this category.

Forensic Science Review, Microscopic Hair Comparison, FBI

B201 The Organization of Scientific Area Committees (OSAC) for Forensic Science Highlights Recent Standards and Baseline Documents for Many Disciplines

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After attending this presentation, attendees will understand the latest standards and guidelines reviewed by the OSAC for Forensic Science, recent baseline documents added to the OSAC Subcommittees webpages, and a summary of OSAC's 50+ Research and Development Needs. The OSAC is comprised of 34 operating units and more than 200 task groups populated by more than 560 OSAC members and 250 affiliates, all working on specific standards activities. The OSAC facilitates collaborations among forensic science practitioners, researchers, statisticians, and measurement scientists to bolster existing standards and guidelines, develop new standards from scratch, highlight the "state of forensic science disciplines," and identify research needs for the forensic science community.

This presentation will impact the forensic science community by educating attendees on the standards and guidelines reviewed by the OSAC, describing the purpose of the OSAC baseline documents listed on the OSAC website, and identifying existing research gaps in specific disciplines. Instructions will also be provided describing how individuals can become involved with the OSAC and have an impact on standards development efforts.

The OSAC has been fully operational since January 2015 and continues to transition the fragmented standards development efforts in the forensic industry to a unified process of recognizing valuable standards and guidelines that have both scientific merit and wide-based community acceptance. The OSAC Subcommittees have started to add baseline documents to their specific subcommittee webpages that describe the "state of the discipline" at this point in time while they work to develop new or updated standards for listing on the OSAC Registry of Approved Standards. These baseline documents contain practical information regarding forensic science disciplines that can help forensic scientists, judges, lawyers, researchers, other interested parties, and the general public to better understand the nature, scope, and foundations of the individual disciplines as they are currently practiced. This presentation will highlight some of the key baseline documents listed on the website.

The OSAC continues to implement the OSAC Registry Approval Process of Published Standards and Guidelines, which includes criteria against which existing standards and guidelines will be analyzed before being approved for posting on the OSAC Registries. This includes an analysis of technical merit, openness of the development process (to ensure balanced interests are represented), consensus, harmonization, and impact on the forensic science community. This presentation will highlight all the standards and guidelines currently listed on the OSAC Registries.

Another of the OSAC's objectives is to inform the forensic science community of research needs that are discovered during the OSAC's standards development activities. These research recommendations may be considered by other agencies and organizations when they develop their own agency research needs and when soliciting funding for forensic science research. The 25 OSAC Subcommittees have identified more than 50 research and development gaps that need to be addressed by the forensic science and academic community. This presentation will highlight some of the critical research needs identified in the OSAC.

Standards, OSAC, NIST

B202 Demolishing Divides: A Discourse on the Dearth of Discipline Differences Between DNA and Dactyloscopy

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After attending this presentation, attendees will observe commonalities between different forensic disciplines, will understand that these disciplines struggle with the same issues, and realize that there is much the disciplines can learn from one another by breaking silos and abandoning an "us-against-them" attitude.

This presentation will impact the forensic science community by encouraging forensic science practitioners to reach across disciplinary lines and share their struggles and successes with one another. Many disciplines are facing the same questions, yet often approach them differently. Open and frank discussions concerning the similarities between disciplines can help everyone in problem solving.

Forensic science is often thought of as a conglomeration of separate disciplines, each residing in its own silo. In larger laboratories where each analyst specializes, this perspective can manifest in negative ways, with some analysts exhibiting an "us-against-them" attitude in which members of different disciplines keep to themselves, rarely converse, and at times exhibit hostility and jealousy toward one another. How many times have you heard members of a discipline describe themselves as the "red-headed stepchildren" of the laboratory or lament that "DNA gets all the money and all the good stuff"?

Not only are these attitudes divisive, but they also rob each discipline of the opportunity to grow and gain strength from the knowledge possessed by the others. When disciplines are compared with the intention of discovering commonalities, it may be surprising to realize how many exist. Many disciplines share similar principles. For instance, nearly all forensic disciplines involve comparing an unknown material to a known material and looking for similarities and differences in patterns, whether those patterns are fingerprints, shoe impressions, signatures, or tool marks, or two chemical spectra or DNA profiles. Many disciplines share the same critics who are saying similar things about them. Many are considering the same statistical frameworks for presenting their evidence or struggling with the same questions of how to interpret noise or report conclusions.

Using DNA and latent prints (a field originally known as dactyloscopy) as a model, this presentation will offer insight into the many ways in which these two disciplines are remarkably similar. Latent prints, once considered the "gold standard" of forensic evidence, and DNA, the current reigning champion, are both considered to be rock-solid evidence when there is a high quantity of high-quality information (such as a clear tenprint card or a complete single-donor profile). Yet once the signal becomes degraded (such as in a distorted partial latent fingerprint or a several-donor DNA mixture), the interpretation required increases dramatically, along with the chances for error and the exposure to criticism.

These two disciplines will be discussed from beginning to end, breaking the issues into Deposition, Detection, and Description (which incorporates database use, documentation, and dissemination of findings). Discover the "Dearth of Discipline Differences" in this eye-opening lecture.

Latent Prints, DNA, Breaking Silos

B203 The National Institute of Standards and Technology (NIST) Trace Evidence Data Workshop: Discussions on a Path Forward for Trace Evidence Analysis

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After attending this presentation, attendees will be informed about current efforts at the NIST to expand and improve the accessibility of reference collections, materials, and databases for trace evidence analysis. This presentation will discuss the content of a workshop that took place at the NIST on July 19-20, 2016, in addition to key takeaways, related upcoming projects, and preliminary findings being crafted into a report to be released in 2017.

This presentation will impact the forensic science community by summarizing the content of the NIST meeting, describing future plans for follow-on projects, and reviewing preliminary recommendations obtained from the various discussions that took place. The ultimate goal of this effort is to assist in coordinating a multi-agency push to strengthen data in trace evidence in order to provide better tools to assist practitioners in conducting analyses and interpretation of their casework.

In 2012, the NIST Forensic Science Research Program, with sponsorship from the National Institute of Justice (NIJ), worked to develop a comprehensive list of databases, reference materials, and standard reference collections used by forensic scientists in laboratories at the state and local levels. The main goals were to identify existing data for forensic practitioners and to discover prevailing limitations. The results of the search identified 228 state, federal, and commercially run databases, which are all available to view at http://www.nist.gov/oles/forensics/forensic-database.cfm. Since that time, the NIST has been involved in several efforts to assist in expanding the availability of data and reference materials for various disciplines, including biometrics and firearms.

On July 19-20, 2016, the NIST held an event titled, "Trace Evidence Data Workshop: Improving Technology and Measurement in Forensic Science." This event was part of an effort at the NIST to gather feedback from the practitioners and researchers in the forensic science community on further improving access and expanding the development of datasets useful for trace forensic evidence. The main objectives of the event were to: (1) identify major gaps in the availability and accessibility of data for major areas of trace evidence analysis; (2) discuss possible solutions to addressing current gaps and determine future resource needs; and, (3) develop a road map of next steps to strengthen data in trace evidence.

Over the course of two days, attendees heard presentations from several renowned experts, scientists, and practitioners on the importance of references and data in trace evidence analysis. Many pointed out the struggle that forensic laboratories have faced in maintaining trace evidence units with the reductions in submissions and budgets for staff and training. Stakeholders from various levels of government verbalized the role that data could play in the development of new strategies for trace evidence analysis, in support of new approaches to interpretation, and in the development of standards. Presentations during the event covered broad topics, in addition to focused, moderated panels on fiber, hair, paint, tape, glass, explosives, and other miscellaneous particle analysis. Representatives from the private sector also contributed in a panel discussion.

This presentation will summarize the content of the meeting, describe future plans for follow-on projects, and relate preliminary recommendations obtained from the various discussions that took place. The ultimate goal of this effort is to assist in coordinating a multi-agency push to strengthen data in trace evidence in order to provide better tools to assist practitioners in conducting analyses and interpretation of their casework.

Trace Evidence, Data, Criminalistics

B204 What Can We Learn From the Coverdell Grants? Data Mining the Coverdell Grants to Assess Their Impact on the Forensic Community

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After attending this presentation, attendees will better understand, through the analysis of the data collected from the Coverdell awardees, that much can be learned about the contribution of the Paul Coverdell Forensic Science Improvement Grants Program (the Coverdell program) to the greater forensic science community.

This presentation will impact the forensic science community by providing an evaluation of the Coverdell program.

The Paul Coverdell National Forensic Science Improvement Act (NFSIA) became Public Law 106-561 on December 21, 2000. This Act authorized grants to states to improve the quality, timeliness, and credibility of forensic science services for criminal justice purposes. Beginning in 2002, the Coverdell program has been perennially offered through the National Institute of Justice (NIJ) and is relied upon by many forensic laboratories and organizations throughout the United States and its territories. Coverdell grants may be used to eliminate a backlog in the analysis of forensic evidence, to purchase instrumentation and/or equipment, and to train and employ personnel in forensic science laboratories and medical examiner/coroner offices. As of 2014, more than $184,000,000 has been awarded for more than 1,000 awards. Broadly, this evaluation will include a review of data collected by the NIJ through the life of the program to elucidate the impact the grants have made throughout the forensic science community. Trends studied include: (1) determining whether the Coverdell grant program has supported the implementation of new technologies by analyzing what trends existed in purchases (e.g., did any major shifts in equipment occur that might demonstrate a discipline-wide shift to a new technology and has it contributed to establishing new technologies in the field?); and, (2) if there were measureable effects on laboratory backlogs (i.e., has the program reduced the evidence backlog in the forensic laboratories and/or has the turn-around time decreased?).

To conduct this retrospective study, a sampling of the final budgets for completed Coverdell projects will be examined to determine if and what equipment was purchased. A review of the supplies purchased in relation to technology (not typical laboratory consumables) and relevant Contracts/Consultants (e.g., services related to evaluating or validating new technologies) will also be completed. Reviews will be conducted of the budget narratives to track the discipline(s)/forensic unit(s) for which those purchases were made, as well as the training purchased for laboratory staff. Additional metrics considered will be whether the Coverdell grants were used to pay overtime or hire new laboratory staff; the relevant dates in relation to the program, such as the initial award date, project starting date, and project completion date; and lastly, the source of the funds — whether they were formula or competitive grants.

Paul Coverdell, Backlog, NIJ

B205 Polymer Replication of Cartridge Cases for Proficiency Testing and Evidence Transfer

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After attending this presentation, attendees will understand the polymer replication process as applied to the replication of cartridge cases. Attendees will be able to perform the basic steps required in replication, as well as perform measurements/analysis of casing replicas to quantify the degree of similarity to the original masters.

This presentation will impact the forensic science community by aiding in the production of large proficiency testing sets that have a very high similarity between sets. This will help reduce errors introduced by variations between sets, thereby improving their effectiveness. Casing replicas can also be used for evidence transfer where chain-of-custody requirements inhibit the transfer of forensic evidence between agencies.

For the past several years, the National Institute of Standards and Technology (NIST) has worked on developing a validated polymer replication process for fired bullets. Quantitative analysis of bullet replicas has shown that polymer replication is a viable option for producing quality replicas that are virtually identical to the master bullets.

The NIST is now developing a process for replicating cartridge cases. Similar to bullet replication, it consists of a two-stage process in which a negative mold is first produced using silicone, then a positive cast replica is produced using polyurethane. While the process is similar to bullet replication, there are several key differences due to the specific geometries of the cartridge case surfaces being replicated. These will be described, as well as the measurement and analysis methods used in quantifying the degree of similarity of the replicas to the master cartridge cases.

Proficiency testing sets are commonly used to ensure forensic examiners are trained and qualified to examine forensic evidence. Typically, these testing sets are produced by repeatedly firing well-marking guns to produce the large number of samples required; however, even well-marking guns still have slight variations from one firing to the next. This can negatively influence the results of proficiency testing. Having the ability to replicate a master set of cartridge cases to a high level of detail is extremely beneficial. This allows multiple sets to be produced without significant differences between them. Using the polymer replication process, this can be achieved.[1] Large sets of casings can be produced and distributed, and the true results of the proficiency testing can be compared without any additional error introduced due to variations between sets.

Another important use for polymer replication is the transfer of forensic evidence. In many cases, evidence that is part of an ongoing investigation cannot be transferred to other agencies; however, due to the nature of violent crimes, it is sometimes necessary to exchange evidence with neighboring agencies to determine if there are additional links to other criminal cases. By using polymer replication, replicas of the original evidence can be shared with other agencies while maintaining chain-of-custody requirements.

Reference(s):

1. Koch A., Katterwe J. Castings of Complex Stereometric Samples for Proficiency Tests in Firearm and Tool Mark Examinations. *AFTE Journal.* Vol. 39 (4), 2007.

Polymer Replication, Cartridge Case, Proficiency Testing

B206 We're Not as Good as We Think, But We're Not as Bad as They Say: The Need for an Objective Look at the Challenges and Perceived Issues Within Forensic Sciences

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After attending this presentation, attendees will have an appreciation for both sides of the debate over the validity of the forensic sciences and its role in investigative and legal proceedings.

This presentation will impact the forensic science community by highlighting and addressing the divide that exists between forensic practices and the view of those practices from critical outside perspectives.

The forensic sciences are currently facing unprecedented challenges. Over the past several years, a series of high-profile missteps have exacerbated the perception that forensic scientists operate in a culture devoid of science. Stoked by a series of DNA exonerations, high-profile fingerprint misidentifications, newsworthy accreditation issues, and a litany of rogue scientists falsifying results, some of our most outspoken critics have generalized the entire forensic field as something that is unworthy of participation in our judicial process. On the opposite side of the debate, there are some among forensic scientists who have dug in their heels and taken the position that such claims are entirely false, everything is fine, and there is no reason to change anything that is currently being conducted.

As with any other scientific endeavor, forensic science is not perfect and is in serious need of improvements in a wide range of areas. To think that it will be fine to proceed in a "business as usual manner" is naive at best. Practices are not as good as some would believe and critics do raise some valid points that need to be seriously considered. Fortunately, rational scientific thought is winning out and a great deal of effort is being expended to make improvements throughout the forensic field. Unfortunately, our critics do not seem to be impressed with many of these efforts. Potentially tainted by environments that only highlight the negative, many of the critics appear unwilling to step outside of their spheres to take an objective look at the efforts toward change that are being put forth.

It is the objective of this presentation to demonstrate that both points of view, each heavily influenced by their own forms of confirmation bias, are incorrect. The opposite extremes of each group select only the information that supports their position yielding a two-way unwillingness to objectively evaluate the merits of each other's position. We may not be as good as we think, but we are certainly not as bad as they say.

Forensic Malpractice, Confirmation Bias, Forensic Validity

B207 The Importance of a Combined DNA Index System (CODIS) DNA Hit Follow-Up: A Case Review of Secondary DNA Transfer and the Individual Wrongfully Charged With Murder

Tahnee Nelson Mehmet, MSFS, Santa Clara County, Crime Lab, 250 W Hedding Street, San Jose, CA 95110*

The goal of this presentation is to share the case specifics of likely the first documented case of secondary DNA transfer of an innocent individual at a crime scene. The discovery of this individual's DNA profile on the fingernails of the decedent incorrectly implicated him as one of the perpetrators of the homicide, consequently forcing him to serve several months in county jail before his alibi was discovered. This case highlights the extreme importance of conducting proper follow-up investigations once an individual is associated to a crime scene via DNA evidence.

This presentation will impact the forensic science community by sharing a case review in which DNA transfer wrongfully implicated an individual of murder and by explaining how the presence of an individual's DNA profile, especially when first determined through a CODIS DNA hit, must be properly investigated.

DNA transfer is the presence of an individual's DNA profile on an item even though the individual never directly came into contact with the item. The mode of transport can be secondary or tertiary in nature and has been documented in mock crime scene scenarios in the relevant literature. DNA transfer has long been discussed in the context of forensic evidence and used to theoretically explain the presence of DNA profiles at crime scenes. This concept has become increasing important, especially with the rise of contact DNA testing of very small amounts of DNA.

The homicide case discussed in this presentation is likely the first documented case of secondary transfer of DNA evidence on an actual crime scene sample. The robbery-homicide rattled the quiet community, especially since there were no leads and the victims initially appeared to be completely random targets. The Santa Clara County Crime Laboratory was tasked with examining several items of evidence from the scene, including samples collected from the decedent's body, duct tape used to bind victims, and several disposable gloves found throughout the house. The identity of the individual, Lukis Anderson, whose DNA was transferred to the fingernails of the decedent, was discovered through a DNA hit. At the time of the homicide, he was a transient of downtown San Jose with a petty criminal history. Two other individuals were also associated to the crime scene through DNA hits on various items of crime scene evidence, including duct tape and disposable gloves. The latter two individuals had extensive criminal histories and were known gang members from Oakland, CA, with clear ties to the victim once further investigations were conducted. On the other hand, follow-up investigations revealed that Anderson did not have any known associations with the other two individuals or with the victims. Further investigation by his defense attorney revealed that he was incapacitated at a local hospital at the time of the homicide, thereby proving his innocence.

Following the discovery of this information, Anderson was promptly released from county jail and cleared of all charges. The crime laboratory conducted additional Y-chromosomal Short Tandem Repeat (Y-STR) testing that confirmed Mr. Anderson wasn't connected to this crime via an adventitious, or false, DNA hit. Once news of this circumstance became public record, the media began to speculate on the explanation of the presence of Anderson's DNA on the decedent's fingernails, including accusing the DNA criminalist of using improper techniques and contaminating the fingernails with Anderson's DNA profile. Ultimately, the Santa Clara County District Attorney's Office discovered that the same paramedics that responded to the homicide scene also treated Anderson earlier that evening. It is thought that a medical apparatus called a pulse oximeter, which attaches to the fingertip of the patient, was the mode of transport of Anderson's DNA profile to the decedent. Without the proper follow-up investigations, it is unclear what Mr. Anderson's fate would have been, as he likely could have been wrongfully convicted of murder. Thankfully, the truth was discovered and he was cleared of all wrongdoing. The forensic science community must learn from this case that DNA transfer is not just a theory used to distract juries, and thorough investigations following a CODIS DNA hit should be undertaken by the proper authorities to provide supporting information as to the individual's criminal involvement.

DNA Transfer, CODIS, Wrongful Arrest

B208 Exploring the Relationship Between Quantitative Polymerase Chain Reaction (qPCR) and Short Tandem Repeat (STR) Data for Compromised, Low-Template DNA Samples

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The goal of this presentation is to provide attendees with an understanding of how information obtained from the qPCR reaction, such as the degradation index and Internal PCR Control (IPC) Cycle threshold (C_t), may relate to the resultant electropherogram. This will be accomplished by examining qPCR and STR data obtained from pristine, enzymatically degraded, sonicated, Ultraviolet (UV) -damaged, and humic acid-inhibited DNA extracts.

This presentation will impact the forensic science community by demonstrating that the correlation between qPCR and STR data varies and depends upon the way in which the DNA was compromised. Although results from the qPCR reaction are often used to inform downstream processing and interpretation, the data presented indicate that under certain circumstances, and particularly for low-template, compromised extracts, information obtained from the qPCR reaction is only an approximation of the amount of amplifiable DNA. The information garnered from qPCR becomes less informative as the DNA becomes more compromised and as the extract contains fewer copy numbers.

With the implementation of increasingly sensitive detection systems, compromised (i.e., damaged, degraded, or inhibited), low-template DNA samples are often encountered in forensic casework. During forensic DNA processing, quantitation via qPCR precedes STR amplification. Data obtained from the quantitation reaction, which now include a measurement of total human DNA content, an assessment of the level of degradation, and information pertaining to the amplification of an Internal PCR Control (IPC), may inform downstream processing steps.[1,2] Ideally, with well-paired qPCR and STR amplification kit chemistry, these metrics may be used to optimize the amount of sample added to the amplification reaction or indicate that additional treatment may be worthwhile.

This study seeks to relate and compare information obtained from the qPCR reaction to the resultant STR Electropherogram (EPG) using both pristine and compromised DNA samples obtained from whole blood. Compromised samples were generated via enzymatic degradation, sonication, UV damage, and inhibition with humic acid. For each type of compromised extract, three levels were produced by varying concentration (DNase and humic acid) or reaction time (Fragmentase, UV, and sonication). Extracts were quantified then amplified using 29 cycles at 0.25ng and 0.03ng. Fragment analysis was completed using common electrophoresis settings and an analytical threshold of one Relative Fluorescence Unit (RFU).

Three phenomena were observed: (1) the correlation between the Degradation Index (DI) obtained from the qPCR assay and the peak height slope observed across increasing molecular weight loci in the EPG varied with different types of compromised extracts. Sonicated samples displayed the highest correlation between DI and slope, and the remaining sample types in order of decreasing correlation for 0.25ng templates were: DNase/Fragmentase, UV, and humic acid. For all compromised extracts, this correlation became worse as the template mass decreased; (2) with the exception of inhibited extracts, a decrease in RFU at the lowest molecular weight loci was observed with all compromised extracts as the DI increased despite the target mass remaining constant (0.25ng). The concentration of the 80bp target in compromised samples may be an overestimate of the amount of amplifiable product available for STR amplification. Although the STR amplicons tested range in size from ~80bp-450bp, the low molecular weight region is largely composed of rare autosomal alleles and non-autosomal markers with low discriminating power.[3] Preliminary results obtained with sonicated extracts quantified with a 140bp target and amplified with STRs that range in size from ~100bp-400bp suggest that using a longer qPCR target more representative of the size of the shortest high-frequency STR alleles may result in a more informative EPG for certain sample types; and, (3) with regard to extracts inhibited with humic acid, no correlation was observed between the DI and IPC C_t obtained from the qPCR assay and the resultant EPG. In the worst-case scenario, characterized by an out-of-range or undetected IPC C_t and an undefined degradation index, the corresponding EPGs exhibited no discernable peak height slope

across loci and a drop-out rate of 0% for 0.25ng template samples. Cumulatively, these data indicate that for low-template, compromised extracts, information obtained from the qPCR reaction provides only some predictive value regarding the final EPG and may not be an appropriate method by which to decide whether a sample is likely to produce an EPG with substantive levels of signal.

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DNA Quantification, DNA Degradation, PCR Inhibition

B209 Touch DNA: "Touch" Time and Resiliency

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After attending this presentation, attendees will better understand touch DNA, the variation in quantity of DNA related to touch time, and what type of methodology can best be used to collect touch DNA.

This presentation will impact the forensic science community by providing information regarding the collection of samples of touch DNA from garments and by clarifying the major aspects of these forensic techniques.

With every touch of the skin on a surface, cells are left behind; with every cell, the genetic code can be found. People touch doors, tables, and so many other surfaces every day, often with multiple people touching the same object throughout the course of a day.[1] New research has found that touch DNA analysis can erroneously implicate a person who had no contact whatsoever with the crime scene as the main contributor of the DNA on its handle.

When a swab is used for evidence collection from a surface, the investigator may not know how many people may have touched this evidence in the past or what level of persistence DNA may have on touched objects over time. Though every touch leaves cells containing DNA, most contact leaves only a few cells if minimal pressure is used. These trace levels of DNA may remain undetected or, if detected, may be at such low levels that only stochastic effects and low levels of allele drop-in are observed.[2]

The goals of this study are to evaluate how much time (in term of seconds) is necessary to leave touch DNA on a dress and to determine what type of technique maximizes the DNA recovery by evaluating swabs, cuttings, and adhesive tape to sample an area of interest.

To acquire a greater knowledge of the rate of a detectable wearer as well as touch and background DNA, 30 females wore their brassieres for 12h. Subsequently, the lateral regions of each brassier were handled by one of five male volunteers for different lengths of time: 60s, 45s, 30s, 20s, and 10s.

Every experiment was conducted in triplicate. In the first case, the sample was collected by swabbing. The second case employed cutting the area for testing. The third case was sampled with adhesive tape. The quantity of recovered DNA was determined using real-time Polymerase Chain Reaction (PCR) with Alu-based targets and SYBR® Green detection. The samples were also analyzed using capillary electrophoresis-based Short Tandem Repeat (STR) typing to determine the percentage of recoverable alleles. Touch DNA is an emulation of the Locard exchange principle, in that any time a person is in a location, they may leave DNA evidence of their presence.

The results revealed that the best technique to recover touch DNA is to cut the area of interest. The "toucher" was detected as a single profile in samples handled between 60s and 45s. The "wearer" was present in the mixtures obtained from the 30s to 10s samples, but the "toucher" was always observed as the major contributor.

Greater knowledge of the frequency of detection of reportable wearer DNA and toucher DNA allows scientists to evaluate the likelihood of observing a matching profile if an individual wore a garment rather than touched it in disputed case scenarios. Everyone in the medicolegal community — forensic scientists and technicians, DNA analysts, potential jurors, and judges and lawyers for both the prosecution and defense — must know and understand the potential for mistakes.

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Touch DNA, DNA Recovery, Touch Time

B210 Increasing DNA Recovery With Nylon Flock Swabs and One-Step Spin Baskets

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After attending this presentation, attendees will understand the salient features related to DNA recovery from forensic samples. Since swabbing is a routine method of sample recovery, the important features include collection efficiency, recovery of DNA from the collection device, elution strategies, and purity of the DNA.

This presentation will impact the forensic science community by stressing the importance of collection and extraction. Indeed, it is these processes that contribute substantially to the success of DNA typing, especially for low-quantity samples. Success of DNA typing is related to the amount of target material recovered from an evidentiary item.

While not considered as dynamic as the analytical phase, recovery and extraction of DNA are critical to the success of DNA typing. Successful typing can be increased by recovery of greater quantities of suitable-quality DNA. The features that favor collection of a stain at the crime scene often are the same ones that reduce successful recovery of DNA from a swab medium. The primary collection device in current use, the cotton swab, is designed to recover biological stain evidence with reasonable efficacy and is relatively inexpensive; however, the release of DNA from cotton is rather inefficient. There are other devices, such as the 4N6FLOQSwab™, composed of short nylon fibers arranged in a perpendicular fashion by flocking, which will not trap DNA within its matrix as cotton does. In addition, swab heads are often subjected to multi-step manipulations involving a spin basket device. One improvement on the process is the use of the Nucleic Acid Optimization (NAO™) basket, an insert which allows for one-step processing of the swab tips. This approach yields more DNA, reduces labor/processing time, and decreases the likelihood of sample contamination. These various devices have been compared to assess the efficiency of DNA recovery by direct deposit and/or swabbing of neat blood and diluted blood; dried blood, semen and saliva at various dilutions; and trace touch samples. In all cases, DNA recovery and, thus, typing results obtained with the 4N6FLOQSwab™ outperformed that of the cotton swab. These alternate devices also contribute to an efficient workflow.

This presentation will describe the important features to consider for an efficient process of DNA recovery and will present results from comparative testing. Attendees will benefit by becoming more aware of methods to enhance DNA typing success.

Swab, Basket, DNA Recovery

B211 Alternative Reducing Agents for DNA Extraction

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After attending this presentation, attendees will understand how reducing agents can improve forensic DNA extraction, that there are alternatives to the commonly used reducing agent Dithiothreitol (DTT), and how to easily substitute these agents into an established casework workflow.

This presentation will impact the forensic science community by introducing and describing alternative reagents to improve DNA extraction from semen samples. The reduction of disulfide bonds is a critical step in the extraction of DNA from sperm cells, which may be improved using alternative reducing agents compared to DTT.

A commonly utilized reducing agent in forensic DNA extraction is DTT, which facilitates the isolation and purification of DNA from proteins within a biological sample by reducing disulfide bonds, thereby enhancing proteinase K digestion. Additionally, an alkylating agent, such as Iodoacetamide (IAM) may be employed as a secondary step to prevent reformation of disulfide bonds.

DTT has many disadvantages as a reducing agent, in that it is unstable in air, requires refrigeration, and has an unpleasant odor. During a previous study, it was observed that alternatives to DTT *alone*, such as DTT followed by alkylation with IAM or replacement of DTT with Tris(2-Carboxyethyl)Phosphine (TCEP), improved DNA extraction yields.[1] Therefore, this study focused on identifying other alternatives to DTT to improve/enhance DNA yields from semen samples. Three alternatives to DTT and TCEP were identified and evaluated. These included Dithiobutylamine (DTBA), Glutathione (GSH), and Tributylphosphine (TBP).[2-4] Specifically the following conditions were compared: (1) DTT alone (Standard Operating Protocol (SOP)); (2) DTBA; (3) DTBA followed by IAM; (4) GSH; (5) GSH followed by IAM; (6) TBP; and, (7) TBP followed by IAM. DNA yields were measured using a nuclear DNA-specific quantitative Polymerase Chain Reaction (qPCR).

All reagents tested were water-soluble and easily incorporated into a semi-automated SOP workflow. In brief, the SOP involved lysis of the semen specimen in buffer G2, proteinase K, and DTT, followed by purification on an EZ1™ BioRobot®. Each alternative reducing agent simply replaced DTT during the initial lysis stage. Alkylation by IAM, where tested, involved a separate incubation for 30 minutes in the dark at room temperature.

Results demonstrated that the alternative reducing agents provided increased DNA yields over the SOP. DTBA, DTBA followed by IAM, GSH, and GSH followed by IAM showed significant increased yields of 25%, 25%, 65%, and 61%, respectively, compared to the SOP. In conclusion, DTBA and GSH may be considered superior reducing agents to that of DTT for forensic DNA extraction. In particular, these alternatives may prove invaluable for challenging samples, such as low template, or those which are recalcitrant to extraction, such as spermatozoa.

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DNA Extraction, Dithiobutylamine, Glutathione

B212 An Analysis of an Internal Validation Dataset for the New Core Short Tandem Repeat (STR) Loci

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After attending this presentation, attendees will better understand the open source interpretation tools for analyzing large internal validation data sets.

This presentation will impact the forensic science community by exploring different means to parse the output from internal validation experiments designed and performed in forensic laboratories to demonstrate the reliability, reproducibility, and robustness of the current larger STR DNA genotyping chemistries.

In March of 2015, the Federal Bureau of Investigation (FBI) published the expansion of the original core 13 loci to a new Combined DNA Index System (CODIS) core containing 20 loci.[1] Following validation guidelines outlined by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the European Network of Forensic Science Institutes (ENFSI) DNA Working Group, an internal validation dataset was generated to assess the PowerPlex® Fusion™ 6C STR multiplex chemistry.[2,3]

Organization and analysis of internal validation data is often performed manually within an Excel® spreadsheet format within laboratories. Thorough analysis of validation data sets for the new STR typing kits and/or new technologies within forensic laboratories may appear daunting, but it is essential for robust data interpretation to generate the most accurate results for producing a laboratory's standard operating procedures and technical manuals.

To illustrate the analysis of an internal validation data set, 44 reference and known samples were used in experiments designed to validate PowerPlex® Fusion™ 6C, which is comprised of 23 autosomal STRs, 3 Y-chromosomal Short Tandem Repeats (Y-STRs), and amelogenin. Results obtained from the internal validation experiments provided data to extract and evaluate parameters, such as sensitivity, stochastic effects, sizing precision, allele calling accuracy, repeatability and reproducibility, DNA mixture performance, and contamination detection.

This presentation will describe the process of interpreting internal validation experiments for PowerPlex® Fusion™ 6C using several open source software tools. Data analysis were conducted using the in-house software programs developed at the National Institute of Standards and Technology (NIST) (http://www.cstl.nist.gov/strbase/software.htm), as well as *STR-validator*, a software created by Oskar Hanson at the Norwegian Institute of Public Health.[4]

This presentation will illustrate: (1) the specific data formatting for import into software tools; (2) the calculations of analytical and stochastic thresholds manually as well as in *STR-validator*, which examines multiple published calculation methods; and, (3) the data output of stutter percentage calculations, peak height ratios, base-pair sizing precision, mixture detection, genotyping concordance, reproducibility, and sensitivity.[5-7] The goal is to share the findings of this study with forensic laboratories and introduce the community to open source programs that can be utilized during the internal validation process.

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Autosomal STR Markers, Internal Validation Studies, Software

B213 A Survey on Serological and DNA Typing Methods in United States Forensic Laboratories

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After attending this presentation, attendees will better understand the most common serological tests and DNA typing kits in use in a representative sample of forensic DNA laboratories in the United States. This presentation will discuss the amount of variation that exists with the laboratories' chosen methodologies, the trends toward new methodology and instruments, and the knowledge that forensic professionals want new employees or recent graduates of forensic programs to possess prior to entering the field.

This presentation will impact the forensic science community by informing United States forensic practitioners about their community's trends and the methods in use. This will help determine the variation present among laboratories. The results will also act as guidelines for educators in developing course content in their forensic science programs to maximize students' pertinent knowledge prior to entering the workplace.

There are approximately 400 public forensic laboratories in the United States, of which 59% perform serological and DNA typing analysis on evidence. Each laboratory selects the methodologies and kits, then validates corresponding Standard Operating Procedures (SOPs) based upon their needs and expertise. The differences among laboratories lead to variation and a lack of SOP standardization within the community, which is a major concern, particularly with DNA mixture interpretations. A 2013 study by the National Institute of Standards and Technology (NIST) found discrepancies in how laboratories were interpreting artificially generated mixture profiles. The results highlighted significant variation among the conclusions reached, not only by different laboratories, but also within a single laboratory between different analysts. Understanding and characterizing the basis of this variation will benefit the community.

The variation in public forensic laboratories is also reflected considerably among forensic biology educational programs in the United States, whose purpose is to develop and effectively prepare pertinent content to maximize employment opportunities for their students.

To address the aforementioned concerns, a survey was developed to receive feedback from laboratories on various areas of testing. Topics ranged from collection methods and presumptive and confirmatory tests for different body fluids to the type of kits laboratories use for extraction, quantitation, and amplification. For example, laboratories were asked to select which tests are used for the presumptive and confirmatory testing of blood and to describe their workflow process for blood evidence screening. In addition, there was a section for comments and advice for educators. The survey was distributed to 198 National DNA Index System (NDIS) laboratories through Qualtrics, an on-line survey-making platform. One hundred seventeen responses were collected, but 17 responses were incomplete and therefore excluded from the analysis. With 100 complete responses, the response rate of the survey was 50.5%.

Results from the survey not only highlight the most popular serological methods and DNA kits, but also provided insight to the forensic DNA community. For example, results revealed that phenolphthalein (the Kastle-Meyer test) was the most common presumptive test (71%) and the ABAcard® HemaTrace® was the most common confirmatory test (64%) for blood. Variation was observed more frequently with the laboratories' serological methods and workflow processes, especially for sexual assault evidence. Several laboratories process blood evidence with a visual examination, followed by a presumptive test; if the presumptive test is positive, the evidence is processed for DNA. Sexual assault workflow, on the other hand, is dependent on the type of evidence given the different items present in a sexual assault kit, such as swabs, slides, and clothing. Consequently, the evidence may be processed differently. One possible workflow is to process the evidence first with a microscopic sperm search and, if the search is negative, with a confirmatory test for p30, indicating the presence of seminal fluid. Another approach is to perform a differential extraction to isolate the male DNA, then a microscopic sperm search on the evidence from which the male profile was obtained. Furthermore, laboratories were surveyed on additional analysis capabilities, such as mitochondrial DNA (mtDNA) analysis and Massively Parallel Sequencing (MPS) capabilities.

The survey provided a significant amount of information from several laboratories. This allows for the identification of commonly used methods and an understanding of how these vary across laboratories. This information will inform laboratories on the most popular current, and potentially future, methods used by forensic practitioners, allow laboratory directors to be better prepared for the future, and help educators develop their programs to produce knowledgeable graduates.

Survey, Serological Tests, DNA Typing

B214 The PROVEDIt Initiative: The Development and Release of a Collection of Computational Tools and a Large-Scale Empirical Data Set for Forensic Research and Validation

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After attending this presentation, attendees will be aware of the release of a large-scale database of DNA samples containing up to five contributors. Attendees will also have knowledge of five software-based applications that can be utilized during forensic research, validation, or pedagogical pursuits.

This presentation will impact the forensic science community by providing an easily accessible large-scale database of compromised, mixed DNA samples of varying templates. This will allow all members of the forensic science community to test software implementations and various hypotheses from a single, well-characterized data set.

The interpretation of forensic mixtures is difficult and becomes increasingly more difficult as: (1) the number of contributors increases; (2) the number of copies decreases; and; (3) Polymerase Chain Reaction (PCR) becomes less efficient because of PCR inhibitors or DNA damage. Several interpretation tools, analysis techniques, and interpretation standards/recommendations have been developed and released. In the case of software solutions, all of these systems rely upon assumptions and have computational nuances associated with their algorithms; thus, there is considerable interest in comparing their performances.

In an effort to provide support to the community and to foster growth in both forensic research and operations, the Project Research Openness for Validation with Empirical Data Initiative (PROVEDIt) is announced.

PROVEDIt comprises 25,000 .fsa and .hid profiles as well as a suite of analysis, interpretation, and *in silico* software systems/procedures and models developed in a variety of environments. The profiles and tools are available to the community on Boston University's DNA Mixture Website (www.bu.edu/dnamixtures).

The collection of computational systems includes: (1) Computational Evaluation of Evidentiary Signal (CEESIt) — outputs the likelihood ratio, likelihood ratio distribution, and p-value for an unknown; (2) Number of Contributors (NOCIt) — outputs the a posteriori probability distribution for the number of contributors; (3) Genotype Generator & Evaluation Tool (GGETIt) — a simulator that outputs the minimum number of contributors based on allele counts; (4) Simulating Evidentiary Eletropherograms (SEEIt) — a dynamic model that simulates the entire forensic process and produces well-characterized electropherograms for up to six contributors; and, (5) CleanIt — an automated procedure for filtering bleed-through, complex bleed-through, and minus A from an electropherogram.

The collection of .fsa and .hid profiles includes one- to five-person DNA samples, amplified with targets ranging from 1ng to 0.007ng, with varying levels of damage and contributor ratios.

The usefulness of large data sets is demonstrated by plotting a histogram of the empirical signal for each locus and confirming that signal from one copy is detected. These histograms exhibit at least three seemingly distinct

peaks. For example, for the D8S1179 locus, the first signal group (median 4 Relative Fluorescence Units (RFU)) consists largely of instrumental noise; the second group (median 24 RFU) is the signal obtained when one copy of DNA is amplified; and the third (median 47 RFU) is the signal obtained when two copies of DNA are amplified. Next, the signal obtained when these same samples were injected for twice as long is presented. The same multi-modal pattern is observed but, with doubled injection time, the first and second signal groups appear at 4-11 RFU and 36-65 RFU, respectively. These data suggest that single-copy DNA signal is regularly detected using modern forensic laboratory implementations.

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PROVEDIt, DNA Mixtures, Low-Template DNA

B215 The Development of the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2391d: The Next Iteration of the Polymerase Chain Reaction (PCR) -Based DNA Profiling Standard

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After attending this presentation, attendees will understand the benefits and potential differences of the next iteration of the NIST SRM 2391d: PCR-Based DNA Profiling Standard.

This presentation will impact the forensic science community by demonstrating what is involved in developing and characterizing a NIST SRM, as well as what will be different about this SRM when compared to the current 2391c version.

The first NIST Standard Reference Material (SRM) 2391: PCR-Based DNA Profiling Standard was developed in 1993 and produced in 1995 when it became apparent that a standard reference material was necessary to ensure accurate and comparable measurements between laboratories in the DNA forensic community.[1] In fact, it was eventually used to address the United States Federal Bureau of Investigation's (FBI's) Quality Assurance Standards (QAS) for laboratories conducting forensic DNA testing that were published in 2000 and updated in 2011 (Sect. 9.5.5): "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to a procedure against an appropriate and available NIST standard reference material or standard traceable to a NIST standard."[2] Since then, several iterations of the SRM have been made available as the prior versions were depleted. SRM 2391c is the current version and is expected to be depleted in February 2018 based on reported sales.[3] Work has begun on planning the next iteration: SRM 2391d, so it is available to the community when SRM 2391c is completely exhausted. The historical significance of these standards is important and will be briefly discussed.

There is a great deal of planning and strategizing involved when beginning the process of producing an SRM. The technology and needs of the DNA forensic community are constantly changing and evolving and these need to be considered when selecting samples and determining what techniques will be used for certification. The thought processes behind the development and production of SRM 2391d will be explained and input from the community will be addressed.

Because of the advances in technology and new Short Tandem Repeat (STR) markers recently required for Combined DNA Index System (CODIS), new information will be included and certified with SRM 2391d. The samples that are chosen will have certified genotypes/haplotypes for the commercially available STR markers via characterization by capillary electrophoresis, Sanger sequencing, and next generation sequencing. Concordance between the results of the fragment-based and sequence-based methods will serve as validation for the certified values that will be assigned for each marker run with the chosen SRM samples/components. A summary of what is planned for SRM 2391d will be presented.

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Forensic DNA, Standard Reference Material, STR Markers

C1 The Development and Preliminary Application of the New ForensicSW Software for Image Analysis

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After attending this presentation, attendees will understand the new ForensicSW software for image analysis.

This presentation will impact the forensic science community by describing how ForensicSW can be utilized easily and intuitively as a suitable method of semi-quantitative analysis in Thin-Layer Chromatography (TLC) plate and may be applied to other forms of forensic analysis.

Recently, many analytical methods using image analysis software with more intuitive, simple, and highly practical applications have been developed to produce the quantitative data from qualitative results by combining the image analysis function.[1] Many types of image analysis software have been utilized in various parts of forensic, medical and analysis science as needed and requested, including TLC, drug test, fingerprint, age estimation, soil granules, blood vessel pattern, Computed Tomography (CT), and many others.[2-4]

Image analysis software is frequently used as a semi-quantitative method in TLC, however, many automatic TLC analysis systems are expensive and not as easy to use as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography/Mas Spectrometry (GC/MS); therefore, general use in the small laboratory is limited.[5-8]

Consequently, several open-source image analysis software tools were developed by universities or government institutions for easier use and lower cost; however, as most of the software has been developed for analysis of images of electrophoresis gel, the software needs to be installed and requires large storage space. Additionally, they consist of macro expansion for less installation capacity, and a programming background is required to utilize the software.

This study is related to the development of image analysis software with a new algorithm named ForensicSW. This software can be utilized easily and intuitively for semi-quantitative analysis in TLC plates and applied to other forensic analysis. It is also compared with two other software products, CP ATLAS 2.0 and ImageJ. To explore the possibility of its use in forensic science, fingerprints and letters written in pencil were analyzed using the newly designed software.

ForensicSW is simple and easy to use with an intuitive User Interface (UI) for the semi-quantitative image analysis based on Windows® OS, written in the C++ language, and mounted with only the minimum functions for easy use and rapid analysis of an image.

The area calculation algorithm of ForensicSW is the sum of the horizontal line of the color density in each pixel ranged from 0 to 255, which corresponds to the y-axis of the plot profile. It can provide more accurate results than other software that use an integral sum algorithm. The automatic recognition of background color is a useful function for rapid analysis without further adjustment of the images digitalized under UV_{254}, $UV_{365,}$ visible light, and any other light source. The calibration curve and R^2 values can be obtained with a built-in function for statistics.

The images of TLC plates of five derivatized PEs with four derivatizing reagents were analyzed with ForensicSW and two other software products.

The result of Repeatability Standard Deviation (RSD) and linearity (R^2) from the analysis using three software products was compared. The evaluation of the semi-quantitative method based on the combination of derivatization in TLC and image analysis was acceptable for identification of amine compounds derivatized with derivatization reagents. The RSDs were 0.69 ~ 5.87 analyzed by CP Atlas 2.0 and 0.72 ~ 11.20 by ImageJ. The R^2 values were

R^2>0.99 on average, depended on the concentration of PEs.

The R^2 value of derivatized EP with FMOC-NHS was 0.9732 when analyzed by CP ATLAS 2.0, 0.9185 by ImageJ, and 0.9842 by ForensicSW. The RSDs were 5.96 ~ 6.88 analyzed by CP ATLAS 2.0, 5.12 ~ 8.35 by ImageJ, and 3.71 ~ 3.82 by ForensicSW. It was proven that ForensicSW demonstrated a rapid, easy, and sensitive semi-quantitative analysis result compared to the other two software products.

The similar patterns of plot profile from the image analysis represented in the fingerprints imprinted twice under the same conditions (about 1kg·f for five seconds) on the A4 paper of the same person were demonstrated by ForensicSW. ForensicSW could be helpful to evaluate fingerprints when combined with Automated Fingerprint Identification System (AFIS) through additional research.

Also, ForensicSW was applied to the analysis of handwriting. The characteristics of writing habits such as the writing pressure of each letter with a pencil, was examined with a plot profile by ForensicSW.

Based on the fact that ForensicSW showed acceptable results in R^2 and the RSD value of the TLC image analysis, the algorithm of ForensicSW was suitable for semi-quantitative analysis of TLC images. The applicability of ForensicSW for other areas of forensics can be studied. This new image analysis software has many merits in the field of forensic science. In addition, the UI of ForensicSW is easier and more intuitive for the user than other image analysis software. Therefore, ForensicSW can be considered as a new alternative for the image analysis.

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Image Analysis Software, Semi-Quantitative Analysis, ForensicSW

C2 An Analysis of a Photocopier Hard Drive for Forensically Relevant Artifacts

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After attending this presentation, attendees will feel more confident in their ability to analyze photocopier hard drives and will better understand what files can and cannot be recovered. Attendees will also be more confident using their own forensic tools to retrieve such information.

This presentation will impact the forensic science community by providing information regarding forensic artifacts that can be recovered from a photocopier hard drive during four stages: (1) a blank hard drive; (2) a hard drive with an Operating System (OS) installed; (3) a hard drive with data generated; and, (4) a hard drive that has been initialized or wiped by the photocopier. The goal of this project was to determine which stage yields the most data and to see how accurate the photocopier's wiping process is. It is believed that data files can be recovered due to the nature of hard drives themselves.

In the world of digital forensics, many people fail to recognize photocopiers, or Multifunction Peripherals (MFPs), as having any probative value. These machines actually contain a hard drive to aid in processing or sorting multitasking functions. Thus, the hard drive acts as a storage media and saves the documents sent to it for the various jobs the machine performs. These machines are heavily used in many offices (businesses, government, universities, etc.), and the devices can potentially be a gold mine if the data falls into the wrong hands. Contrary to popular belief, the hard drives are fairly easy to obtain if the photocopier breaks or gets replaced by a newer model. This is due in part to some offices simply tossing out the old copiers and paying no attention to the hard drive left in the machine, thus making the hard drive available to anyone who wants to take the time to remove it.

The project involved removing the hard drive from a Canon® imageRUNNER ADVANCE 4035 photocopier during the four stages of its life cycle and analyzing the content obtained. The hard drive was cloned twice at each stage using a Disk Jockey PRO Forensic Edition to provide an actual and working copy to use during analysis. The results suggest that data generated on the machines was able to be recovered using forensic software programs such as FTK® 5.6.13 and Autopsy® 4.0.0. The files that were obtained corresponded to time stamps for the various jobs performed, phone numbers for faxes, email addresses, and other log files. In one case, an exact document matching the original was found as a PDF file. After initializing the photocopier, the data was overwritten by the machine and only the files associated with the working OS remained.

Photocopier, Hard Drive, Forensics

C3 A Text-Independent, Automatic Speaker Recognition System Evaluation With Males Speaking Both Arabic and English

Safi S. Alamri, MS, PO Box 91904, Riyadh 11634, SAUDI ARABIA*

After attending this presentation, attendees will better understand how to compare text-independent samples of speakers using different languages against a single-language reference population.

This presentation will impact the forensic science community by illustrating how conducting a study to better understand a design may be beneficial in further developing software that can complete accurate, text-independent, automatic speaker recognition for bilingual speakers against a single-reference population. All samples were taken from a text-independent speaker recognition system and enhanced to optimal performance. The data obtained were processed by a BATVOX 4.1, which deploys the Mel Frequency Cepstral Coefficients (MFCCs) and Gaussian Mixture Model (GMM) methods of speaker recognition and identification. The results of testing through BATVOX 4.1 was a likelihood ratio for each sampled voice that was evaluated and the problems experienced. This presentation provides a brief overview of the area of text-independent, automatic speaker recognition system evaluation with males speaking both Arabic and English. The purpose is to compare text-independent samples of speakers using different languages against a single language reference population.

Automatic speaker recognition can be classified into speaker identification and speaker verification. Speaker identification deals with determining who the speaker is in the provided sample. The speaker usually lacks identity, so it is assumed the unknown speaker must come from a set of known speakers fixed from the system. Speaker verification deals with determining whether or not the speaker is the claimed person. In this research, the emphasis is on text-independent automatic speaker recognition; however, the process can be classified as either text-dependent or text-independent. But it depends on the cooperation of the involved parties and the available data. The text-dependent application is designed to identify the speaker through the recognition systems regardless of what they say.[1]

Automatic speaker recognition has several applications, both commercially and forensically. Some of the commercial applications entail telephone banking, voice mail, prison call monitoring, voice dialing, and biometric authentication.[2] The emphasis on the current study is on forensic applications, which includes systems such as BATVOX. It can be applied to both investigative and evidential purposes. The systems have two main processes, which are feature extraction and classification. Feature extraction takes small portions of samples that will be stored and used later for speaker identification. The most common technique for feature extraction is the MFCCs.[3] Classification is a two-phase process that starts with speaker modeling, which is the features of a new speaker, then uses speaker matching, which includes the features saved in a database.[4]

For text-independent applications, there must be a speaker model in place. The speaker model is a recognition system that has trained speaker samples stored in a database that used acoustic feature vectors extracted from each trained samples comparison to any given sample. This is what allows the text-independent application to have no restrictions on the words the speaker can use, but also makes it a more challenging method of automatic speaker recognition because of differences in linguistic content and potential phonetic mismatch.[5] Many models are available, such as Hidden Markov Model, MFCCs, Vector Quantization, GMM, Neural Networks, and Radial Basic Functions.[6]

In this study, the MFCCs and GMM-Universal Background Model (UMB) models are used. Nonetheless, MMFCs are the most notable features in automatic speaker recognition. The goal of the MFCC is to model the vocal tract's spectral envelope, consisting of the formants and a smooth curve connecting them, and using them as an identifier. This takes place by taking the spectral envelope and applying a filter based on human perception experiments, which applies filters to the spectral envelope and creates the spectrum known as the Mel-Spectrum. Cepstral transformation is then performed on the Mel-Spectrum, and the outputs are the MFCCs and speech then represented as a sequence of cepstral vectors.[7] The GMM is a weighted cumulative of the features observed from a sample when compared to the trained model, the outcome being the Log Likelihood (LL). The higher the value of the LL the higher probability that the mode and evidence are the same speakers. The GMM is a representation of the cumulative observed features from the speaker taken from the underlying model. Forensic automation speaker

recognition was created to make it easier and more accurate to conduct speaker recognition. This involved creating an algorithm that then makes a quantitative analysis of the speech signal.[8]

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Automatic Speaker Recognition, Likelihood Ratio, Arabic and English

C4 Closing the Performance Gap in Forensic Speaker Recognition

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The goal of this presentation is to educate the broader forensic community about current capabilities in forensic speaker recognition and National Institute of Standards and Technology (NIST) activities to support progress in this field.

This presentation will impact the forensic science community by providing an overview of what is possible in the field of forensic speaker recognition, an update on research activities, and a description of future directions.

Speaker recognition is the process of determining whether two (or more) speech samples (live or recorded) are from the same person or different people. While speech recognition seeks to answer what is being said, speaker recognition attempts to answer who is talking. Forensic speaker recognition isn't called upon very often as a forensic technique, but when it is, there are a variety of methods in use.[1]

In forensic casework, analysts are asked to perform speaker comparison tasks from evidentiary recordings that typically exhibit a high degree of variability. The forensic speaker recognition research community has spent a great deal of time and resources in an attempt to study and address these sources of variability. Most of the benefits have played out in algorithm development and, when tested with the type of high-quality speech data that is usually unseen in forensic settings, technology performance has improved greatly.[2] Yet, very often these tools do not perform well when confronted with the type of data encountered in forensic casework.[3] This performance gap is real and significant when compared to performance under optimal conditions. The path forward to address this gap has taken time to come into view.

The Organization of Scientific Aea Committees (OSAC) Speaker Recognition Subcommittee and the European Network of Forensic Science Institutes (ENFSI) Forensic Speech and Audio Analysis Working Group have been developing guidelines and best practice documents, separately, for various aspects of forensic speaker comparison.[4] Their work also seeks to answer some of these lingering questions about how to harmonize practice across the discipline due to the myriad forms of variability present in case data and the differing approaches used in comparison.

Ultimately, any forensic system must be tested under the conditions seen in casework.[5] This means we need to assess performance of these systems using a variety of data types.

While the use of automation in forensic practice has grown, laboratories tend to rely on human practitioners for various aspects of the examination process. How any of these systems — human, automation, hybrid — perform under forensic conditions, remains unclear.[6-8]

To illuminate future paths for forensic speaker comparison, NIST will focus on a series of research activities in the near term, which will be described in detail during this presentation: (1) improvement of underlying technology; (2) assessment of performance across listener types; (3) comparison of performance between listeners and state-of-the-art automated systems; (4) focus on data that bears a close resemblance to that seen in forensic conditions, through the use of: (a) new experimental data collections dedicated to a different set of variables than in the past; (b) operational data for: (i) system testing; (ii) new development of reference datasets; and, (iii) system calibration; and, (5) development of a new Speaker Recognition Evaluation focused on issues inherent to forensic practice.

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Speaker Recognition, Reference Data, System Performance

C5 "HAND-ling" *Daubert*: A Photographic Comparison Case Study

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After attending this presentation, attendees will have an understanding of: (1) the importance of forensic photographic comparisons; (2) a case example in which skin detail was used in such a comparison; and, (3) the *Daubert* challenges that were presented at the court-martial.

This presentation will impact the forensic science community by demonstrating, through a case study, how images retrieved from a computer's hard drive can be linked to a suspect. The challenges encountered in a forensic photographic comparison and the associated legal proceedings will also be discussed as they pertain to a specific case example.

Photographic comparisons are a part of forensic image analysis. In such examinations, the analyst will determine whether the subject or object that is depicted in a questioned image is the same as the subject or object depicted in a known image/photograph. While any number of objects depicted in imagery (photographs) may be the focus of a photographic comparison, skin detail has become of particular interest in forensic casework. The importance of skin detail becomes paramount when a suspect's face is not visible in an image (photograph). For instance, in the present case, an individual's hands and forearms were visible, but a face was not.

In this case, 318 images (photographs) were recovered from a computer hard drive. These images depicted child pornography and the sexual assault of a child by an unknown individual. The individual photographed the assault to include his hands and forearms in the process. A suspect was identified, and sixteen known images (photographs) of a suspect were submitted. It was requested that the known photographs of the suspect (hands and forearms) be compared to the photographs of the assault containing an unknown individual's hands and forearms. A photographic comparison was conducted on the skin detail present on the individual's hands and forearms visible/depicted in the photographs. The photographic comparison conducted visually presented the similarities between the individual in the recovered images (photographs) and the suspect in the known images (photographs).

Prior to the military court-martial, the defense counsel requested a *Daubert* hearing in an attempt to limit the admissibility of the photographic comparison analysis. As such, preparations were made to demonstrate in what manner the photographic comparison met the *Daubert* criteria of: testability, peer review, error rates, standards, and the degree of acceptance in the community. The *Daubert* criteria response materials were presented to the judge and included numerous research articles supporting photographic comparison analysis. The result of the *Daubert* hearing was successful in that the judge ruled that the photographic comparison analysis met the *Daubert* criteria, citing the methods were sound and reliable. The court-martial continued, allowing the photographic comparison to be admitted. The court-martial concluded with the accused being sentenced to life in prison.

Through examining this case example, the ability of photographic comparisons to link a suspect to recovered images is established. Additionally, examiner performance is demonstrated through the analysis conducted and the compelling testimony to educate the court on the photographic comparisons process as it relates to the *Daubert* criteria and the examination involved in this case.

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Photographic Comparison, Image Analysis, *Daubert*

C6 Tool Testing and Comparison: Recovery of Snapchat™ Pictures From Mobile Device Unallocated Space

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After attending this presentation, attendees will expand their general understanding of the value of utilizing multiple forensic tools and techniques to recover deleted graphical content from mobile devices, specifically those utilizing the Snapchat™ application, which is designed to clear transmitted content from the receiver's mobile device after a designated time frame.

This presentation will impact the forensic science community by providing a comparison of the abilities of various software packages in recovering deleted Snapchat™ content from mobile device unallocated space.

Forensic analysis of mobile devices is one of the most quickly evolving areas of Digital and Multimedia Sciences (DMS). With the development and release of mobile devices occurring at a very rapid pace, Digital Forensic Examiners (DFEs) and mobile forensic software companies are faced with the task of determining how to extract and interpret data from the constantly evolving hardware and software of mobile devices. As each new iteration of mobile device and/or mobile device Operating System (OS) is released, it must be determined how to not only extract data from the device, but how to convert the raw data into a format that makes sense to the end user. The use of mobile device applications, or apps, further complicates data analysis of mobile devices. Not only is the base OS of mobile devices under constant development, but individual application developers release and update apps at a surprising pace.

Snapchat™ is a mobile device application that allows users to send and receive multimedia content, such as pictures and video, between specified individual contacts. The transferred multimedia is termed a "Snap." Settings within the sender's Snapchat™ application determine how long the sent content will be viewable on the receiver's mobile device, from one to ten seconds. After the time limit has expired on the receiver's device, an attempt is made by the Snapchat™ software to delete the data. Security features of the Snapchat™ application are also designed to prevent users from taking screen captures of received content through other mobile device applications.

A previous presentation on this topic provided an overview of the examination of an Android-based mobile device submitted for examination to the United States Army Criminal Investigation Laboratory (USACIL) in a case involving the Snapchat™ application. Upon completion of this case, it was determined that at that, time, traditional mobile device forensic software packages were unable to extract any deleted Snapchat™ pictures from the mobile device; however, traditional computer forensic software was successful at recovering the pertinent content. This presentation will provide the results of a research study developed as a result of this case, comparing various (computer and mobile device) forensic software packages and their ability (or lack thereof) to recover deleted Snapchat™ content.

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Snapchat™, Data Recovery, Digital Evidence

C7 Forensic Image and Video Enhancement: Methodology for Improved Efficacy and Error Mitigation

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After attending this presentation, attendees will develop an understanding of how the fidelity of image processing results can be profoundly impacted by the order in which image and video enhancement filters are applied. This presentation will entail discussion of commonly available forensic image and video enhancement techniques, fundamentals regarding their inner workings, and the complex interactions that can occur when multiple image processing techniques are applied in succession. Through this discussion and a series of practical examples, attendees will be prepared to evaluate a proposed order of operations for image enhancement, which seeks to maximize the effectiveness of forensic image enhancements while reducing spurious artifacts created throughout the image processing pipeline.

This presentation will impact the forensic science community by raising awareness of potential causes of image artifact creation and by offering methodology to mitigate such errors so that the best available information can be extracted from image data used in forensic investigations. The research presented will aid in the development of improved quality assurance standards for laboratories and individuals practicing forensic image and video enhancement. Furthermore, attendees who do not engage in image enhancement but are nevertheless impacted by the practice, such as law enforcement and legal professionals, will gain a better understanding of modern image enhancement techniques as well as their strengths and limitations.

Image enhancement is an accepted practice in the field of digital and multimedia forensics and heavily relied upon in many forensic disciplines, such as crime scene reconstruction, photogrammetry, questioned documents, and biometric analysis, including facial and fingerprint identification. Images used in these applications often undergo several concurrent image processing operations. It is significant to note that when multiple processing operations are applied to an image, like a falling stack of dominoes, each operation that an image undergoes will have an effect on any future processing on the image. Even when applying identical enhancement techniques, at the same exact settings, to the same image, applying them in the incorrect order can lead to an overall loss in image fidelity, loss of data, and the creation of features that are non-existent in the original image data, including artifacts such as image noise or false edges.

Visual components of digital images are, in principle, matrixes of numerical values. Image processing operations use algorithms to manipulate these numerical values mathematically. Since these algorithms operate in predefined ways, it is possible to predict their behavior. By studying the underlying processes of enhancement algorithms, it is therefore possible to predict how they react in relation to different image properties and thereby establish an ideal order for their application.

Currently, the digital and multimedia forensics community has not universally agreed upon the order in which image enhancements should be applied. Since the needs of every enhancement case is unique and can require different combinations of image processing operations and settings, it has often been seen as impossible, or at the least, impractical, to establish an order of operations for image enhancement. This presentation advocates that an order of operations for forensic digital image enhancement is both possible and readily applicable to forensic casework.

Image, Video, Enhancement

C8 Structure and Format Analysis of Lossy Compressed Audio Files

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After attending this presentation, attendees will have a better understanding of multimedia metadata analysis and how it can benefit investigations which audio authenticity and source attribution are important questions.

This presentation will impact the forensic science community by revealing the latest results in a large study of digital audio recordings that are lossy compressed such as MPEG-1 and/or MPEG-2 Audio Layer III (MP3), Advanced Audio Coding (AAC), and more.

This presentation introduces a study on the format and structure of digital audio files, with an emphasis on lossy compression algorithms like MP3, Windows® Media Audio (WMA), and AAC. Lossy compressed audio files are common in real forensic cases, can be produced with digital audio recorders, digital video cameras, mobile phones, tablets, computers, and other digital systems, and their forensic authentication can be crucial in the courtroom or other extrajudicial investigations. As was presented at the 2016 AAFS Annual Scientific Meeting, in conjunction with other analyses largely involving time and frequency domain measurements/plots (e.g., Quantization Levels/ Bit Depth, Long-Term Average Spectrum, Modified Discrete Cosine Transform coefficients, etc.), a framework for digital audio authentication includes analysis of the file structure and format as well as investigation of the suspected recording device itself, when available.[1-3] Previous papers presented the results of MP3 and WMA digital lossy audio format analysis for forensic purposes.[4,5] This study reports an investigation of more than 50 different digital audio recorders and mobile phones from over ten years of data collection, validates the previously mentioned studies on MP3 and WMA, and extends the research on iPhones recordings and AAC files as well. In the interest of authentication and establishing digital provenance of recordings, examples of traces left by different digital audio editors (e.g., Adobe® Audition, GoldWave, Sound Forge™ Pro) and converters (e.g., ffmpeg) are also presented.

Preliminary results indicate that while, in most of the cases, the original files contain references of the make, model, recording timestamp, and/or OS version, the digital lossy recompression process affects files' headers and removes this information. The following tables illustrate different examples of the structure analysis results for: original MP3 and WMA files from Olympus® and Sony recorders; original AAC files from different iPhone® OS versions; and digital audio converted/edited MP3, WMA, and AAC files. The conclusion of this study is that structure analysis can be very effective in forensic audio authentication. It can be used to: (1) reveal similarities or inconsistencies between evidence and original reference recordings produced with the suspect digital audio recording system; (2) to verify the evidence file(s) against original reference recordings created with the suspect recorder or another same make and model device; and, (3) to identify the recording system when a database of original recordings is maintained and available.

The following tables provide examples of the material and results collected in this study.

Table 1. MP3

OLYMPUS®VN-5200PC, VN-8100PC	**OLYMPUS® DM-7, VP-10**	**OLYMPUS® DM-520**	**SONY ICD-UX533**	**Adobe® Audition MP3 edited file**
Ofs: 0 -> ID3 Ofs: A -> XOLY Ofs: 15 -> dss Ofs: 20 -> *model* Ofs: 3A -> *timestamp*	Ofs: 0 -> ID3 Ofs: A -> XOLY Ofs: 15 -> mp3 Ofs: 20 -> *model* Ofs: 3A -> *timestamp*	No make, model, timestamp, or ID3 tag	Ofs: 0 -> ID3 Ofs: A -> GEOB Ofs: 17 -> SfMarkers Ofs: AE5 -> GEOB Ofs: AF2 -> IcdRInfo Ofs: AF6 -> Info Ofs: B00 -> ICDUX533 Ofs: B35 -> TIT2 Ofs: B4B -> TPE1 Ofs: B72 -> TENC Ofs: B7D -> SONY IC RECORDER MP3	Ofs: 0 -> ID3 Ofs: A -> TCON

Table 2. WMA

OLYMPUS® DM-520, VN-722PC, WS-822 **8KHz, 8kbps, mono**	**OLYMPUS® DM-520, WS-550M, WS-560M** **22KHz, 32kbps, stereo**	**OLYMPUS® DM-520, DM-620, LS10, WS-210S, WS-311M, WS-750M, WS-760M** **44KHz, 128kbps, stereo**	**Adobe Audition** **WMA edited file**
Ofs: 0 -> 0&² Ofs: 3A -> OLYMPUS® Ofs: 4F -> dss Ofs: 5A -> *model* Ofs: 568 -> Windows® Media Audio V8 Ofs: 59C -> 8kbps Ofs: 5AE -> 8kHz Ofs: 5BC -> mono Ofs: 74 -> *timestamp*	0 -> 0&² 3A -> OLYMPUS® 4F -> dss 5A -> *model* 568 -> Windows® Media Audio V8 59A -> 16kbps 5AC -> 22kHz 5BC -> mono 74 -> *timestamp*	0 -> 0&² 3A -> OLYMPUS® 4F -> dss 5A -> *model* 568 -> Windows® Media Audio V8 598 -> 128kbps 5AC -> 44kHz 5BC -> stereo 74 -> *timestamp*	0 -> 0&² 40 -> Tool Name 56 -> Adobe® Audition 78 -> WMAFilter 96 -> ToolVersion CA -> WMFSDKVersion 10C -> WMFSDKNeeded 142 -> IsVBR 253 -> IsVBR 26D -> DeviceConformanceTemplate 130B -> 128kbps 131F -> 44kHz 1355 -> passCBR

Table 3. AAC (iPhones®)

OS 7.1.1	**OS 8.0.2**	**OS 8.4.1**	**OS 9.2**	**Adobe® Audition** **AAC edited file**
Ofs: 4 -> ftyp Ofs: 8 -> M4A Ofs: 10 -> M4A Ofs: 14 -> mp42 Ofs: 18 -> isom Ofs: 20 -> wide Ofs: 8B495 -> moov Ofs: 8B49C -> lmvhd Ofs: 8B509 -> trak Ofs: 8B511 -> tkhd Ofs: 8B56D -> mdia Ofs: 8B575 -> mdhd Ofs: 8B594 -> 1hdlr Ofs: 8B595 -> hdlr Ofs: 8B5A1 -> soun Ofs: 8B5B1 -> CoreMediaAudio Ofs: 8B5C6 -> minf Ofs: 8B5CE -> smhd Ofs: 8B5DE -> dinf Ofs: 8B5E6 -> dref Ofs: 8B5F6 -> url	Ofs: 4 -> ftyp Ofs: 8 -> M4A Ofs: 10 -> M4A Ofs: 14 -> mp42 Ofs: 18 -> isom Ofs: 20 -> wide Ofs: 14808 -> moov Ofs: 1480F -> lmvhd Ofs: 1487C -> trak Ofs: 14884 -> tkhd Ofs: 148E0 -> mdia Ofs: 148E8 -> mdhd Ofs: 14907 -> 1hdlr Ofs: 14908 -> hdlr Ofs: 14914 -> soun Ofs: 14924 -> CoreMediaAudio Ofs: 14939 -> minf Ofs: 14941 -> smhd Ofs: 14951 -> dinf Ofs: 14959 -> dref Ofs: 14969 -> url	Ofs: 4 -> ftyp Ofs: 8 -> M4A Ofs: 10 -> M4A Ofs: 14 -> mp42 Ofs: 18 -> isom Ofs: 20 -> wide Ofs: 24FDD1 -> moov Ofs: 24FDD8 -> lmvhd Ofs: 24FE45 -> trak Ofs: 24FE4D -> tkhd Ofs: 24FEA9 -> mdia Ofs: 24FEB1 -> mdhd Ofs: 24FED0 -> 1hdlr Ofs: 24FED1 -> hdlr Ofs: 24FEDD -> soun Ofs: 24FEED -> CoreMediaAudio Ofs: 24FF02 -> minf Ofs: 24FF0A -> smhd Ofs: 24FF1A -> dinf Ofs: 24FF22 -> dref Ofs: 24FF32 -> url	Ofs: 4 -> ftyp Ofs: 8 -> M4A Ofs: 10 -> M4A Ofs: 14 -> mp42 Ofs: 18 -> isom Ofs: 20 -> wide Ofs: 1644B -> moov Ofs: 16452 -> lmvhd Ofs: 164BF -> trak Ofs: 164C7 -> tkhd Ofs: 16523 -> mdia Ofs: 1652B -> mdhd Ofs: 1654A -> 1hdlr Ofs: 1654B -> hdlr Ofs: 16557 -> soun Ofs: 16567 -> CoreMediaAudio Ofs: 1657C -> minf Ofs: 16584 -> smhd Ofs: 16594 -> dinf Ofs: 1659C -> dref Ofs: 165AC -> url	Ofs: 4 -> ftyp Ofs: 8 -> mp42 Ofs: 10 -> mp42 Ofs: 14 -> isom Ofs: 97331 -> moov Ofs: 97338 -> lmvhd Ofs: 973BD -> trak Ofs: 973C5 -> tkhd Ofs: 97421 -> mdia Ofs: 97429 -> mdhd Ofs: 97449 -> hdlr Ofs: 97455 -> soun Ofs: 9746A -> minf Ofs: 97472 -> smhd Ofs: 97482 -> dinf Ofs: 9748A -> dref Ofs: 9749A -> url Ofs: 974A6 -> stbl Ofs: 974AD -> gstsd Ofs: 974AE -> stsd Ofs: 974BE -> mp4a Ofs: 974E2 -> esds

OS 7.1.1	OS 8.0.2	OS 8.4.1	OS 9.2	Adobe® Audition AAC edited file
Ofs: 8B602 -> stbl Ofs: 8B609 -> gstsd Ofs: 8B60A -> stsd Ofs: 8B61A -> mp4a Ofs: 8B63E -> esds Ofs: 8B671 -> stts Ofs: 8B689 -> stsc Ofs: 8B6A5 -> stsz Ofs: 8E78D -> stco Ofs: 8E7A9 -> meta Ofs: 8E7B5 -> hdlr Ofs: 8E7E7 -> mean Ofs: 8E7EF -> comappleiTunes Ofs: 8E803 -> name Ofs: 8E80B -> iTunSMPB Ofs: 8E817 -> data Ofs: 8E89B -> day Ofs: 8E8A3 -> data Ofs: 8E8CB -> too Ofs: 8E8D3 -> data Ofs: 8E8DF -> comappleVoiceMemos Ofs: 8E8F5 -> iPhone® OS711	Ofs: 14975 -> stbl Ofs: 1497C -> gstsd Ofs: 1497D -> stsd Ofs: 1498D -> mp4a Ofs: 149B1 -> esds Ofs: 149E4 -> stts Ofs: 149FC -> stsc Ofs: 14A18 -> stsz Ofs: 15140 -> stco Ofs: 1515C -> meta Ofs: 15168 -> hdlr Ofs: 1519A -> mean Ofs: 151A2 -> comappleiTunes Ofs: 151B6 -> name Ofs: 151BE -> iTunSMPB Ofs: 151CA -> data Ofs: 1524E -> day Ofs: 15256 -> data Ofs: 1527E -> too Ofs: 15286 -> data Ofs: 15292 -> comappleVoiceMemos Ofs: 152A8 -> iPhone® OS802	Ofs: 24FF3E -> stbl Ofs: 24FF45 -> gstsd Ofs: 24FF46 -> stsd Ofs: 24FF56 -> mp4a Ofs: 24FF7A -> esds Ofs: 24FFAD -> stts Ofs: 24FFC5 -> stsc Ofs: 24FFF9 -> stsz Ofs: 25CA4D -> stco Ofs: 25CA71 -> meta Ofs: 25CA7D -> hdlr Ofs: 25CAAF -> mean Ofs: 25CAB7 -> comappleiTunes Ofs: 25CACB -> name Ofs: 25CAD3 -> iTunSMPB Ofs: 25CADF -> data Ofs: 25CB63 -> day Ofs: 25CB6B -> data Ofs: 25CB93 -> too Ofs: 25CB9B -> data Ofs: 25CBA7 -> comappleVoiceMemos Ofs: 25CBBD -> iPhone® OS841	Ofs: 165B8 -> stbl Ofs: 165BF -> gstsd Ofs: 165C0 -> stsd Ofs: 165D0 -> mp4a Ofs: 165F4 -> esds Ofs: 16627 -> stts Ofs: 1663F -> stsc Ofs: 16667 -> stsz Ofs: 16E63 -> stco Ofs: 16EAB -> meta Ofs: 16EB7 -> hdlr Ofs: 16EE9 -> mean Ofs: 16EF1 -> comappleiTunes Ofs: 16F05 -> name Ofs: 16F0D -> iTunSMPB Ofs: 16F19 -> data Ofs: 16F9D -> day Ofs: 16FA5 -> data Ofs: 16FCD -> too Ofs: 16FD5 -> data Ofs: 16FE1 -> comappleVoiceMemos Ofs: 16FF6 -> iPhone® OS92	Ofs: 97515 -> stts Ofs: 97535 -> stsz Ofs: 9A5F1 -> stsc Ofs: 9A619 -> stco

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Forensic Audio, Metadata, Digital Evidence

C9 Media Forensics and Microphones

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After attending this presentation, attendees will better understand microphone transducer principles that can be applied in audio forensic examinations. Basic specifications/limitations, performance of pressure vs. pressure-gradient types, beamforming, and the influence on recorded audio will be discussed.

This presentation will impact the forensic science community by highlighting microphone performative characteristics which are important for those working with media forensics.

Nearly all acoustic recordings include a microphone in the recording chain. Recordings may stem from dashboard cameras, cell phones, smartphones, car phones, dispatcher headsets, video cameras, surveillance systems, dictaphones, computers of all sorts, and others.

It is very seldom that the result of a forensic examination of a recording device includes any findings related to the microphone/microphones; however, the microphone may add valuable information to findings of the recording.

A microphone is not just a microphone. Devices responsible for the majority of recordings that become subject to forensic examination are either electret condenser types or Micro-Electro-Mechanical Systems (MEMS). The sound quality in good electret microphones is clearly superior to the MEMS types; however, the MEMS are extremely inepensive and can more or less directly deliver a digital code (density modulation). As with most coding, this may leave traces in the signal.

Any microphone can be specified by different data (i.e., sensitivity or how much output for a given Sound Pressure Level (SPL), self-noise (all microphones have a noise floor), max SPL (the SPL at which the microphone will start clipping/distorting), and frequency response (how wide is the range of the given microphone).

The directivity of microphones are especially of great interest. Two ways of coupling the diaphragm to the surrounding air are responsible for the directivity.

Pressure microphones (the sound can only access the diaphragm from one side) are by definition omnidirectional; however, this also depends on the practical mounting in/on the device or in the room. For instance, mounting on a surface changes the directivity dramatically, depending on the size of that surface. Pressure microphones are the least sensitive to wind and vibrations. In regard to frequency response, it decreases to DC and lets one consider whether to open or close doors in rooms due to the pressure variation.

Pressure gradient microphones can provide a variety of first-order directional characteristics: figure eight (bi-directional), super cardioid, cardioid, subcardioid, etc. The final directivity is primarily determined by the relationship between the front and rear inlet. The pressure gradient microphones normally have "looser" diaphragm to reproduce some amount of low frequencies. This fact also leads to a higher sensitivity (approximately 10-15 dB) to vibrations, wind-noise, and handling-noise.

Pressure gradient microphones exhibit proximity effect. This means that the low frequencies are boosted as the microphone gets closer to the sound source (i.e., point source). On the other hand, sound sources moving away from the microphone lose bass. This is practical in phone calls; however, it also provides information on distance when examining the spectral changes of recordings' content.

When applying more than one microphone capsule in a device, arrays can be formed.

Two-channel recordings contain directional information depending on the directivity and angling of the microphones (XY stereo) or the distance between them (AB stereo and near-coincident set-up). Here, there is considerable information to extract. This also provides possibilities of signal enhancement when one channel can be utilized to improve the other (i.e., cross-lattice filtering and the like). Shot-spotting systems also form a type of array, although with much larger distances between the microphones.

Arrays can be used for directivity beamforming. Today, most computers and smartphones contain two or three microphones for this purpose. This means that certain frequencies coming from certain angles are either boosted or cut. This is why it is essential to gain as much knowledge as possible of the recording devices to include/exclude sound sources. Accordingly, the difference in the individual microphone's sensitivity provides information for the authentication of the device or for the scene.

Casework (test sound-files to be presented) has shown that the characteristics of the recording devices, and the microphone in particular, have provided evidence or led to the better choice of enhancement technique.

Which (fixed-line) phone was used for the emergency call? Why was the background sound lower than expected? The saturation of the input was pure microphone clipping; hence, de-clip was possible. Crime scene analysis has been made possible because of the wide frequency range of the microphone.

Examiners sometimes neglect the characteristics of the microphone; however, microphone specifications and implementation influence the signal recorded. For the purposes of selecting the right microphone for forensic recordings, rescuing bad recordings, authenticating recordings, and extracting valuable crimescene information, practitioners in media forensics should acquire a basic knowledge regarding microphones.

Microphones, Forensic Recordings, Audio Forensics

C10 An Analysis of Apple® iOS® Version Effects on Format and Metadata Structure of Audio Files Recorded Using the Native "Voice Memos" App

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After attending this presentation, attendees will better understand the critical aspects of audio format and metadata analysis that can inform audio authentication examination of recordings made with Apple® iOS® Voice Memos app and how a similar approach can be applied to other recording devices. A preliminary determination of an iPhone® Voice Memos recording's originality and integrity can be assessed through the use of a decision tree that takes into account how different editing processes and recording interruptions affect the resulting audio file.

This presentation will impact the forensic science community by disseminating a decision tree for authenticity analysis of audio recordings from iOS® Voice Memos. This procedure demonstrates the initial results of a large study into changes in the format and metadata structure of audio files recorded using the native Voice Memos app. This presentation will also impact the forensic science community by shedding light on how certain recorder operations and interruptions modify the native format and structure of embedded metadata within the Voice Memos recordings.

This presentation will describe the ongoing study into the authentication of audio recordings originating from an Apple® iPhone® using the Voice Memos app, which comes built-into the iOS® operating system. The data collection procedure for producing test recordings from iPhones® will be described. These test recordings will account for various capture, editing, and interruption scenarios, based on the inherent functionality of the Voice Memos app. Metadata analyses of these test recordings were conducted, and any modifications to the format and structure as a result of an editing process or recording interruption were documented. For example, the bitrate of the recorded audio information may change as a result of an editing process having been applied to the original recording or due to an interruption in the recording caused by an incoming call. Based on the collected data and metadata analyses, a decision tree, aimed at helping guide authentication examinations involving recordings purported to have originated from the iPhone® Voice Memos app, was developed and will be shared during the presentation. Additionally, a study based on the practical application of the decision tree to real-world examples was undertaken and will be discussed.

The materials used during this study involved 20 recordings made with various iPhones® running on various versions of the iOS® operating system. The materials were prepped for testing employing the various types of editing processes and record interruptions that can be conducted directly on the phone on some recordings, while leaving others in their original, unaltered state. These materials were then disseminated to test subjects and examiners, who each worked in blind testing the recordings for authenticity against the proposed decision tree. In order to assess the efficacy of the decision tree, the accuracy of examiner decisions made in blind were tabulated. Results will be shared at the presentation and recommendations for further research will be discussed.

Audio Forensics, iPhone® Forensics, Digital Evidence

C11 An Analysis of Digital Forensic Units

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After attending this presentation, attendees will have learned about the operations of digital forensic units, what can be improved upon, and what tactics have succeeded.

This presentation will impact the forensic science community by providing a basis point for analyzing digital forensic units and also confirms the findings of previous research within the digital forensic community.

Computer technology is growing rapidly, and law enforcement has seen an increase in the number of criminal cases that involve digital evidence. Nearly all crimes, including both cybercrimes and traditional crimes, include some type of digital media.[1,2] In fact, it is becoming more common to see multiple forms of digital evidence in a single criminal case, such as texts from a mobile phone, travel coordinates on a GPS, and saved photos on a computer.

Law enforcement agencies are having a difficult time processing all of the digital media in an effective and efficient manner.[1] As the volume of digital data increases, so does the amount of time it takes to examine the data for evidence, resulting in a backlog. In addition, this backlog is exacerbated when there are multiple parties involved with multiple digital devices.[3] Thus, law enforcement is overwhelmed with the number of cases that involve digital evidence, as well as the number of devices and variety of devices which may be involved in a single case, adding to the backlog of cases.[2]

In order to overcome the backlog created by cases involving digital evidence, some states have created computer crime or digital forensic units that investigate and/or process the digital evidence obtained in criminal cases. For the purposes of this study, specialized cybercrime units were defined as units that work only on digital media forensics and complete the forensic investigation within their own unit. Non-specialized units were defined as any other unit that did not fit the specialized unit criteria. The current study assessed the effectiveness of specialized vs. non-specialized units.

Two surveys were completed: a phone interview and an online questionnaire. Twelve specialized and eight non-specialized units completed the phone interview. Eight specialized and eight non-specialized units completed the online survey. The data was aggregated and anonymized so the respondents felt comfortable reporting information about their units. Respondents answered a variety of questions about the unit, such as the unit's history (e.g., Why was it created? What were the original goals?), their past and/or current backlog, number of cases worked, and types of digital evidence examined, just to name a few. The study suggested the specialized units operated more effectively than the non-specialized units. This study also revealed the lack of knowledge regarding standard procedures/best practices in digital forensics, as well as the lack of consistency in the standards reported among the cybercrime units community. Finally, the current study also supported previous research regarding the need for more training, funding, and personnel in digital forensics.

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Cyberforensics, Digital Media, Cybercrime

C12 Image Correction and Enhancement With the Apparatus for Actual Measurement of Image Degradation Properties in Security Cameras

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After attending this presentation, attendees will better understand the method to measure actual image degradation properties in security cameras as well as better enhanced and/or corrected images that can be obtained using the measured properties.

This presentation will impact the forensic science community by providing information about methods and an apparatus for measuring degradation properties in security cameras. The experimental results showed better images by using the measured actual properties in comparison with modeling methods. This information will be useful for forensic image analysts.

Videos or images that are recorded by security cameras can be objective evidence leading to criminal investigations; however, in some cases, the recorded images cannot be effectively utilized due to insufficient image quality. Thus, image processing, such as image enhancement or image correction, is expected under the circumstances. In the image processing procedure, some types of functions or parameters describing image degradation characteristics in cameras are required; for example, Point Spread Function (PFS) is used for image deblurring.[1] The Gaussian function is commonly used as an approximate model for PSF; however, the optimal deblurred image cannot be obtained with the Gaussian PSF because there is a difference between the actual PSF of a camera lens and the Gaussian function. Therefore, another approach for image enhancement/correction method is proposed in this presentation, which uses actual measured image degradation properties in security cameras.

An apparatus to measure the image degradation properties in security cameras was developed. The apparatus consists of an industrial high brightness Liquid Crystal Display (LCD) panel (19 inches, 1500cd/cm^2), three-joint flexible arm, a single board computer (Raspberry Pi 2 model B), a wireless control circuit, a small stand on casters, and a battery. The total weight was approximately 28kg. After putting the apparatus into place in view of a security camera, a series of basic graphics, such as dots, lines, color patches, and digits, were displayed on the LCD panel by the single computer board. During that time, the images of the LCD panel are recorded by the security camera. Because the recorded images of the basic graphics were distorted according to the degradation properties of the security camera, the degradation properties were obtained by analyzing the recorded images. This is the principle of the proposed method and the following are expected: (1) image deblurring using the measured PSF; (2) geometric correction of lens distortion; and, (3) measurement of color characteristics of the camera and color correction. Furthermore, the apparatus can be used for identification of digits/characters on a car license plate image and scale estimation of objects in images.

Experiments were performed using a security camera (Victor VN-H137B) in terms of 1, 2, and 3 as mentioned above. By displaying dot patterns, the PSF was successfully measured. When the Wiener deblurring filter was adopted to a blurred image taken by the camera, the restored image with the measured PSF was clearer than those with the Gaussian PSF or the circular PSF. By displaying a lattice pattern, the property of the geometric lens distortion was successfully measured. A corrected image was synthesized with the measured lens distortion data. Furthermore, the composed non-linear chromatic transfer characteristics of the camera and the recording device were successfully measured by displaying 861 color patches.

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Security Camera, Image Enhancement, Camera Properties

C13 Automatic Removal of Face Features in Video for Purposes of Visual Anonymity Using Statistical Learning and Meaningful Face Color Detection

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After attending this presentation, attendees will understand how the use of color information may permit the fast detection and removal of faces from video sequences.

This presentation will impact the forensic science community by demonstrating the effectiveness of the proposed method for preserving the anonymity of individuals in real-life videos.

The need for personal anonymity in video surveillance footage imposes the use of face hiding techniques on these videos.

The manual removal of people's faces is time consuming, while automatic face detection techniques exhibit low performances when applied on videos recorded in emergency situations. Motion induced by shaking cameras, people in motion, etc., blurs the faces and makes most of these algorithms unable to correctly detect them.[1]

For these reasons, an automatic technique for face removal is proposed based on the color characteristics of the faces, not on the face features usually considered by the face detection methods. This has the advantage of making the method invariant to deformations of the face geometry (rotations, side views, partial occlusions, etc.).

The proposed approach consists of: (1) representing image colors using their Hue, Saturation and Intensity attributes (the so called HSI color space), instead of the usual Red, Green, Blue (RGB) space. This representation is more intuitive and perceptually relevant and has the additional advantage of separating the pure color information (represented by the hue and saturation values) from the illumination information (i.e., the intensity); (2) statistically learning the meaningful (statistically robust) ranges of hue and saturation associated to the color of human faces. Intensity information is not taken into account because the goal is to detect faces independently on the illumination conditions of the scene (dark or bright). Such ranges have proven to be quite robust to ethnic variation of the features, thus being equally successful in any subject population; and, (3) selectively bluring image pixels whose hue and saturation values fall in the learned range.

Of course, besides faces, several other objects in the scene may have a color in the learned range. In order to prevent the blurring of non-face objects, the previous algorithm is refined by introducing two additional steps: (1) use a background extraction algorithm to discern between background and foreground parts of the scene, then apply the blurring method only to the foreground pixels; and, (2) use an anisotropic blurring filter. This will preserve the main image edges, while blurring the small details (e.g., faces). The scale of this filter is a critical parameter of the method.[2,3]

The results of the proposed algorithm are promising. Moreover, since only color information is used, computations are fast and a real-time implementation has been produced. The algorithm is parallelizable and an open-source implementation has also been developed.

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Face Removal, Face Detection, Color Features

C14 Open Source Automatic Facial Comparison Algorithms' Potential Application in Forensics

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After attending this presentation, attendees will understand several open source initiatives that exist for facial comparison as well as a test on forensic images.

This presentation will impact the forensic science community by introducing algorithms that are open source for facial comparison and discussing how they can be used in research.

The face is considered to be one of the main organs used in biometrics. It is a relatively stable part of the human body that has proven to be a great instrument in forensics. Over the past decade, the automatic facial analysis algorithms are at the center of the interest in the biometrics community. Recent facial comparison algorithms have illustrated significant growth regarding their performance; however, none were able to fill the gap between software and the significant high human accuracy. OpenFace is an algorithm that is trying to fulfill the aforementioned need. The center of this research is to determine the efficiency of the automatic OpenFace system as it relates to relevant forensic data. OpenFace is an open source toolkit based on the FaceNet algorithm, which has been created by Google®; however, it has been developed and shared as open source by Brandon Amos at Carnegie Mellon University. The main advantages of OpenFace are its online availability (which makes it a low-cost option), the limited need for human resources, and its reported performance on the Labeled Faces in the Wild (LFW)benchmark.

OpenFace requires specific products in order to operate, which are all open source as well; namely, OpenCV®, Torch, GitHub™, Docker, Dlib™, and a few libraries used in Python™. These products contribute to the three main tasks of OpenFace, which are verification, recognition, and clustering of faces on stable images and on real-time web videos. FaceNet, and hence OpenFace, are based on Euclidean embedding per image. Furthermore, they use the squared L2 distance in order to determine the face similarity of the query pair of faces. Then, by the use of one of the well-known techniques of K-means or agglomerative clustering, the recognition of the similar faces is achieved. Additionally, there is a triplet-based loss function used during training, which assures that the output of the algorithm is a compatible 128-D embedding. This sets a positive respective distance limitation. In general, this function minimizes the relevant distance when the same face is present on both query images and maximizes the relevant distance when different faces are present on the pair of query images. OpenFace could have multiple forensic applications. It could be used for facial recognition of suspects based on a query image and a reference; it could provide a connection of criminals by the use of images between multiple crime scenes; and it could be used with surveillance cameras in order to recognize specific individuals, as terrorists or a person reported as missing. Moreover, it could be used for the identification of corpses in mass disasters.

Based on the four OpenFace models and LFW benchmark, the verification of the reported results is achieved. The nn4.small2.v1 model outperforms the other three models in accuracy. For that reason, the further examination of nn4.small2.v1 model is selected. The experiments on OpenFace with the use of LFW-raw, LFW-deep funneled, SCface, and ForenFace datasets reveal that as the resolution of the input images is getting worse, the performance of nn4.small2.v1 OpenFace model is getting low. Moreover, the performance of OpenFace detector depends on the quality of the input images. This is due to the fact that it is unable to detect a face or align the input low-analysis image. Furthermore, the runtime of OpenFace depends on the specifications of the machine in use and the quality of the image. This includes the fact that the detector processes images of low resolution more slowly. Hence, the intervention of the quality of the query images to the efficiency of OpenFace is forthright. Therefore, OpenFace proved inadequate in its current condition for forensic application. Notwithstanding, there are possible proposed improvements that are promising.

OpenFace, Open Source, Automated Facial Comparison

C15 Accurate Video Reconstruction and Metadata Extraction From a Self-Executing Video File

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After attending this presentation, attendees will understand how video files can be wrapped inside proprietary Windows® executable video players and how the native video data can be extracted to ensure accurate playback and analysis.

This presentation will impact the forensic science community by demonstrating that native video data and metadata can be extracted from proprietary Windows® executable video players, thus allowing for a proper forensic analysis of the video.

Forensic video analysis has evolved in recent years to eschew the use of proprietary video players wherever possible in favor of independent playback using open source multimedia frameworks, such as FFmpeg, that can decode native video data. When analyzing native formats directly rather than through opaque proprietary third-party viewers, accurate metadata, such as storage aspect ratio and frame-level timing data, can often be established explicitly in a reliable and repeatable way, thus allowing for accurate playback and analysis; however, this approach is hindered when video data and player data are wrapped together as a single executable file and the native video data is not immediately identifiable.

This presentation will introduce two ways in which native video data can be contained within Windows® executable files. The first method is when the video data is written directly into the payload of the video player executable, so extraction relies simply on recognizing the video format start codes and carving out the data. With the second method, the executable is simply a data decompression utility, while its payload contains compressed video data along with other compressed files required for playback, such as the player executable itself, associated Dynamic Link Libraries (DLLs), and text files containing supplementary video information. As a result, directly carving out the (uncompressed) video file is not possible.

This second case will be discussed in detail using the "MP4Extract" class of video files as a case study. It will be shown, for this class of videos, how the compressed data can be identified within the executable, carved out and decompressed to yield a non-conforming (i.e., non-playable) MPEG-4 (Part 2) format video file. The metadata of the resulting video file can be fully decoded so all proprietary video information, such as camera number, accurate frame-level timing data and supplementary overlay data, can be exported for reference purposes. Furthermore, the extent of the video file's non-conformity will be presented and a solution to produce a fully conformant, readily playable video file will be outlined.

The approach outlined in this study also has broader implications for forensic video analysis. In particular, application to the authentication of digital video and metadata analysis of proprietary video file formats more generally will be discussed in the time remaining.

Data Carving, Metadata Analysis, Video Reconstruction

C16 Pokémon™ GO® Forensic Artifacts: An Exploratory Study

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After attending this presentation, attendees will have obtained the results of research regarding the availability of forensic artifacts maintained by mobile devices specific to the Pokémon™ GO® application.

This presentation will impact the forensic science community by providing potential sources for data relevant to criminal investigations maintained within user data associated with the Pokémon™ GO® mobile device application.

Digital Forensic Examiners (DFEs) are responsible for extracting data from a growing number of different electronic device types and performing analyses on a multitude of different resultant data types. It is up to members of the Digital and Multimedia Evidence (DME) field (DFE's and researchers) to search out and interpret new sources of potentially important data to aid in future investigations. This responsibility includes mining for potentially pertinent data stored within mobile device applications, such as the game Pokémon™ GO®.

Pokémon™ GO® is a location-based augmented reality mobile game released in July 2016. There was enormous controversy upon the release of this game due to potential privacy issues and threats to personal property and personal safety. Even with the concerns, Pokémon™ GO® quickly became one of the most popular mobile device games for both Android™ and Apple® iOS®. Active game players are required to physically visit specified locations in order to refill energy, gather objects necessary for game play, and capture Pokémon™ characters. Global Positioning Satellite (GPS) technology tracks the location and progress of game players to determine their physical proximity to designated Pokéstops (locations for players to collect Pokéballs and other items necessary to continue game play and to capture Pokémon™ characters) and Gyms (locations set by game developers to join together with additional players as teams and battle for virtual control of the designated Gym).

Pokémon™ GO® game play requires users to utilize a Pokémon™ Trainer Club, Google®, or Facebook® account for login identification and to provide permissions for the game to monitor player GPS location. The Pokémon™ GO® application also stores information on game play activities within mobile device memory. This presentation will provide the results of an exploratory study into forensic artifacts left behind on Apple® iOS® and Android™ mobile devices specific to the mobile device game Pokémon™ GO® and explore how these artifacts may be used to aid in criminal investigations.

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

Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, DFSC, OPMG, DA, or DoD.

Pokemon™, Digital, Forensic Artifacts

C17 Digital Stratigraphy: Analyzing File Allocation Methods to Uncover Concealment Behavior

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The goals of this presentation are to: (1) increase knowledge of file allocation methods and traces; (2) review contextual analysis of file allocation traces (digital stratigraphy); and, (3) raise awareness of potential limitations of digital stratigraphy.

This presentation will impact the forensic science community by illustrating how advances in the practice of file system analysis will enable forensic examiners to perform contextual analysis of file allocation traces in order to differentiate between concealment behavior and normal system activity.

When computing devices are used to conceal digital evidence by deleting, reformatting, wiping, or backdating files, it can be challenging to prove concealment behavior versus normal system activity.

There is a common misconception that new files are saved onto storage media in a predictable fashion. Certainly, in the simplest scenarios, such as on a memory card in a digital camera when files are saved in quick succession, there can be a predictable next-available allocation; however, there are situations that will cause file allocation to deviate from predictable or deterministic allocation strategies. In one case, a forensic examiner misinterpreted gaps between allocated files as indications of file wiping.

Furthermore, file initialization can exhibit itself in various ways when different applications, operating systems, and file systems are involved. In one case, a new file system entry was initialized but nothing was saved to disk, leaving untouched data from a prior deleted document in the space allocated to the new file system entry, making it seem like backdating (i.e., a file system entry created in 2015 seemingly containing information dated 2014). To avoid confusion, great care must be taken when interpreting the provenance of deleted data that is recovered from a partially initialized file.

Understanding file allocation methods can provide insight into such concealment behavior, but real world computer use introduces complexity that complicates forensic analysis. Each file system tells a story about the use of that storage media, and analyzing the allocation of files over time can sometimes provide insight into deletion or other concealment activities. As a result, general understanding of file allocation methods can only be used as a starting point, and it is necessary to take the overall context into account when analyzing traces of such concealment behavior. Contextual analysis of file allocation traces is called digital stratigraphy because it has similarities to the concept of stratigraphy in archaeology.

Using examples of forensic analysis from past cases, this presentation demonstrates how digital stratigraphy has been used to address questions of concealment behavior. The challenges, successes, and limitations of this form of forensic analysis are discussed.

File System Forensics, Digital Concealment Behavior, Digital Stratigraphy

C18 Paired Apple® Watch® Forensics

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After attending this presentation, attendees will be more familiar with paired iPhone® examinations. The implications for iPhone® examinations when paired with an Apple® Watch® will also be discussed.

This presentation will impact the forensic science community by providing information on the Apple® Watch® and paired iPhone® forensic artifacts.

An Apple® Watch® paired with an iPhone® provides not only precise time but also unique functions operating with installed applications and embedded equipment. There were some differences from conventional iPhone® examinations that could be recognized based on some research on an Apple® Watch® and the paired iPhone®. Factors changing the procedures are categorized into three groups, which are named influence, independence, and uniqueness for descriptive purposes. Influence is referred to as the pairing of an Apple® Watch® and an iPhone® and the influences the two devices have on each other. The interaction between them and the storage of Apple® Watch® data on the paired iPhone® are two examples. Independence is defined as separately processing and storing data on an Apple® Watch® and the paired iPhone®. Uniqueness encompasses Apple® Watch's® unique functions added to the paired iPhone®. These factors would bring new steps, new analyses, and new information, which is used for an iPhone® analysis to iPhone® examination processes. In the seizure phase, an Apple® Watch® should be isolated in order not to alter both data stored on the Apple® Watch® and the paired iPhone® because they communicate with each other via a wireless network. Also, the step to seize the Apple® Watch® needs to be added to the phase. In the collection phase, Apple® Watch® data should be extracted from the two devices; therefore, the step to extract the data from the Apple® Watch® needs to be conducted in this phase.

In this research, a manual extraction method (e.g., taking pictures or notes) was employed for the Apple® Watch®; also, an iTunes® backup method was used for the paired iPhone®. In the analysis and examination phase, the extracted data should be confirmed. The data from the Apple® Watch® were easily understood because the extracted data were pictures of applications displayed on the screen; however, there was extra work to figure out the data from the paired iPhone® because the backup contained a lot of files. Additionally, health data such as heart rates and active calories, which were sourced from the Apple® Watch®, could be analyzed by making more detailed charts than the charts displayed on the iPhone®. Also, information remaining on the Apple® Watch®, which has been deleted from the paired iPhone® could be applied to an analysis of the iPhone® in certain instances. The research concluded that pairing an Apple® Watch® and an iPhone® makes iPhone® examinations more complex and creates valuable data for forensic examiners.

Apple® Watch®, iPhone®, Wearable Device

C19 Experience Validating Disk-Imaging Tools With Computer Forensic Tool Testing (CFTT) Federated Testing

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After attending this presentation, attendees will be aware of the CFTT Federated Testing forensic tool-testing environment utility that is used for validating disk-imaging tools.

This presentation will impact the forensic science community by increasing awareness of the capabilities of Federated Testing when applied to real imaging tools. This presentation will provide examples of Federated Testing on actual disk-imaging tools, in the same manor as a digital forensics laboratory would conduct validation testing. In addition, by documenting the resource commitment required to perform the tool testing, forensic practitioners will be able to estimate the cost in time and effort to test disk-imaging tools in their laboratory. This presentation will aid the forensic practitioner choosing to use Federated Testing by providing examples of using Federated Testing to test actual disk-imaging tools, much as a digital forensics laboratory would conduct validation testing.

The CFTT project at the National Institute of Standards and Technology (NIST) develops methodologies for testing computer forensic tools. Currently, there are CFTT methodologies for testing the following: disk imaging, write blocking, deleted file recovery, file carving, forensic media preparation, and mobile devices.

A variety of tools in each of these categories have been tested and observed flaws in the tools have been reported by the National Institute of Justice (NIJ) and the Department of Homeland Security (DHS). These results can be used as a basis for identifying the types of likely failures that occur in forensic tools. Currently, CFTT has implemented testing disk imaging into Federated Testing.

Using Federated Testing has several advantages: (1) it relieves a forensic laboratory of the task of developing a test plan for tool testing because Federated Testing generates a test plan based on selections made by the user describing how the laboratory uses the tested tool: (a) a list of test cases (based on user input); (b) tools and detailed procedures for creating test drives (adding known content); (c) detailed procedures for running each test case; (d) tools to evaluate test results; (e) tools to generate a skeleton test report that can then can be finished in the style favored by the laboratory; (2) the test reports can be shared with other laboritories; and, (3) completed test reports can be submitted to CFTT for administrative review and if no issues are found, the report is passed on to the vendor for comment. The final report is published by the DHS.

In this round of testing, the following tools were tested, making slight variations in feature selection:

Tool	Version
FTK	3.4.2.6
Guymager	0.8.1
Logicube Falcon	2.4U1
Logicube Falcon	3.0U1
Paladin/ewfacquire	6.09/20160403
Paladin/dc3dd	6.08/7.1.614
X-Ways	18.8
dc3dd	7.2.641
Ditto FieldStation	2016 Mar01
Tableau TD2u	1.1.2.3948-4270f9c

Temporal and physical resources to measure the level of commitment that was required to test each tool were tracked. It was found that with two PCs, a single practitioner could set up test drives in just a few hours. The drives

can be set up faster if more Personal Computers (PCs) were devoted to the task. After the test drives are set up, running the tests takes less than two days. The most time expended is actually taking the generated skeleton test report and adding laboratory-specific information.

If a laboratory uses (or just wants to test) more than one imaging tool, the drive set up only needs to be executed once and can be reused for additional tool testing.

Digital Forensics, Tool Testing, Disk Imaging

C20 An Anatomy of a Knockoff

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After attending this presentation, attendees will be more knowledgeable of the motivations to create knockoff mobile devices, the forensic characteristics of knockoff mobile devices, and how much they differ from the real thing.

This presentation will impact the forensic science community by directly analyzing a topic that is typically underresearched (i.e., knockoff devices) due to the international and criminal aspects of this described consumer supply chain problem.

This case study involves the technical examination of three international Samsung™ devices purchased through an official Samsung™ reseller. The devices ordered were Samsung™ Galaxy™ S5 phones, model SM-G900F. These devices have a Snapdragon™ processor and are typically used on mostly European carrier networks. Upon receiving the order, each devices' boxes were found to be in a pristine state in which they appeared to be factory sealed within the expected packaging, with stickers denoting the device model SM-G900F. During an examination of two of the devices while in Samsung's™ Download Mode, it was immediately noticed that the devices identified themselves as SM-G9006V and that each device had its warranty bits tripped. Further inspection while in the Android™ operating system revealed that the devices reported a device type of SM-G900F and the bootloader threw an error when an SM-G900F update file was attempted. Having believed that the research team may have been responsible for the warranty bit trips, the third device was unpackaged and inspected. It too exhibited the same behavior and its warranty bit was also tripped.

The Samsung™ GS5 SM-G900F and Samsung™ GS5 SM-G9006V have very similar hardware sets and it appears that in this case, the model SM-G9006V is used as a "knockoff" device for the model SM-G900F. The team was able to identify more knockoff devices with similar characteristics purchased from other authorized resellers, indicative of a more regional supply chain problem. Based on the research team's observations, the existence of a supply chain problem in both Europe and Asia manifesting itself through authorized Samsung™ resellers in the United States is assumed. This study focuses on the technical changes made to mask the original hardware and hypothesizes on the possible motivations for knocking off these particular device models. Using the Periodic Mobile Forensics tool suite, a suite of tools previously discussed at AAFS Annual Scientific Meetings, the research team examined these SM-G900F knockoff devices and will report on its hardware, the shape of the bootloader, the system partitions, and the state of the secure booting integrity. Results will be discussed.

Periodic Mobile Forensics, Android™, Knockoff

C21 A Forensic Investigation of Drones

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After attending this presentation, attendees will learn what type of data can be extracted from drones after they have been used.

This presentation will impact the forensic science community by highlighting several methods for the digital investigation of drones. Since the number of drones is rising, they are also being used in criminal activities.

Drones come in all shapes and sizes and are becoming more accessible to individuals. Drones are increasingly popular among criminal organizations due to their ease of operation and the different functions they offer. Criminals use drones to explore unseen locations and to transport illegal cargo.

Because little is known about what data is stored on different types of drones, it is important to focus on an exploratory study. The Netherlands Forensic Institute (NFI) wanted to know what research questions regarding drones to take into consideration. For this purpose, six different types of drones popular in 2016 were purchased. These include drones from the DJI®, 3DR®, and Align, and Yuneec brands. These drones have assistance systems, making it easy to control the drones. The auxiliary systems themselves handle a large amount of data that can be useful for forensic examination.

Data extraction is divided into two categories: destructive testing and non-destructive testing. For destructive testing, chip-off may be used. Non-destructive testing consists of extracting data from forensic software using a Joint Test Action Group (JTAG) method. This study uses the least intrusive method, namely Forensic Toolkit® (FTK®). The study consists of an external examination, which looks at possible access ports on storage media (e.g., USB, SD card) and an internal examination, which looks at the printed circuit boards inside the drones. Raw data that is acquired is converted from the drones and ground stations to a readable file. There are many converter programs that are specifically designed for these specific drones. A disadvantage of many of these converter programs is that data must be uploaded to a site, which is not possible for case-related data.

The Align M690L is the only drone that stores any data unless a data logger has been purchased. The other drones include all data that have been extracted and analyzed using standard forensic software. It includes data about various assistance systems such as Global Positioning System (GPS), Gyro, Accelerator, and a barometer. The GPS coordinates can be plotted on a map to provide an escape route. The 3DR® IRIS+ contains waypoints that are pre-programmed and can be downloaded at any time. Imports logged on the remote controls are visible so that a statement can be made about a possible driver.

For several drones, the route flown can be extracted; however, the owner could not be found from the data extracted from the drone. Conventional forensic methods such as DNA and fingerprints of the drone might be used to find the owner of the drone. In further research, chip-off will be tested to determine if more information pertaining to the drone owner can be obtained.

Drone Forensics, UAV, Digital Investigation

C22 Joint Task Action Group (JTAG) Phone Forensics

William Charles Easttom II, MBA, Chuck Easttom Consulting, 5605 Woodspring Drive, Plano, TX 75093*

After attending this presentation, attendees will better understand what it means to use JTAG techniques to access the raw data from a phone. Attendees will also receive an overview of when JTAG is the appropriate approach to phone forensics. This presentation will cover the types of techniques, equipment needed, and skills required to JTAG a phone. This presentation will also cover the limitations of JTAG and help forensic examiners know when this is the appropriate technique and when it is not. The goal of this presentation is to provide attendees with an understanding of JTAG phone forensics techniques.

This presentation will impact the forensic science community by defining how JTAG phone forensics techniques are a very critical topic in the area of mobile forensics.

It is a common occurrence for digital forensic investigators to require data from a phone that is locked or even physically damaged. When one of these situations occurs, common phone forensics tools are not adequate for the task of extracting data from the phone. In many cases, investigators in this situation will determine data cannot be extracted from the phone and will simply stop the investigation; however, there are techniques that allow a forensic examiner to directly access the computer chip on a phone (at least for an Android™ or Windows® phone), then to extract that information in a hexadecimal format.

Many law enforcement agencies lack personnel trained in JTAG techniques. Many forensic examiners assume that JTAG is very complex, exceedingly difficult, and is beyond their skillset. Some even suppose that an electrical engineer is required to JTAG a phone; however, these assumptions are inaccurate. There are a variety of inexpensive kits designed for testing of chips that can be applied to JTAG techniques on a phone.

Forensic investigators need a better understanding of the process, techniques, and procedures in order to begin to leverage JTAG techniques in their phone forensics investigations. This presentation will provide that fundamental knowledge that will allow forensic examiners to take the next step to implementing JTAG in their own investigations.

This topic is very important to digital forensics. As any examiner can attest, it is common to find a phone related to a case but be unable to access the data on that phone. This impedes investigations in all areas. Phone forensics itself impacts not just traditional cyber crime investigations but also violent crimes, drug trafficking, and even terrorism investigations. The ubiquitous nature of smart phones makes phone forensics one of the most critical aspects of digital forensics. Both civilian and law enforcement forensic examiners need to expand their technical skillset in order to provide the possibility of extracting data from a phone, even if that phone is locked or damaged.

JTAG, Mobile Forensics, Phone Forensics

C23 Joint Task Action Group (JTAG) Data Extraction and Analysis

Jenise Reyes-Rodriguez, BS, NIST, 100 Bureau Drive, Gaithersburg, MD 20899; and Richard Ayers, MS, 100 Bureau Drive, MS 8970, Gaithersburg, MD 20899-8970*

After attending this presentation, attendees will better understand the importance of JTAG data extraction, analysis research, and testing conducted within the Computer Forensic Tool Testing (CFTT) project at the National Institute of Standards and Technology (NIST).

This presentation will impact the forensic science community by providing awareness of the capabilities and limitations for data extraction and digital forensic tools supporting JTAG binary file analysis.

As mobile device usage and sophistication continues to grow, the need for rigorous research and testing conducted across a variety of forensic tools and techniques is critical.

JTAG, an Institute of Electrical and Electronics Engineers (IEEE) standard, began as a method to verify the design and testing of printed circuit boards after manufacturing. The JTAG interface provides forensic examiners with numerous advantages, such as a non-destructive byte-for-byte memory dump from supported mobile devices whose data contents are typically examined using a traditional digital forensic tool. Additionally, the JTAG interface provides examiners with the ability to bypass mobile devices with disabled USB ports and the ability to extract data from devices that may have been subjected to liquid, thermal, or structural damage.

JTAG research conducted within the CFTT program begins by populating a set of supported mobile phones with a known data set containing active and deleted data. A known data set yields a way for results to be measured across several extraction solutions, techniques (i.e., solder, jig), and tools capable of parsing JTAG binary files. The memory contents are acquired across supporting JTAG extraction tools using a variety of connectivity techniques (e.g., solder, solderless). Data extractions begin with methodically disassembling a supported mobile device and identification of the copper Test Access Ports (TAPs) located on the Printed Circuit Board (PCB). The TAPs across various makes and models of supported mobile devices will vary in location and size. The TAPs are generally about the size of the tip of a thumbtack. Once the TAPs have been identified, various techniques can be utilized to establish connectivity and begin data extraction from the mobile device's internal memory, resulting in a JTAG binary file. After JTAG extraction, the data contents are examined by importing the JTAG binary into supporting analysis tools and comparing the results against known content.

The goal of the research and testing within the CFTT program is intended to provide the forensic community with an understanding of the capabilities and limitations of various JTAG extraction techniques and analysis tools. These results provide insight into any pros and cons across a combination of supported hardware, techniques, and tools capable of performing JTAG data analysis.

This presentation provides a summary of findings and lessons learned during the research and testing process of tools capable of extracting and analyzing memory contents from a mobile device using the JTAG interface.

Certain trade names and company products are mentioned in the text or identified. In no case does such identification imply recommendation or endorsement, nor does it imply that the products are necessarily the best available for the purpose.

JTAG, Mobile, Forensics

C24 Personality Characteristics of Computer Hackers: A Systematic Review and Meta-Analysis

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After attending this presentation, attendees will have a better understanding regarding personality characteristics of computer hackers.

This presentation will impact the forensic science community by increasing understanding of the personality characteristics of computer hackers through a systematic review and meta-analysis.

According to Symantec™, there was a 23% increase in the number of data breaches as well as an all-time high of 24 discovered zero-day vulnerabilities, with a combined 295 days passing until vendors released patches for the top five zero-days in 2014.[1] In addition, small- to medium-sized organizations were the victims of 60% of all targeted attacks. Verizon® reported an estimated 79,790 security incidents and 2,122 confirmed data breaches in 2014, and 55% of insider incidents involved abuse of privileges.[2] Regarding software security, Veracode™ reported that the government sector only remediates 27% of application vulnerabilities and 80% of healthcare sector applications suffer from cryptographic issues.[3] Finally, Abellera reports that the 16 critical infrastructure sectors in the United States (c.f., Presidential Policy Directive 21) are constantly defending against cyber-attacks.[4]

It is clear that escalating cyber threats and vulnerabilities are a serious concern for both small and large organizations as well as the private sector and general public. For the past decade, empirical studies exist that focus on the human element of computer crime; that is, trying to understand the personality characteristics of computer hackers.[5-7] Studies suggest that not only are there personality differences between computer criminals and non-criminals, but there are personality differences between different types of hackers, such as virus writers and identity thieves; however, researchers have yet to combine the results of these studies in order to better understand the overall relationship between computer criminal behavior and personality.[6,7]

After a decade of research, enough studies exist for a systematic review of the literature in order to conduct a meta-analysis.[8] A meta-analysis is a statistical approach to combining the results of multiple studies in order to understand the estimates of the effect or relationship under investigation. The specific goal of the current study is to conduct a meta-analysis of the literature, by including published and unpublished studies, to improve the overall understanding of the personality characteristics associated with computer criminal behavior. The systematic review of the literature will include unpublished works in order to account for publication bias, which should also result in a more accurate representation of the overall effect size estimate.

The results will be discussed in addition to the limitations of the study, final conclusions, and suggestions for future research.

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C25 Detecting Causality Through Fine-Grain Logging in Digital Investigations

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After attending this presentation, attendees will understand the impact of attribution on multi-app systems such as Android™. Storage and access of sensitive system data like contacts on SQLite databases by applications does not leave any trace of the causal relationships that will attribute the target app to the database object(s). Attendees will also learn how this problem can be solved by enforcing fine-grained access control on the SQLite database using static bytecode rewriting. The methodology is backed by a practical experiment that analyzed the 64 most-downloaded free apps on Google® Play™ and evaluated them based on application crashes, static, and runtime overhead.

This presentation will impact the forensic science community by discussing how fine-grained logging mechanisms can aid investigations by providing clear causal relationships between apps and database objects. Practical scenarios will be illustrated on how these casual relationships can be vital in supplementing memory and disk forensics to enhance digital investigations.

In a digital forensics investigation, how data gets written onto a device is as important as who writes the data. Often, research has shown that malicious software can write incriminating evidence on digital devices, thereby putting users at risk, as in the child pornography case against Michael Fiola.[1] The problem presented in this study illustrates how data written onto SQLite databases via the Android™ native providers cannot be linked to any specific application. While READ/WRITE accesses on the database are restricted and granted based on exclusive user permissions, general log files and typical digital evidence sources do not affiliate any app to the written data at any time.

With WRITE permission for instance, update, insert, and delete database operations can be performed by an application with very little data available to support attribution. This is primarily because SQLite is a single-user system and is not designed to keep track of who performs what operations on a system. For forensics investigation, this makes it very difficult to ascertain if a particular entry in the database is added or updated by the user or by a malicious application.

Thus, to aid digital investigation, a fine-grain logging technique that uses bytecode weaving to statically weave in extra auditing code after the return of specific Android™ database Create, Read, Update, and Delete (CRUD) functions (insert, update, delete, and query) is presented. The presented technique uses aspect-oriented programming to instrument Android apps. This instrumentation process does not require any modification to the operating system and/or framework code, thereby making it easily adoptable by average users. The experiment also revealed the system incurs an average of 15 seconds of static overhead and 58 nanoseconds runtime overhead across a range of test apps.

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Causal Relationships, Fine-Grain Logging, Bytecode Weaving

C26 Supervisory Control and Data Acquisition (SCADA) Forensics: Network Traffic Analysis for Extracting a Programmable Logic Controller (PLC) System and Programming Logic Files

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After attending this presentation, attendees will understand the message format of the Allen-Bradley Programmable Controller Communication Commands (PCCC) protocol and how the network traffic of the protocol can be analyzed for extracting ladder logic program and system files. Ladder logic is a popular programming language for a PLC that is an essential and critical component for the automation of industrial processes such as gas pipelines, chemical and nuclear plants, and power generation and distribution.

This presentation will impact the forensic science community by providing an overview of a parsing and recovery technique for a ladder logic program and system files from the network traffic of the Allen-Bradley PCCC protocol. The protocol is supported and widely used by the PLCs of the vendor, Allen-Bradley, for transferring configurations and programming logic and control data (such as sensor readings, current state of actuators, counters, and timers). When the programming software, such as Studio 5000® and RSLogix 500™, transfers a ladder logic program from an engineering workstation to a PLC, it transfers system files along with the program.

In order to extract these files, the technique takes into account the PCCC message format allowing the identification and filtering of packets based on the protocol header fields. The fields include byte-count, file-number, file-type, element-number, and sub element number. In particular, the file-type field is used to identify the type of data in a packet. For instance, "O" represents output file data. Each packet contains headers of three industrial protocols in the following sequence: Ethernet/IP, Common Industrial Protocol (CIP), and PCCC. The technique for recovering the files involves parsing the PCCC header, then further filtering out the packets containing the chunks of system and program logic files, which are then combined in a logical sequence to reproduce the files being transferred through the network.

A prototype implementation (tool) of the technique will also be provided in the presentation. The tool can play an important role in the forensic investigation of a SCADA system. For instance, a forensic investigator can determine the presence of any ladder logic code and system files in a network packet capture and further obtain the whole code from the capture in a file for further analysis. The investigator can compare the hash values of the code extracted from the network trace to the original code of the PLC created and downloaded from PLC programming software.

Ladder Logic, SCADA Forensics, Network File Carving

C27 Assessing the Psychological Well-Being and Coping Mechanisms for Law Enforcement Investigators and Digital Forensic Examiners of Child Pornography Investigations

Kathryn C. Seigfried-Spellar, PhD, Purdue University, Computer and Information Technology, 401 N Grant Street, West Lafayette, IN 47907*

After attending this presentation, attendees will understand the significant differences of psychological well-being, job satisfaction, and coping mechanisms for individuals working as either digital forensic examiners and/or investigators of cases involving child pornography.

This presentation will impact the forensic science community by highlighting this first study to assess the psychological well-being, as well as attitudes toward psychological services, for individuals working as either digital forensic examiners and/or investigators of cases involving child pornography.

The United States has seen an increase in the number of law enforcement officers investigating child pornography cases as well as the number of digital forensic examiners involved in the analysis of child pornography-related digital evidence.[1] Specialized task forces or units that focus on child pornography cases exist. Some are nationwide, such as the Internet Crimes Against Children (ICAC) task force, whereas others exist at the state or local level. In some units, there is a clear division of labor — computer forensic examiners analyze and index the child pornography collections, while the investigators work directly with the offender and the victim/family; however, in other units, the investigators are also the examiners, meaning they not only work alongside the offender and/or victim, but they also analyze the digital evidence in child pornography cases. Overall, criminal justice occupations are associated with high work stress leading to psychological illnesses (e.g., depression, anxiety) and high turnover rates, especially for officers dealing with child investigations.[2,3] Previous research has assessed the level of job satisfaction and coping mechanisms for digital forensic examiners; however, research has yet to compare the psychological well being of individuals who work as either digital forensics examiners, investigators, or both, while working child pornography cases.

The current study was the first to compare the psychological well being, job satisfaction, coping mechanisms (i.e., strategies for dealing with stress), and attitudes toward psychological treatment (e.g., therapy) for individuals working as either digital forensics examiners and/or investigators of child pornography cases. This study had three specific goals: (1) explore the psychological wellbeing and job satisfaction; (2) identify any differences in coping mechanisms; and, (3) assess attitudes toward psychological treatment as well as the availability of psychological resources for examiners/investigators working child pornography cases.

A snowball sample of law enforcement officers and digital forensic examiners were solicited to participate in the anonymous, online survey through a variety of means, including the ICAC task force listserv. Based on their current, self-reported duties, 20 respondents were classified as digital forensic examiners only, 71 respondents as investigators only, and 38 respondents as both digital forensics examiners and investigators of cases involving internet child pornography. Results suggested significant differences in the psychological well being, job satisfaction, and coping mechanisms for individuals working as either digital forensic examiners and/or investigators of cases involving child pornography.

The results will be fully discussed as well as suggestions for future research.

Digital Forensics, Law Enforcement, Child Pornography

C28 A 3D Study of Facial Mimicry: A New Approach for the Identification of Missing Children

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After attending this presentation, attendees will better understand how to improve the possibility of facial recognition of missing subjects by exploiting the memory for facial expressions.

This presentation will impact the forensic science community by highlighting an important 3D facial analytical method that can extract invariant features from the 3D face template. The results could be very helpful in the interpretation of 2D images, given that the 3D verification of the mimicry in childhood/adolescent samples revealed the persistence of highly individualizing facial expressions.

The assessment of facial morphology is mainly used in the facial identification of missing subjects when photographs and videos are available; however, in everyday life, faces are in constant motion and often a person is recognized for distinct facial expressions. Facial morphology also is altered with age and environmental conditions. Even a simple instantaneous contact captures the image of a moving face. For this reason, researchers have begun to consider how facial motion affects memory for faces. The face provides a distinctive indication of the individual identity and previous studies demonstrated that memory for a moving face can help personal recognition.[1] Moreover, in previous studies conducted with 3D methods, adults undergoing reconstructive facial surgery retained the same mimic movements one year after surgery, despite the changes to the underlying bones. This study proposes a method for personal identification of missing children, at a period of time after their disappearance, using 3D analysis of facial movements.

New technologies enable presentation and manipulation of digital videos, techniques that are fundamental in the efforts to identify subjects, including minors. The identification of facial expressions can implement the effectiveness of recognition of missing persons. Three boys and three girls aged 7-14 years of age in T1 and 14-21 years of age in T2 were recruited for the study. Facial surface data were acquired by a 3D laser scanner (Cyberware 3030RGB™) and transferred to Geomagic Design™ X software for data elaboration. In previous studies, the development of a specific protocol resulted in a mean scanning error of 1mm–1.2mm and a recording error of 0.3mm–0.4 mm on repeated scans of human subjects. The patients were registered in a resting position and after movements of the upper, middle, and lower area of the face. They were frowning, grimacing, smiling, and pursing their lips. To study the modifications of these faces during growth, an anthropometric evaluation was conducted using 3D points to discern distance measurements in a resting position.

Statistically significant results were obtained in longitudinal and oblique 3D measurements ($p<0.05$). In T1 children, the motion was easier and spontaneous in the lower area of the face, while the movements of the upper area were not intuitive. The amplitude of the movements of the lower area of the face was augmented with age. In T2, spontaneous movements in the upper area of the face appeared more evident (i.e., grimace). This is consistent with the literature. Two observers separately evaluated the soft tissue displacements during the facial movements of T1 and T2. The 3D morphological study of the mobility of the soft tissues of the lower part of the face of the subjects demonstrated that the same parts of the soft tissue were involved in the same areas of the face. A unique and characteristic facial morphology was generated, different in every subject, but similar and consistent in the subject during repeated trials. In particular, the mouth, cheeks, and nosewing presented the same morphological displacement, and nose-lip sulks and asymmetries of the lower area of face were consistent over time. The maxilla-facial bone structures, especially in children, vary significantly over time, but facial movements do not.[2]

The 3D technology is useful for facial recognition. Nevertheless, facial modifications may pose problems for automatic individual identification and recognition, and more work needs to be conductede on a larger sample, including more variations in age categories.

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3D Mimicry, Facial Recognition, Missing Children

C29 Behavioral Forensic Analysis, Biometrics, and Social Media — A New Investigative Tool

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After attending this presentation, attendees will understand that tracking online behavior makes it possible to understand, to deeply explore, and to predict the evolution of the users' activities. This study introduces the different modeling strategies and their peculiarities. Also being proposed is an integrated demolition tool.

This presentation will impact the forensic science community by providing information regarding how the use of a solid framework of computational social science combining biometry and online users' behavior for the detection and classification of specific behavioral patterns is useful in investigating single and mass phenomena.

The wide availability of social and mobility traces on online Social Media (SM) represents an opportunity for the quantitative analysis of human behavior for investigative and forensic purposes.

The recent advances in the computational social science field may provide an unprecedented set of tools able to disentangle social dynamics at both the micro and macro level, from behavioral and cognitive profiling of single users to forecasting massive social trends and viral phenomena.

Currently, modeling human behavior has become a priority, especially as it applies to social network behaviors as well as the proper use of behavioral biometrics to define and detect strategically hidden identities. Therefore, it is strategic to combine identification tools based on behavior implemented within structures that allow tracking, control, and the potential demolition of assets of dangerous activities. The study of human behavior, especially applied to behavioral analysis in the forensic field, offers new and interesting application perspectives; this study explores two main areas of application by combining them.

This stydy's goal is to use the solid framework of computational social science to combine biometry and online users' behavior for the detection and classification of specific behavioral patterns. By means of the proposed approach, behavioral analysis can become an active tool. On the one hand, the precision and speed of anomaly detection and user identification can be improved. On the other hand, through mathematical modeling, user behavior at the individual and social level can now be forecast. Furthermore, the application of mathematical tools for the understanding of users' behaviors on SM can reveal critical information to be exploited for security purposes.

Users online, tend to join polarized groups of like-minded people and to acquire information that only adheres to their belief system. Such a tendency, known as confirmation bias, plays a pivotal role in online social dynamics and reflects the users' personality traits.

The analysis, therefore, allows us, on the one hand, to explore behavior of the media in order to extrapolate information susceptible and sensitive information. On the other hand, it provides a useful tool for the identification of the subject. By tracking online behavior, it is possible to understand, to deeply explore, and to predict the evolution of the users' activities. This study introduces the different modeling strategies and their variences and proposes an integrated demolition tool.

Behavior, Forensic Analysis, Behavioral Biometrics

C30 Advances in Automated Content Carving

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WITHDRAWN

C31 Challenges in Determining End-User Actions Based on Cloud Repository Metadata

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After attending this presentation, attendees will better understand the challenges involved in drawing conclusions based on metadata from cloud repositories, such as Google® Drive™ and Dropbox™ Paper, in addition to how end-user actions affect the available metadata.

This presentation will impact the forensic science community by providing a clear and concise summary of how end-user actions impact the metadata maintained in cloud-based environments and how this information can be used to make reliable conclusions in digital forensic investigations.

Metadata, or "data about data," is a critical artifact relied upon in many digital forensic investigations and extensive research has been conducted by the digital forensic community to better comprehend how dates of files relate to actions taken by the user; however, cloud metadata is stored and updated differently than metadata on a physical file system, and, thus, the need to understand this tracking system is of the utmost importance. Therefore, this presentation will describe recent research and analysis performed on data stored in Google® Drive™ and Dropbox™ Paper repositories, with a specific emphasis on the analysis of metadata to determine user activity.

The use of various cloud repositories to create, edit, and store documents is becoming more and more commonplace, and the data in these repositories has become increasingly relevant in both civil and criminal digital forensic investigations. Google® Drive™ and Dropbox™ Paper were researched in order to determine what types of metadata are stored and how they relate to common user actions. To replicate as many user scenarios as possible, the web-based interface was used in conjunction with the downloaded applications on a Windows® desktop computer and an Apple® mobile device. Several typical user actions were completed, such as creating, editing, downloading, and uploading documents, while changes made to the metadata were documented.

This presentation will describe how various actions performed by the user can change the metadata between different platforms: the web-based interface, the application on a Windows® machine, and the application on an Apple® iPhone®. For example, while Google® Drive™ maintains date metadata to an extent within the web interface, the created time of the file is overwritten by the time of download, regardless of how it is downloaded; however, the modified date remains unchanged except for the fact that it is represented in Pacific Standard Time despite Google® Drive™ settings being set to Eastern Standard Time. Unlike the original Dropbox™, Dropbox™ Paper allows for the creation and editing of documents and only shows the dates of recent edits within the web-based interface. Once the file created within Dropbox™ Paper is downloaded, the modification date is overwritten with the time of download. Many other situations will be discussed to enhance analyst comprehension of how metadata is affected by common end-user actions.

Forensic analysis of cloud metadata is especially challenging, as new features and versions are constantly being introduced by cloud providers. To overcome the challenges of ever-changing metadata tracking systems, digital forensic analysts need to be aware of the versions and features relevant to their case and, if necessary, verify their understanding of the relationship between user actions and the changes in the metadata in the same environment. Without this comprehension, analysts may be unable to make knowledgeable and accurate conclusions when translating the metadata of a cloud repository file into user-attributable actions.

Cloud Forensics, Google® Drive™ Metadata, Dropbox™ Paper Metadata

C32 The Acquisition and Analysis of Evidence From Cloud and Internet of Things (IoT) Services

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After attending this presentation, attendees will better understand the qualitative differences between working with traditional and cloud-based evidence sources. Attendees will also gain methodological insight into the inherent limitations of the currently prevalent client-side analysis of cloud services as well as a preliminary notion of how IoT forensics is likely to take place.

This presentation will impact the forensic science community by clearly demonstrating: (1) the need to develop new approaches to investigating cloud services; (2) a summary of early results in the field, including new data sources that are not available on client devices; and, (3) several new types of capabilities that forensic tools already need.

The rapid transition to a cloud-centric delivery of Information Technology (IT) services is having a profound, and currently underappreciated, impact on the acquisition and analysis of digital evidence. Namely, the switch from deploying "software as a product" to using it "as a service" changes both the legal environment and the technical requirements for search, seizure, and evidence analysis operations. Considering the technical side, it is shown that digital forensics needs to move away from the idea that physical acquisition is the gold standard and transition to the concept of acquiring evidence from an authoritative source, such as a cloud service. It is argued that digital forensics, as a whole, is on the verge of transitioning to a new mode of operation in which large-scale automated data analysis will dominate and mainstay data recovery and reconstruction techniques will rapidly diminish in importance.

Several case studies of cloud acquisition and analysis tools that work with cloud drive and online collaboration services to illustrate both the limitations of client-side analysis and the new opportunities presented by rich historical data collected by services will be discussed. Examples include direct acquisition from Dropbox™, Box, Google® Drive™, and Microsoft® OneDrive®, and analysis of native Google® Docs artifacts, including the extraction of user biometric signatures based on keyboard dynamics.

This presentation will also briefly consider the forensic analysis of IoT devices, which are placed in several capability classes. The argument will be made that for a large class of low-end devices, the only meaningful forensic analysis will be based on the data acquired from the cloud service backing the devices.

Based on the above reasoning and experiences, the argument is made that the next generation of forensic tools will be primarily focused on working with network Application Programming Interfaces (APIs) and the forensic community will need to develop completely new capabilities for (semi-) automated reverse engineering and will describe the semantics of network protocols and data APIs.

Cloud Forensics, Cloud Evidence, IoT Forensics

C33 Representing and Exchanging Cyber-Investigation Information in a Standard Format

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After attending this presentation, attendees will have learned about the community-driven standard format for representing digital evidence and other cyber-investigation information. Uses and benefits of this standard are presented and initial implementations are demonstrated.

This presentation will impact the forensic science community by demonstrating how the adoption of this standard by forensic laboratories, tool developers, and other members of the digital evidence community will enable sharing, increase efficiency, enhance analysis, and reduce linkage blindness.

Any type of investigation can have a digital dimension, ranging from computers as a source of information in homicides and terrorist attacks to computers as instrumentalities of fraud and cyber-attacks. Offenders, violent and white collar alike, including drug dealers making simple use of cell phones and organized criminals making sophisticated use of computer networks, use technology in various ways. As a result, cyber-investigations are complex, multifaceted, and have specific applications within broader contexts of criminal justice systems, enterprise governance, and military operations.

Furthermore, investigations of cybercrime can involve multiple investigating entities, forensic tools, and jurisdictions that each have pieces of information needed to solve the case. It is important to bring these pieces of information together to support forensic analysis and reduce the risk of overlooked linkages. Current approaches to sharing cyber-investigation information are ad hoc, inconsistent, and inefficient. Where standardized structures are used, they are typically focused on only an individual portion of the overall cyber-investigation process, they do not integrate well with each other, or they lack coherent flexibility. Many existing information-sharing activities involve conversion of proprietary formats and are human-to-human exchanges of unstructured or semi-structured descriptions of cyber-investigation traces and analysis. Correlating results from digital forensic tools can be a time-intensive and error-prone process, largely due to the inconsistent export formats.

This presentation details a community-driven standard format to support interoperability between organizations, jurisdictions, investigations, and tools. This initiative is called Cyber-investigation Analysis Standard Expression (CASE). The intended benefits for this standard format include increased efficiency when combining and deduplicating output from multiple tools and reduced linkage blindness between investigations and jurisdictions.

Core features of this standard are presented, including how traces, actions, relationships, and provenance are represented. Examples are provided of the initial JavaScript™ Object Notification (JSON) serialization of the underlying information model. Use cases for the standard format are discussed, including collaboration and sharing, correlation and analysis, and tool comparison and interoperability. An initial proof-of-concept implementation of CASE is demonstrated using the open source "plaso" forensic framework.

Attendees are invited to join this community effort, using the standard in their organizations and tools, and proposing improvements to the standard.

Digital Evidence, Cyber-Investigation, Information Sharing

C34 Vehicle Forensics: A Tale of Two Cars

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After attending this presentation, attendees will recognize the capabilities and potential for vehicle forensics to uncover valuable digital evidence. Attendees will also understand the basic capabilities of the only commercial vehicle forensics tool currently available.

This presentation will impact the forensic science community by demonstrating the ability of vehicle forensics to unearth valuable digital evidence in an innovative manner.

The analysis of mobile devices and hard drives has been the focus of the digital forensics world for years; but, there is another source of potential evidence that is not often considered: vehicles.[1,2] In fact, vehicle forensics is so new that attorneys, investigators, or law enforcement may not even realize the wealth of digital information cars can provide. Many of today's digitally "connected cars" have infotainment and telematics systems that function like computers, storing information that they process, including user data from mobile phones and devices that have been synced to the system.[2,3] These systems have been said to store unique identifying information originating from mobile devices such as call logs, contacts, sent/received text messages Short Message Service (SMS), and even social media feeds that remain on the system even when the phone is detached.[2,4] User artifacts such as these can point to suspects or witnesses and/or play a role in corroborating or disproving someone's alibi. There has been little-to-no research conducted regarding how long these artifacts remain on the system and whether or not the user can remove those artifacts.

Additionally, there has been minimal research conducted regarding the types of user artifacts that can be left on vehicle systems by mobile devices. This is understandable since vehicle forensics is new and there is currently just one commercially available tool: Berla Corporation's iVe.[2,4] Furthermore, due to the differences between various makes and models of infotainment systems, it stands to reason that the user information obtained from one system by iVe may be different than that obtained from another. Little research has been done regarding certain systems potentially providing more information than others.

This research seeks to answer the following research questions: Which user artifacts can be found on vehicle infotainment systems?; How long will those artifacts remain on the system?; Will any of those artifacts remain on the system once the devices are removed or unpaired through the on-screen interface?; and will different infotainment systems allow for recovery of different artifacts? For this study, two different makes and models of vehicle infotainment systems were used for the data acquisition process: a Uconnect® system and another with a Toyota® Extension Box. It was found that the Toyota® system provided a significant amount of user information (contacts, call logs, media files, locations, and unique identifiers), while the Uconnect® system only provided locations. Additionally, it was found that even when these paired devices were unsynced from the Toyota® system using the on-screen interface, a significant amount of acquirable information remained behind on the system: contacts, call logs, and media files were recovered from devices that were not currently connected to the system. When devices were unpaired from the Toyota® system using the on-screen interface, the mobile phone identifying information (i.e., International Mobile Equipment Identity (IMEI)) was unable to be recovered, but contacts, call logs, media files, last sync time, and phone version could still be seen. Even after selecting the "Remove Personal Data" option on the Toyota® system, the same identifying information (contacts, call logs, etc.) could still be retrieved; call logs dating back several years were still recoverable from the system. While this study provides valuable insight into artifacts that can be found using vehicle forensics, future work should include the performance of physical acquisitions on vehicle systems, as well as analyzing data from other types of infotainment systems.

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Vehicle Forensics, Infotainment Systems, Digital Artifacts

D1 Can Gait Recognition Using Coupled Non-Linear Oscillators Overcome Intra-Individual Variability?

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After attending this presentation, attendees will understand how to formulate and implement gait recognition schemes using Central Pattern Generator (CPG) -based coupled non-linear oscillators for overcoming the problems of false rejection due to intra-individual variability.

This presentation will impact the forensic science community by providing a novel gait recognition scheme using a coupled non-linear oscillator model. This study will also initiate discussion regarding which gait-relevant parameters can potentially be used to solve the problem of intra-individual variability.

Gait recognition is a recently developed and still evolving technique by which individuals can be recognized based upon their gait. Two different approaches are used: (1) silhouette-based; and, (2) model-based.[1-5] The first, using silhouette information, is being tested for forensic identification purposes in Japan.[1] The second uses body-model information, such as joint coordinates or angles, and is not yet in general use. Because conventional model-based methods mainly compare similarity of joint dynamics, gait recognition is adversely affected by intra-individual variability arising from environmental or mental changes.[3,4] False rejections (i.e., incorrectly identifying the same person as "different") therefore sometimes occur due to these alterations. It is a difficult but necessary and urgent task to overcome these problems to allow expanded application of this technology. To simulate and reconstruct foot joint dynamics, previous studies have successfully demonstrated the usefulness of non-linear oscillator models, which are one of the representations of the CPG framework.[6] Gait recognition schemes incorporating those models may offer the solution to false rejections arising from intra-individual variability.

This study compares different methods using a coupled non-linear oscillator model.[6] This study involves lower extremity joint trajectories (two hips, knees, and ankles) in a model which includes three Degrees Of Freedom (DOF) for one hip, one DOF for one knee, and two DOF for one ankle. Evaluations were performed using 3D joint trajectories to focus the possibility and usefulness of the suggested method. To fit these parameters to a non-linear oscillator model, a Levenberg-Marquardt algorithm was used that evaluates errors as sum of squares of joint coordinate differences. This study examined and compared four ways of selecting features described below: (1) using all fitted parameters; (2) using some fitted parameters; (3) using simulated joint trajectories; and, (4) using dynamic properties featured as a phase-response curve, which consists of the relationships between phase changes and perturbation timings when a perturbation is added.

The 3D joint trajectories of 20 subjects were measured by Microsoft® Kinect® v2 sensors. The data was obtained from four walks obtained during each of a two-day experiment. The error rate of conventional methods used to analyze the data from two days of experiments was greater than the error rate obtained from the date of a one-day experiment. This confirmed that intra-individual variability can cause false rejections. The results of these studies and suggested methods will be presented and error rates obtained from each of the four methods of selecting key

joint relevant features will be examined. The results will provide information regarding which joint parameters can or cannot be used to solve the problem of intra-individual variability.

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Model-Based Gait Recognition, Intra-Individual Variability, Coupled Non-Linear Oscillators

D2 A Forensic Analysis of Flora Damage Caused by an Environmental Disaster at Mariana, Brazil

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After attending this presentation, attendees will understand several of the techniques used for the characterization of damage to flora that occurred in a large area.

This presentation will impact the forensic science community by detailing the environmental expertise activities undertaken to assess the extent of flora damage in large areas.

On November 5, 2015, the worst Brazilian mining accident occurred in the city of Mariana, Minas Gerais, Brazil. This occurred as a result of a dam (Fundão) breach that produced a mud torrent for more than 600km along Vale do Rio Doce, affecting all the cities and communities along the way and impacting approximately 1.2 million people between the dam and the sea. An estimated 18 victims died; one person remains missing.

The Forensic Unit of Environmental Crimes of the Federal Police was assigned to examine the damaged area. Experts from this group were divided into teams according to specific types of damage, including vegetation, animals, drownings, causes of the dam collapse, and more. The purpose of this presentation is to describe the activities undertaken to assess the damaged vegetation.

The wave of mining waste caused contamination of mining tailings over the watercourse gutter limits, thereby destroying the existing vegetation. This wave reached the grassy vegetation, herbaceous-shrubby vegetation, and treed areas. Damage to vegetation encompassed a region approximately 117km in length, extending from the Fundão dam head to the beginning of the Risoleta Neves Hydroelectric Plant dam lake.

High-resolution satellite images of the damaged area were unavailable following the catastrophe. Later images were available from the Landsat 8 satellite, but the spatial resolution (15m) of these images limited the ability to quantify the extent of the damage.

Commercially available micro- Unmanned Aerial Vehicles (UAVs) were not an option to image and assess the damage as the range of these vehicles was limited to 6km, a distance incompatible with the location of the affected area. Thus, manned helicopter-based aerial surveillance was conducted over the affected riverbeds. A total of 940 aerial photographs were obtained by using a GoPro® HD2 camera equipped with a wide-angle lens to survey a large area in a single flight path. The photographs recorded approximately 80% of the available area viewed. Using these data, experts employed a photogrammetric technique of Structure from Motion (SfM) for generating 3D terrain models and, from these models, obtained georeferenced orthophotos (spatial resolution of approximately 30cm). Distortions due to geophysical relief and photographic angles were accounted for in these models by using a Geographic Information System app.

Simultaneously, a multispectral classification of high-resolution satellite images, taken before the dam disaster, was used to define the topography prior to the incident and provide "baseline" information. The processed information was verified by the use of data gathered in the field and with other prior aerial photographs. Atlantic forest, eucalyptus forest, woods, crops, pastures, and disturbed areas (roads and built-up areas) of physiognomy vegetation were characterized.

It was concluded that the extent of the destruction totaled 1,176,44 hectares (ha), distributed as: 240,880ha of Atlantic forest; 45,000ha of Atlantic forest with eucalyptus; 174,300ha of natural vegetation; 39,110ha of pasture areas; 1,380ha of commercial forests (eucalyptus), and 86,006ha of disturbed areas. This presentation offers attendees a learning experience regarding the methods available to quantify the extent of affected vegetation of large-scale environmental disasters.

Photogrammetry, Flora Damage, Remote Sensing

D3 An Environmental Forensic Analysis at the Doce River Estuary: A Tool for Damage Assessment in a Major Tailings Dam Break in Brazil

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After attending this presentation, attendees will better understand the important aspects of environmental forensic examinations as applied to industrial disasters of major proportions.

This presentation will impact the forensic science community by illustrating modern techniques and an integrated approach to geoprocessing and environmental chemistry.

The goal of this presentation is to present the forensic examinations used to assess the extent of environmental damage at the mouth of the Doce River in Brazil, caused by mining tailings discharged by the disruption of the Fundão dam.

Estuary, a coastal environment of transition between river and sea, is considered an area of high environmental sensitivity, since it is a breeding and feeding area for several river and marine species. On November 21 2015, the Doce river estuary was affected by a plume of tailings originating from a mining company dam that had broken 16 days earlier. Some consider this the greatest dam collapse in mining history.

To assess the damage to the estuarine ecosystem, a forensic team from the Brazilian Federal Police conducted environmental examinations at the mouth of the Doce River. The examined site consisted of an area of natural occurrence of various endemic species of mangroves, typical wetlands plants, and endangered animal species. The marine zone adjacent to the mouth of the river is a reproduction and migration area of marine cetaceans and turtles, many of them endangered.

Forensic experts monitored the area before and after the arrival of the tailings plume, using boats, aircraft, and Unmanned Aerial Vehicles (UAVs). Field inspections and laboratory analyses were conducted to check water quality indices. Experts measured the dispersion of the tailings plume through the sea by daily helicopter overflights arranged using photographic and Global Positioning System (GPS) data. The plume advance was also tracked by images produced by orbital sensors (WorldView 02, WorldView 03, GeoEye-1, and Landsat 8, the latter having a specific band for sediment analysis in water). UAVs were used to obtain high-resolution images of a nearby coastal Federal Conservation Unit, another highly sensitive area in the region.

Normally, turbidity levels of the mouth of the Doce River were lower than five Nephelometric Turbidity Units (NTUs). The tailings plume resulted in the turbidity of mouth waters exceeding 2,000 NTUs, with peaks exceeding 5,000 NTUs and well above the legal Brazilian limit (100 NTUs). Water pollution resulting from mining tailings caused serious environmental damage, such as modification or elimination of wildlife niches, refuges, and breeding sites. Mortality of aquatic animals and migratory marine birds was also observed. Socioeconomic activities in local villages were affected due to the interruption of the drinking water supply and the decrease of tourism and fishing.

Expert examinations allowed a precise characterization of the site before and after the arrival of the tailings plume, thus enabling quantification of the environmental and socioeconomic damage caused by dam break. The current forensic examinations were crucial for proving the environmental crime caused by the dam break, revealing the deleterious effects of pollution on the Doce River estuary.

Dam Break, Estuary, Pollution

D4 Low Price or High Performance?

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The goal of this presentation is to increase awareness of the role of economics regarding choices made in materials, designs, or workmanship of everyday devices that humans interact with and depend upon for satisfactory performance and personal safety.

This presentation will impact the forensic science community by discussing how human injury or death is always a tragedy, but these tragedies are exacerbated when their cause can ultimately be attributed to intentional choices made between low cost or high performance in materials, designs, or workmanship of the everyday devices relied upon to provide service. This presentation provides an overview of a session devoted to this topic and sets the basis for the concluding panel discussion.

Reliable performance of safety helmets, air bags, automotive seats, and seat buckles, as well as vehicle body designs, etc. are taken for granted by virtually all consumers. Yet reports often reveal that the risk of injury or death from these and other everyday devices of modern society is more than would ordinarily be expected. Although risk is a necessary accompaniment of a modern technological society, elevated risk arising from economic choices made in the materials, design, or workmanship of these devices merits the attention of consumers, regulators, and legislators.

This session features a compendium of presentations by experts who have spent a considerable portion of their professional careers investigating human injury or deaths that have occurred in conjunction with devices posing elevated risks to society from economic-driven compromises to football helmets, automobile seat backs, buckles, and fuel tanks as well as body and chassis design.

Information presented in this session is useful for forensic investigators as it will highlight design or material shortcomings in such devices and direct the attention of the investigator to these weaknesses, thereby promoting efficient, thorough determination of mishap etiology.

This session is important for attendees with regulatory or legislative responsibilities because lacking government regulation, organizations often choose a path which minimizes expenses to maximize profits at the cost of human safety. Decisions attending design of the Ford® Pinto® or General Motors® (GM) ignition lock provide noteworthy examples. Furthermore, information presented during this session will provide a basis for all attendees to improve their ability to understand mishap etiology.

This session will conclude with a panel discussion that attempts to raise societal awareness of the decisions governing material selection, device design, or manufacturing processes that compromise the performance of everyday items upon which society depends and takes for granted. This panel discussion may also result in technology-based suggestions for reducing such risks. Elected representatives in attendance may use the information provided as a point of departure for introducing new legislation designed to enhance societal safety and protect the public from economic-based compromises to devices upon which humans depend for safe performance.

Material Compromise, Design Flaw, Economics

D5 Vehicle Fuel Systems: Low Performance at a High Cost to Motorists

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The goals of this presentation are to explain how and why a variety of automotive safety defects exist, along with effective countermeasures and safer alternative designs. The effects of low cost vs. high performance in automotive designs will be demonstrated by analyzing and discussing fuel leakage and ignition designs attending otherwise preventable human injuries.

This presentation will impact the forensic science community by illustrating how to recognize and prevent unsafe fuel system designs as well as by introducing forensic evidence associated with fuel system failure, including puncture and separation of fuel system components, ignition sources, fire burn patterns, and effects on vehicle occupant survivability. Attendees will learn how and why fire occurs in readily survivable collisions in passenger cars, light trucks, and heavy trucks.

Vehicle fuel systems have presented extreme hazards to motorist safety since the creation of motor vehicles. One gallon of gasoline has sufficient energy to propel a vehicle many miles; therefore, it can have instantaneous life-threatening consequences on occupant survivability if ignited due to fuel leakage occurring during an otherwise survivable collision.

Many early vehicle designs employed gravity-feed fuel tanks located between the engine and windshield. This design often caused non-collision-related fires due to fuel leakage onto hot engine and exhaust components. Fuel tanks were then relocated underneath seats and elsewhere inside occupant compartments and, with rare exceptions, along the exterior vehicle perimeter. This general practice continued well into the 1960s and, in some designs, even later.

The earliest efforts involving automobile fuel system evaluation involved ground vehicle safety testing conducted by the United States Air Force in the 1950s, eventually leading to the 1966 creation of Federal Motor Vehicle Safety Standard (FMVSS) 301. By the late 1960s and early 1970s, sufficient research had been performed at the University of California Los Angeles in the Department of Transportation (DOT) Experimental Safety Vehicle program and at independent laboratories, such as Dynamic Science Inc., to develop properly mounted, crashworthy vehicle fuel systems that were sufficiently cost-effective to enable wide-scale adoption in mass-produced vehicles sold to the public.

The theme of low price vs. high performance clearly reared its head as, unfortunately, automakers have, to the present time, resisted efforts to implement improved high-performance fuel systems in favor of those with low cost, despite the continuing evolution of design improvements in racing vehicles and military aircraft fuel system integrity. These high-performance design improvements are significantly more advanced than those found in current mass-produced automobiles. Such design improvements are not only far more crashworthy, they often contain fire-preventive materials or protection systems that were validated via analysis and testing by researchers. This knowledge was disseminated in the Department of Defense *Crash Survival Design Guide* and other technical safety engineering literature published decades ago.

Unfortunately, the quest for low price is manifested in numerous examples in which automakers built vehicles that ignored well-established fuel system safety design guidelines. Specifically, fuel tanks have been located in vulnerable areas aft of the rear axle, inside cabs on pickups and utility vehicles, and outside frame rails (i.e., “sidesaddle”) on millions of light-duty pickup trucks manufactured through 1987. The lessons that should have been learned from these low-cost, dangerous designs were ignored by automakers who continued to produce unsafe vehicles well into the new millennia. These designs persist with fuel tank locations aft of the rear axle, fuel tanks with no check valves or tethers on filler necks, and fuel tanks lacking effective impact shields or interior bladders to resist fuel leakage if the tank is compromised. This has resulted in a significant number of fire deaths and recalls involving late-model production vehicles. With rare exception, there are no automaker efforts to test production fuel systems in real-world collision conditions, such as vehicle-to-vehicle crash testing at foreseeable highway speeds. Government efforts to improve FMVSS 301 still do not include designs to mitigate fuel tank leakage caused by “underride effects” commonly seen in many vehicle-to-vehicle collisions. Fuel system failures have also

been caused by contact with guardrails, signs, and other common roadside fixed objects. None of these events are considered or evaluated by the DOT or any automaker.

Fuel system design flaws persist in heavy trucks as well, as plainly evidenced by designs clearly showing fuel tanks mounted outside the frame rails of large commercial trucks. Little evolution has occurred in this design since the 1950s. Diesel tanks constructed of thin-walled aluminum are extremely vulnerable to mechanical compromise, content leakage, and disastrous fire. While comparatively safe in bulk liquid form, diesel fuel readily ignites when atomized into a fuel/air mist as commonly occurs during a collision. Fuel tanks on these trucks have exposed filler valves that are vulnerable to impact by vehicles and relatively benign collisions against fixed objects. Collision-induced malfunctions in vehicle electrical systems can provide ignition sources for some time following the impact(s) of the initial collision.

Fuel System, Fire, Safety

D6 Richard III — Finding a King: High Performance and Low Cost

Sarah V. Hainsworth, PhD, University of Leicester, Dept of Engineering, Leicester LE1 7RH, UNITED KINGDOM; and Kevin Schurer, PhD, University of Leicester, School of History, University Road, Leicester , UNITED KINGDOM*

After attending this presentation, attendees will understand elements of planning a major undertaking and using information to guide decision making.

This presentation will impact the forensic science community by illustrating how a major multidisciplinary project can be planned and managed to achieve a successful outcome.

The Richard III project received worldwide attention and clearly demonstrated how multidisciplinary teams can collaborate to demonstrate the efficacy of skillful planning together with modern forensic techniques to efficiently identify the location of a 500-year-old British king's remains. This endeavor was managed like a textbook example of a forensic missing person case from the outset.

The first step involved identification of the known facts and listing the information known with certainty regarding the circumstances surrounding Richard III's death. These facts were that Richard III was in Nottingham when he learned of Henry Tudor's (Henry VII) landing in Milford Haven. Richard III's army rode into Leicester on August 20, 1485, passing through the North Gate, continuing along the High Street, and spending the night at the Blue Boar Inn. The next morning, he rode out of Leicester across Bow Bridge toward Market Bosworth, 12 miles to the east of Leicester. On August 22, he was killed in battle at Bosworth while fighting against the army of Henry Tudor.

After Richard III was killed, his body was stripped naked, thrown over the back of a horse, brought back to Leicester, where his corpse was taken through the town to the Newarke (a religious precinct and home to a college of priests), then displayed in the Church of the Annunciation of the Blessed Virgin Mary. Two or three days later, Richard III's body was taken from the church and hastily buried in the choir of a Franciscan friary church known as Greyfriars.

The Greyfriars monastery was dissolved in 1540 and the exact location of Richard's grave was lost; however, analyses of historical records have suggested the approximate location of the friary and its likely situation in relation to the modern urban landscape of the town of Leicester.

These analyses indicated that the likely site of Greyfriars was under a car park belonging to the Social Services at Leicester. Open spaces constituted 17% of the total friary precinct. Because both access to this site and financial resources for research were limited, only approximately 1% of the friary precinct could be excavated. The key decision involved where to utilize scarce resources needed for expensive excavation (typically trenches dug by large hydraulic earth-moving machinery) on the site. Ground-penetrating radar was used to survey all three of the most likely open spaces in the hope that one of these trenches would identify some portion of the friary walls. Excavation of the first two trenches identified only anomalous spreads of rubble and modern service lines. Six hours and 43 minutes into excavation of the third trench, a skeleton was discovered. Only after much additional research did the significance of this find become evident: the site where this skeleton was found meant that the skeleton was likely a person of significance.

The second step involved identifying the known anatomical facts concerning Richard III. Richard was 32 years of age at the time of his death. Contemporary accounts described him as being of slender build, "small of stature," and having "unequal shoulders, the right higher than the left." Because Richard III died in battle, his body should have had forensic evidence of trauma consistent with combat of this era.

A key element to the efficient success of this project involved managing the team from Leicester, who were involved with identifying, organizing, and disseminating historical information obtained from the relevant academic literature. Team members were responsible for interacting with other individuals from Leicester City and the Richard III Society to aid in locating his remains. A further aspect to the project involved management of the intense media interest and public scrutiny of the endeavor.

This presentation will discuss the elements of: planning a major undertaking; identifying, collecting, and assimilating both known facts and information that may not be accurate; operating under a constrained budget;

managing a large multidisciplinary project and the various personnel involved; and controlling the dissemination of information at key times. All of this resulted in a highly successful outcome that exceeded expectations.

Richard III, Multidisciplinary, High Performance

D7 A Comparison of Modern Youth Football Helmet Design Impact Attenuating Performance With Measurements Made in 1992 for the Helmet Design of a Fatally Injured High School Player

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The goal of this presentation is to demonstrate the means for comparing and assessing designs of current youth football helmet impact performance in front, side, and rear locations versus impact results of a high school football helmet design tested decades earlier (i.e., 1992) in relation to the forensic analysis of a fatally injured 17-year-old high school football player.

This presentation will impact the forensic science community by informing attendees that helmet Severity Index (SI) test data certification measures of new and reconditioned youth football helmets should be made available to the public so parents, coaches, school administrators, and forensic researchers can compare SI impact safety performance levels and identify those helmets that provide uniformly optimum safe levels, throughout all regions of a youth helmet, when tested at realistic impact energies consistent with the speed of players. This type of evaluation would be beneficial for improving youth football player safety and would assist in improving helmet designs.

Head injury and concussion risk to high school and youth football players has recently received attention, including interest by the United States Congress, due to research by Omalu and McKee regarding repeat impact brain damage to professional football players diagnosed with Chronic Traumatic Encephalopathy (CTE).[1-4] Testimony by the National Football League (NFL) before Congress in 2009 suggested "optimism" regarding the safety of newer helmets, compared to older helmets used by CTE-injured players.[1] Unfortunately, research on contact-impact safety of current youth football helmet designs reveals similarities to non-uniform, dangerously high head impact loads seen in older helmets, such as those worn by athletes diagnosed with CTE, when compared to 1992 tests on the helmet of a deceased high school player.[5-7] Energy-absorbing padding in the frontal area of a forensically examined football helmet consisted of a dual-density elastomeric foam (Fig. 1.) Linear contact-impact testing used a certified National Operating Committee on Standards for Athletic Equipment (NOCSAE) drop-impact fixture. Repeat impacts were conducted at 5.5m/s (6.6 second 40-yard dash) with energy of 108 Joules (J). Figure 1 illustrates 1992 helmet compliance with NOCSAE head SI limit of 1,200, but the frontal helmet padding provided the least amount of protection in comparison to the side and rear. More importantly, the SI frontal measures were at or above current National Highway Traffic Safety Administration (NHTSA) Head Injury Criteria (HIC) Injury Reference Value (IRV) of 700 (denoting approximately 7% of the United States population would be at risk of severe head injury with this HIC value).[8] Data also demonstrated a wide disparity of SI measures from front-, side-, and rear-impact locations by approximately 30%. A safe design should not have such wide disparity in injury measures for a given impact energy, but should provide low SI values uniformly at all helmet impact locations.

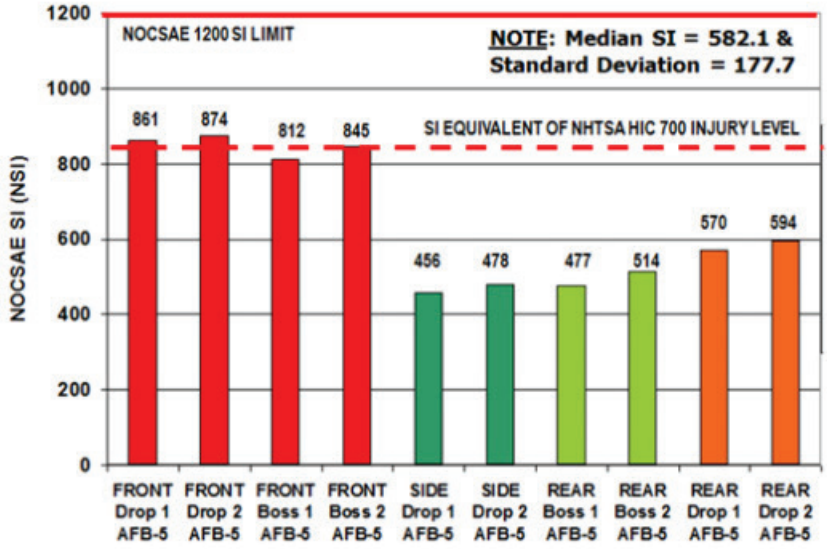

Fig-1. NOCSAE Severity Index Data from 1992 Helmet Design Used by a Fatally Injured High School Player

Comparison of the 1992 NOCSAE SI test data measures for the helmet (Fig. 1) of the fatally injured high school player with recent NOCSAE SI test data of a 2014 Youth Helmet design (Fig. 2) demonstrate that both helmet designs have impact regions exceeding NHTSA HIC 700 IRVs. The danger region of the helmet shown (Fig. 1) was the frontal area, the injury location of the high school player; the danger region of the helmet show in Fig. 2 was the rear (occipital) area. In both cases, the high SI measures deal with response from elastomeric foam impact attenuating "Energy Absorbing" (EA) materials. A positive, or "optimistic," aspect of the 2014 Youth helmet was the incorporation of a newer EA "Thermoplastic-Urethane" (TPU) waffle-pattern material in the front of that helmet (blue frontal material in helmet on left of Fig. 2). While this newer TPU material provides some improvement for direct contact-impact, recent studies by Saczalski et al. indicate head injury risk from rotational accelerations remains a problem with these materials, and that the rotationally induced danger and injury measures are still not part of the NOCSAE helmet safety performance criteria injury evaluation.

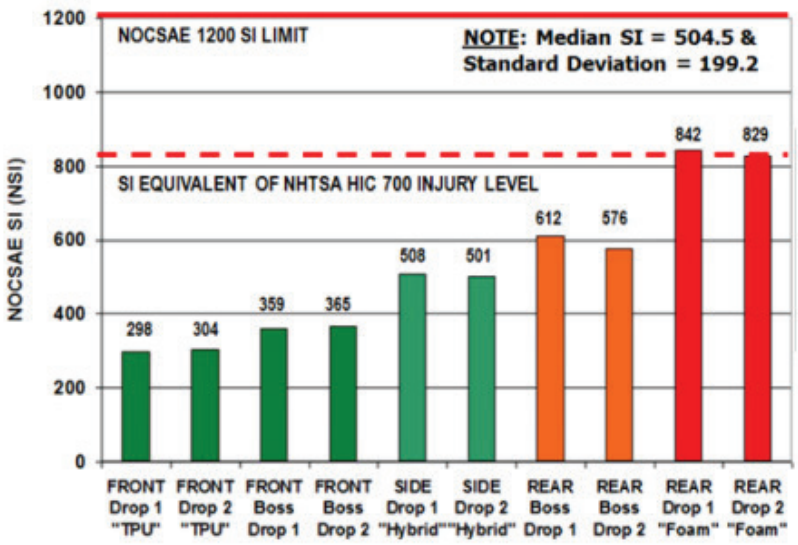

Fig-2. NOCSAE Severity Index Data from 2014 Peewee Youth Helmet Design Approved for Peewee Play

Summary: "Optimism" regarding the efficacy of football helmets to attenuate concussive level impact forces should, unfortunately, be tempered by the reality of recent research, rather than relying solely upon the assurances of NOCSAE and football helmet manufacturers using outdated safety certification testing and injury reference levels.[1,5,6] Ultimately, the NFL and the sport of football need a more rigorous engineering approach to design safer helmets, such as "multi-variable" methods enabling performance assessment for linear impact and rotational injury

measures, plus ambient, high-humidity, and temperature conditions.

Impact: NOCSAE helmet test data should be made readily available so the public can compare SI impact performance levels and identify helmets providing uniformly optimum safety in all regions, when tested at realistic impact energies consistent with actual player speeds. This would benefit football player safety and facilitate improvements in helmet design or materials.

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Youth Football Helmets, Football Helmet Testing, Concussion Risk

D8 Visibility and Lighting Aspects of Perceiving a Liquid Slip Hazard on a Walkway Surface: A Case Study

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The goal of this presentation is to discuss how visibility and lighting influence human ability to visually perceive a liquid slip hazard on a walkway surface.

This presentation will impact the forensic science community by illustrating how conspicuity, expectancy, lighting, and measurable floor surface properties, such as gloss, influence the probability that an individual will visually perceive a liquid slip hazard in time to avoid a slip-and-fall accident.

A common cause of slip-and-fall accidents is liquid on floors. Accident avoidance in such cases, in the absence of prior warning, requires that the individual is able to visually perceive and avoid the hazard before stepping on it. At a minimum, the visual detection of a liquid hazard on the walkway requires that it be either more or less luminous than its immediate background and with sufficient contrast to be distinguishable from its background; however, it will be shown that even though a hazard may be in plain sight and visible, to be seen in sufficient time by all individuals, it must also be conspicuous relative to its surroundings. An object is *conspicuous* if it attracts or tends to attract the attention of an observer so as to be readily discovered. Conversely, an object is *inconspicuous* if it is not readily noticeable or discoverable. The term *conspicuity* refers to the capacity of an object to stand out in relation to its background so as to be readily discovered by vision. Liquid hazards that are more conspicuous are going to be perceived more quickly and therefore at greater distances than hazards that are less conspicuous. At the extremes, hazards that are highly conspicuous should be seen at the greatest possible distances and hazards that are perfectly camouflaged will not be seen at all. For example, colored liquids are generally more conspicuous than liquids that are clear.

It will be shown that the type and orientation of overhead lighting are significant factors affecting the conspicuity of liquids. In particular, some clear liquid hazards can only be seen as a reflection of overhead lighting, and then only when the overhead lighting is in an optimum orientation relative to the individual who will benefit from perceiving the hazard.

A case study involving a slip-and-fall accident at a retail shopping facility exemplifies the points made. At issue was whether the plaintiff should have been able to visually perceive a pool of clear detergent on what defense documents described as a “wet-look” commercial vinyl tile floor. It will be shown that a clear liquid hazard on a “wet look” high-gloss floor would be visually camouflaged under the lighting conditions that are typical in a large retail facility. Additionally, an individual exercising reasonable care regarding where they are walking would not visually perceive an unexpected clear liquid hazard on the surface of a “wet-look” high-gloss floor.

Numerical characterization of wet-look high-gloss floors will be discussed. “Gloss” is quantified in Gloss Units (GU) using an instrument called a glossmeter. Gloss quantifies the shininess of a surface; waxed and polished smooth tile floors have high gloss and unwaxed non-polished floors have low gloss. The GU for the subject “wet-look” dry floor was measured at 75GU-80GU. When wet with clear detergent, it measured 69GU-72GU. The unfinished tile measured 11GU and a typical commercial ceramic tile measured 2.2GU.

It will become obvious that some floors and floor finishes are better suited for exposing liquid slipping hazards by rendering them more conspicuous. The analogous case of ice on outdoor pavement will also be discussed.

Visibility, Lighting, Slip-and-Fall

D9 A Forensic Engineering Review of Vision in Automobile Crashes: Part I

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After attending this presentation, attendees will better understand the general complexity of the human visual system and how fundamental characteristics affect human performance under common conditions, often with fatal results.

This presentation will impact the forensic science community by assisting attendees in assessing future cases they may encounter and by offering guidance in distinguishing confounding factors in visual system investigations.

Study of the human visual system and how its fundamental characteristics affect performance under common conditions is an element of Human Factors Engineering. It has a role in defining the design aspects of the human mobile environment as well as deficiencies due to human visual system limitations. Although there is a dominant theme in forensic engineering dealing with the design of products for human use, and associated cost and performance issues, there is also a subset that is less obvious — the overall mobile environment.

Humans have altered their mobile environment, one that was formerly dominated by low-speed walking and running performed for thousands of years, to (over a few generations) one that now also includes driving, skiing, and flying, all accompanying high-speed permutations. Humans have also created other fast-paced environments in which we rely on our visual system to function; however, the human visual system is simply not designed or evolved for optimal performance in such high-speed and high-complexity regimes. Currently, artificial intelligence systems are being tasked to intervene, presently with mixed results, and eventually take over substantial parts of these visual and cognitive functions.

In nearly every investigation involving a visual perception mishap, the following key questions arise: (1) What was there to be seen?; (2) Why was this not seen?; and, (3) How did this contribute (if at all) to the mishap? The short answer is that the human visual system is extremely complex and the human visual system is not a camera!

There are literally tens of thousands of design standards for automobiles, roads, controls, visibility, materials, lights, luminaires, and all their subsystems. Despite these design standards, hundreds of thousands of accidents, crashes, and mishaps occur each year across the globe.

This brief overview of multiple forensic engineering visibility cases will provide different explanations regarding why the scenes and situations were not sufficiently defined by the participants to avoid mishaps. This sets the basis for the matrix of causative factors to be discussed in Part II of this presentation.

This presentation will touch on fundamental concepts, including Gibson's visual world, the visual field, optical projection to the retinal structure, foveal vision, peripheral vision, processing by specialized receptor sensors, translation into bio-electrical signals, feed-forward to the visual cortex, processing and integration by neural-brain system, short-term memory, further cognitive processing and perceptual responses transmitted to specific muscle response groups, and feedback to complete a control loop.

Additionally, the discussion will include (as time permits) inclusion of the following elements as part of the eight-case analysis: perception-response time, attention and limited attention capability, illusions and perceptual traps, scanning of the visual world, human-designed visual aids, mirrors, devices, geometry, contrast, conspicuity, luminance, illumination, pattern recognition, driving search patterns, visual flow, focus of expansion, eye tracking, saccades, scans, fixations, blinking, combining images, contrast sensitivity, line of sight, foveal vision, peripheral vision, perception of color, rods and cones, human error, risk assessment, "inattentional blindness," driver expectancy, time to collision, looming, and alerted and non-alerted observers. These topics will assist attendees in assessing future cases they may encounter and offer guidance in distinguishing the confounding factors in visual system investigations.

Human Factors, Visual World, Attention

D10 A Forensic Engineering Review of Vision in Automobile Crashes: Part II

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After attending this presentation, attendees will be aware of the general complexity of the human visual system and how fundamental characteristics of this system affect human performance under common conditions, often with fatal results. This second of a two-part presentation examines specific instances of collisions and crashes and incorporates the principles and elements outlined in Part I.

This presentation will impact the forensic science community by helping attendees identify specific characteristics attending the cause of these vision-related incidents and the interaction of potentially causative factors. The ability of attendees to investigate other incidents involving human visual systems will be aided by attending this presentation.

The presentation of these cases will include discussion of the matrix of causative factors that may be reasonably considered in the analysis.

The cases include: (1) the State vs. JT involving an auto pedestrian fatal collision. A driver hit two elderly pedestrians in a crosswalk, despite the fact that he should have clearly seen these two pedestrians. In this case, a medical condition led to a scene-scanning behavior that allowed adequate driving behavior, except when faced with this critical situation; (2) Estate of L vs. CPM, a pedestrian vs. a backing asphalt truck resulting in a fatal collision. The driver should have seen the pedestrian, but did not. Elements to be considered in this case include mirror system design, signaling, night environment, illumination, and attention and illumination issues; (3) B vs. S, a high-speed skier who impacted another skier in a serious injury collision. The skier should have seen the impending cross traffic. Elements include perception reaction time, gradients, visual field, and foveal vision; (4) P vs. CJ, a young female who was assaulted in a complex playground with hidden playground features. The argument was made that the child should have been seen. Elements include line of sight, contrast, conspicuity, masking, and position of viewer; (5) B vs. ICG, an underground mine scoop was moving in a mine passageway and was involved in a fatal collision with another scoop. The impacted scoop was supposedly visible, yet an analysis of the line of sight, contrast, visual field, attention, and perception reaction demonstrated essentially blind driving. (6) H vs. IT, a pedestrian walked into a right-turning truck in a crosswalk collision. The pedestrian should have seen the large moving truck and trailer. Visibility issues were assessed, as well as distraction, and inattentional blindness-related issues. (7) H vs. R, a motorcycle rider on an unfamiliar road approached a modified T intersection and went straight, resulting in a crash. It was deemed that he should have seen the intersection, yet an analysis of the signs, scene, and visibility factors revealed a perceptual illusion (a visual trap); (8) K vs. ST, a pickup struck the back of a semi-truck on a clear road, resulting in multiple fatalities. An analysis considered the looming effect, attention, foveal vision, and perception reaction time as well as other motorists that avoided the hazard.

In these and other similar cases, no single factor fully explains the visual system or cause of mishap nor can the investigator place himself/herself in the eyes of any of the participants or witnesses involved. The matrix of factors presented will assist in developing potential explanations. Furthermore, cameras, whatever their modern features, cannot capture the events as they happened from the involved individual's perceptions. They can only memorialize the observations of the forensic engineer or investigator, who must understand the visual system to render opinions to assist the court.

Attendees will benefit from the identification of specific dominant characteristics of the proximate causes of the incidents and the interaction of other factors. This information may assist them in investigating other visual system cases.

Inattentional Blindness, Distraction, Line of Sight

D11 A Computer Simulation for Use in Impact Force Evaluations: Studies on the Correlation Between Impact Force and Finger Bone Fracture

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After attending this presentation, attendees will better understand the relationship between impact force and finger bone fracture to establish a safety assessment index. In addition, attendees will learn the potential use of the Finite Element Method (FEM) for evaluating human finger bone fracture risk.

This presentation will impact the forensic science community by providing an example of computer simulation for human body injury assessment and by illustrating how safety assessment can be used to prevent accidents from a variety of devices.

Establishing relationships between external force and human injury is needed to estimate intent and certify negligence in various incidents or accidents. Recent research revealed that there exists a relationship between bone density and certain types of fracture risk. Techniques that evaluate fracture risk by individual bone shape and bone density are in the process of being developed and some are being used in medical care in Japan. Non-injurious evaluation of individual bone fracture risk is important, but difficult to achieve given variations in individual physical characteristics. This is particularly important when considering finger and hand injuries involving interactions with power windows in motor vehicles. Accurate estimates regarding the relationships between external force magnitude and finger injury risk are needed, but few studies address even the basic biomechanics of this issue due to the low risk to human life and perceived relative unimportance.

This study used a pig tail model of human finger bones during static compression and dynamic drop tests to determine the relationships between external force and injury level. Varying edge shapes were used to consider this covariate in the injury risk determinations. Furthermore, Finite Element Methods (FEM) were also employed to estimate bone fracture risk so relationships between human fingers and pig tail bones could be developed.

The results demonstrated that actual fracture loads (approximately 1,100N to 1,400N) varied significantly with impact velocity, contacting edge shape, and the amount of soft tissue present. FEM simulation demonstrated estimated finger failure loads of approximately 870N to 980N. The role of these finger fracture parameters was confirmed in addition to the usefulness of computer simulation as an effective tool for the evaluation of finger bone fracture risk.

Human Injury, FEM Analysis, Finger Fracture

D12 Basic Research on the Development of Crash Dummy Skin for Risk Evaluation of Fracture and Skin Injury

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After attending this presentation, attendees will better understand techniques for evaluating the dynamic mechanical properties of soft tissue.

This presentation will impact the forensic science community by providing a physical model of personal injury evaluation that accounts for the dynamic mechanical properties of soft biological tissue.

It is known that the presence of soft biological tissue such as skin and muscle has a large effect on the presence or absence of bone fracture resulting from a dynamic load such as impact from a blunt object; however, this effect has not been quantified since data quantifying the dynamic mechanical properties of soft tissue is unavailable. In addition, the dynamic properties of soft tissue are essential to evaluate damage due to external forces. The Hybrid III 50th Percentile Anthropometric Test Dummy (ATD) is commonly used to measure human injury in vehicular accidents. This is used not only in automotive frontal collision tests, but also in human body damage evaluations that were caused by impacts, drops, falls, vibration, etc.; however, the mechanical properties of the simulated soft biological tissue in this model have not been reproduced with high accuracy. The next-generation dummy has a much higher human being enhancement degree than existing dummies, and the soft biological tissue will be incorporated; therefore, substitute materials reproducing a soft biological tissue must be examined. This study seeks to quantify impact properties and dynamic viscoelastic properties for the development of alternative substitute materials which can reproduce the dynamic mechanical properties of soft biological tissue.

This study used urethane gel as a physical material of ATD skin. The surface of the urethane gel was covered in a simulated epidermis. The dynamic viscoelasticity was measured using a rheometer. Two-axis loading of this simulated skin revealed good correlation with the dynamic viscoelasticity measured from the human skin of 20-year-old men.

Dummy Skin, Dynamic Viscoelasticity, Human Body Damage Evaluation

D13 Multivariate Analysis Applied to Sawed-Off Shotgun Pellet Dispersion

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After attending this presentation, attendees will understand how statistics can model the dispersion of birdshot fired from a 12-gauge sawed-off shotgun and will also learn that the spread of lead birdshot depends only slightly on the barrel length and mainly on the firing range.

This presentation will impact the forensic science community by providing attendees with an appreciation of the fact that, in contrast to previously published works, the dispersion of lead birdshot is best described by a second-order polynomial expression rather than a linear relationship.[1-3]

Statistical techniques have been used to establish a mathematical relationship enabling estimation of the distance from which a shotgun was fired with buckshot ammunition.[4] In the present study, pellet dispersion accompanying the discharge of lead birdshot was analyzed. Shotguns are easy to obtain in several countries as they are considered hunting devices, including sport hunting or pest elimination such as hare, mice, or mink plagues occurring in the fields of southern Chile. Due to their low cost and capability to inflict injury, shotguns (especially those with substantially shortened barrels, also known as "Recortadas" in Chile) are sometimes the preferred weapons for criminal activities. A long-held belief was that pellet dispersion varied greatly with barrel length, hence the motivation for the present study. The objective of this study was to analyze the effects of barrel length and pellet size on pellet dispersion at several distances using two 12-gauge FAMAE™ shotguns. A two-level factorial design was used to analyze the effect of pellet size (5cm-7.5cm), barrel length (33cm-72cm), and the firing distance on pellet dispersion (evaluated as the area of the figure enclosing all leads on the target, the square root of such area, and finally the Matto and Nabar effective radius method, which requires the measurements of x and y coordinates for every single impact on the target).[1] The results demonstrated that pellet size has a dominant influence on pellet dispersion, but barrel length has a nearly negligible influence on pellet dispersion. The effective radius was the best parameter, reaching R2 values of 99.9% vs. 89.x% of the area of the minimum figure around the birdshots and 99.x% for the root of the area.

The second stage involved the development of a response surface methodology to obtain a graphical relation between barrel length and firing range, assuming a second order polynomial as response evaluated for the same functions described above for the pellet dispersion. The chosen method for the experimental design was a Box-Behnken model covering ranges of 33cm, 45cm, and 72cm for the length of the shotgun barrel and 5m, 10m, and 15m for the firing range. After conducting the experiments using only Nº 5 pellets (the most commonly used in Chile), a second degree equation describing lead pattern as a function of the two variables studied was obtained, and an Analysis of Variance (ANOVA) test demonstrated a very good fitness for the effective radius, with R2 values of 99.7% of agreement. Results again exhibited that pellet dispersion is not directly influenced by the length of the weapon's barrel but depends strongly on the distance from muzzle to target. According to these results, the senior method of Matoo and Nabar revealed this is a very good way to estimate the firing range for 12-gauge shotguns, but by using a parabolic curve instead of the linear approach initially proposed. Surprisingly, the square root of the area enclosing the impacts is a very easy method to calculate the approach to the distance of the firing distance; meanwhile, the ANOVA test showed that it is a statistically acceptable approach to the firing range.

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Multivariate Analysis, ANOVA, Pellet Dispersion

D14 Wildlife Forensic Examinations in the Largest Dam Collapse in the History of World Mining

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After attending this presentation, attendees will better understand the important aspects of wildlife forensics as applied to environmental disasters.

This presentation will impact the forensic science community by informing attendees about modern techniques and integrated approaches in wildlife and environmental forensic examinations.

The goal of this presentation is to describe the assessment and extension of the environmental damage caused by a huge mining dam break that occurred on November 5, 2015, in Minas Gerais, the state with the highest concentration of industrial mines in Brazil.

The dam, owned by two of the biggest mining enterprises in the world, has been considered the biggest disaster of its kind in mining history, given the volume of waste released (50-60 million cubic meters of tailings extending along an affected area of 600km and costing an estimated $ 5.2 billion (United States dollars) in environmental damage.

Since Brazilian law considers water pollution an environmental crime, the Brazilian Federal Police established an investigation into the causes and consequences of this disaster. From November 2015 to March 2016, a multidisciplinary team of environmental forensic experts made technical inspections along 600km of the Doce River to determine the damage on local wildlife caused by the waste discharge. Furthermore, the experts examined more than 70 technical reports written by government agencies and consultancy companies in charge of monitoring the effects of the catastrophe. Moreover, the Federal Police forensic service established technical partnerships for the exchange of information with the Brazilian federal environmental agency (Instituto Brasileiro do Meio Ambiente E Dos Recursos Naturais Renováveis (IBAMA)), research centers such as the Continental Fish Center of the Brazilian Institute for Biodiversity Conservation (ICMBio), and universities.

The forensic investigation determined the environmental impact on crustaceans, amphibians, reptiles, mammals, birds, invertebrates, and domestic animals. At least 29,300 fish carcasses (more than 13.9 tons) were found and collected at more than 60 sampling points along the river. The disaster occurred just before spawning, killing a large number of fish that went up the river to reproduce. Thus, the forensic analysis also estimated the effects of the pollution on the depletion of fish stocks for the following years.

In addition, more than 1,500 water and sediment samples were submitted for ecotoxicological tests during the first four months following the disaster. This set of data was analyzed to monitor acute and chronic damage caused by the tailings plume to the aquatic fauna.

Some of the species that suffered acute and chronic effects are officially classified as endangered or critically endangered, such as the endemic marine catfishes *Genidens genidens* and *Potamarius grandoculis*, the blue land crab (*Cardisoma guanhumi*), and the sea turtles *Dermochelys coriacea* (leatherback sea turtle) and *Caretta caretta* (loggerhead sea turtle). Forensic veterinarians and biologists analyzed approximately 130 records of animal health care and performed dozens of necropsies and histopathological analyses.

The current forensic examinations were crucial for proving the environmental crime caused by the dam break, showing the deleterious effects of pollution on the Doce River wildlife. In addition to registering and interpreting the evidence to press criminal charges, the forensic reports warned about the importance of continuous monitoring of chronic effects in aquatic, terrestrial, and migratory animals.

Dam Break, Wildlife, Environmental Forensic

D15 Data Integrity in Forensic Science and Engineering

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The goal of this presentation is to increase awareness of the existence of data fabrication or falsification perpetrated by a small subset of scientists and engineers and to help the ethical forensic investigator develop robust analyses and conclusions that account for this unfortunate reality.

Scientists and engineers have historically been among the most trusted and respected professionals in society, but increasing reports of data fraud generate cause for alarm. This presentation will impact the forensic science community by raising awareness of this unethical behavior and will review the ways in which data is fabricated or falsified.

The Internal Revenue Service is an effective supervisory agency ensuring honest and accurate self-reporting of income upon which taxation is based. No such organization exists for the data self-reported by scientists or engineers. None has been needed because these professionals have historically told only the truth, and for this reason society holds scientists and engineers in high esteem and implicitly trusts the data they report.

Unfortunately, a small subset of scientists and engineers has deliberately invented or altered data for ill purposes. This behavior is particularly troublesome in matters concerning forensic investigations in which wrongful conviction (or failure to correctly convict) in a criminal case or an inappropriate settlement in a civil case may result. Regardless of the reason, any attempt to alter the reality of an experiment or observation is wrong and unethical. Such alterations take many forms and fall chiefly under the subheadings of fabrication and falsification.

Fabrication refers to ill-intentioned "invention" of data where none exists. This blatant act occurs by: (1) "dry-labbing" — reporting the results of calculations or experiments that never occurred; (2) adding "invented" data to actual experiments (so that mean values are altered) — this can also include physically or digitally adding objects to crime scene images (where no such objects existed in reality) or the opposite — removing or materially repositioning such objects; or, (3) reporting actual data, but from an inflated sample size (thereby misrepresenting the population estimated by a falsely reported sample size).

Falsification, while equally wrong and unethical, is subtler and has more manifestations. Falsification refers to taking actual experimental data and modifying it for ill purposes. This includes, for example: (1) undocumented measurement system "recalibration" to achieve a desired outcome; (2) biased (and undocumented) study subject selection; (3) unjustified data rejection; (4) exaggerated experimental measurement precision; (5) wrongfully identifying results as "typical" when, in fact, the results were the best ever observed; and, (6) confusing sample or subject identification numbers to obscure tracing.

It is important to add that discussions, including disagreements, regarding data interpretation are healthy and acceptable in ethical forensic science and engineering; however, the data itself should never be in question.

In summary, data fabrication and falsification are despicable acts diametrically opposed to the ethics of moral people and violate the trust society places in scientists and engineers. Such acts waste time and money, can endanger human life, and besmirch the vast majority of forensic scientists and engineers who behave according to high ethical standards. The panel discussion concluding this session will explore the means by which forensic scientists and engineers can be alert to acts of fabrication or falsification by others and thereby improve the credibility of their reports and the robustness of their conclusions.

Data Fabrication, Data Falsification, Data Interpretation

D16 The Deaths of Children in Moderate-Speed, Rear-End Impacts — Are They Unfortunate Accidents or Manslaughter? Discussions on the Ethical and the Moral Obligations of Manufacturers and Government to the Consumer

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The goal of this presentation is to demonstrate safety defects in vehicles and safer alternative designs. The effects of low costs resulting in predictable seat and belt failure in foreseeable survivable crashes and the effects on adult and child vehicle occupants are presented in tests and case studies.

This presentation will impact the forensic science community by informing attendees regarding the predictable dangers of unsafe seats, slackened belts, and intrusion of rear seat survival space, as well as by presenting associated forensic evidence.

The *Code of Ethics for Engineers*, published by the National Society of Professional Engineers, states in the first section entitled Fundamental Canons that "Engineers, in the fulfillment of their professional duties, shall: (1) Hold paramount the safety, health, and welfare of the public; (2) Perform services only in the areas of their competence; (3) Issue public statements only in an objective and truthful manner; (4) Act for each employer or client as faithful agents or trustees; (5) Avoid deceptive acts; and (6) Conduct themselves honorably, responsibly, ethically and lawfully so as to enhance the honor, reputation and usefulness of the profession."

Is there any valid reason why the above Fundamental Canons should not be adhered to by the manufacturers of products, like automobiles, that are intended for use by trusting consumers? Likewise, is there any valid reason why government agents, who are assigned to regulate and insure the safety of such products so that they provide the utmost level of protection and safety to the trusting citizens who purchase them and use such products, should not also follow similar Fundamental Canons?

Even if unwritten, the ethical and moral practices following implicit adherence to such Fundamental Canons by manufacturers and the governing regulators should be obvious as far as understanding the importance of the obligations needed to provide the utmost protection and care for its trusting citizens, in particular the most innocent citizens such as the infants and children, who are often exposed to the dangers of defectively produced products that are added to the stream of commerce.

Unfortunately, there are several real-world examples of failure of manufacturers and government regulators to follow such Ethical Canons. This discussion will present one such example entitled, "The Death of 8-Month-Old JF: A Properly Seated and Restrained Infant in a Middle Row Who Was Fatally Injured in a Moderate-Speed Rear-Impact Crash to the Family Minivan, While All Other Occupants, Including the Third-Row Seated Occupants Located Closest to the Impact Escaped Without Serious Injury."

In 1954, United States Air Force (USAF) tests proved that human tolerance to frontal impact was 46G. In 1958, a human volunteer in a crashworthy seat withstood 83G in a rear-impact test without loss of consciousness or significant injury. Static and dynamic testing from the 1960s to the present by researchers have consistently demonstrated the clear need for strong, crashworthy seats capable of absorbing predictable occupant crash loads. Weak, collapsing seats allowed by meaningless "safety standards" continue to defeat seat belts and demonstrate predictable life-threatening consequences to front and rear seat vehicle occupants in otherwise readily survivable crash tests and real-world collisions.

Based on the foregoing human tolerance testing, Federal Motor Vehicle Safety Standard (FMVSS) 210 requires vehicle seat belts to withstand 6,000 pounds static frontal loading. FMVSS 207 ignores the presence of a human in the seat and only requires 275 pounds of static load capacity, much less than the American Society for Testing and Materials (ASTM) safety standard for office chairs. There are no FMVSS regarding occupant protection for rear impacts or rollovers. This is why vehicle seat and seat belt failures predictably occur in minor rear crashes, resulting in severe injury and death to adults and rear-seated children. Yet automakers admit that stronger, crashworthy seats would cost "around a dollar."

Since 1996, automakers and the National Highway Traffic Safety Administration (NHTSA) advised placing children in rear seats to avoid airbag hazards, while ignoring predictable seat, belt, and intrusion failures. Research published in 1997 by the Insurance Institute for Highway Safety proved that children are 61% more likely to be severely injured or killed in rear impacts than in other collisions. Despite more than 50 years of crash test research and petitions proving the dangers of weak vehicle seats that defeat safety belts, no action has been taken to prevent these hazards.

Seat Failure, Child Fatality, Rear Impact

D17 Data Integrity in Forensic Engineering — Spoliation and Loss of Evidence

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After attending this presentation, attendees will be aware of the various practices used to further the legal success or outcome of one party at the expense of another.

This presentation will impact the forensic science community by exposing examples of the trickery practiced by unscrupulous parties in civil and criminal litigation involving willful or careless loss, destruction, or alteration of evidence.

Case 1: Loss of evidence — an electrician was electrocuted in his own workshop while testing a motor that he had rewound. He connected the motor to the test terminals and was immediately electrocuted at 480 volts. The two 50-ampere test clips he used had a protective insulating cover (boot) designed to provide insulation and protection from electrocution when handling. The attorney for the deceased claimed that the insulating boot was defective (i.e., had unspecified cuts or holes which permitted contact with the spring-loaded test clips. The plaintiff filed a product liability complaint against the manufacturer of the boot. The expert witness, upon being retained and asked to examine the boot, clips, and cable, was informed that this evidence was "lost" by the deceased's attorney. The only evidence available was autopsy photos of the deceased's hand. This was sufficient to generate a favorable outcome for the defense.

Case 2: Spoliation and failure to collect evidence — downed power line cases that involve electrocution or electrical injury require that utility companies that own the conductor that came down often do not collect the downed conductor or identify it properly. Most large utility companies have incident investigation procedures that call for such evidence to be retained. Often the conductor is discarded, "misplaced," or re-used by splicing (and thus non-identifiable). In many jurisdictions, the State Public Utilities Commission requires the utility companies to thoroughly investigate and record all incidents involving public contact cases. The utility investigators sometimes willfully take no or deficient notes or photos, even though their investigation procedures require these actions. Some conductors that were spliced and re-used, if they can be identified, must be removed to be examined inch by inch at the cost of the parties involved. Sometimes, such identification and removal cannot be conducted without an order from a judge.

Spoliation, Loss of Evidence, Electrocution

D18 Scientific Data Integrity and Engineering Ethics: Test Manipulation, Data Alteration, Elimination of Safety Regulations, and the Theft of Scientific Records

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The goals of this presentation are to alert forensic professionals in the ways that testing, data, and safety standards have been manipulated and the manner in which multiple records of taxpayer-supported research have been intentionally suppressed, stolen, or destroyed. This loss of critical information negatively affects public safety and efforts to improve technology. This loss also allows defective, dangerous products to continue to be produced, placing the public at risk. None of this could happen if those involved followed the *Code of Ethics for Engineers*.

This presentation will impact the forensic science community by increasing awareness of how automakers, government officials, and others who are responsible for generating and maintaining objective and reliable scientific information have failed in many instances to properly carry out this responsibility in an ethical manner.

Since the 1940s, countless research projects were performed to advance scientific and engineering knowledge affecting the performance and safety of transportation vehicles for personal, occupational, or recreational pursuits. The present era, especially since the 1960s, showed the advent of many performance and safety regulations and related compliance testing. This includes thousands of scientific tests performed by manufacturers and independent researchers working for government agencies such as the Department Of Transportation (DOT), Department Of Defense (DOD), National Aeronautics and Space Administration (NASA), Consumer Product Safety Commission (CPSC), Occupational Safety and Health Administration (OSHA), the insurance industry, National Operating Committee on Standards for Athletic Equipment (NOCSAE), and others. These include functional, durability, and static tests on consumer goods, ground vehicles, aircraft and spacecraft components and structures, braking/handling/rollover tests, full-scale crash and sled tests of production and prototype vehicles, and full-scale dynamic flight tests of production and experimental aircraft and spacecraft.

The First Canon of Engineering and Scientific Ethics states: One's first duty is to the public. The vast majority of the foregoing test work was conducted in an ethical and scientifically reliable manner, resulting in improvements in the performance and safety of ground vehicles, aircraft, spacecraft, and consumer goods. Unfortunately, during the course of this work, there have also been numerous examples of engineering ethics violations. This has typically occurred when sufficient money and political influence affect interpretation of the often huge gaps between "state of the art," a "reasonably safe, reliable design," and what is "legal to sell" under minimal, often outdated, safety regulations. This conflict has affected forensic investigations and the safety and welfare of the public. These ethical violations and poor engineering judgements have occurred despite civil and criminal laws intended to prevent dangerous products from being sold to unsuspecting consumers. Such violations have also delayed and otherwise affected the safety interpretation, rule-making, and enforcement process. Despite *Daubert* and other gatekeeping rules, unethical "junk science" occurs with regularity in courtrooms.

Examples include: (1) suppression/destruction/sale of scientific records and/or experimental prototypes from taxpayer-funded research, which were supposed to be returned to government safekeeping; (2) manipulation of test conditions, alteration or fabrication of test data (e.g., conducting a "proof" rather than a "test"; (3) diminishing or eliminating safety standards, with no possible public safety benefit, ignoring the National Transportation Safety Board and others opposing such changes; (4) stopping research on improving regulations that the responsible safety agency had admitted were "flawed and inadequate"; (5) conflict among government agencies (e.g., one DOT agency declaring a safety device unreliable and unsafe with another finding "no defects," despite multiple failures in crash testing and field investigations; and, (6) the theft or destruction of original taxpayer-funded test records or classified information from otherwise entrusted archives with subsequent cover-ups by institutions and individuals charged with upholding public safety.

The foregoing was witnessed or learned of during work on government safety research and defect investigations, private sector forensic investigations, and while performing testing with manufacturers, independent test laboratories, and government test facilities. One independent researcher physically searched government archives for extensive

testing he had performed for that agency, relevant to critical safety issues affecting millions of vehicles; he was told "no such testing exists." If the author of unclassified test reports cannot gain access to them, how would another researcher, or ordinary citizens, possibly learn of their existence, let alone the implications for current and future safety issues?

Data Integrity, Engineering Ethics, Test Manipulation

D19 Checking Canine and Handler Credentials Entering a Crime Scene and Evidence Contamination

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After attending this presentation, attendees will understand the techniques of checking cadaver canine credentials before entering a crime scene, what the cadaver canine credentials are, and how they affect the case. Cadaver canine handlers receive national certification by taking courses that are accepted by the National Incident Management System (NIMS). Attendees of this presentation will discover what happens when a cadaver canine personnel spoils the scene by intentionally dropping physical evidence onto the scene that is not related to the case, only to have it turned over to police personnel as physical evidence.

This presentation will impact the forensic science community by demonstrating the importance of relying on the real physical evidence at hand and by illustrating how to treat unrelated spoiled evidence brought into the scene by someone else.

This case involves a single motor vehicle accident. The vehicle traveled off the right side of roadway, missed the guardrail, went down a ravine, and struck the embankment. The creek was full of water and no body was recovered with the vehicle. A cadaver canine with handler was called into the scene to check for the driver. The cadaver canine personnel "planted" a human toe on the scene when no one was looking. The canine alerted the handler to the human toe. The toe was submitted as evidence to police personnel. Eight weeks later, the body surfaced approximately one mile down the river. This body had work boots on and all toes were accounted for by the medical examiner. Two medical examiners were working on this case and neither would sign the death certificate due to foul play, not knowing whose toe was in police possession. It was difficult to confirm if the body was correctly identified as the driver of the vehicle, because the body was decomposed. As the driver in question had only visited the dentist twice in the driver's life, dental records were unable to confirm the driver's identity. Cross-parenting DNA was then utilized to determine the identity of the driver. The next step was to determine whose toe the police had and whether it pertained to the crash.

The investigator examined the body and the interior of the vehicle for cross-matching evidence to confirm the driver's movement inside the vehicle. Also, the investigator needed to determine how the body exited the vehicle after impact with the embankment. The vehicle had a clothing imprint transfer to the "A" pillar. This "A" pillar was cut from the vehicle and shown to the medical examiners to assist in the investigation. The investigation turned to the canine cadaver handler and a search warrant was issued for the canine handler's residence. The toe was not a part of the collision. The canine cadaver handler was charged with planting physical evidence on several crime scenes in many different states.

When evidence is found, it is accepted as fact without question by the forensic community. Once accepted, it is part of the investigation and submitted to the pertinent parties. In this case, the evidence was accepted and ruled out. In general, tampering with evidence leads to disciplinary action. The body that was found with ten toes was a crucial finding, leading to the discovery of evidence planting t the scene. Accepting the toe as physical evidence made it difficult to complete what should have been an easy investigation of a single-death motor vehicle crash.

Canine cadaver handlers have certifications for both the canine and the handler. These certifications should be checked upon entry to the scene. If the scene involves water, there are water Human Remains Detection (HRD) certifications available for both the handler and the canine. Canines have a special enhanced ability to recover human remains when trained to do so. Canines do an outstanding job in covering large amounts of area in detecting human remains, with the canine and the handler generally working together as a team. The certifications are not transferable between handlers.

Cadaver Canine Credentials, Scene Spoilation, Trusting Scene's Real Evidence

D20 May the Best Liar Win! Lies, Fraud, Intimidation, Deception, Bias, Error, and Incompetence at Work Within Our Courts

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After attending this presentation, attendees will better appreciate what can go wrong with scientific analyses and testimony, what enabling mechanisms are at work, what motivates practitioners to indulge in unethical and/or illegal behavior, and what strategies may be employed to avoid or minimize such problems.

This presentation will impact the forensic science community by alerting attendees to the diversity and extent of forensic expert intimidation, bias, and dishonesty. The forensic science community will be motivated to consider more effective alternatives to the current purely adversarial, process-governed system.

Expert forensic testimony is so widespread that it now often forms the backbone of criminal cases, and almost always plays a critical role in civil litigation. Some disciplines rely on conclusions derived primarily from hard data (test results or recorded observations, for example) while other disciplines rely on subjective opinion that is based upon experience, hypotheses, individual theories, and judgement. Virtually every discipline involves at least some subjective judgment, and that leaves plenty of scope for an apparently scrupulous process to go awry.

The continuing viability of industries, huge sums of money, and entire lives are often at stake. The outcomes of trials change the lives not only of the parties involved, but potentially of huge numbers of people for many years to come. With so much at stake, it is imperative that society ensure the integrity of the system — it should be beyond reproach. Unfortunately, we are falling short of that ideal. The court's role as gatekeeper and the application of *Daubert* and *Frye* standards have advanced keeping unreliable expert testimony out of the courtroom, but even these safeguards fall far short of desired ideals. Frye, for example, requires that testing protocols and underlying scientific principles be accepted by the relevant scientific community — but how does one identify who the relevant scientific community is? The application of a *Frye*-type standard in 16th century Italy resulted in the incarceration of Galilei Galileo because he said the earth was round and rotated around the sun, while the "relevant scientific community" dismissed his work as fantasy. How can we be sure that similar errors are not being made in courtrooms today?

Incompetence can largely be overcome by remedial education and stringent staff selection policies. Errors will never be eliminated completely, but can be minimized by the adoption of procedures such as independent peer review; however, there are far more sinister forces at work within the injustice system (lies, fraud, intimidation, deception, bias, etc.) and these can be difficult to detect and very difficult to eliminate.

Historically, the legal system appears to have dismissed the aforementioned acts as so rare as to be insignificant; however, a review of cases, and especially criminal cases, reveals that the problem is not as isolated as the layperson may assume. Perhaps there is more motivation to eliminate such acts in civil litigation in which huge sums of money are often involved, and less motivation in the criminal arena, where the high volume of cases and society's perception of criminal defendants as thieves, junkies, rapists, murderers, and general "street scum" results in far less scrutiny. The post-conviction process does little to discourage these behaviors as the review system is geared toward errors of process, rather than errors of fact or opinion, and the attitude of the courts is often one of "tough, the defendant should have hired his own expert and challenged this testimony first time around – he gets only one bite of the cherry." These undesirable expert behaviors have essentially been encouraged by the attitude of the legal community, by experts themselves, by the adoption of poor procedures, and by the creation of innocuous names for unethical or illegal activities. Generating fictitious test data (fraud) is usually described as "dry labbing," and false and misleading testimony is often described as "he misspoke," even when it leans solely in favor of the hiring party.

Case studies will be used to illustrate the points discussed and perhaps identify the motivations of the guilty parties. It will be demonstrated that while some instances of wrongdoing are the acts of individuals, others indicate conspiracy. It is concluded that the systemic failures that enable these practices to continue will not be changed without significant and concerted effort on the part of the legal system and lawmakers.

Systemic Failure, Witness Intimidation, Witness Dishonesty

D21 Proper (and Improper) Handling of Data and Analysis in Forensic Linguistics

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After attending this presentation, attendees will be able to evaluate proper or improper handling of data in forensic linguistics (forensic stylistics, forensic computational linguistics, or forensic natural language engineering).

This presentation will impact the forensic science community by providing principles for proper data handling for forensic linguistics and other pattern recognition techniques and ways to recognize when data is being improperly handled or analyses are improperly conducted.

Improper handling of linguistic data and analysis impedes the progress of forensic linguistics and its acceptance as a legitimate forensic science. Data management can affect admissibility based on case law and federal/state rules of evidence because proper data handling enables methods to meet legal standards. This presentation covers principles of data management in linguistics, computer science, and forensic science, including ground truth data, human subjects protection, data scarcity, data ill-formedness, data contamination, and statistical analysis. Linguistic (text) data has specific requirements for proper management, but these principles apply to many kinds of forensic techniques.

What kind of data is needed? Ground truth data has known characteristics relevant for a specific task. In authorship identification work, ground truth data would be a set of texts whose authorship is known and verified. It is important to secure ground truth data, but difficult to do. At least one State Superior Court excluded the Federal Bureau of Investigation's (FBI's) Behavioral Analysis Unit (BAU) Communicated Threat Assessment Database (CTAD) due to ground truth problems (*New Jersey v McGuire*). Some advocate using the internet for authorship Identification (ID), but electronic authorial suspicions arise precisely because screen names are pseudonymous.[1-2] Alternative ground truth datasets do exist and are still needed for validation testing.[3-5]

What regulations apply to data sources? Human Subjects Protection (45 Code of Federal Regulations (CFR) 46) defines the standard practice for linguists, but sharing forensic linguistic data must follow both CFR restrictions as well as policing and legal policies. Ethical issues arise in the collection of suicide notes, threats, and predatory chats, including data collection methods, legality, and chain of evidence. Further, discussion of any case while it is still in adjudication is unethical and a potential obstacle to a fair trial. In *Tennessee v Potter*, a forensic stylist was excluded from testifying but presented a talk about the case while the case was in trial. In the JonBenet Ramsey case, a forensic stylist working for the prosecution provided his analysis to *The New York Times* during the grand jury.

What qualities of text data affect method? Three qualities are important: scarcity, ill-formedness, and contaminant-free. Data scarcity is a fact of forensic casework, so methods must select analytical levels to exploit information in minimal amounts of text. Forensic data is measured in the tens and hundreds, not hundred-thousands of words. Analytical levels are thus constrained. In the smallest samples, lexis isn't reliable for authorship ID but grapheme and syntax are.[4, 6-11] Lexis, grapheme, and syntax are standard analytical levels; prescriptive grammar is not.

Ill-formedness is another fact of forensic casework, so analytical procedures must perform on messy input while still preserving it. Spelling, syntax, or punctuation should not be "corrected" by the linguist because this changes the data, but some have. Standard sociolinguistics preserves data, no matter how ill-formed it may seem to the analyst.[12] In a habeas corpus case, two forensic stylists were engaged by the plaintiff and calculated wildly different sentence lengths; when questioned, one explained that he had "corrected the punctuation" of the data. This is not standard practice in linguistics or forensic science.

Correcting ill-formedness is close to contamination, which occurs when multiple unknowns are assumed to be from the same source and treated as known examples of one unknown source. Data should remain contaminant-free. Samples, whether blood or text, should never be mixed, although stylists regularly mix texts.[13] Alternatively, multiple unknowns can be hypothesized to come from one source, but not assumed to be; they can be tested for internal consistency as a single-source, but only if the expert report makes it clear that such a test has occurred, as in *BWI v John Doe*.

Finally, what statistical practice is required? Statistical analysis should proceed by normal rules for particular statistical procedures. While it is true that a few statistical procedures will still work well even if a requirement is violated, the multiplication rule will not work accurately if its requirement of independence is violated. In an immigration case, a computational linguist applied the multiplication rule on dependent data so that he could get, in his own words, the probability that the attorney requested, below .05. After being questioned about this, the analyst called it, in print, "statistical hand-waving."

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Forensic Linguistics, Text Mining, Data

D22 Forensic Microscopy and Reverse Engineering in Asbestos-Containing Building Product Identification

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The goal of this presentation is to inform attendees how microscopic analysis of building products can be used to identify the manufacturer of a product.

This presentation will impact the forensic science community by informing attendees regarding how forensic microscopy can be used to identify the manufacturer of a product by reverse engineering a sample (determining its constituents) of the product, then comparing the resulting list of ingredients with product formula information obtained from a number of sources.

In the late 1980s, President Reagan signed into law the Asbestos Hazard Emergency Response Act (AHERA) to address the problem of asbestos in building materials in the nation's schools. To fund asbestos abatement or removal from existing structures, several school systems and state attorney generals sued the manufacturers of asbestos-containing building products. Determination of which specific manufacturer had produced a particular asbestos-containing building product was an important part of this endeavor. The analysis of thousands of individual samples and matching them to their manufacturer (commonly called Product Identification or Product ID) was a large forensic investigation involving thousands of samples from buildings across the United States and Canada.

There were two general objectives of this project. The first was to collect, decipher, and collate information concerning the formula components of asbestos-containing products, and the second was to develop, modify, and apply forensic microscopic methods to identify components in samples of building products.

There were several sources of manufacturers' product formula information. These included court-ordered releases in a number of cases, the 1990 Federal Register publication of the Environmental Protection Agency (EPA) Asbestos Information Act, Mealey's Litigation Reports, and analysis and deformulation of samples from building applications in which the manufacturer claimed ownership. In one of the first cases, a court order from Maryland's attorney general resulted in thousands of pages of formula documents from former asbestos product manufacturers. Although it was a daunting task to organize this massive amount of information so that it could be used efficiently, it soon became apparent that the majority of the products could be classified on the basis of the type of asbestos present and which of about two dozen binders and fillers were used to construct the products.

Forensic microscopy and chemistry methods were developed or modified from classical procedures to identify the product ingredients, which included: perlite, vermiculite, mineral wool, bentonite clay, kaolin clay, Portland cement, precipitated lime, gypsum, sand, limestone, diatoms, talc, starch, cellulose, mica, wollastonite, titanium dioxide, lithapone, sodium silicate, calcium silicate, magnesite, and sodium nitrate. These methods were used to deformulate known samples of product materials.

A comprehensive Excel® spreadsheet database of the asbestos-containing building material product compositions and their manufacturers was constructed. This database was used with forensic microscopy analysis and chemical testing to identify the manufacturer of the majority of asbestos-containing building products. Development of the database could not have been conducted without extensive investigation into brand names and industry-wide or company-specific terms used to identify each of the components listed on a manufacturer's batch production sheets. For example, an ingredient listed as "Staramic" is a trade name for starch and an ingredient listed as "Snowflake F" is wet-ground limestone (calcium carbonate). This research effort to identify ingredients listed in manufacturers' documents led to the publication of a dictionary of terms in 1999.[1]

This presentation will provide a detailed description of the processes by which the product formula database was developed and specific examples of the practical application of the forensic microscopy techniques used.

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Building Product, Microscopy, Asbestos

D23 Why Engineers Are Named in Construction Defect Lawsuits: When Conflicts Arise Between the Building Code, Engineering Judgment, and Common Sense

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After attending this presentation, attendees will better understand some of the common reasons engineers are named in construction defect lawsuits, various aspects of the building code, situations in which using engineering judgment may be problematic, and the challenges facing forensic engineers when a conflict arises between engineering recommendations, engineering plans, and the applicable building code.

This presentation will impact the forensic science community by sharing experience gained through involvement in nearly 500 construction defect claims (and expert testimony in more than 200 construction defect lawsuits). This presentation will also increase engineers' awareness of various conditions that should be assessed at the time of design and construction. Multiple case studies will be presented and the relevant findings and opinions on each matter will be discussed.

The building code provides relevant guidance for design professionals and contractors. Experience has shown that many design professionals and contractors are not well acquainted with some portions of the building code. In these instances, the variation between the engineering design and the applicable building code can become problematic. Engineers are frequently named in lawsuits when conflicts arise between the building code, engineering judgment, and common sense.

Many parties acknowledge that when multiple standards specify differing requirements for the same aspect of construction, the more stringent (or restrictive) standard shall apply; however, experience has shown that the more stringent standard is not always the "applicable" standard. The Building Code states: "Where there is a conflict between a general requirement and a specific requirement, the specific requirement shall be applicable." In more complicated litigated matters, engineering plans sometimes provide details which are less stringent than the adopted building code or general notes to a set of plans. In these cases, the specific requirement may be the "applicable" requirement, but not the more stringent (or restrictive) requirement implemented for construction. In situations such as these, engineering judgment and common sense should be used.

When variations between engineering design and the adopted building code exist, the arguments issued by opposing parties generally relate to engineering judgment. In some instances, engineering judgment may be inconsistent with the current or applicable building code. Perceived deviations, or inconsistencies, within engineering plans will frequently lead to lawsuits because forensic engineers may argue the relative importance of the building code versus engineering judgment.

Based on the review of numerous cases, differences are frequently found by outside parties who scrutinize the pre-design engineering recommendations, the engineering plans and the adopted building code. Differences between engineering plans and building codes can be one of the primary causes for a construction defect lawsuit.

Engineer Lawsuit, Building Code, Engineering Judgment

D24 The Fire Marshal and Forensic Fire Investigation

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After attending this presentation, attendees will be able to identify the legal and statutory duties of the local fire marshal in a science-based fire investigation, understand a number of ethical and forensic implications associated with reporting a fire, discuss the jurisdictional limits of a fire marshal, and be able to identify some ethical and forensic limitations associated with data collection and reporting when the legal or statutory duty of the local fire marshal does not extend to root-cause or engineering failure analysis. This presentation is accompanied by a case study.

This presentation will impact the forensic science community by providing case facts meant to highlight how the duties of the local fire marshal can provide important testimony regarding culpability, yet still fall short of determining responsibility in civil or criminal litigation. Increased liability is of interest in situations in which there is a loss of life or property due to non-compliance with codes and standards, when the nature of the responsibility is a deliberate omission or an unintentional act. The information presented will be beneficial to fire investigators and public officials responsible for life safety in the local community.

Insofar as forensic fire investigation, it is the duty of the fire marshal to determine the cause, origin, and development of a fire within a jurisdiction and report findings to the state. His or her conclusion will determine whether a crime may have been committed, as in cases of arson or criminal negligence. If there is no evidence a crime may have been committed, then the fire marshal classifies the fire as natural, accidental, or undetermined. This will close the official investigation and no further attempt will be made to gather empirical data. Information reported is pertinent; however, it is limited to data required to fulfill the fire marshal's obligation to the state.

Fire investigations more commonly result in civil litigation than criminal prosecution. Although the local fire marshal or another public service agent may be deposed or must otherwise testify in a civil matter, it is the root-cause failure analysis performed by an engineer or private sector fire investigator that will usually point to civil responsibility. Insurance companies and independent forensic firms are better funded and can often bring a particular case further along to determine specific liability. An investigative engineer can avoid misinterpretation of evidence at the fire scene by studying the local fire marshal's incident summary, photo documentation, and investigative report. The fire marshal receives emergency dispatch notifications and begins data collection concurrent to the arrival of fire and rescue services. He or she should be able to provide a first-hand incident summary, from the initiation of emergency response to the termination of the incident at headquarters, and a written report based on the guidelines, published in the National Fire Protection Association document NFPA 921, for conducting a science-based fire investigation.

The case study that will be presented details the total destruction of a 4.5-million-dollar home in rural Connecticut. This case will show the progression of litigation after criminality was dismissed by the local fire marshal. Facts to be presented will illustrate how reports made by the Office of the Fire Marshal can provide data on origin, development, and proximate cause; confirm exculpatory statements during civil litigation through exclusion; and articulate how the scientific method was used to determine fire cause and origin within the scope of his or her investigation. Ethical implications regarding specific requests for data or statements that exceed the parameters of such an investigation (e.g., complete failure analysis of an appliance or system and testimony regarding the degree of engineering certainty in determining root causation) will be discussed.

Fire Marshal, Fire Investigation, Failure Analysis

D25 Vaginal and Colorectal Injuries From a Personal Watercraft Jet Stream Versus Falling Into the Water at Speed

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The goal of this presentation is to demonstrate, with a case study and analysis of the relevant literature, how vaginal and colorectal injuries caused by the high-pressure water jet emitted by personal watercraft become more severe with increasing power of these machines and how the pattern of these injuries differs from those typically observed due to falling into the water at speed.

This presentation will impact the forensic science community by demonstrating how the severity and type of vaginal and colorectal injuries can differ due to proximity to the high-pressure water nozzle of personal watercraft versus entering the water at speed by falling off a craft but not landing near the nozzle.

A Personal Watercraft (PWC) is generally defined as a vessel which uses an inboard motor powering a water jet pump as its primary source of motive power. The majority of PWC's manufactured in the United States are designed to be operated by a person sitting on the vessel in a straddled configuration, like a snowmobile or a motorcycle. According to the United States Department of Transportation, 1.2 million PWC's were in use in the United States in 2014. Since their advent, PWC's have become faster and more powerful. Current models can have 300HP engines and the pressure from the water jet emitted from the PWC nozzle can exceed 800psi.

Although relatively rare, serious orifice injuries may occur to the vagina, perineum, anus, rectum, or colon when the rider or passenger of a PWC falls (or is ejected) from the back of this watercraft and lands in proximity to the nozzle with both legs abducted, such that these anatomical structures are directly in the path of the PWC's high-pressure water jet.

A case study is presented whereby two young women (a 17-year-old female seated immediately behind the operator and a 16-year-old female seated behind the 17-year-old) were passengers on a 2007 model year PWC. When the 21-year-old male operator accelerated rapidly from rest with no warning, both female passengers (wet from prior water immersion) were ejected from the rear of the PWC. They landed in the water near the jet nozzle, each with both legs abducted. The 17-year-old sustained a 6cm defect at the left pararectal space inferior to the vaginal mucosa; detachment of the perineal body; a 5cm-long vaginal laceration extending caudally along the left lateral posterior vagina, and a 4cm superficial deficit in the left ischiorectal fossa. The 16-year-old sustained a significant anal tear at the midline 12:00 o'clock position anteriorly and completely through the internal and external sphincter, as well as a posterior anal tear through the interior and external sphincters.

A question that often arises in litigation related to PWC orifice injuries is whether the injuries were more likely to be caused by the pressure from the jet nozzle or if it would have occurred regardless, due to falling into the water while traveling at a given velocity.

Freeman, et al. reported that the average New Injury Severity Score (NISS) a scale based on threat-to-life values for reported PWC falls and other water sports and activities was about half of those values reported for PWC jet nozzle injuries.[1] This study adds to that database by analyzing the extent and type of injuries reported in the literature and in the subject case to determine if certain types of injuries are more likely related to the jet stream or simply falling into the water at speed. By examining the structures disrupted and the severity of each disruption, it may be evident that more force would be required to produce a given injury, even if threat-to-life is not increased. For example, it would take more force or pressure to lacerate the tough fibrous anal sphincter than to make a tear in the inner wall of the colon or vagina.

Analysis of the injuries from this case study, together with a review of similar injuries in the literature, lead to the conclusion that as power and thrust of the PWC increases, injury patterns will become less like those typically seen from falling into water at speed and the severity of injuries observed will increase.

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Personal Watercraft, Colorectal Injury, Vaginal Injury

D26 The Time Needed to Exit a Stationary Vehicle and the Forces Required, at Highway Speed, for a Passenger to Open a Vehicle Door Wide Enough to Exit

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After attending this presentation, attendees will better understand how to quantify exit time from a stationary vehicle and the forces required to open the door of a vehicle that is traveling at highway speed.

This presentation will impact the forensic science community by providing quantitative data regarding the time for a passenger to exit a stationary vehicle as well as providing quantification of the force required to hold an automobile door open wide enough to allow a person to exit while traveling at highway speeds.

In an accident sequence, a pickup truck occupied by two adult males lost control and rolled over. The passenger sustained fatal injuries. Rather than being ejected during the rollover, it was alleged that the passenger elected to jump out of the pickup truck while it was traveling in excess of 60mph just as it began losing control immediately before the rollover. Because the scenario allowed for only a very short time interval between the beginning of the rollover event and the passenger's claimed egress, both the length of time required to accomplish this type of maneuver and the magnitude of force that must be simultaneously applied to the door to keep it open to exit were the objective of this study.

The time and effort required to force a door open and hold it open in order to exit a vehicle traveling at highway speeds could not be directly measured due to the requirement to ensure human subject safety and well-being. As such, the problem was addressed in two parts: measure the time to exit a stationary vehicle, then measure the force it takes to overcome the aerodynamic tendency for the wind to close the door, while traveling at highway speeds. Since that force must be overcome and maintained to hold the door open in the dynamic situation, it was important since it would hinder a passenger from exiting the vehicle by increasing both the complexity and effort required. As such, the exit times acquired in the static vehicle situation would be expected to represent the lower bound for the actual time for a passenger to self-eject from a moving vehicle.

The dynamic test involved driving an exemplar pickup on the freeway and opening the door at various speeds. The vehicle was instrumented to measure vehicle speed, door-opening force, and door-opening angle. The passenger was seat belted and the action of opening the door was also videotaped. Since the force to hold the door open should not depend on the specific subject, only one subject opened the door in the moving vehicle test. Seven instrumented runs were performed at speeds ranging from 37mph to 63mph. The door was opened to approximately 50 degrees, with the range from 49 to 57 degrees. The maximum force at 37mph was 20 lbs. and the force at 63mph was approximately 75 lbs. Generation of the force required to keep the door open required some form of occupant interior bracing. Of course, for a stationary vehicle, the force required to hold the door open is essentially zero.

For the static vehicle door open timing, the exemplar pickup was instrumented with a contact switch on the dash for time zero and a contact switch was placed on the ground where the subject opening the door would initially step to represent the end of the maneuver. These trials were also videotaped. Four adult male subjects ranging from 40 to 68 years of age were recruited to participate. The physical characteristics of the participants are summarized in the table below.

Subject	Height (in)	Weight (lbs.)	Age (years)
1	70	213	68
2	72	215	58
3	67	200	46
4	71	260	40

Rather than attempt to replicate the physical characteristics of the passenger in the case, it was decided to use a variety of subjects with different ages, heights, and weights to arrive at a range of times that could be considered realistic.

The subjects were instructed to contact the switch on the dash, open the door, and exit as fast as possible, landing on the ground contact switch. The time between switch contacts was recorded. Each subject performed the task at least three times and there were a total of 18 recorded runs. For each subject, the first attempt at exiting the vehicle was typically their longest time, followed by generally decreasing times as they became more practiced. All the exit times ranged from 1.4 seconds to 3.0 seconds and, by their last attempt, all subjects were able to bring the time down to 1.7 seconds or less.

Besides presumably not having practiced rapidly self-ejecting from a moving vehicle, in addition to opening the door and moving to exit the vehicle, as in the static vehicle condition, exiting a moving vehicle would require the passenger to find a way to brace to force the door open, then maintain that level of force while positioning to exit the vehicle. As such, it can safely be concluded that the time required to exit a moving vehicle would be longer than those times obtained in the static vehicle trials since such a maneuver would be both more complex and require greater effort to complete.

It is concluded that without pre-planning or practice, it would be challenging to successfully exit a vehicle traveling at highway speeds, and it would be extremely unlikely that such could be accomplished quickly.

Accident Reconstruction, Door Opening, Egress Time

D27 Engineering Investigations of Quadriplegic Diving Accidents

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After attending this presentation, attendees will better understand how forensic engineering principles are applied to quadriplegic diving incidents to determine how the incidents occurred and how Newton's Second Law and the Equations of Motion are applied to a headfirst dive into water. Attendees will also better understand how forensic engineering investigations assist the pursuit of justice in litigation and will be aware of some measures that can help to reduce or prevent occurrences of quadriplegic diving injuries.

This presentation will impact the forensic science community by demonstrating the collaboration of forensic biomedical engineering analysis and forensic mechanical engineering analysis in quadriplegic diving accident litigation. In this presentation, legal perspectives will be presented by counsel and forensic engineering sciences will be presented by forensic engineering experts.

Spinal cord injuries from diving accidents in swimming pools and natural bodies of water are costly, debilitating, and life altering. Due to the extreme and permanent nature of the injuries suffered, protracted and expensive lawsuits frequently arise from these incidents. In the forensic analysis of a quadriplegic diving incident, the biomedical engineer and mechanical engineer are uniquely situated to offer insights with respect to the quantification of forces and accelerations. Because of the multidisciplinary aspects of diving incidents, the biomedical engineer is able to tie the engineering aspects together with the medical diagnoses by the health care providers and forensic pathologists. Various head impact scenarios are analyzed from an engineering perspective and supported with analysis of physical evidence and/or experimentally verified test data.

The forensic investigations into cervical spine trauma from diving injuries in this presentation required both a forensic biomedical engineering analysis and a forensic trajectory analysis through air and water to the moment of impact to determine the causes of injury and identify other contributing factors. Through these analyses, the forensic experts determined whether the physical evidence was consistent with the injured diver's narrative of the precipitating events or if there was evidence of other causal factors.

The first information that needs to be obtained in a quadriplegic diving accident case is the biomedical information of the cervical spine trauma. The forensic biomedical engineer must analyze the fractures to the head and cervical spine sustained from the incident to determine the physical characteristics at impact that caused the fractures (i.e., the mechanism of injury), including: (1) the minimum amount of force at impact necessary to cause the injuries; (2) the velocity of the diver at the moment of impact; (3) the angle of the diver's body at the moment of impact; and, (4) the angulation of the diver's head to the diver's body at the moment of impact.

In the descending portion of a headfirst dive into water, a diver's body is an object in a freefall trajectory. By applying the equations of motion pertaining to freefall to the characteristics of the impact in a parametric analysis, possible dive solutions can be calculated. A dive into shallow water can originate from a jump from a standing position or from a moving start. A jump does not normally occur in a slip-and-fall or in a push. A dive caused by a push into shallow water typically has an increased dive angle because the push causes the diver to over-rotate.

Based on the above-listed evidence from the trauma, in conjunction with other forensic evidence, the biomedical engineer and mechanical engineer collectively assessed whether the divers' trajectories were consistent with: (1) a slip and fall into shallow water; (2) a push into shallow water; or, (3) a dive into shallow water.

These forensic investigations demonstrated how spinal cord injuries occurred from diving accidents. At the end of the presentation, measures will be identified that help to prevent occurrences of quadriplegic diving injuries and the consequential personal injury litigation.

Quadriplegic Diving Accident, Cervical Spine Injury/Trauma, Personal Injury Litigation

D28 Fatal Launch: Fireworks Fatality and the Determination of Generated Recoil Force

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After attending this presentation, attendees will better understand the construction and lethality of consumer-grade fireworks as well as how to measure force production.

This presentation will impact the forensic science community by describing the method for quantifying the magnitude of recoil generated by consumer-grade fireworks, presenting recoil force data from such fireworks, and reporting a case study proving how lethal this force can be.

Every year, fatalities occur due to improper use of fireworks. In 2015, one of these fatalities occurred through the use of a reloadable 60-gram canister shell fireworks mortar.

A 30-year-old male was with friends consuming alcohol and methamphetamine while shooting off fireworks. The decedent decided to launch a mortar off of his chest. From a standing position, the decedent placed the mortar to his chest and lit the shell. The firework ignited and the decedent stumbled back and fell unresponsive to the ground. Cardiopulmonary resuscitation was initiated by bystanders. Emergency services responded and continued advanced cardiac life-saving measures, then transported the decedent to a local hospital where he was pronounced dead.

During external examination prior to autopsy, an 11cm x 8cm contused, red-brown abrasion was on the upper left side of the chest with smaller surrounding abrasions. There were abrasions and contusions of the back of the head, the lower left side of the abdomen, and the upper extremities. Internal examination revealed fractures of the sternum and the anterior aspect of left ribs 3, 4, and 5, contusions and lacerations of the lungs, and contusions and lacerations of the heart and great vessels.

To determine the amount of recoil force generated by the firework, five different 60-gram mortar shell tubes from two different fireworks companies were tested. The mortars varied in price, quality, and construction. The mortars tested were either a two-piece fiberglass/High Density Polyethylene (HDPE) tube with a particle board base (similar to the variety used by the decedent) or a solid HDPE plastic single mold. The mortar base was 11.43cm square. The mortars were placed, without restraint, on a flat, 11.43cm square and 1.27cm thick aluminum plate. The plate was mounted on two Link ICP® quartz force sensors. An identical aluminum base plate was mounted to the bottom of the sensors and was placed, without restraint, on level concrete pavement. A Data Acquisition (DAQ) system converted the analogue voltage output of the sensors to a force reading. A total of 22 shells were tested in this manner.

Each of the five mortars tested produced a unique and somewhat consistent force signature. Force amplitude among the shells/mortars tested varied greatly, with peak values ranging from 742N to 2,410N (mean = 1,589N, *SD* = 497N). The average force production varied between 271N and 686N (mean = 442N, *SD* = 114N). Among the shells tested, the entire force event lasted between 16ms and 28ms (mean = 21ms, *SD* = 3ms). The impulse (integral of force with respect to time) ranged between 6.0N-s and 12.6N-s (mean = 9.0N-s, *SD* = 1.6N-s). The estimated kinetic energy of the shells varied between 145 to 624 Joules (J) (mean = 320J, *SD* = 113J). The findings illustrate that a considerable force can be rapidly generated by these fireworks. This would put significant sudden force on the ribs in a small area, causing the ribs to fracture, resulting in lacerating and contusing the lungs, heart, and great vessels.

Fireworks Fatality, Recoil Force, Kinetic Energy

D29 Updating the Injury Reconstruction Methodology: An Exemplar Motor Vehicle Crash (MVC) Case Involving Restraint and Intrusion Issues

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After attending this presentation, attendees will understand how the study of free, publicly available online databases of MVC data can assist with the reconstruction of injuries for research and/or forensic analysis. A sample crash case will illustrate this process.

This presentation will impact the forensic science community by informing attendees of an important update to the method of injury reconstruction, which is a less common subdivision of accident reconstruction.

In 1994, Nahum and Gomez published a Society of Automotive Engineers (SAE) technical paper describing the steps recommended for reconstruction of injuries, a parallel to accident reconstruction which focuses on occupant kinematics in addition to the vehicles and roadway.[1] The paper also included a nice overview of biomechanical research related to injury production. American Academy of Forensic Sciences (AAFS) attendees will understand how utilizing online federal crash databases serves as a modernization and enhancement of the methodology described in this seminal work. This will be demonstrated via examination of a forensic case involving the questions of if and how proper restraint utilization could have mitigated injuries to the unrestrained driver of a particular model pickup truck which struck the rear of a tractor trailer.

Approximately 15 states allow for the so-called "Seat-Belt Defense" in which it can be argued in court that a driver is partially responsible for his or her injuries when that person was not properly restrained — even when they are not at fault in the accident. The subject case involves such a defense. Crash severity can be described in terms of the information gleaned from the police report, the event data recorder (a.k.a. black box), and photos from the accident scene. In this case, the pickup was travelling 52mph one second before impact and brakes were applied 0.5 seconds before impact. The damage was severe as it suffered a 43mph change in longitudinal velocity. Although police indicated the 5′11″, 264-pound, 39-year-old male driver was restrained, the event data recorder and eventual inspection showed otherwise. He suffered fractures of the right femur and bilateral tibial plateaus, a T-1 wedge fracture, right foot fractures, and a mesenteric tear, in addition to numerous contusions and abrasions. What injuries could have been prevented had the driver elected to follow state law and properly restrain himself?

To answer this question, the facts were compared to the data and videos publicly available through the New Car Assessment Program (NCAP) frontal crash tests of this model vehicle (specifically NCAP tests 7121 and 7099) performed for the United States Department of Transportation National Highway Transportation Safety Administration (NHTSA). These tests involved 35mph crashes of the same model truck into a solid barrier. In each case, a restrained, instrumented 50-percentile male Anthropomorphic Test Dummy (ATD) was placed in the driver's seat and an instrumented 5th-percentile female ATD was restrained in the passenger seat. The NCAP tests indicated less than a 5% chance of femur fracture in these crashes with proper restraint use (three-point seat-belt and airbag). The online databases of the National Automotive Sampling System (NASS) were searched for investigations of real-world frontal crashes involving the same model vehicle with even higher changes in velocity. In NASS case 77301100, the pickup truck struck a square bridge support column and experienced a 51.6mph change in velocity. The 6′, 190-pound, restrained 30-year-old male driver only suffered bruised knees. While this data seemingly supports a seat-belt defense, the importance of an actual vehicle inspection will be demonstrated.

Online crash data can be extremely valuable, but issues such as underride, override, and intrusion may trump the crash data during injury reconstruction and exploration of the seat-belt defense. In this case, the pickup truck underrode the trailer and struck with such violence that displacement of the engine and transmission forced the firewall, dashboard, and steering wheel well into the occupant compartment negating the normal benefit of properly utilized three-point restraint.

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Injury Reconstruction, Injury Biomechanics, Seatbelt

D30 Age Effects on Bending Fracture Patterns in Ovine Femora

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After attending this presentation, attendees will better understand the effects of age on fracture patterns in controlled, concentrated four-point bending tests on fresh ovine femora.

This presentation will impact the forensic science community by utilizing ground-truth experimental data and simple Finite Element (FE) analyses to aid in the interpretation of fracture patterns under concentrated four-point bending of ovine femora.

Most research in forensic biomechanics of long bones has focused on the failure pattern from three-point bending and how tension wedges are consistently produced. This involves a transverse crack that initiates on the tensile surface of the bone and forms a Y-shaped fracture pattern from the tensile to the compressive sides of the bone. From these data, many forensic practitioners claim to cite impact direction based on the three-point model as noted.[1] This becomes problematic because cases of compression wedge fractures under non-controlled impact testing have been reported where the typical Y-shaped fracture pattern has become inverted.[2] Furthermore, reports cite a consistent pattern of compression wedge fractures under a slow, concentrated four-point bending configuration.[3] Recently, 40% of ovine femur failures have been reported to be compression wedge fractures.[4] Also, it has been shown that under torsion, age and rate effects influence spiral fracture patterns of long bones.[5] It is therefore forensically relevant to determine how compression wedges in long bone fractures might be produced under a concentrated four-point loading configuration, and what effects, if any, specimen age may have on the production of these compression wedge fracture patterns.

The objectives of the present study were to: (1) execute controlled, concentrated four-point bending tests of ovine femora; (2) identify fracture patterns in association with specimen age; and, (3) study the fracture patterns with the help of FE modeling.

Fourteen freely supported ovine femora were failed under a concentrated four-point bending configuration.[3] Five femora (one day to one week old) were classified as young and nine femora (one to two years old) were classified as old. In this test configuration, the distance between the two outer supports was 60% of the specimen length, while the distance between the two inner loading probes was 10% of the specimen length. A servo-hydraulic testing machine was used to fracture the bones. Failure was achieved at a rate of 2Hz over an 8mm and 10mm displacement of the loading probes for young and old bones, respectively. All bones were loaded on the posterior surface of the bone. The experiments were filmed with a high-speed camera at 40,000fps. After experimentation, specimens were examined for gross fracture morphology.

Prior to mechanical testing, a whole-bone Computed Tomography (CT) scan was taken of an old ovine specimen. These 3D images were reprocessed using volumetric techniques in Mimics®, given a tetrahedral mesh in 3-matic®, then imported into Abaqus 6.11 where loads and boundary conditions were applied to simulate the above-mentioned four-point bending tests.

In the young group, 60% (3/5) of the specimens failed with a tension wedge. The remaining specimens exhibited complete transverse fractures; however, in the old group 67% (6/9) of the specimens produced compression wedge fractures, while the remaining specimens showed oblique fractures located slightly outside an inner probe where compression wedges initiated. High-speed footage confirmed fracture initiation occurring on the tensile side of the bone in all cases. All experimental measures (i.e., initial bone stiffness, bone displacement at failure, energy input during failure, and failure loads) have shown significant differences between groups at a level of $p<0.001$.

The observed experiments demonstrated that under concentrated four-point loading, young bones produced transverse or tension wedge fractures while older bones produced compression wedge fractures. The direction of compression wedge fracture was perpendicular to the line of maximal principal stresses generated from the FE model of the older bone, slightly outside the inner probes. To date, FE analyses of younger bones have not been

completed to help explain the production of the transverse and tension wedge failures in the young bones. The premise is that changes in the structural and material descriptions of these bones will help in these explanations.

Reference(s):

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3. Martens M., Van Audekercke R., De Meester O., Mulier J. Mechanical behavior of femoral bones in bending loading. *J. Biomech* 1986; 19(6):443-54.
4. Reber S.L., Simmons T. Interpreting injury mechanisms of blunt force trauma from butterfly fracture formation. *J. Forensic Sci*. 2015; 60(6):1401-11.
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Forensic Biomechanics, Long Bone Fracture, Four-Point Bending

D31 Electrocution Precipitated by a Flying Duck

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After attending this presentation, attendees will better understand the importance of considering all unusual evidence in the vicinity of an electrocution and why laboratory high-voltage tests should be applied when appropriate before an opinion or hypothesis is rendered in cases involving a downed power line.

The presentation will impact the forensic science community as well as the, legal, judiciary, insurance, electric utility industry by highlighting the importance of considering all evidence including birds or animals in the vicinity of an electrocution.

The forensic engineering case and facts: A male resident in a bungalow was watching television with his family when suddenly the power went out. After hearing two explosions outside, he went outside in his socks and observed a wire lying across his white pickup truck. While standing on the concrete sidewalk, he touched this small uninsulated solid copper #6 American Wire Gauge (AWG) wire and was immediately electrocuted. Exit burn marks from both feet in socks were found on the concrete sidewalk next to his truck. Entrance burn marks were found on his hand. The small downed copper wire was unprotected by a fuse but connected to a large conductor utility main feeder. The wire also draped over a four-foot dry wood fence on the decedent's property and across his truck; it then lay on an asphalt road, across a concrete gutter and curb, where arcing to ground severed the wire. Other severed wire parts were found draped over another wood fence.

A deceased mallard duck was found farther downstream along the circuit. The duck presented a severe abdominal injury and perforation consistent with electrical burns, which exposed its entrails. There were no electrical burn marks found on its wings or anywhere else on its body. The initial investigators and experts dismissed any relationship between the duck and this incident. Although the actual remains of the duck were no longer available, photographs of the duck were available.

Questions arose regarding how a duck could suffer a fatal injury to its abdomen similar to those evident in the photographs and what did that have to do with a downed power line. To address these questions, experiments were conducted in a high-voltage laboratory with a deceased mallard duck at 12,400 volts. This presentation will explain the complex mechanisms regarding how the duck precipitated this accident in addition to the other ancillary factors (e.g., inadequate electric utility fuse protection of laterals, incorrect circuit drawings, and incorrect conductor sizes).

Electrocution, Downed Power Line, Duck Bird

D32 Using Finite Element Modeling to Extend Non-Contact Age Estimation of Blood Stains to Complex Blood Stain Morphologies

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After attending this presentation, attendees will gain insight regarding the possible use of finite element modeling to enhance spectroscopic methods for non-contact age estimation of blood stains.

This presentation will impact the forensic science community by providing an improved non-contact method for the age estimation of blood stains.

Many crimes involve the transfer of biological materials, including hair, tissue, and bodily fluids such as blood. Knowledge of the age of such biological traces is of great importance; a temporal relation of a biological trace to the pertinent crime may render the sample as "activity level" evidence. As a result, there is a clear need for age estimation methods for biological traces.

An innovative non-contact method for estimating the age of blood stains was recently developed. To this end, Visible Near Infrared (VIS-NIR) spectroscopy reflection measurements were used in conjunction with a physical one-dimensional light transport model describing the wavelength-dependent reflection profile of a blood stain. In this model, blood stains were represented as a two-layered system: a top layer of blood deposited on a substrate, with layer-dependent optical properties. In addition, the chemical reaction governing the aging of blood stains was assumed to consist of three phases: full oxygenation of the hemoglobin to oxyhemoglobin; subsequent auto-oxidation to methemoglobin; and a further denaturation to hemichrome. The stoichiometry of these three phases was assumed to vary with time, corresponding to the chromatic changes observed in aging blood stains. By probing the relative quantities of these chromophores using reflectance spectroscopy, the present approach provided an estimate of blood stain age. This was achieved by means of a least-squares fit of the light-transport model to the measured reflection spectrum of a blood stain.

Despite the excellent predictive capabilities of this approach for special cases of blood stain morphologies, namely complete layer separation and no layer separation, more complex morphologies remain challenging. The diversity of blood stain morphologies is a direct consequence of the continuum of possible porosities, rheologies, and geometries of various blood stain substrates. This multitude of blood stain morphologies corresponds to different regimes of light propagation, which in turn represents the limits of validity of the currently employed light-transport model. This has implications for the applicability of this approach in forensic practice as the number of conceivable morphologies of blood stains exceeds the two mentioned special cases.

This presentation explores how the theory of light-transport can be further developed to probe complex blood stain morphologies, thus enhancing the versatility of the approach. To this end, finite element modeling is used in conjunction with phantom measurements to simulate and investigate the effects of blood stain morphology on light propagation. The findings of this study form the basis of an extended light-transport model for improved applicability and dating accuracy of the developed non-contact approach.

Blood Stains, Age Estimation, Non-Contact

D33 3D Surface-Scanning Techniques: Current Use, Limitations, and Improvement Propositions

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After attending this presentation, attendees will understand the role of different 3D surface-scanning technologies in investigating various types of cases. Attendees will also learn the limitations and problems linked to these technologies and will understand the necessity of developing and evaluating a suitable and robust methodology for the 3D comparison of marks and objects.

This presentation will impact the forensic science community by explaining the issues linked to the current use of the different 3D technologies in forensic medicine. Studies planned to answer the methods needed for the analysis and comparison of wounds and blunt objects using 3D surface scanners will also be presented.

Recent developments in forensic imaging led to an increasing use of 3D surface-scanning techniques, especially for traffic accident reconstruction and the physical correlation between resulting injuries and causative instruments. Although different 3D technologies, such as laser scanning, surface-scanning and photogrammetry, have great potential for such use in forensic investigations, they have limits as will be exemplified in the following case study.

A traffic accident victim, treated at the University Centre of Legal Medicine in Lausanne two years ago, highlights some of the methodological shortcomings regarding the means by which analyses and comparisons are actually conducted using 3D surface- scanning technologies. Regarding this victim, an accident-relevant mark on the victim's face and neck was documented using a 3D surface scanner and photogrammetry. Tires of the vehicle involved were scanned and a morphometric comparison was produced between the resulting different 3D models. Only a partial superimposition of the patterns was observed; however, the implication of the car was proven and this should have led to a complete superimposition. An explanation for this discrepancy is that the mark on the victim's face was deformed due to massive lesions of the facial bones. This case raises questions like: Would one have excluded the tire as being the object that left the trace if one were not sure about it, in which cases can one exclude that an object has produced a mark, and what level of certainty can one reach for a conclusion in cases in which there is an imperfect correspondence between the patterns and the object?

To address these questions, research was conducted to study the use of a fringe-light 3D surface scanner to determine whether an incriminated object could have caused a certain wound. Seven volunteers inflicted a total of 23 "injuries" on watermelons using 15 different blunt objects. Then, 3D models of the injuries and the instruments were acquired using a GOM ATOS Compact Scan 5M. An operator experienced in 3D surface-scanning and blinded to the origin of the experimentally created "injuries" scanned the test watermelons, then compared the resulting 3D models following the Analysis Comparison Evaluation–Verification (ACE-V) methodology widely used in forensic science. In 57 % of the cases, a correct non-exclusion of the injury-causing object was made, but in 26% of the cases, the exclusion was wrong.

The actual case example and the accompanying laboratory study exemplify the difficulties involved when 3D models are used to reconstruct a wound and perform a correlation with the object that produced the wound. This highlights the need to develop and assess a suitable and robust methodology. Further research is needed to address these issues and to strengthen the scientific basis of 3D comparison of marks and objects. A collaborative effort has been established between the University Centre of Legal Medicine in Lausanne and the School of Criminal Justice. This effort seeks to formalize and study the different steps of the comparison process, following an ACE-V approach already employed for comparison purposes in forensic science (fingerprint comparison, footprint comparison, etc.). This methodology strongly emphasizes the importance of the analysis phase that enables the expert to assess the informative value conveyed by the mark before any comparison is made. Based on this approach, the strength of the conclusion that can be expected from further comparisons (exclusion, identification, etc.) can be quantified.

The goal is to develop a methodological framework for the study of object marks on the body, encompassing 2D and 3D surface technologies, and for the comparison of questioned marks with reference features produced by suspected objects.

Forensic Imaging, Morphometric Comparison, Methodology

D34 The Use of Strava in Bicycle Collision Analysis — A Case Study

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The goal of this presentation is to illustrate by example how Strava (a social network for athletes) was used to validate the conventional reconstruction methods applied to a bicycle collision analysis.

This presentation will impact the forensic science community by demonstrating the basics of Strava's online user activity page and by illustrating how time-base data can be employed to supplement the existing tools employed by the forensic investigator to analyze a bicycle collision.

The accuracy of traffic collision analysis depends on the quality and quantity of information gathered by law enforcement investigators. Often, additional information helps validate a conclusion by enabling an additional independent analysis that leads to the same conclusion. This is exemplified in the following case study used to understand a mishap involving a bicyclist.

This particular case involved road construction at the bottom of a particularly steep downhill section of a four-lane roadway having a posted 40mph speed limit. Road work included trenching across the road. The trench was concealed overnight with 6′ x 8′ steel plates. Because the steel plates rested on top of the traveled roadway surface, a tapered layer of asphalt was applied to the periphery of these plates to provide a graded transition from the roadway to the steel plate surface. Several road signs were positioned upstream of the steel plates in both directions to warn vehicle operators of the approaching surface irregularities.

A bicyclist's established daily home-to-office-to-home commute was composed of a regular route consisting of approximately seven miles in the morning and approximately nine miles at night. The accumulated average was approximately 50 miles per work week, excluding a typically longer weekend ride. Rainfall was one of the factors that would alter the rider's choice of transportation mode.

The incident occurred on the first clear, dry evening after nearly two weeks of inclement weather, during which time road construction had begun on the route taken by the rider in the evening. The rider was, therefore, unaware of any potential road hazard ahead. There were no eyewitnesses to the incident. The rider was found wearing a helmet, lying on his left side, both cycling shoes still attached to the pedals, and straddling the bicycle. The forward facing bike light was still illuminated and the taillight was flashing. The rider suffered multiple skin abrasions, but the more significant injuries included a fractured left clavicle, ribs, hip, and skull.

Investigators found an approximately 36-foot-long trail of metal, clothing, and tissue scuff marks on the roadway angled toward the curb and leading to a blood stain. The overall slide distance measured 44.2 feet. Using sliding friction (0.6g) and accounting for the upward roadway slope (1.3%), the rider's speed was calculated to be approximately 28.5mph when the front wheel struck the steel plate.

While the bicycle was equipped with a non-Global Positioning System (GPS) type cycle-computer, the rider routinely used Strava on his smart phone. Strava is an application for tracking runners and cyclists on a social network for athletes. Among other features, Strava users can track equipment use, personal goals, individual rides, accumulated mileage, and standings among other riders on similar route segments; however, this smart phone was passcode protected and thus attempts to retrieve data related to the incident from the Subscriber Identity Module (SIM) card were unsuccessful.

Alternatively, the Activity Feed from two prior months was analyzed on the Strava account to determine a behavior pattern with regard to the rider's speed where the incident occurred. The Activity Feed is a timeline of the rides uploaded to a user's account. Clicking a ride link loads a page featuring a map view of a planned route and specific details such as ride time, distance, average speed, elevation, and energy output. Selecting the Analysis tab on the ride page provides time-base detail, such as current speed, estimated power output, heart rate (if captured), and ambient temperature. Using the mouse pointer and swiping either left or right, a dot on the route map advances forward or backward to indicate the rider's location on the route and their corresponding time-base data is featured below the map.

Using the Analysis tab, the incident rider's maximum speed on the steep downhill section was between 30.9mph and 36.7mph, with an average of 33.8mph. Narrowing the search to the approximate area in question revealed the

rider's speed was between 27.3mph and 34.7mph, with an average of 30.7mph.

The speed of the bicyclist was calculated to be approximately 28.5mph at the time his front wheel struck the steel plate on the road. This calculated incident rider's speed was then validated by noting a behavior pattern established by analyzing the rider's Strava account data.

Strava, Bicycle, Reconstruction

D35 Data Integrity Issues for Micro-Computed X-Ray Tomography (µ-CT) in Forensic Applications

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After attending this presentation, attendees will understand the different artifacts that can influence µ-CT issues and metrological issues that affect the data.

This presentation will impact the forensic science community by creating greater awareness of the issues that need to be taken into account when presenting µ-CT data.

X-ray computed tomography has been widely used in forensic applications for a number of years, in particular as a means of performing virtual autopsies to determine cause of death. µ-CT has also been used to investigate tool marks on bones and for the analysis of the remains of Richard III. µ-CT has a number of advantages over traditional X-ray computed tomography; most notably, it offers greater resolution and magnification. There are a number of artifacts that can occur in CT including noise, beam hardening, scatter, helical, ring and metal artifacts. Some of these artifacts can be reduced by the use of filters and collimators. There are also important considerations for the accurate dimensional use of µ-CT; thus the recording of magnification and resolution and the metrology of measurements is critically important for accurate forensic use of this technique. Nevertheless, µ-CT does allow the measurement of internal dimensions in a way that is not accessible by other techniques.

In µ-CT, a beam of X-rays is generated and focused, usually into a cone-beam. This beam of X-rays travels through the sample and as it does so, the intensity of the X-rays is attenuated. Attenuation depends on the energy (frequency) of the X-ray radiation, the material's density, element number, and the length of penetration of X-rays through that material. X-rays unattenuated by the sample reach the detector. The intensity of these X-rays is registered by the detector and converted to a digital signal. The intensity of X-rays at a particular pixel forms the radiographic image. Many radiographs are taken as a sample is rotated through a small angle while exposed to the beam.

Laboratory µ-CTs use a "white" polychromatic beam to generate as many photons as possible to obtain statistically reliable images within a reasonable time, but this can lead to beam hardening artifacts. Beam hardening is manifested on an image as dark streaks. Beam hardening arises from lower energy photons being more readily attenuated than higher energy photons. This means that beam transmission is complex and not a simple exponential decay as seen with monochromatic X-ray beams. Filters and collimators can be used to reduce the effect of beam hardening artifacts; the range of available filters and collimators will be discussed in relationship to the analysis of gunshot residue.

The radiographs obtained in the µ-CT scanning are reconstructed by software to form a 3D volumetric model. The model consists of 3D voxels (volumetric pixels) of varying gray levels depending on the attenuation at that point. The gray values correspond to varying material properties. Detection of edges between two materials is achieved by interrogating gray level thresholds. Surface points are extracted from this by defining a sampling interval on the surface model. The surfaces can then be used for defining and analyzing sample dimensions and whether or not another material is present.

The magnification and resolution of µ-CT images depends on the relative distances between the source, the sample, and the detector. In practical terms, how close the sample can go to either the source or detector depends on its size because the sample must be rotated by 360˚ to form the volumetric 3D surface. The spatial resolution of µ-CT can be down to 1µm.

Metrology from measurements in µ-CT needs to be performed with care as the dimensional analysis of samples with known geometries shows that there are different relative errors in different analysis directions. The current understanding of the dimensional fidelity with respect to reference standards will be discussed.

The use of micro-computed tomography for investigation of a number of forensic issues will be considered, including measurement of tool marks on bone, detection of voids in welds, development of the internal structure of blow flies, and detection of metallic fragments from gunshot residue and Improvised Explosive Devices (IEDs).

The use of phantoms for determining the composition of debris will also be illustrated.

Artifacts, μ-CT, Metrology

D36 A Methodologies Comparison of Crimes Against the Environment in the Brazilian Amazon

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After attending this presentation, attendees will understand the productivity gain in forensic analyses in crimes against the environment using drones (remote piloted aircraft) with image survey capabilities.

This presentation will impact the forensic science community by discussing the benefits of using drone-acquired and post-processed images in the characterization of criminal environmental damage by generating orthomosaic images and digital elevation models using drones to not only obtain data that proves, but also better quantifies, environmental crimes.

Current forensic methods for planning and conducting the actual analysis of environmental damage involve the use of satellite images; however, satellite images of the Brazilian Amazon region are of insufficient image quality due to the large territory surveyed and the frequency of cloud cover. Limitations of these particular images underscore the need for improved imaging and analysis tools to assess the presence and extent of crimes against the environment in this region.

A cooperative effort between the Federal Police and the Ministry of Science and Technology (via the Financier of Studies and Projects) has provided funds to purchase equipment, including a drone, and develop new methods for assessing crimes against the environment. Often, criminal activities consist of extracting natural resources from protected areas and selling these resources as though they were obtained from authorized sources. These resources include, but are not limited to, wood, minerals, animals, or plant materials. The type and size of these illegal activities define the time, personnel, and budget required by the police to record the environmental damage. Experts conduct a field examination in the area where the alleged illegal activities have occurred. Various measuring devices are used to quantify the area, perimeter, and volume of illegal activity (e.g., logging of trees or removal of sand). Because of the area and duration of required police operations, in addition to the precision of acquired images, security and operational costs are main concerns. The use of drones to obtain high-resolution orthomosaic images allows experts to perform environmental measurements faster, with better quality, and are sometimes the only way to assess the environment damage due to terrain inaccessibility.

The comparison of methods is based on a visit to six environmental crime sites in the Brazilian Amazon forest, where two teams of forensics experts map the areas and collect relevant data. One team used traditional assessment tools, including a precision Global Positioning System (GPS), walking or driving the area, and measuring the depth of illegal mining by hand. The second team used drone flights to acquire images of the area, from which post-processing of the resulting orthomosaic images were performed and the environmental crimes were quantified.

Drone technology was associated with high-productivity gains. For example, an on-the-ground team needed three hours to quantify the extent of illegal sand extraction from an area, whereas comparable measurements were available following a 30-minute drone flight. This does not include the time required to process the drone images needed to generate the generation of orthomosaics used for these measurements.

The conclusion is that the use of drone technology to assess environmental crimes allows reduced costs, greater measurement accuracy, measurements in virtually all areas (especially those otherwise inaccessible), and the ability to virtually revisit the site and identify new features of interest.

Brazilian Amazon Forest, Environmental Crime, Drone Image Acquisition

D37 Technical Guiding Elements for Forensic Analysis of Tailings Dam Breaks: A Case Study From the Brazilian Federal Police

Leonardo Souza, Rua Nascimento Gurgel 30, Belo Horizonte, Minas Gerais, BRAZIL*

After attending this presentation, attendees will better understand current efforts being made regarding a forensic science subject that is less developed, particularly in Brazil.

This presentation will impact the forensic science community by presenting the singularities of a catastrophic event that occurred in Brazil, revealing the main points of interest for future investigations of similar cases.

Currently, the economic viability of global mining necessarily implies a cost reduction in tailings dams. In such a context, upstream raising techniques are often taken as a recurrent solution, in spite of being a riskier one, almost always demanding the adoption of quality-controlled operations and more rigid and conservative security protocols.

In the interest of criminal inquiry led by the Brazilian Federal Police (BFP), forensic science experts were asked to take part in the case of a recent catastrophic event related to a massive-scale tailings dam failure that occurred in Brazil last year (November 5) the outcome of which was drawn the attention of the international press. After strenuous work, the forensic Brazilian experts group developed new technical guiding elements to drive the initial work of similar cases. Such elements would comprise various dam stages (project, construction, operation, and deactivation) and would consider, among other points: the general characterization of the damming structures and their internal and external draining devices; the presence of spare safety structures; the establishment of technical premises for keeping good permeability conditions at the tailings according to its properties and its disposal techniques; the use of separated and independent reservoirs according to different types of tailings; the potential disruptions in the structural security throughout its working life; excessive raising of rates; proneness to liquefaction at valve-controlled tailings according to scientifically approved testing methods; occasional modifications in geotechnical properties of soil used in stability analysis, in accordance with on-site inspection; a periodic review of dynamic loads and instrumentation gauges on the basis of the dam functioning.

The failure that took place at the "Fundão" dam, located in Brazil in the city of Mariana, state of Minas Gerais, also caused a stir in the scientific community, revealing the urgent need for improvements in the local protocols devoted to environmental licensing, together with revisions of Brazilian technical standards so as to ensure greater security levels in this raising type of dam. The disaster occurred in the area surrounding the district of Bento Rodrigues, near the so-called "Germano" mine, an open-cast ore mine controlled by the Samarco Company, which belongs to a joint venture from VALE S.A. (a Brazilian company) and BHP Billiton Limited Plc (an Anglo-Australian company) and was created to explore low iron content material in that site. The disaster result in nearly 20 casualties, including not only dam workers, but also towns people residing immediately downstream, in addition to causing extensive harmful environmental effects related to the slurry wave that flowed more than 600km along streams and river basins, surpassing the state limits, and reaching the Atlantic Ocean.

It was concluded that the accident was caused by not only one factor, but a combination of the previously referred to factors, which were maximized by the absence of an in-depth approach in the Brazilian standards, as well as by the lack of a governmental well-prepared supervisory body, in compliance with the current increasing demands for tailing dams in this country.

Tailing Dam, Break, Analysis

D38 Takata Airbag Inflator Module Failure and Metal Fragment Analysis: Death Number Nine

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After attending this presentation, attendees will better understand how Polarized Light Microscopy (PLM), Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS), Fourier Transform Infrared Microspectroscopy (FTIR), and 3D X-ray computed tomography were used to determine the source of a metal projectile recovered from the driver of a 2006 Ford® Ranger® after a fatal accident.

This presentation will impact the forensic science community by providing information to assist in the investigation of fatalities that were the result of airbag inflator module shrapnel.

The Takata airbag module recall is the largest automobile manufacturer recall in history. A brief history of airbag design, construction, and airbag recall history will be provided to attendees to provide the background of the investigation. While being driven, a 2006 Ford® Ranger® airbag deployed when the vehicle collided with an object in the road resulting in the death of the driver.

At autopsy, a metal fragment, approximately 22.6 millimeters in diameter, was recovered from the deceased Ford® Ranger® driver. MVA Scientific Consultants was asked by the Lancaster County coroner's department to determine the source of the metal fragment. Visual examination was performed of the submitted airbag module, which consists of a plastic cover (the piece in the center of the steering wheel covering the folded undeployed airbag), the airbag itself, and the airbag inflator. An airbag initiator is located within the airbag inflator. The base of the airbag initiator points toward the steering wheel nut (away from the driver) and contains the electrical connector used to activate the airbag when a collision is detected. The metal fragment from the deceased and the airbag module (less the airbag) were examined. Images supplied by the coroner's office revealed a second metal fragment impaled on the steering wheel nut that resembled the metal fragment obtained from the deceased. Micro chemical testing using the hanging drop method (chloroplatinic acid) and PLM were used to confirm the presence of ammonium ions on this metal fragment. Other residues, such as strontium, were detected on the airbag inflator components using SEM/EDS. Adhesive tape residue on the metal fragment recovered from the deceased was consistent in physical characteristics and chemical composition with tape residue on the airbag inflator as determined by FTIR microscopy. Fracture surfaces on the metal fragment from the deceased and on the metal fragment impaled on the steering wheel nut demonstrated that the two fragments were originally from the same piece of steel (the initiator). Pressures produced during detonation of the airbag inflator were sufficient to distort the steel inflator housing. 3D X-ray computed tomography of a detonated exemplar airbag inflator revealed the internal structure, initiator position, and construction of the Takata inflator. The metal fragment collected from the deceased was the result of the steel initiator fracturing then coring through the steel inflator module housing, rupturing the airbag and penetrating the neck and spine of the deceased.

Reference(s):

1. Roger W. Barrette, Adam M. Hyde, Richard S. Brown. Investigation of Hit and Run Crashes, Chapter 14, *Traffic Crash Investigation* 11th Ed., J. Stannard Baker, Lynn B. Fricke, Eds. Northwestern University Center for Public Safety, Evanston, IL. 2014.

Microscopy, Takata Airbag, Death Investigation

D39 Vehicle Interior Surface Witness Marks Observed in the Analysis of Traffic Collisions

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The goal of this presentation is to exemplify how witness marks observed on vehicle interior surfaces can be used, together with the knowledge of vehicle collision type, to determine restraint use and injury causation.

This presentation will impact the forensic science community by demonstrating how physical evidence created during occupant impacts with interior vehicle structures during frontal, rear, lateral, or rollover collisions can be vital in correctly identifying seating position, restraint function, and cause of injury.

Human occupant motion within a vehicle as a result of the forces experienced during a collision will often result in forceful impacts between the occupant and vehicle interior (or exterior, if the occupant is ejected). The marks left on seat belt webbing and hardware (or lack thereof) is regularly utilized in analyzing restraint use and function. This presentation will focus on vehicle interior surface evidence from occupant interactions during motor vehicle collisions.

In collinear collisions, occupants generally move in a direction opposite the vehicle's velocity change vector (i.e., toward the impact location). Therefore, collision type (frontal, lateral, rear, or rollover) will determine where to look for telltale physical evidence. When two objects collide, forces are transferred between the two objects at the point of contact. The force applied to the human body from contacting the vehicle interior may cause injury. Likewise, the equal but opposite force applied by the occupant to the vehicle interior may cause disruption, damage, or failure of the impacted vehicle interior structures. The physical evidence (witness marks) often observed as a result of these interactions includes blood stains, tissue deposits, hair deposits, fractured glass, steering wheel rim deformation, seat back deformation, and plastic trim deformation, as well as scuffs, abrasions, and cracks on various vehicle interior surfaces.

Occupant motion within the vehicle interior will depend on dynamics, the occupant's initial position, and restraint design and use. Effective restraint designs mitigate injury risk through the reduction of impact force magnitude and distribution of those forces over a larger body area. Oftentimes, the presence and location of witness marks will aid in the determination of restraint use and restraint effectiveness.

In frontal crashes, the velocity change vector is directed from front to rear, so an occupant's initial (primary) motion will be toward the front interior surfaces. Interior surfaces offering evidence of contact by front seat occupants include the sun visor, windshield, A-pillar, steering wheel, dashboard, glove box, knee bolster, and center console. If airbags deploy, then the airbag fabric may also reveal evidence of occupant interaction. Rear seat occupants are exposed to impact with the front seat assemblies, B-pillar, or center console, depending on occupant seating position and the precise orientation of the velocity change vector.

In rear-end crashes, the velocity change vector is directed from rear to front, so the rear interior surfaces are among those potentially exposed to occupant impact. For front seat occupants, these surfaces include the front seat assembly and head restraint, the rear seat, the rear roof pillars, the rear cargo area, the rear window, and the rear roof header. For more severe impacts, potential contacts may also occur with cargo from the trunk or intrusion by the striking vehicle. Although their primary motion is not toward the front, rear seat occupants can be contacted by a front seat assembly if it collapses rearward into their space. For rear seat occupants, these surfaces include all those listed for the front seat occupants, except that the front seat assembly is a factor only if it collapses and intrudes into their occupant space.

In near-side or far-side lateral crashes, the occupant's primary motion will be toward the side of the impact. Interior surfaces to examine for potential contact evidence include, but are not limited to, the doors, window glass and window frame, roof pillars, center console, and deployed airbags.

The most common type of rollover collision is a barrel-type rollover. In barrel-type rollovers, the occupants tend to move in a direction away from the roll axis. In doing so, these occupants will also be moving toward, and eventually impact in a direction toward the ground in what are typically fairly low velocity events because the majority of the energy possessed by the vehicle continues along the roll trajectory. Consequently, depending on

the number and orientation of ground contacts during a particular rollover crash and depending on the preservation of the occupant survival space, the interior surfaces available for occupant contact are many, and include those identified in front, rear, and side crashes. Although not limited to rollovers, contacts with the headliner are frequently observed in a rollover. Additional interior surfaces into which moving occupants come in contact during collisions include doors and door handles, center consoles, dome light covers, and deployed airbags.

In all types of crashes, seat belt use and structural integrity of the occupant compartment, which are foremost in occupant containment, have roles in the location, type, and significance of the physical evidence observed on the vehicle interior.

Vehicle Interior, Occupant Contact, Witness Marks

D40 An Array of ZnO and SnO_2 Heterojunction Semiconducting Metal Oxide Gas Sensors Used as a Tool for Explosive Detection

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After attending this presentation, attendees will recognize the current technological difficulties involved regarding explosives detection, the importance of explosive gas sensing for threat detection, and the new possible technologies for sensing explosive gases.

This presentation will impact the forensic science community by demonstrating the threat to national and global security posed by terrorists' use of explosives and the efforts to develop new methods of disaster prevention through the detection of explosive gases.

Terrorists frequently use explosives and represent an imminent threat to national and global security. Recent events highlight the necessity of explosive detection, demonstrating the need for developing and applying new sensors for explosive gas detection. Currently sniffer dogs provide the highest sensitivity when detecting explosives; however, the costs associated with the training, together with the limited information produced from the sniffer dog, establishes the need to improve current technology within explosive detection. Semiconducting metal oxide gas sensors can be incorporated into electronic noses, which provide a cheap, portable, and highly sensitive device, therefore making them a reliable method when detecting explosives.

Using unmodified, admixed, and 2-layered sensors consisting of ZnO and SnO_2, an array of seven heterojunction semiconducting metal oxide sensors was produced. A heterojunction is the combination of two dissimilar metal oxides with differing band gaps. Creating a heterojunction with metal oxides ZnO and SnO_2 with wide band gaps of 3.4 and 3.6, respectively, has improved sensing properties. Therefore, having already yielded some promising results, ZnO and SnO_2 are a good heterojunction to be tested against the range of explosive-associated gases.

The sensors were produced by screen printing the metal oxide inks onto 3mm by 3mm alumina substrates with gold interdigitated patterned electrodes on the top and integrated platinum resistance heater tracks underneath. All seven sensors were tested against gases associated with explosive materials at 300°C, 400°C, and 500°C. Characterization techniques were performed in order to establish if any structural changes occurred to the array due to the exposure of the gases or temperatures. All sensors produced underwent X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDX), and Raman spectroscopy, both before and after being exposed to the test gases. No changes to the structure of the metal oxides were detected.

Both the admixtures and the 2-layered semiconducting metal oxide gas sensors have been shown to enhance sensor response when detecting explosive associated gases. The admixed semiconducting metal oxide gas sensors have increased responses to NO_2, 2-ethylhexanol, nitromethane and ammonia when compared to the unmodified metal oxides; whereas, the produced 2-layered sensors, proved highly successful when detecting 2,3-dimethyl-2,3-dinitrobutane (DMNB). The data collected was processed against a support vector machine in order to comprehend the sensors application into an electronic nose. The technique produced a high data classification when classifying the gases used within the study. Therefore, the array produced has successfully discriminated the test gases from one another; consequently, showing the potential use of the array implemented into an electronic nose for the use of explosive detection to be an effective method.

Gas Sensing, Explosives, Heterojunctions

D41 A Discussion of Testing Methods to Understand the Interaction of Power Brake Boost and Power Steering Boost in Ford® Hydro-Boost Systems in Panic Braking Situations

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After attending this presentation, attendees will better understand the mechanism of operation of hydro-boost brake systems, the development of test protocols for laboratory and field characterization of hydro-boost brake system operation, and how to analyze the resulting test data.

This presentation will impact the forensic science community by illustrating how to test and analyze a complex system to understand its operation.

Following a serious motor vehicle collision, it is not uncommon for the driver to describe a control system (steering, braking, or other vehicle component) failure. This presentation benefits the forensic engineering community by demonstrating a systematic method to investigate a specific type of vehicle control issue that can manifest in panic situations immediately preceding a collision.

Some light-duty trucks use a hydro-boost power braking system, so named because the power steering pump is used to provide a hydraulic boost for both steering and braking assistance. During certain types of heavy brake application, such as occurs during panic braking, the amount of boost to the power steering system is limited. This situation results in a significant increase in steering effort, a condition that many drivers perceive as locked steering. The increased steering effort, or the perception of locked vehicle steering, can lead to dangerous vehicle handling. Unfortunately, boost available to the power steering system does not return to normal until the brake pedal has been released, which typically occurs after the problem causing the panic braking has either manifested or is no longer a threat.

This issue can occur in any vehicle that uses hydro-boost power braking. Historically, this behavior has been most prevalent in early model-year 200X Ford® pickups. For the present example, a 2001 Ford® F350 Super Duty diesel was used.

The demonstrations were documented with video cameras and the vehicle was fully instrumented to measure steering angle, rate and effort, brake pedal force and travel, power steering fluid flow and boost pressure, and power brake boost pressure. A robotic steering machine was used to produce consistent and precise steering maneuvers. Reproducible braking inputs were provided by an automatic pressure-controlled brake pedal applicator that could be timed with steering inputs.

The vehicle's hydro-boost system, as originally configured, was characterized in a step-wise fashion as listed: (1) measure fluid flow rate and pressure over a range of engine speeds; (2) measure fluid flow rate over a range of brake pedal pressures; (3) measure the steering response to a step-steer input at ten different steering rates after low pressure brake application; and, (4) measure the steering response to a step-steer input at ten different steering rates after high-pressure brake application.

Finally, the behavior of the factory-installed hydraulic system was compared to a modified hydraulic system in which the power braking and power steering systems are augmented. This was accomplished by using a portable hydraulic pump, driven by a small gasoline engine, in addition to the necessary hydraulic plumbing and valving, to boost the power steering system. Operator-controlled use of this valving allowed boost to the power steering system to be rapidly switched from the vehicle's power steering pump to the external pump, leaving the vehicle's power steering pump to supply only the power brake system.

Repeating the step-wise characterization of the hydraulic system behavior, as noted in the steps listed above, using the modified system allowed comparison between the original and test configurations. Most importantly, interactions between the power braking and steering systems in the original system were demonstrated and compared with the modified system under identical circumstances but for the removal of that interaction.

This study demonstrated that during hard brake application, the power steering pump cannot supply sufficient flow at sufficient pressure to supply needed hydraulic assist to both the braking system and the steering system. During hard braking, the brake system consumes most of the hydraulic energy and an insufficient amount remains

to provide normal steering assist. This results in a dangerous condition in which there is a significant increase in steering effort, which drivers can perceive as locked steering.

Hydro-Boost, Power Steering, Power Brakes

E1 Bloodstain Pattern Analysis Using 3D Laser Scanning Technology

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After attending this presentation, attendees will understand: (1) the use of 3D laser technology for the documentation of bloodstain patterns; and, (2) limitations of the 3D laser scanner for reconstruction purposes and bloodstain patterns.

This presentation will impact the forensic science community by providing research results for the use of 3D laser scanning technology in the area of crime scene documentation and reconstruction.

Currently 3D laser scanning technology is used to document crime scenes and physical evidence. Patterned evidence is one type of physical evidence commonly encountered at a crime scene and can be used to reconstruct the crime scene. Pattern evidence is made in a repeated and predictable manner, especially bloodstain patterns. By using the 3D laser scanner for documentation of physical evidence and the location of blood spatter in relation to the rest of the evidence, it may be used to reconstruct a bloodshed event. This project examined the ability of the 3D laser scanner to adequately document prepared bloodstain patterns; therefore, the use of the laser scanner would allow processing of the scene to take less time while maintaining the high accuracy needed for the reconstruction of the scene, specifically bloodstain pattern analysis.

White butcher paper was taped to a white board and pig blood was thrown against the paper, mimicking impact spatter stains and a transfer pattern. Traditional photographs were taken of the bloodstain patterns using a Nikon® D3300 digital camera with 18mm-55mm and 55mm-200mm lenses. A Leica® ScanStation C10 laser scanner was set up following the user manual. Throughout the designated space where the bloodstain patterns were placed, two six-inch targets were utilized in addition to the twin target. For the first scanning process, the scanner was five feet away from the bloodstained wall and a medium-resolution scan was performed. After the initial medium-resolution scans of the entire room, three highest-resolution scans were used for the bloodstain patterns. A second scanning was conducted with the laser scanner 17 feet away from the blood patterns. The scanning data was downloaded for compilation and image preparation by the Cyclone software into a ModelSpace view, which creates unified images of all data points with a point spacing of 0.01 for pixilation quality. Within the formulated image, manipulation of excess regions was eliminated and cleaned up before moving on to TruView™. The ModelSpace view created in Cyclone was utilized for the next step. Each scan that was registered was incorporated into one overall TruView™ site map. Within the TruView™ there were embedded images from the traditional photography. The 3D laser scans resolution and pixel quality were compared to the embedded photographic images.

After completion of the scans in Cyclone and TruView™, the resolution of the laser scans was not as high as the traditional photographs taken of each bloodstain pattern. With the distorted resolution in TruView™, the quality of the scanned image made it very difficult to determine the origin of the bloodstain patterns and, therefore, the photographs of the patterns must be embedded in the TruView™ for reconstruction purposes. Perhaps in the future, advanced algorithms or software may allow for the increase in the resolution of the bloodstain patterns when using the 3D laser scanner.

3D Laser Scanning, Bloodstain Patterns, Crime Scene Reconstruction

E2 A Combined Method of Detection for Organic and Inorganic Gunshot Residue (GSR)

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After attending this presentation, attendees will better understand current GSR presumptive test limitations and alternative GSR presumptive testing methods using reliable colorimetric color spot tests that do not affect subsequent analysis using common instrumentation methods.

This presentation will impact the forensic science community by proposing an alternative GSR presumptive test method using reagents with long storage life, based upon color mechanisms with color production specific to compounds in GSR, allowing simultaneous testing of inorganic and organic GSR. This will impact crime scene analysis domestically and internationally for deployed forensics units to rapidly analyze samples and provide a rapid and dependable response.

GSR is comprised of burned and unburned smokeless powder particulates and metallic particles that are expelled from the muzzle of a firearm in a vaporous plume; these are deposited onto the face, hands, and clothing of the shooter and can be used to potentially identify a suspect in a criminal investigation. Currently, crime scene technicians use an aluminum stub with a carbon adhesive to collect these particulates, further subdivided into Organic (OGSR) and Inorganic (IGSR). Presumptive GSR tests use colorimetric determination to identify the presence of some OGSR and IGSR while confirmatory GSR tests use Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy (SEM/EDX) to analyze the type of metal present. The SEM/EDX is used to visualize specific topographical morphology of the metallic particulates and confirm an elemental composition of the traditional lead-barium-antimony composite. Presumptive IGSR tests have limitations due to their specificity to lead and not to GSR and nullification in the case of lead-free primers. Presumptive OGSR tests produce false positives to environmental contaminants and are also non-specific, producing color responses to common nitrated compounds. Simultaneous testing for I/OGSR currently requires that the already miniscule sample amount be split into two portions, impacting downstream analytical techniques.

The goal is to develop a presumptive I/OGSR colorimetric test that does not interfere with the classic SEM/EDX analysis of IGSR and would allow the simultaneous detection of GSR without splitting the sample and thus utilizing all components available. Organic color spot tests such as nitrous acid, 4-nitrosophenol, and sodium borohydride are explored. The tests are evaluated in four stages on differing samples: (1) on individual compounds contained in GSR and the environment for specificity; (2) on limited mixtures of three to four inorganic and organic components to evaluate effectiveness of color production and duration; (3) on all-inclusive mixtures of known inorganic and organic GSR components to evaluate using the SEM/EDX for the presence of inorganic components; and, (4) on real-world samples for proof of theory and determination of limit of detection.

The three colorimetric tests above passed the first three stages; however, 4-nitrosophenol and sodium borohydride passed all stages with specificity, visible differentiation of color production, and did not adversely affect the elemental composition of lead-barium-antimony for lead ammunition and zinc-titanium-potassium-copper for lead-free ammunition. This provides the foundation that a simultaneous test for I/OGSR can be used both in the field for rapid determinations and, subsequently, in the laboratory for confirmation of the elemental analysis.

Gunshot Residue, Colorimetric, Spot Test

E3 Automated Fingerprint Identification System (AFIS) -Based Likelihood Ratios for Latent Fingerprint Comparisons

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After attending this presentation, attendees will understand the different variables, such as match score, match minutiae, and minutiae matched, that influence match results obtained during an AFIS database search.

This presentation will impact the forensic science community by providing attendees with a possible explanation regarding the workings of the AFIS system by analyzing the inter-dependency of features in determining a true match/non-match and the accuracy of the system.

Latent fingerprints are one of the most common pieces of evidence found on a crime scene that represent accidental or unintentional prints collected as part of a criminal investigation. They are caused by the friction ridge skin deposition on a surface, hence requiring the use of chemical processing to be visualized with the naked eye. While fingerprint evidence itself is very reliable, the comparison and identification of fingerprints depends on various factors, such as the substrate quality, surface, duration, environmental factors, and examiner experience. These factors can result in reduced clarity, content, and even distortions as compared to a fingerprint taken under controlled conditions. Since the release of the National Academy of Sciences (NAS) Report in 2009, the field of fingerprint analysis has come under much scrutiny.[1] Specifically, the need for more research into the determination of the accuracy and reliability of the identifications made by fingerprint examiners has been raised.

One method used for the comparison of latent fingerprints to known prints is through an AFIS. The performance of the AFIS was measured using the AFIX Tracker® software where the variability of the data produced was analyzed using the match score, minutia marked, fingers matched, and matching minutiae. The Biocop database was used which contained 962 ten-print cards and latents developed through ninhydrin, cyanoacrylate fuming, and black powder. The quality of the prints was assessed using the National Institute of Standards and Technology (NIST) Fingerprint Image Quality (NFIQ) score in which each latent was scored from one-five to determine its quality. A 70-30 approach was then used in which 70% of the (known) prints were used to test the accuracy of the system and 30% of the prints were unknown prints. A Bayesian network was constructed to perform statistical analysis of the matches obtained while comparing a latent print to a known (ten-print) card where the match score, match minutiae, match status, fingers matched and NIST scores were the nodes analyzed. A preliminary analysis of the results revealed that true matches were only found when more than 25 minutiae were marked on the latent, yielding a match score of $6x10^5$ or higher while 90% of the match scores for the non-matches ranged from 55,000-1x105. This research project may help explain different fingerprint results obtained based on the match score, database size, and number of minutiae marked.

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Latent Fingerprints, AFIS, Accuracy

E4 The Use of Near Infrared Spectroscopy (NIRS) to Discriminate Wood Species and Determine Their Origins

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After attending this presentation, attendees will better understand the use of recent fast and clean wood species discrimination methods using samples. Traditional methods use microscopy and require more time and specific knowledge related to wood anatomical features that are needed for species identification and discrimination.[1]

This presentation will impact the forensic science community by providing test results gathered from a native Brazilian wood species, which is based on the data obtained by the discrimination of both protected and unprotected wood species and the determination of their origins.[2,3] This presentation will add to forensic research the possible use of portable NIRS as a tool that has the potential of being deployed in the field to improve both fraud detection and the investigation of environmental crimes related to the timber trade.

Unfortunately, the timber trade sometimes involves species of prohibited trade, by either international treaties or national environmental rules. Prohibited trade species present anatomical similarities and general features common to other commercial species, hindering the fraud combat actions involving this activity. The Brazilian Nut (*Bertholletia excelsa* Bonpl.), for example, is a protected species and has similarities to other genres of unprotected commercial species belonging to the same botanical family. Loggers often try to sell Brazilian Nut under the guise of another similar unprotected species or try to sell one wood species allowed to be commercialized from origin "A," while, in truth, it comes from origin "B."

Spectral NIR information analysis was conducted using statistical multivariate analysis in order to discriminate between non-protected commercial species of the Lecythidaceae family, and Brazilian Nut, as well as to determine different origins. Techniques used were Partial Least Squares Discriminant Analysis (PLSDA) and analysis of the K- Nearest Neighbor (KNN). Discrimination models were designed for the following species: *A. decandra, A. lineata, B. excelsa*, *C. micranta*, *C. ubra*, *Eschweilera* sp., and samples of a single species from different origins were also analyzed for *A. decandra* and *A. lineata*.

A total of 1,615 spectra were obtained from 323 samples, between 6,270 and 4,172cm^{-1} wavenumbers, using the portable microPhazir™ (Thermo Scientifics) and the software Method Generator, version 4.0 R2. Root Mean Square Error of Cross Validation (RMSECV) and Root Mean Square Error of Prediction (RMSEP) were used to choose the best pre-processing parameters as well as for choosing the best spectral ranges for discrimination. The number of latent variables and the limits of discrimination between classes were also evaluated for the PLSDA models.

The KNN model displayed correct identifications in 96.3% of the samples. The PLSDA models presented correct identifications between 83% and 100% in five of six species evaluated and 24.21% for *A. decandra*.

A. lineata from two distinct sources were identified with a success rate of 82% for origin A and 92.5% for origin B by the KNN model. Using PLSDA models, the success rate was 44.0% for origin A and 100% for origin B. As to *A. decandra,* samples from two distinct origins were identified with a success rate of 100% by the KNN model. The success rate was 100% for origin A and 52.63% for origin B using PLSDA models.

The NIRS and statistical methods used were successful in discriminating most species analyzed and were able to determine different origins of samples in the two analyzed species, thus presenting a promising tool to detect fraud in the exploration and wood trade.

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Wood, Discrimination, Spectroscopy

E5 Development and Validation of an Analytical Protocol for the Characterization of Lubricant Evidence

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After attending this presentation, attendees will have a fundamental understanding of the significance of lubricant evidence as it pertains to sexual assault investigations. Additionally, attendees will be familiar with a rapid analytical protocol involving Direct Analysis in Real Time-Mass Spectrometry (DART®-MS) for the chemical interrogation of lubricant evidence.

This presentation will impact the forensic science community by demonstrating a unique analytical methodology for the forensic analysis of lubricant evidence. As the forensic analysis of lubricant evidence is a relatively new concept in sexual assault investigations, the following research seeks to highlight the potential for the developed classification scheme to aid not only in the identification of unknown lubricants following a sexual assault, but also add credibility in instances where questioned vs. known comparisons are possible.

Unfortunately, sexual assaults are a reality in modern society, with recent statistics revealing that roughly one in five women will experience a sexual assault in her lifetime. As condom usage has also increased in instances of sexual assaults, further emphasis must be placed on the analysis of lubricant evidence to provide an evidential link between the victim and assailant. Conventional techniques for lubricant analysis, such as gas chromatography/mass spectrometry and Fourier transform infrared spectroscopy are adept at identifying the major components or base of the lubricant; however, due to the significant concentration of the base constituents, these techniques frequently have trouble isolating and identifying the minor components that may provide more discriminating information.

For this research, a classification scheme for the characterization of lubricants was developed using DART®-MS. DART®-MS is an ambient ionization technique capable of rapidly characterizing samples in any physical state with high resolution and accurate mass detection, while requiring minimal sample preparation. This technique was employed to rapidly analyze more than 100 water- and silicone-based personal and condom lubricants, generating more than 500 mass spectra in the positive-ion mode. Multivariate statistical analysis in the form of agglomerative hierarchical clustering, principal component, and linear discriminant analysis was used to interpret the resultant mass spectral data. Statistical analysis of the mass spectral data revealed six groups within the lubricant samples that enabled discrimination not only between the three broad classifications (i.e., water/silicone-based personal and condom lubricants) but also within these marketing groups based upon the presence or absence of key additive components (i.e., flavors, sensatory, etc.). Approximately 98% of the data was correctly classified using the leave-one -out cross-validation approach. Samples will continuously be analyzed and implemented into the statistical model, to eventually generate the necessary taxonomy to develop a publicly available lubricant database that may aid in forensic casework.

Lubricant, DART®-MS, Sexual Assault

E6 Distinguishing Condom Lubricants From Personal Hygiene Products (PHPs) Using Direct Analysis in Real Time-Time-of-Flight/Mass Spectrometry (DART®-TOF/MS)

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After attending this presentation, attendees will understand the capabilities of analyzing and identifying samples from sexual assault evidence using DART®-TOF/MS. The power of this technique to differentiate between condom lubricants and PHPs, such as shampoos and lotions, is demonstrated through statistical treatments such as Principle Component Analysis (PCA), Analysis of Variance (ANOVA), and Linear Discriminant Analysis (LDA).

This presentation will impact the forensic science community by serving as a supplemental method that can analyze samples using mass spectrometry that cannot not be analyzed using traditional instrumentation such as a Gas Chromatograph/Mass Spectrometer (GC/MS).

Due to the increased awareness that DNA can be recovered from seminal fluid testing in sexual assault cases and used to identify the assailant, condoms have been used extensively to conceal and prevent conviction. From this, condom lubricants were tested in sexual assault investigations; however, residuals from such components can sometimes resemble common hygiene products found on the skin, such as lotions and soaps. Thus, the purpose of this study is to be able to differentiate condom lubricants from personal hygiene products.

DART®-TOF/MS is a rapid non-destructive method in which the sample is softly ionized and the analyte molecules are desorbed by excited-state helium gas stream. This technique can be operated in either positive or negative mode with protonation of the analyte via reaction with protonated water clusters or electron capture via reaction with oxygen radicals, respectively. This instrument is capable of giving the user a full mass profile within seconds of sampling, with little to no destruction to the sample itself. Sampling is simply done via a glass capillary tube with merely a small coat of the sample on the tip. Thus, for multiple sample analysis and ease of comprehension of data, DART®-TOF/MS is an excellent method for the rapid and non-intrusive evaluation of sexual assault evidence. Unlike traditional GC/MS techniques, coelution issues of and the inability to analyze silicone-based samples are not a mitigating factor in DART®-TOF/MS as there is no column present. Samples are analyzed via direct mass analysis.

In this study, 30 samples consisting of 12 lubricants, 10 PHPs, and 8 different condom types were analyzed. The 12 lubricants were divided further into three distinct groups-corresponding to water, silicone, and oil-based lubricants. The PHPs included shampoo, lotion, sunscreen, soap, petroleum jelly, and oils. Samples were analyzed in both positive and negative mode to obtain a cumulative chemical profile for each sample. Samples were diluted to 1:1 and 1:10 ratios, where the solvents used were methanol for water, oil, and PHPs and hexane for silicone-based lubricants. The diluted samples were analyzed to simulate low-quantity samples that might typically be recovered from the victim. Following sample collection and analysis, PCA, ANOVA, and LDA treatments were performed to visualize and discriminate lubricant samples versus normal hygiene products and to determine if the correct classification can be attributed to an unknown sample.

The goal of this study is to differentiate lubricants from PHPs to further aid in the analysis of sexual assault evidence using the rapid features of DART®-TOF/MS. The ultimate goal is to create a protocol in which lubricants can be examined as common pieces of evidence in a sexual assault case.

DART®-TOF/MS, Lubricants, Personal Hygiene Products

E7 High Resolution Accurate Mass (HRAM) Liquid Chromatography/Mass Spectrometry (LC/MS) Screen for Prostaglandins Found in Consumer Products

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The goals of this presentation are to: (1) identify a class of pharmaceuticals found in unapproved consumer products; and, (2) evaluate the differences in detection and fragmentation between an ion trap mass spectrometer and the high-resolution Thermo Q-Exactive™.

This presentation will impact the forensic science community by bringing awareness to the fact that prostaglandins can be found in consumer products and are detected differently based on the MS being used.

Prostaglandins are used in both human and veterinary pharmaceutical products to treat a variety of symptoms including Raynaud's disease, erectile dysfunction, and glaucoma. Prostaglandins are also used in products to induce childbirth and increase eyelash length. Previously, the Forensic Chemistry Center developed a screening method for prostaglandins to analyze potential counterfeit and unapproved consumer products using LC/MS/MS with a Thermo Linear Trap Quadrupole (LTQ) MS with low-energy Collision-Induced Dissociation (CID) for fragmentation. Incorporating new technology into the screen, the method has been updated using a Thermo Q-Exactive with Higher-Energy Collisional Dissociation (HCD) to obtain new fragmentation patterns of the prostaglandins.

Standards were injected into a Thermo Q-Exactive™ MS with an HCD cell using an Electrospray Ionization (ESI) source, which was used to analyze the standards in both positive and negative mode. The sheath gas was set to ten, the auxiliary to five, and sweep gas was zero. The spray voltage was set to 3.5eV. Fragmentation was conducted utilizing a 4Da window in the targeted mode and the normalized collision energy varied from 10% to 55%. An Agilent® 1200 Ultra High-Performance Liquid Chromatography (UHPLC) with a C18 column was used to introduce the prostaglandins into the MS.

Nine of the prostaglandin standards were analyzed via infusion using an ESI source, which will represent four different prostaglandin classes: E1, E2, F2α, and I2. Once the analyte of interest was detected, fragmentation was performed using All-Ion Fragmentation (AIF), as well as using a targeted mass window. It was found that using the AIF mode created MS/MS spectra with too many peaks that were not associated with the ion of interest. Therefore, a specific LC/MS method was developed, listing the masses of the prostaglandins and incorporating a small mass window for fragmentation, in order to obtain the most useful MS/MS spectra for compound identification. Because prostaglandins found in pharmaceutical products have very low dosage levels, it is important to optimize the mass spectrometric conditions to obtain the highest sensitivity for a given analyte. All of the standards were run in positive mode only, then negative mode only, and peak intensities were measured to optimize sensitivity. Infusing the standards, bimatoprost provides a higher intensity in positive mode using the Q-Exactive™, but a higher intensity in negative mode using the LTQ, indicating that the current LC/MS method may need to be re-evaluated. In addition, new limits of detection will be determined using the new Thermo Q-Exactive™ MS. As expected, utilizing HCD on the Q-Exactive™ gave different fragmentation patterns with more fragment ions for each of the prostaglandins studied than those obtained using CID, eliminating the need to collect MS3 data. An updated LC method combined with optimized MS conditions was applied to detect prostaglandins in consumer products using the Q-Exactive™.

Mass Spectrometry, Prostaglandins, Unapproved Consumer Products

E8 Bioaffinity-Based Concepts in Forensic Serology

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After attending this presentation, attendees will understand that bioaffinity-based methods for the analysis of body fluids offer simplicity compared to traditional forensic analyses of such samples. In addition, attendees will also understand the concept of using such body fluids to identify originator attributes in a quick and straightforward manner.

This presentation will impact the forensic science community by providing new methods for the analysis of body fluids in order to generate essential information directly at a crime scene. Ultimately, these systems can be incorporated into field-deployable devices (similar to glucometers) or connected to hand-held smart devices, which will allow for rapid analysis of body fluids that can be used and interpreted by operators with no scientific training, thus revolutionizing the "front end" of forensic science.

The analysis of biomarkers has been used in the field of forensics for many years in the form of DNA (usually from blood) for identification purposes; however, the process of matching DNA samples is very time consuming and causes backlogs in many states. While this is a useful tool, it may not be the best method of analysis during an active criminal investigation. There are many other biomarkers present in blood that can be analyzed in a much shorter amount of time by utilizing bioaffinity-based cascades. Cascades were developed, and are in the process of being developed, for the purpose of identifying personal attributes from individuals, such as age, biological sex, and general health conditions. These cascades were developed for both blood and fingerprint analysis. The cascades created for blood analysis focused on the determination of the age of the originator and the time since deposition of the sample.

Fingerprint analysis has focused on pictorial comparisons since the process was adapted for forensics. Advances in this area only progressed insofar that automated fingerprint identification systems can be used in certain cases (with an expert checking the results). Because of this, a fingerprint may be determined to be too smudged or smeared to be of use; however, what is often overlooked is that the patterns used to match fingerprint samples are created by sweat/sebum emulsions excreted from the fingertips. Like all bodily excretions, the emulsions have their own unique chemical composition, meaning there are biomarkers present for analysis. One of the cascades developed in this lab focused on the analysis of amino acids in the samples. The cascades developed for fingerprint analysis focused on the determination of biological sex. There is also ongoing research targeted at the development of a larger variety of cascades able to determine other attributes from blood and fingerprint samples.

Bioaffinity, Fingerprint, Blood

E9 Single Analyte Bioaffinity-Based Assays for Body Fluid Analysis

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After attending this presentation, attendees will understand that single analyte bioaffinity-based assays will expedite the process of identification for forensics investigations that involve body fluids such as sweat and blood. The use of the single analyte assays would not only hasten the process, but would allow for a narrowing of the suspect pool without needing a database for comparison and without the potential for compromised attributes. In addition, attendees will come to understand that blood and fingerprints can be more useful for forensic analyses by using methods other than DNA and profile/image comparisons.

This presentation will impact the forensic science community by demonstrating that single analyte bioaffinity-based assays are able to be used on body fluids for a quick response in the identification of the originator and would hopefully be able to be used for on-scene analysis requiring little to no scientific expertise.

Biomarkers are widely used for identification purposes in the field of forensic science. This is usually done with DNA from blood or fingerprints. Even though DNA analysis is incredibly accurate, there have been drawbacks to this method. The major drawback is that DNA analysis is a time-consuming process, leading to a long wait time for results and backlogs in criminal investigations. Another drawback to this process is that there needs to be a matching profile for a positive identification to be made since the method is comparative. Single analyte bioaffinity-based assays are able to analyze biological markers (biomarkers) in blood in order to identify attributes of a person to expedite the identification process during a criminal investigation. These assays are also able to be performed on certain analytes in fingerprints, since fingerprints are formed by sweat and sebum, which contain the biomarkers similar to those found in blood.

Fingerprints are also widely used for forensic purposes, albeit in a narrow role of pictorial comparison based on the ridge patterns of the print itself. Fingerprint analysis is also a lengthy process that causes (backlogs similar backlogs to blood) due to the need for highly trained personnel. In addition, similar to DNA, there needs to be a matching image since this is also performed in comparison to a database. This mindset causes fingerprints that would be usable for other analyses, being smudged or a partial, to be overlooked and not examined. The use of a single analyte bioaffinity-based assay allows the use of these prints and results that exhibit characteristics of the originator without the need for a database or a comparison.

By using single analyte assays, a major problem of multi-analyte cascades is eliminated: compromising results from multiple analytes affected by the same attribute. These attributes provide identification of the person or information about the blood spot or fingerprint itself. For example, the results determine age, race, biological sex, or other characteristics, leading to quicker and more efficient investigations. Ideally, these assays would be able to be a part of a field kit that would be used by law enforcement personnel directly at the crime scene to further simplify and accelerate the process of identification.

Identification, Biomarkers, Body Fluids

E10 A Comparison of Tenprint Examiner and Latent Print Examiner Minutiae Annotation

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The goal of this presentation is to compare tenprint examiners and latent print examiners as both examine fingerprints with the goal of making a decision of identification or exclusion upon each comparison. They do so with different training, different experiences, and under different working conditions that can affect their final determinations.

This presentation will impact the forensic science community as it explores the general differences between tenprint examiners' and latent examiners' thought processes during comparison and offers possible explanations for the differences present in minutiae annotations between these two groups of examiners.

The lack of standardization and different training methods within the fingerprint discipline causes differences in the examiners' thought processes used to make a comparison determination while using the Analysis, Comparison, Evaluation-Verification (ACE-V) methodology. Working environment, training, and experience between the tenprint examiners and latent print examiners possibly affects these thought processes. This study involved 28 participants, 8 tenprint examiners, and 20 latent print examiners, ranging in experience from six months to 33 years, and in age from 26 to 62 years. Participants work in 11 states, and span the fingerprint discipline from city, state, and federal employees to independent consultants. These volunteers participated in a two-part study designed to determine if tenprint examiners and latent print examiners differed in their consistency of marking minutiae of the same fingerprint presented to them in two different environments: independent mark-up and comparison analysis mark-up.

This study found there was no statistically significant difference between these two groups when looking at the percentage of matching minutiae markings between the two tests. Unlike what was predicted, there was statistical significance to show that all examiners tend to mark more minutiae during independent mark-up than they do in a comparison analysis environment.

The lack of standardization in minutiae annotation caused great variety regarding if any and how many minutiae were marked throughout this study. It appeared that some examiners marked each and every minutia they could see on each of the fingerprints presented in test packet #1; some fingerprints had 80-90 minutiae marked. Other examiners performed no mark-up on fingerprints that were presented independently. Still other examiners marked just enough minutiae to support the value determination and made no further marks after their value decision threshold was met. Standardization of mark-up procedure would allow for more universal documentation to support conclusion decisions and would allow better inter-agency communication regarding shared comparisons.

The higher accuracy and conclusion rates in the latent examiner group is believed to be attributed to the higher average experience of latent examiners.

Fingerprint Mark-Up, Fingerprint Comparison, Minutiae Annotation

E11 Analysis Using Applied Biosystems® Quantifiler® Trio With a Y-Screening Technique at the West Virginia State Police Forensic Laboratory

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After attending this presentation, attendees will understand how the Applied Biosystems® Quantifiler® Trio DNA Quantification kit can be used with a Y-screening technique.

This presentation will impact the forensic science community by discussing how Quantifiler® Trio can be used with a Y-screening technique in addition to or in place of traditional serology to assist in determining which samples will advance to DNA testing. This presentation will also discuss the efficiency and cost effectiveness of Quantifiler® Trio as a Y-screening technique.

DNA evidence can be key to identifying the perpetrator in sexual assault cases. Forensic examination of the victim is performed in which intimate samples are collected. As these samples are collected from the victim, these samples can be expected to contain high levels of victim DNA. In cases in which a female is sexually assaulted by a male, screening tests can assist in determining what samples possess male DNA and should therefore go on to DNA-based human identification testing. These screening tests, commonly referred to as semen serology tests, include the Acid Phosphatase (AP) test, the Abacus ABAcard® p30 test, and the microscopic identification of human spermatozoa. The AP test is a presumptive test for the presence of the acid phosphatase enzyme, present in seminal fluid.[1] The p30 test is a confirmatory test for the presence of prostate -specific antigen, also known as the p30 molecule, which is produced by the male prostate gland.[1] Spermatozoa can be identified on prepared slides using the Christmas tree Staining procedure. Both AP and p30 can be found in body fluids other than semen.[1] The p30 test has a sensitivity of 4 ng/mL.[2] Quantifiler® Trio can also assist in determining what samples possess male DNA and should advance to DNA testing through either a differential separation or simple extraction. Quantifiler® Trio can be used as a Y-screening tool to screen for male DNA in casework samples as it includes two autosomal targets (small and large), as well as a Y-chromosome target. Although there is some cross reactivity of Quantifiler® Trio with the DNA of higher primates, the supported quantification range is from 5pg/µL to 100 ng/µL, making Quantifiler® Trio more specific and more sensitive than forensic serology tests.[3]

In completing this analysis, several studies were performed. A Y-screening technique developed for the West Virginia State Police Forensic Laboratory was used throughout this study. A sensitivity study was performed in order to determine if this Y-screening technique using Quantifiler® Trio was more sensitive than traditional serology and also to determine at what DNA concentration the profile obtained was no longer probative. A cost-benefit analysis was performed in order to compare the costs of traditional serology to the costs of Y-screening. A contamination study was performed by running reagent controls, free of human DNA, with each test that was performed to test for any extraneously introduced DNA contamination. A mixture study was performed by creating known mixtures of male and female DNA to determine at which mixture ratio and concentration Y-Chromosomal Short Tandem Repeats (Y-STRs) should be performed instead of autosomal STRs. A non-probative study was performed using mock casework samples to demonstrate that the Y-screening technique will perform as expected on casework samples. Finally, a second mixture study was performed by preparing mock samples that contained mixtures of male and female DNA.

The sensitivity study revealed that this Quantifiler® Trio Y-screening technique was more sensitive than the AP test, the p30 test, and the microscopic identification of spermatozoa. It also illustrated that even at the lowest concentration point used in the study (with concentrations ranging from 16ng/µL to 0.015625ng/µL), a probative profile was still obtained.

It can be concluded that the Y-screening technique is more sensitive than traditional serology and may be expected to be adopted by laboratories, including the West Virginia State Police Forensic Laboratory, in the future for the testing of sexual assault evidence.

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DNA, Y-Screen, Serology

E12 Analysis of Blood Spatter Formation on Stain-Resistant Fabrics

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After attending this presentation, attendees will understand how stain repellent affects the formation and appearance of bloodstains on fabrics and household textiles.

This presentation will impact the forensic science community by providing information that can aid bloodstain pattern analysts in the examination and interpretation of bloodstains on porous surfaces. This knowledge will limit misinterpretations of the type and cause of bloodstain patterns.

Bloodstain patterns provide valuable information on the physical events that occur during a crime and assist investigators in the reconstruction of an incident. Bloodstain pattern analysts examine, categorize, and interpret bloodstains based on their shape, size, and patterns; however, the surface on which the bloodstains are present can affect the stain formation. Much of the research for understanding the formation of bloodstains on different surfaces was only conducted on non-porous surfaces. The forensic science community understands the formation of stains on these surfaces because only the roughness of non-porous surfaces has an impact on the stain; however, the majority of crime scenes involve bloodstains on porous surfaces such as apparel, household textiles, upholstery, and carpets. Unfortunately, the science of bloodstain pattern analysis is not able to provide the same level of confidence in the analysis of the bloodstains on these surfaces because of the complex structure of textiles. It is important for a bloodstain pattern analyst to consider both the type and texture of the fabric and understand how these two characteristics affect bloodstains.

The purpose of this research was to examine and compare the characteristics of impact bloodstain patterns on different fabrics, including those that were treated with stain repellent. Stain resistant fabrics are treated to repel and release stains. Ten common fabrics that may be encountered at a crime scene were chosen. Five of these fabrics are common clothing items: khaki pants, denim, a silk tie, a dress shirt, and a polo shirt. The other five are common household fabrics: carpet, upholstery, pillow cases, a tablecloth, and an outdoor furniture fabric. For each type of fabric, a section of the fabric was chemically treated with a stain-repellent spray. A rat trap was utilized to create medium- to high-velocity impact blood spatter stains. White butcher paper was used as a control. Comparisons were made between the fabric and control bloodstains, bloodstains on chemically treated fabrics and regular fabrics, and different textures of fabrics. Ten representative spatter stains were selected on each piece of fabric to analyze. The height and width of each individual stain was measured in millimeters and photographs were taken. The spatter size range and average was determined for each fabric. The comparison microscope was also used to compare individual stains present on the stain-resistant fabrics to those present on the regular fabric of the same composition and the different types of patterns were recorded. It was hypothesized that there would be a difference in the absorption of the blood spatter on the stain-resistant fabrics compared to non-stain-resistant fabrics of the same composition. This could affect the analysis of the pattern, causing the analyst to misinterpret the events of the crime scene. The documentation of the stain sizes, shapes, and characteristics on the different fabrics will aid bloodstain pattern analysts in understanding how stain-resistant fabrics affect bloodstains.

Bloodstain Pattern Analysis, Blood Spatter, Stain Resistant

E13 A Retrospective Analysis of the Prevalence and Characteristics of Child Maltreatment in Southern Italy

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After attending this presentation, attendees will understand the urgency of child maltreatment in Southern Italy by: (1) providing a qualitative interpretation of the circumstances; and, (2) identifying causes and risk factors that can determine the "susceptibility" of children to be victims of violence.

This presentation will impact the forensic science community by providing knowledge of the risk factors associated with child maltreatment that may be helpful in making an early diagnosis and creating prevention strategies. It is very important for healthcare professionals to increase their awareness for early identification of maltreatment.

Worldwide child maltreatment is recognized as a significant public health problem, with the potential for a lifelong impact on victims without proper treatment. Child abuse represents a cause of infant mortality and is a sentinel event in a community, reflecting the effectiveness of prevention strategies, social security policy, and primary care for children.[1] Seventy-five percent of abuse is not diagnosed because physicians fail to recognize signs of abuse. This lack of diagnosis causes a missed opportunity for early intervention, and many children suffer repetitions of abuse.[2] The European Report on Preventing Child Maltreatment estimates that more than 18 million children in the region younger than 18 years of age suffer from maltreatment during their childhood, and at least 850 children younger than 15 years of age die from abuse each year.[3] Italy does not currently have a national surveillance register on child abuse and the present data are incomplete.

With this in mind, childr victims of maltreatment and their parents residing in Calabria (from 2004-2014) were analyzed. A questionnaire was developed and used as an investigative tool to gain different types of information (on victims, types of maltreatment, perpatrators, and sociocultural stratification of the victim's family).

The sample comprised 1,225 children aged 0 to 18 years. The examination of the sample showed two trends: (1) nearly all of the sample (94.7%) was school age, with an evident spike in the range seven to nine years (40.2%); and, (2) a greater number of females (59%) were among the victims of maltreatment.

In the study, the percentage of children with a reported history of low birth weight (1.4%) and preterm infants (1.15%) is low. The percentage of children with disabilities and/or inappropriate mental-physical development is 13.8%. The common psycho-physical profile of the abused child is: a restless, aggressive, child with a sudden mood change, who has eating behavior disorders, sleeping disorders, etc. (behavioral disorders 35%), with clear signs of neglect (49.4%). In terms of typologies of maltreatment, neglect is the most common type (39.6%), followed by psychological abuse (29%). The percentage of sexual abuse victims is 18.7% physical abuse is 10.3%, while the cases of Munchausen syndrome-by-proxy is a minimum quota, involving only 2.2% of the sample. The different typologies of maltreatment were analyzed by age.

Approximately 85% of child maltreatment happens in the "family domestic" context and the mothers are responsible for neglect in 73.6% of cases. Parents' education level was classified as: middle (47%), high (3%), and low (50%).

In conclusion, given that >80% of maltreatment happens in the home, the attention must be focused on the dynamics of relationships within the family. The research, the regular collection of data, and the proper training programs for pediatricians (but also for sanitary personnel in primary care centers, who are often the first to see victims) are essential to minimize the probability of future violence and the long-termsocial/health consequences.

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Child Maltreatment, Risk Factors, Prevention

E14 Putting a Name to a Face: An Updated Methodology for the Application of Forensic Facial Reconstruction of Unidentified Skeletal Remains

Katelyn Norman, BFA, 702 Boston Post Road, Madison, CT 06443*

After attending this presentation, attendees will better understand the psychology of familiar face identification, the fine art concepts related to facial geometry, portraiture, caricature, and the "uncanny," the phenomenon by which a humanoid likeness may seem frightening or unsettling, and how these concepts, combined with new and traditional anatomic data, may improve the likelihood that a facial reconstruction will lead to an identification of unidentified skeletal remains.

This presentation will impact the forensic science community by identifying and addressing the problems with current practices of forensic facial reconstruction and by providing an updated methodology for the practice of forensic facial reconstruction, informed by traditional practices, the latest research, and related fields of study.

Forensic facial reconstruction is the practice of approximating the likeness of an unidentified person based on careful assessment of the skull, consideration for average tissue-depth data, and appreciation for soft tissue anatomy. Whether from software, clay, or pencil and paper, the ability to produce a portrait from a skull can be a powerful tool for generating public interest and, ultimately, an identification; however the literature on this topic highlights numerous problems with the current practices and outcomes of forensic facial reconstruction, including non-reproducibility, outdated tissue depth data, inconsistent success in achieving a likeness, an inability to predict soft-tissue features such as hair style and weight, and discrepancies in artistic ability. This presentation seeks to identify the problems with the current practice of forensic facial reconstruction, to identify related bodies of knowledge that may inform the practices and methodologies of forensic facial reconstruction, and to propose an updated methodology for synthesizing these interdisciplinary concepts with existing practice so that facial reconstructions more reliably result in a positive identification

Augmenting the traditional practices and methodologies for forensic facial reconstruction by incorporating knowledge from related fields of study will result in greater success in the identification of unidentified skeletal remains from artists' facial approximations. This methodology was designed paying particular attention to the neurology and psychology of familiar face identification, the fine art theories on likeness and the uncanny, as well as a synthesis of existing techniques with relevant anatomical data from anthropology, dental, plastic surgery, and maxillofacial literature.

This new methodology was applied to radiologic images of the skulls of several subjects, with the subsequent drawings presented to the subject's family for evaluation, as a preliminary means of evaluating the merit behind the design. Unique to this study is the completion of multiple facial reconstructions by a single artist as well as the inclusion of the next of kin in the evaluation process.

Facial Reconstruction, Identification, Skeletal Remains

E15 Initial Efforts to Create Local Libraries of Alcoholic Beverages Using Raman and Attenuated Total Reflectance/Mid-Infrared (ATR/Mid-IR) Spectroscopy

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After attending this presentation, attendees will better understand the role of vibrational spectroscopy techniques and chemometrics in forensic sciences, specifically addressing the classification problem and the importance of developing local spectral libraries.

This presentation will impact the forensic science community by illustraiting the potential of Raman and ATR spectroscopies as versatile techniques to create local spectral libraries as a lab or field tool available to the forensic scientist.

The goal of this work is to present the initial efforts toward the development of a Raman and ATR spectral library of local alcoholic beverages as well as to evaluate chemometric techniques such as Principle Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), or Discriminant Analysis (DA) in the classification and clustering of the beverages.

Vibrational spectroscopies, including Raman, ATR/Mid-IR, and Near-IR, hold great promise as forensic tools. The versatility of these tools provide field analysis with no sample preparation required and thus no sample destruction, results that are attractive since the evidence can be conserved for further analysis. Vibrational spectroscopies, in conjunction with classification and clustering chemometric techniques such as PCA, HCA, and DA, are helping to address questions posed by police, prosecutors, and courts in regard to the identification and classification of objects into certain categories or the association between two or more items. The main objective is to contribute in the same direction by exploring the creation of vibrational spectroscopy (ATR/Mid-IR and Raman) libraries and evaluate the capabilities of chemometric techniques such as PCA, HCA, and DA to classify alcoholic beverages that are commercially available in local stores. Vibrational spectroscopy techniques offer the possibility of multichannel detection in which thousands of wavelengths are collected simultaneously, offering unique chemical information characteristic of the structure. The above-mentioned chemometric techniques can use multichannel information of known samples to create mathematical training models that are later used to predict chemical information in unknown samples with similar characteristics.

A series of Raman and ATR/Mid-IR spectra were collected of different types of alcoholic beverages: (vodkas, whiskeys, liquors, or rums) using a portable BRAVO® hand-held Raman spectrometer or an ALPHA® ATR/Fourier Transform Infrared (FTIR) spectrometer. Instrumental parameters were kept constant for all measurements at 4cm^{-1} resolution, averaging five scans, 1,000ms integration time and 300cm^{-1} to 3,200cm^{-1} spectral range for Raman spectroscopy. ATR spectra were collected using a single reflection diamond ATR accessory with a resolution of 4cm^{-1} in the range of 4,000cm to 400 cm^{-1}. Both Raman and ATR spectra will be processed using either default Bruker's OPUS 7.5 software or the Statistics and Machine Learning Toolbox™ in conjunction with MATLAB® 9.0.

The results from this initial effort evaluates the capabilities of Raman and ATR/Mid-IR in conjunction with the applied chemometric techniques in terms of identification and classification of alcoholic beverages. Questions such as, "Is Raman or ATR along with DA, PCA, or HCA capable of classifying a sample as belonging to the group of vodkas, whiskeys, liquor, or rum?" and "Can Raman or ATR in addition to DA, PCA, or HCA identify an unknown sample as a match to one of the samples present in the training set?" will be addressed.

Raman Spectroscopy, ATR, Classification

E16 New Suggestions and Modifications in the Extraction, Analysis, and Detection of Eight Organic Post-Explosion Traces

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After attending this presentation, attendees will better understand the importance of applying quality parameters in debris collection from the crime scene, careful sample collection, extraction, and suitable analytical techniques ensuring successful analysis for the studied explosives, especially the thermal labile explosive Pentaerythritol Tetranitrate (PETN).

This presentation will impact the forensic science community by: (1) modifying one of the extraction methods used in extracting explosives traces from soil; (2) providing new methods for analyzing mentioned extracts; and, (3) highlighting some of the important roles on which the collection of debris and detection of explosives are based.

Trace analysis of explosives is one of the most challenging fields in forensic chemistry. It generally includes separation and identification of unknown explosives traces. Accordingly, combinations of selective and sensitive techniques are required.[1] The present study focuses on detecting and analyzing explosives traces in two statuses: artificial explosions extracts and real post-explosion extracts. Explosive materials from the three groups of high explosives, nitramines, nitrate esters, and nitro compounds, were used in this study. The individual explosives tested were NC, NG, Tetryl, TNT, RDX, PETN, sheet explosives, and plastic explosives. Samples were extracted from sand, filtered, cleaned up, and subjected to spot testing, Thin Layer Chromatography (TLC), Ion Trap Mobility Spectometry (ITMS), and Gas Chromatography/Electron Ionization, Mass Spectrometry (GC/EI/MS).

This study achieved successful results in extraction and analysis of explosives traces via: (1) using acetonitril as a solvent in a short extraction procedure by a solvent/solid ratio 1:1.5 which is lower than the referral method; (2) filtration to remove all solid particles from organic extract. Filtration was performed by using a Buchner funnel with an efficient vacuum pump. The organic extract was subjected to a further normal filtration step before conducting a sample concentration; (3) clean-up by Porapack™ SPE.[2] Porapack RDX seppak solid phase extraction column, followed by TLC; and, (4) detection and analysis using: (a) spot test; by suggesting a scheme for colorimetric identification of the investigated explosives; using the following reagents: NaOH and Griess[2], 5% DPA (diphenylamine) in methanol acidified by H_2SO_4[2], piperidine, and KOH; (b) TLC; by suggesting seven mobile phases, and reporting new R_f values; (c) ITMS as one of the most advanced and fast detection techniques now in the field of explosives trace analysis and detection.[3] The ITMS was used in this study for fast detection of explosives and detection of common explosive traces TNT and NC concentration and time relation in hand swabs; and, (d) GC/MS the present study also suggests a new GC/EI/MS method for the separation and detection of post-explosion traces of TNT and sheet explosives containing PETN, which avoided the disadvantages of common GC/EI/MS methods in detecting PETN. The GC column used was capillary column HP-5MS, 30m length, 0.25mm ID, and 0.25µm film thickness. The Minimum Detection Limit (MDL) was detected for the studied explosives. The following values were reported for the MDLs: 90ng for TNT, 150ng for RDX, and 120ng for PETN.

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Analysis of Explosives, Detection of Explosives, Trace Analysis

E17 Forensic DNA Phenotyping Through Massively Parallel Sequencing (MPS): Improving the Prediction of Eye, Hair, and Skin Color Through Quantitative Measurement

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WITHDRAWN

E18 Greenhouse Evaluations of Volatile Plant Defense Against an Invasive Agricultural and Environmental Biothreat Agent, *Raffaelea lauricola*, and Possible Implications for Canine Detection

Alison Simon, BS, 11200 SW 8th Street, CP304, Miami, FL 33199; and Kenneth G. Furton, PhD, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand the processes of the biothreat *Raffaelea lauricola* and how avocado trees respond through altering volatile secondary metabolites. Attendees will also understand how research is progressing to characterize these volatiles so that mimic training aids can be created to expand the use of canine detection for this protection of national resources and the economy.

This presentation will impact the forensic science community by presenting a method of strengthening pre-existing canine detection in forensic science and food safety through the chemical analysis of the Volatile Organic Compounds (VOCs) in the headspace of an invasive biothreat agent for the protection of natural resources by characterizing the VOCs of a living target's odor.

Biological threats are increasingly gaining attention because of the hazard they pose to national resources, including agriculture and the environment. The fungus *Raffaelea lauricola* is a biothreat vectored by the invasive beetle *Xyleborus glabratus*, or Redbay Ambrosia Beetle (RAB). RABs are attracted to members of the Lauraceae family, including commercial and private avocado trees as well as six wild species. Avocado trees are of particular interest because they are Florida's biggest tropical fruit crop, comprising $55 million of the state's annual economy. Since the RAB introduction into the United States about a decade ago, the fungus killed more than 12,000 commercial avocado trees and an estimated half million wild trees in the southeastern region of the nation. The introduction of the RAB and similar vectors into the United States can be halted through improved detection techniques using canines handled by the United States Department of Agriculture (USDA), who police agricultural ports of entry. The resulting introduction of the RAB and now-rapid spread of the fungus puts commercial avocado groves at risk in California, Mexico, and Central and South America. The fungus lives in the tree's xylem, or vascular tissue. Once a tree is inoculated, it shuts down the vascular tissue in an attempt to halt the spread of the fungus. Unfortunately, this also stops the spread of water and nutrients vital to the tree's life, resulting in tree death within approximately six weeks in a process referred to as laurel wilt disease (a reference to the disease's symptoms).

Due to the rapid spread of *R. lauricola* and the quick death of trees, early detection is essential. The only current method of pre-symptomatic identification is canine detection. Despite the high risk to food safety associated with biothreats and invasive species, canine detection use has been limited in this field. The lack of widespread application for canines in food safety targets is largely due to the lack of mimic training aids. Without mimic aids for biothreats, live training aids must be used; however, live aids are high risk and often prove difficult to obtain because of rarity, legality of attaining and transporting the species, and method of containment. In the case of *R. lauricola*, containment is difficult because fungal spores are easily spread. In order to create a mimic training aid, the VOCs in the headspace of infected trees must be fully characterized.

The current study evaluated VOCs of inoculated young avocado trees in a greenhouse setting using Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) in order to follow the progression of infection through VOCs in a controlled environment. Nine compounds were previously detected in greater than 80% of avocado trees, all of which are sesquiterpenes, secondary metabolites produced in indirect plant defense and interactions with fungi. Young trees were inoculated with plugs of *R. lauricola*, while healthy trees were spatially separated from the others. The trees were sampled each day, starting before inoculation and ending with tree death. Inoculated trees change their VOC production as part of their inherent defense system, slowing the production of certain categories of VOCs and producing others not seen in the healthy trees. Notably, these changes are detected before symptoms can be visually observed. The characterization of VOCs in this study will be essential in creating a mimic training aid for avocado trees infected with *R. lauricola*. Use of detector canines can then be expanded to help protect national resources and the economy.

Biothreat, Canine Detection, Food Safety

E19 Veterinary Forensics and Large Animal Deaths

Cheryl F. Nelson, DVM, 1735 Pinckard Pike, Versailles, KY 40383*

After attending this presentation, attendees will understand the role of the forensic veterinarian in preventing and uncovering abuse, inhumane destruction of animals, and their relationship to insurance fraud and animal cruelty.

This presentation will impact the forensic science community by discussing the importance of veterinary forensic techniques to help solve cases. Practical applications including scene photos, necropsy findings, and veterinary records will be presented with an emphasis on a 2008 case as well as cases from 1973 to 1992.

Prior to 1932, four states had felony laws for animal abuse. In 1966, a federal law regulating the treatment of animals in research and exhibition was passed. In 1986, veterinary forensics was recognized pertaining to insults inflicted on humans by animals and on animals by humans. Soon after, animal cruelty was included in the Diagnostic and Statistical Manual of Mental Disorders Third Edition (DSM III) as a mental disorder. In 1988, the United States National Fish and Wildlife Lab in Ashland, OR, was established as the crime laboratory for the Convention on International Trade in Endangered Species (CITES).

By 2000, 25 states had made animal abuse a felony. In 2006 and 2007, three texts pertaining to animal forensic investigations were published and the Michael Vick dog fighting case was in the news. In the Vick case, search warrants resulted in the discovery of six to eight dogs buried in two mass graves. Consequent bone evidence indicated dog fighting which provided key evidence in the Vick case.

In 2008, the International Veterinary Forensic Sciences Association (IVFSA) was formed. Conferences and training in veterinary forensics began to be offered at the University of Florida. By 2014, all 50 states had made animal abuse a felony and 43 states had made animal abuse a felony on the first offense. The Federal Bureau of Investigation (FBI) added animal cruelty as a category in the agency Uniform Crime Report.[1]

On May 23, 2008, in Escambia County, AL, a neighbor saw smoke and reported a barn fire. By the time firefighters arrived, the barn and hay were fully engulfed. As they began to check for hot spots, they noticed that among the charred remnants of a stall was a deceased horse. Here collaboration and cooperation were key. The fire department realized this was a complicated scene and notified the Alabama State Veterinarian and the Alabama State Fire Marshall.

A member of the Alabama Department of Agriculture and Industries arrived on scene, and performed a gross exam and preliminary necropsy. He noted free blood in the chest cavity, which would not be normal in death due to fire. The horse's body was then taken by police escort to the Auburn University Diagnostic Laboratory for a complete necropsy. Two bullet wounds were identified, resulting in shrapnel and a bullet being recovered from the horse's heart. The case continued to develop and on June 10, 2008 the owner was charged with multiple counts of felony attempted theft by deception, arson, and animal cruelty.

In other cases, from 1973 to 1992, a substantial number of expensive race horses and show horses were brutally abused and inhumanely destroyed. Horses were killed in ten states. Only with the 1989 re-opening of the murder investigation of the candy heiress, Helen Vorhees Brach, was much of this brought to light. These cases led to 36 indictments and 35 convictions of fraud and cruelty.[2-4]

Dogs and cats account for 64% and 18% of cruelty cases, respectively, while the remainder involve cruelty to horses and livestock. In 2010, a $6 million (euro) insurance fraud scheme involving pets was uncovered in the United Kingdom.[5] Veterinarians must show where human forensics can apply to animals and work diligently to learn more about the differences between human and veterinary forensic pathology.

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Veterinary, Forensics, Large Animals

E20 Characterization of Controlled Odor Mimic Permeation Systems (COMPS) Containing Live Training Aids for Utilization by Detection Canines

Alison Simon, BS, 11200 SW 8th Street, CP304, Miami, FL 33199; and Kenneth G. Furton, PhD, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand: (1) current processes of training detector canines for food safety; (2) how training aids are created for live target odors; and, (3) how COMPS can be used as effective alternatives for which no other containment system is available.

This presentation will impact the forensic science community by presenting a method of strengthening pre-existing canine detection in forensic science and food safety through the characterization of the Volatile Organic Compounds (VOCs) in the headspace of canine training aids containing an invasive biological threat for the protection of natural resources.

Food safety is gaining attention on the national platform because of the risk that various biological threats pose to these national resources and the economy. Since 1984, the United States Department of Agriculture (USDA) successfully used canine detection to decrease the number of biothreats entering the country, but it cannot completely stop these threats due to the volume of activity at various ports of entry. One of the biggest reasons detection canines are not applied more to the food safety field is the lack of efficient and safe training aids. Generally, for biological threats, training aids are live, which poses many dilemmas, such as shelf-life, cost, and difficulty of obtainning and containing the target odor. For invasive species, additional risks include the rarity of the species as well as the legality of obtaining and transporting it. Common alternative training aids include nests, burrows, and objects left behind by the target, such as scat, carcasses, or feathers; however, for many invasive species and pests, these alternatives are not possible. This study used the invasive fungus *Raffaelea lauricola* as a proof of concept to illustrate how COMPS can be used as effective containment devices for biological threats such as fungi, whose spores are easily spread, but for which no other alternative exists.

R. lauricola entered the United States through Georgia in the early 2000s on infected wooden shipping pallets, carried by its vector, *Xyleborus glabratus*, or the Redbay Ambrosia Beetle (RAB). This fungus and its host spread through eight states in the southeastern region of the nation, infecting members of the Lauraceae family, including commercial and private avocado trees. In the decade since it entered the United States, *R. lauricola* has killed 12,000 commercial avocado trees in Miami-Dade County, FL alone. The fungus also poses a huge risk to the 250,000 privately owned avocado trees in Miami-Dade County. Thanks to the rapid spread of the pathogen, the food safety of California and Mexico are also at risk. *R. lauricola* causes laurel wilt disease, which kills trees within six weeks of inoculation and rapidly spreads to its neighbors through the process referred to as root grafting. The trees over-respond to the threat by shutting down their vascular system in an attempt to stop the spread of the disease; however, this ill-fated attempt at defense shuts down the water and nutrient transportation systems of the vascular tissue, leading to tree death. An infected tree displays dark discoloration of the xylem, wilted yellow to brown leaves, and frass on the trunk from increased insect activity. The only current method of early disease identification is canine detection.

The detector canines trained to locate infected avocado trees are trained using COMPS-containing infected wood. This study demonstrated that COMPS effectively contain the fungal spores while allowing for the release of target odors. First, VOCs released from the COMPS were detected and compared to those released from samples of live infected trees. Then, the dissipation rate was determined so shelf-life could be established. Externally Sampled Internal Standard-Solid Phase Microextraction/Gas Chromatography/Mass Spectrometry (ESIS-SPME/GC/MS) was used as the extraction and analytical method. The characterization of these COMPS demonstrated that COMPS can be used as effective containment devices for many biological threats for which alternative training aids do not exist. This containment method can be used to expand the field of detector canines to protect against the rising threats to food safety.

COMPS, Canine Detection, Food Safety

E21 Detecting Human Remains in Fatal Fires Using Cadaver Dogs

Mary E. Cablk, PhD, 2215 Raggio Parkway, Reno, NV 89512*

After attending this presentation, attendees will better understand the science behind canines detecting human remains in fatal fires as well as deployment parameters and limitations of canines to fatal fire incidences.

This presentation will impact the forensic science community by providing a critical look at cadaver dogs as a potential resource for search and recovery of fatal fire decedents from case studies and scientific research.

Properly trained cadaver dogs have demonstrated the ability to locate human remains in fatal fires efficiently, safely, and where visible signs of remains were unobserved. They have been deployed to wildland fires, arson, explosions, suicides, and accidental fires resulting in suspected or known fatalities, with successful outcomes. These successful detections and recoveries are reported in popular media and shared as firsthand anecdotes among handlers. While such reports serve as a means to inform general knowledge of the use of cadaver dogs, none can be considered to be rigorous assessment from a scientific view. Controlled experiments using scientific protocols to evaluate the capability of dogs trained to locate human remains has not been reported in the scientific literature, which contributes to the potential for a biased interpretation of accuracy (e.g., unknown false positive rate). Nonetheless, human remains detection dogs are clearly finding human remains in fatal fires.

What dogs use to determine human remains target odor remains unknown, and while focused research has been published on the general topic, none pertains to burned remains specifically. Bones are known to be composed of a mineral phase similar to hydroxyapatite and a collagen matrix. Research has reported on the alteration and decomposition of bones exposed to thermal treatment, focusing on bone mineral modification and ashing, in which the organic component is dehydrated and decomposed, but has not focused on odor signature for olfactory detection. Even with thermal decomposition, properly trained cadaver dogs not only identify ashed bones, but differentiate them from animal remains. The science behind matching targets (burned remains, cremains) versus target generalization (decomposed human remains, bones) will be discussed.

Post-fire repopulation of communities includes great pressure from individuals whose homes and other property were lost. Authorities deem it untenable that families return to their properties where they may find deceased relatives or neighbors. In addition, there is a law enforcement investigation aspect to unwitnessed deaths. In 2015, cadaver dogs were dispatched to and located remains in two different wildfires in California. Less than one year later, cadaver dogs cleared several hundred burned properties in another fatal wildfire. Cadaver dogs dispatched in Los Angeles, CA, also located the remains of multiple individuals in an urban environment in 2016. Cadaver dogs were dispatched to conduct searches in these fatal fires with three different search criteria: (1) subjects possibly missing (homeowners/residents); (2) unknown structure habitation (transient population); and, (3) no subjects reported missing in residential neighborhoods. Where there were decedents, the dogs located those individuals. No instances of dogs missing decedents were reported.

The criteria in place for qualified canines for primary dispatch was certified, single-purpose, passive-alert dogs, with a disaster-experienced handler. Second-tier qualifications included cross-trained dogs that were also trained to locate live people, but still requiring passive alerts for human remains. Dog teams meeting the primary dispatch criteria produced positive outcomes. Not all dog teams are appropriately trained for and meet the criteria to qualify for fatal fire incidents.

Searching for decedents in burned areas poses numerous hazards to the canines and handlers. These include environmental, material, and physical hazards. Unlike searching for live victims, recovery is not a life-threatening emergency, so the acceptable risk to searchers is approached differently. Risk also applies to the dog, which is the sensing device, and must be maintained as any other biosensor or biological detector.

Cadaver Dog, Fatal Fire, Burned Remains

E22 Crime Scene Videography Revisited: Combining Digital Single-Lens Reflex (DSLR) Camera's Videography Capabilities With Photography

Kevin J. Parmelee, PhD, Somerset County Prosecutor's Office, Forensic Lab, 40 N Bridge Street, Somerville, NJ 08876*

After attending this presentation, attendees will better understand how current DSLR technology can be used to document crime scenes more effectively with a single device that combines the use of videography and photography.

This presentation will impact the forensic science community by providing a new methodology for documenting crime scenes using current DSLR technology. This information may impact the development of future guidelines and standards for using sequential capture of video and photography in documenting crime scenes.

This presentation will provide the technical use of DSLR cameras to capture both still and video images consecutively at a crime scene using one device. Photography provides a means of recording visual information and details regarding how a crime scene and evidence appeared. The visual documentation of crime scenes and evidence is commonly accomplished using photographic technology. Although capturing video may not be mandatory at all crime scenes, it provides additional context of the scene that is not available with still images. As the technology of DSLR cameras advanced, the option of capturing video with the DSLR became available and the quality of the images and footage advanced as well. The advancing technology now allows for the capture of still images and video, and the opportunity now exists to capture them sequentially at a crime scene using a single device.

Using one DSLR camera, the crime scene investigator can capture still images and video in sequence, which reduces the time associated with operating two devices, allows for a more convenient methodology for capturing both still and video images, reduces issues of contention that images may have been altered, and promotes the opportunity to capture video at a greater variety of crime scenes.

A study was conducted using an older DSLR camera, Nikon® D90, and a newer DSLR, Nikon® D610, to determine whether common camera/video technology will meet the requirements of current guidelines, enhance the efficiency for capturing images and video at crime scenes, and meet court admissibility requirements.

The scope of this presentation evaluates a test of two Nikon® brand DSLR cameras, which offer both still and video methods of capturing images, against current guidelines. Other camera brands that offer similar technology and options for crime scene investigators may be useful as an alternative. Additionally, a new methodology for capturing both still and video images will be presented that conforms with current and proposed guidelines set forth by the Scientific Working Group Imaging Technology (SWGIT) and the Scientific Working Group on Digital Evidence (SWGDE).

Videography, Photography, Documentation

E23 Introducing a Methodology to Process Digital Images for Investigation, Intelligence, and Evaluation Purposes: Combining Forensic and Managerial Perspectives

Simon Baechler, PhD, School of Criminal Justice, University of Lausanne, UNIL-Batochime, Lausanne, Vaud 1015, SWITZERLAND*

After attending this presentation, attendees will have increased their competence and performance in processing digital images for various policing, crime intelligence, and justice purposes. Attendees will learn how to efficiently and effectively manage and process flows of digital images coming from various sources. Attendees will also learn about an approach and a dedicated toolbox that are in line with forensic science principles and forensic best practices. They will be made aware of use case scenarios which may directly inspire their forensic practice and research.

This presentation will impact the forensic science community by making the argument that digital images gathered from various sources should be duly considered as forensic data, and that forensic science should take more leadership, if not ownership, in processing digital images for investigation, intelligence, and evaluation purposes. This presentation provides an original and economical approach that may inspire different organizations facing the issue of managing complex flows of images. This presentation also showcases that collaboration of forensic scientists with other stakeholders is a key success factor.

Digital images, both photographs and videos, available in the framework of criminal investigations come from an ever-broader range of sources, such as witnesses, offenders themselves, Closed-Circuit Television (CCTV), smart phones, the internet, police body cameras, drones, mug shots, covert police operations, traffic enforcement cameras, etc. All these images can be viewed as forensic remnants of crime events and obviously represent an opportunity for forensic science, policing, and justice; however, they raise an acute methodological and management challenge as they represent a novel, rich, evolving, and complex source and flow of data.

Currently, most policing organizations exploit these images both as they occur and on the job without a defined strategy that incorporates forensic science principles and forensic best practices. Even if successful in some cases, this current practice would certainly benefit from a scientific and more structured approach. The collection, storage, processing, comparison, and evaluation of digital images fall more often than not outside the supervision of forensic scientists, a situation that has to come into question.

This presentation advocates that digital images should be duly considered as forensic data and that forensic science should take leadership, if not ownership, of the process and management of digital images. This presentation exposes a methodology developed and implemented in a police service to handle digital images effectively, efficiently, and in respect to forensic science principles (such as Analysis, Comparison, Evaluation-Verification (ACE-V), for instance). That methodology is intended to serve investigation and intelligence, as well as evaluation purposes. The bottom-up development of the methodology and its operation implies a collaborative approach, bringing together forensic scientists (both researchers and practitioners), uniformed police, the criminal investigation division, and crime intelligence analysts.

This presentation details forensic and managerial solutions that were designed to structure the collection and storage of images coming from a wide range of sources and to handle a growing qualitative and quantitative flow of images. This presentation presents the database, the original analysis, and the search-and-comparison toolbox that supports the process. That toolbox was developed in-house and does not require costly technological solutions. Evaluation of the strength of evidence in regard to digital images is raised as a key issue where empiric solutions currently prevail and further research is obviously needed.

The methodology contribution is illustrated through case scenarios related to a large scope of crime types, from serious crimes to burglaries or card frauds. This presentation finally expands on computer-vision-based techniques that may be of interest for forensic science applications.

Digital Images, Methodology, Management

E24 You Didn't Tell Me That! Thank You for Letting Me Know That! — The Importance of Evidence Recognition and Documentation of the Death Scene

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After attending this presentation, attendees will learn the importance of law enforcement's description of the death scene during the initial death notification to the medicolegal office as it regards whether to release a scene or respond to a scene. Attendees will also understand the importance of evidence recognition and scene documentation in assisting the forensic pathologist in determining the manner and cause of death.

This presentation will impact the forensic science community, particularly medicolegal death investigators and law enforcement officers, by stressing the importance of communication and collaboration between these two professionals as well as evidence recognition, evidence collection, and documentation of the death scene.

Medicolegal death investigators receive death notifications not only from hospitals and nursing homes but also from law enforcement personnel who are present at a death scene. The death investigator triages these telephone calls to decide whether a scene response is necessary by asking the police officer such questions as when the decedent was last known alive, past medical history, and a description of the death scene. There may be instances when law enforcement may miss important scene details and the death investigator may release the case as non-jurisdictional or when the police officer describes crucial information about the death scene that initiates a response by the medicolegal death investigator.

Autopsies cannot be performed in a vacuum. Failure to have relevant information will result in misdiagnosis and possible issues with the integrity of the case. Medicolegal death investigators are observers for the forensic pathologist. It is imperative that the death investigator recognize evidence that helps the forensic pathologist determine manner and cause of death. This evidence may be of transient, functional, or unpredictable forms. Documentation of these types of evidence are documented through photographs and report narratives.

It is important that the death investigator receive accurate scene description from law enforcement. Receiving this information from police will help the death investigator make a determination as to whether to respond to a scene. Once an investigator is at a death scene, is it crucial that he/she recognize evidence and know how to collect and document it. This presentation will discuss these issues through a discussion of an ethylene glycol poisoning death, the hanging death of an 11-year-old, and an indoor hyperthermia death.

Notification of Death, Death Scene Investigation, Evidence Recognition

E25 How Thorough Medicolegal Death Scene Investigations and Autopsies Impact Public Health and Safety

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After attending this presentation, attendees will be able to establish the importance of the medicolegal death scene investigation and autopsy with toxicology in order to accurately complete death certificates. Death certificate data is used to monitor public health and safety, allocate resources, and develop initiatives.

This presentation will impact the forensic science community by demonstrating why a thorough medicolegal death investigation, including scene, autopsy, and toxicology testing, is necessary to accurately certify the death.

The incidence of drug intoxication mortality continues to rise with more than 47,000 people dying from a drug overdose in 2014. Since 2000, the drug intoxication death rate has increased 137%, including a 200% increase in the rate of drug intoxication deaths involving opioids.[1] Opioids are currently the leading cause of drug intoxication deaths as these drugs were involved in more than 61% of overdose deaths in 2014.[1] These data from the Centers for Disease Control and prevention (CDC) are derived from death certificates and are the only national source data on drug intoxication deaths.

While death certificate data provide a national picture of drug intoxication mortality, there are limitations to these data due to variations in death certification practice, as well as variations in death investigation practice, which impacts all data on drug intoxication deaths regardless of the data source. Inconsistency in death certification practice influencing the utility of the data include reporting metabolites (e.g., morphine rather than heroin) and not identifying the specific drugs involved (e.g., "multidrug intoxication") on the death certificate. Death investigation practices may also vary, including when decedents are tested for the presence of drugs, substances tested for, and circumstances under which the tests are performed. These factors may vary by jurisdiction, decedent, and temporally (e.g., routine fentanyl testing). Both death investigation and certification practice is reflected in the reported data, which in turn impacts not only monitoring trends but also initiatives to prevent deaths as well as resources allocated.

In order for public health and safety to accurately monitor the opioid epidemic, information reported (including on death certificates) on opioid deaths by medical examiners and coroners is critical. Therefore, assuming jurisdiction in these cases is essential and should include both a scene investigation and complete autopsy, including toxicology. A trained medicolegal death investigator should look for evidence of medication, both prescription and illicit substances, at the scene. This evidence should be thoroughly inventoried. Information should be obtained by observing the actual bottles and not from medication lists obtained from family members on scene. The presence of paraphernalia, such as needles, should also be documented, photographed, and collected, when possible, for future testing; however, the absence of medications or paraphernalia on scene has a low predictive value for drug intoxication, which should not eliminate suspicion of drugs causing death.[2] Medical history is also essential to document, as well as prescription history, which may be obtained from a Prescription Drug Monitoring Program (PDMP) if the investigator has the authority to access it.[3] Evaluations have shown PDMPs to be a valuable tool for medicolegal death investigation.[4]

Death scene investigations may be hampered by scene tampering particularly for deaths involving the use of illicit or illegally obtained substances. For example, illicit substances may be removed before investigators arrive and, in some cases, bodies may be moved from the place where the death occurred. Witnesses and family may share limited details surrounding the deaths. This may impact both cause of death and manner of death. Drug intoxication is a leading mechanism of suicide among men, and the leading cause among women. Of all causes of death, drug intoxication is most likely to have a manner that could not be determined.[5]

Medical examiners, coroners, and medicolegal death investigators are at the front lines of this evolving crisis and are in a unique position to gather the information needed for monitoring the new and emerging drugs of interest, as well as monitoring consistent drugs of abuse, such as heroin, cocaine, and methamphetamine. For drug

intoxication deaths, in addition to all the other information gathered, the death scene may influence when decedents are tested, what drugs are tested for, and the interpretation of the toxicological tests. Information from the death scene needed to assess for risk and protective factors may go beyond that needed for determining cause and manner of death.

Improving the quality of death investigation and certification will maximize the utility of our existing national registration of deaths for public health surveillance and research and contribute to the design of programs to prevent drug intoxication deaths.

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Opioid Deaths, Overdose, Death Certification

E26 The Transgender Consideration: The Importance of Reassessing Unidentified Human Skeletal Remains to Provide New Investigative Directions

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After attending this presentation, attendees will better understand: (1) challenges associated with investigating the death of an individual whose biological gender was originally incorrectly determined; (2) investigative detours that will take the attendees to new places and introduce them to new communities; and, (3) that it is imperative to pre-examine unidentified decedent cases.

This presentation will impact the forensic science community by illustrating the importance of unidentified decedent case reassessment and how inaccurate information can impact the direction of an entire investigation 25 years later.

This presentation reviews an unusual case study that illustrates the importance of revisiting scientific and investigative methods that were used in attempts to identify unidentified decedents from many years ago. As technology advances, so does subculture awareness, access, and acceptance.

In 1988, decomposing and partially skeletonized human remains were found in a wooded area in south Lake County, FL. Based on the decedent's hair, clothing, autopsy results, and anthropological studies, the body was identified as that of a Caucasian female. Additional information was provided that this woman most likely had one or more children. A thorough investigation was completed by law enforcement, but the decedent was never identified. The cause and manner of death were certified as "Undetermined." Throughout the following years, this decedent's information was disseminated through different websites that specialize in aiding with identification.

As part of an initiative of the Florida Department of Law Enforcement Missing Endangered Persons Information Clearing House and the Florida Medical Examiner's Commission, the District 5 medical examiner began re-evaluating unidentified human skeletal remains cases that had been in storage, some dating back to the 1970s. Although the majority of these remains had already been examined by an anthropologist, it was determined that a subsequent examination, along with the extraction of samples for DNA testing, could further assist with identification.

In 2013, the District 5 Medical Examiner's Office was notified by the C.A. Pound Human Identification Laboratory that a second anthropological examination revealed that the aforementioned decedent was not a female as previously thought, but a male. This determination was confirmed through DNA analysis. It was further believed that the decedent was, at some point, receiving estrogen and relaxin, which resulted in changes that exhibited bone resorption. This bone resorption is often associated with childbirth but is also found in pre- and post- operative transgender people. The results of the second anthropological analysis, in addition to the DNA confirmation of gender, redirected the entire investigation. The focus of the investigation shifted from a local, rural community to the closest metropolitan areas that supported a transgender community.

Anthropology, Forensic Investigation, Transgender

E27 Medicolegal Death Investigation: Staged Scenes or Setting the Scene — When Appearances are Deceiving

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After attending this presentation, attendees will have a better understanding of death investigations. From the initial death report, presentation at a scene, or investigative follow-up, attendees will have a greater appreciation of the steps which would ultimately assist in determining a correct conclusion relative to cause and manner of death. Case examples of homicides or accidental deaths masked as suicides, naturals, or overdoses will be presented, and investigative strategies' which facilitate finding the right answer will be discussed.

This presentation will impact the forensic science community by demonstrating the impact of proper medicolegal death investigation when confronting and evaluating staged scenes.

Death investigation begins with the report of a death to the Medicolegal Death Investigator (MDI). The initial interaction of the MDI with the individual or agency reporting the death sets the stage for what follows. Asking the right questions determines the nature and extent of the response and consideration a given case will receive. Communication matters, and the thread of questioning based upon the expertise and experience of the MDI can lead to whether or not a scene visit is made, a case is brought into the office, or a case released to a signing clinician.[1]

When scene visits are made as a result of this initial report and interaction, the MDI must be vigilant, aware of indicators observed at the scene, and continue the dialogue with law enforcement officials, emergency medical staff, family members, and other witnesses in gathering and documenting details and information that lead to the correct outcome.

An important element in the MDI process includes the awareness of "red flags" in the interview dialogue(s) or on scene, which alert the investigator to the potential of other investigative paths to pursue. Red flags can include: (1) interviews with law enforcement, arson squad, medical personnel, family, witnesses, etc., which lead to conflicting, inconsistent, or missing details and information; (2) body position, location, and presentation of the decedent at the scene; (3) postmortem findings at the scene (i.e., rigor and livor mortis) — are they appropriate for body position?; (4) physical examination and documentation of findings, including trauma and clothing — are they consistent with history and presentation?; (5) items surrounding the decedent — are items in their proper locations and relative positions?; and, (6) the presence (or absence) of biological secretions on body, blood spatter, etc. — are the findings consistent with expectation relative to surfaces of the body and/or items in the area surrounding the decedent?

Examples of red flags in an apparent suicidal overdose case of a 39-year-old female, a gunshot wound Suicide of a 25-year-old male, and a reported natural death of a 34-year-old female (i.e., no apparent violence or foul play involved in the case) which appeared during the MDI of these cases will be elaborated upon and discussed. In each of these cases, different causes and manners of death than those initially presented were determined. Investigative insights, details, and specifics in these cases leading to new conclusions will be presented.

Finally, the Cuyahoga County Medical Examiner's Office (CCMEO) provides support services to the community, allowing for storage of cases of reported natural deaths while waiting for the attending clinician to sign the death certificate. When these cases are brought to the MEO, as with any case received at the office, it is incumbent that the office reviews and inspects the case prior to it being released. The case is presented of an 84-year-old decedent sent to this office for storage, which, when inspected onsite was discovered and determined, following further investigation, to have been a homicide.

The common denominator in all of these cases is the proper application of an aware, thoughtful, and comprehensive investigative and communication process which leads to correct conclusions in medicolegal death investigations.

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Medicolegal Investigation, Death Investigator, Staged Scene

E28 Identification of Desiccated Remains 64 Years Postmortem

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After attending this presentation, attendees will better understand techniques employed to rehydrate desiccated human remains from a 1952 United States Air Force Douglas C-124 Globemaster II plane that crashed into an Alaskan glacier. Fifty-two service members were on board when the plane crashed into the side of Mt. Gannett and, based on the location and weather conditions, only minimal parts of the wreckage can be retrieved each year as the glacier moves and melts. Different methods of obtaining fingerprints from the remains along with the identification of the two service members impacted will be shown and discussed.

This presentation will impact the forensic science community by offering real-life examples of processing difficult and unique decedents' hands for postmortem fingerprints. Techniques and results will be explained and discussed.

Postmortem processing of fingerprints often has challenges related to the conditions of the decedents' hands. In standard cases, decedents can be printed with ink and paper or powder and an adhesive for identification purposes. In the case of badly damaged, desiccated, or decomposed hands, other methods must be employed. These methods will be outlined and the effects demonstrated.

Desiccated remains offer a unique difficulty in processing for postmortem prints. There are commercial products and tissue softeners available to assist in this procedure, but these techniques often take time and can damage the remains as they soften. By using the boiling technique, thereby obtaining high-quality friction ridge impressions and identifying two military members not previously accounted for through other forensic means, was possible. This technique required only hours, instead of days or months, making it a viable alternative to other commercially available means. This technique requires only water and a heat source, making it deployable to the field without the need for chemical transport or hazardous waste.

Presented here is a case study in processing desiccated remains that describes the successes of obtaining prints from badly damaged and aged hands. Difficulties encountered with antemortem record collection will also be discussed, as it pertains to many cold case studies. Unlike DNA in which references can be obtained from surviving family matters, fingerprints rely on antemortem records. These antemortem records can become compromised, leaving even the best-obtained postmortem prints unidentified forever.

The boiling technique is a viable method of rehydrating desiccated remains for postmortem printing. It shortens the time spent working on the body and provides a usable result. There are limitations in what the boiling technique can do. The hands typically revert back to their dehydrated state within a minimal amount of time (minutes, or even seconds), but it eliminates the need for any chemicals or detergents. Sodium hydroxide baths, conditioners, embalming materials, and detergents can all assist in processing desiccated remains, but this method was found to be easy, quick, efficient, and safe.

Desiccated, Boiling Technique, Fingerprints

E29 The Missing Foreigner: Remains Found Eight Years Later Submerged in the Trunk of a Vehicle Full of Pluff Mud

Kelly Kraus, BS, Charleston County Coroner's Office, 2326 Eagle Creek Drive, Charleston, SC 29414*

After attending this presentation, attendees will understand the importance of a complete and thorough medicolegal death investigation using a multidisciplinary team approach and multiple resources in unusual or atypical homicide cases. In the United States, the type of death investigation and resources vary from state to state. This presentation will demonstrate how and why these complex cases must be thoroughly investigated by medicolegal death investigators. Information, resources, and suggestions will be provided to assist the attendees in investigating complex homicides with a multidisciplinary team approach.

This presentation will impact the forensic science community by providing information regarding unusual or atypical homicides and the medicolegal death investigation process used to investigate such cases.

Homicides resulting in submergence are fairly uncommon and much can be learned from these cases when they do occur. Presented here is a case study of a 31-year-old Middle Eastern male who died from homicidal violence. The decedent's skeletal remains were found in the trunk of a car submerged in water and pluff mud eight years after his death after the police received a tip from a citizen pulled over for a traffic citation. This case study highlights the importance of a multidisciplinary team approach that can include police officers, detectives, a dive team, a forensic services unit, deputy coroners, a forensic anthropologist, and a forensic pathologist while investigating these complex medicolegal death investigations.

This presentation contains case information regarding the extensive death scene investigation, which invoved multiple locations (including rivers and pluff mud), the review of pertinent medical records, the review of social service records, the autopsy findings, the anthropology findings, and relevant interviews conducted by the medicolegal death investigator and law enforcement personnel utilized prior to ruling the death a homicide.

The skills needed to investigate these types of deaths will be discussed as well as resources available to gain that knowledge and information, including interviewing, forensic photography, chain of custody, identification, notification, and death certificates, among others. Other opportunities and resources involved in the specialty are also discussed, including detectives, rescue and recovery units, forensic pathologists, anthropologists, and medical records.

The physical findings noted in the case as well as similarities and differences which may be found in homicides that are dumped in a trunk of a vehicle and then submerged in water will be discussed as will be the importance of medicolegal death scene investigation. This case study is a useful tool in explaining and discussing the importance of an immediate and timely death scene investigation and the information that may be obtained. The case also highlights the importance of obtaining and reviewing all social records as well as the importance of interviews, which may be conducted by medicolegal death investigators and/ or law enforcement personnel with family, friends, coworkers, medical providers, and others. A team approach to investigating these complex and suspicious death investigations is required in order to accurately determine the circumstances surrounding the deaths.

Homicidal Violence, Skeletal Remains, Pluff Mud

E30 Unusual Suicide: Staged Homicide in a Carbon Monoxide Setting

Brittney W. Martin, BS, 4050 Bridgeview Drive, Ste 500, North Charleston, SC 29405*

After attending this presentation, attendees will understand the importance of a complete and thorough medicolegal death investigation using a multidisciplinary team approach in an atypical suicide case. In the United States, the types of death investigation and resources vary from jurisdiction to jurisdiction. The presentation of this case study will demonstrate how and why these complex cases must be thoroughly investigated by medicolegal death investigators. Information, resources, and suggestions will be presented to assist in investigating complex suicides with a multidisciplinary team approach.

This presentation will impact the forensic science community by providing information regarding unusual suicides and the medicolegal death investigation process used to investigate complex staged death scenes.

A documented, staged homicide used to cover up a suicide death is uncommon in the United States and much can be learned in how to appropriately process the scene regardless of pre-investigation assumptions. Presented is a case study of an intelligent 27-year-old who methodically planned and staged his death to make it appear as a homicide. In the staged crime scene, the decedent's legs and arms were bound and tied together in a vehicle inside his detached garage. Evidence to suggest carbon monoxide poisoning was present. This presentation also supports why a thorough investigation of both the scene and surrounding areas is crucial in all investigations. This crime scene was located in the garage; however, inside the residence, there was evidence of a fire, a destroyed phone, and several windows that were caulked shut.

The lack of common physical findings in the setting of a decomposed body and how the decomposition of the body affects not only the initial examination but also the toxicology findings will be discussed. The toxicology findings in this case came back negative for carbon monoxide and this significantly affected the investigation. This case highlights the importance of obtaining and reviewing all medical, social, education, and criminal records as part of a complete investigation and illustrates how those records may be used in determining the manner of death.

This presentation includes case information regarding the extensive death scene investigation, which involves the review of pertinent medical records, review of social records, autopsy findings, toxicology findings, and relevant interviews conducted by the medicolegal death investigator and law enforcement personnel utilized prior to ruling the death a suicide.

The skills needed to investigate these types of deaths will be discussed, as will the resources available to gain that knowledge and information including interviewing, forensic photography, chain of custody, identification, notification, and death certificates, among others.

Staged Crime Scene, Restraints, Carbon Monoxide

E31 Homicide Victims in Freezers — A Report of Two Cases

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After attending this presentation, attendees will: (1) gain an appreciation for the special concerns when dealing with a frozen body; and, (2) understand additional issues related to the investigation of homicides involving frozen bodies.

This presentation will impact the forensic science community by providing information related to the evaluation of two homicide victims whose bodies were placed into freezers.

Many forensic pathologists are familiar with the challenges encountered when investigating a death involving a frozen body. These cases are relatively frequent in geographical locations with cold winters, where it is not uncommon to investigate numerous frozen bodies over the course of a winter season. A major challenge associated with frozen bodies, which can be "rock-hard," involves the fact that, in order to adequately examine the body via routine internal examination autopsy performance, the body must first be allowed to thaw sufficiently.[1] A troublesome problem that commonly accompanies the thawing process is rapid decomposition, which, as in frozen animals, typically occurs from the "outside in," as opposed to the typical "inside out" decomposition process that occurs in non-frozen bodies.[1,2] Although frozen bodies are not uncommon in certain jurisdictions, encountering frozen bodies that have been previously placed in a freezer are much less common than those resulting from exposure to cold outdoor environments. Presented herein are two unrelated homicides in which the bodies were placed in freezers by the perpetrators of the crimes.

An 81-year-old wealthy widow, estranged from family members, was living in her upscale home, which she shared with a middle-aged single man, who was a part-time mortician and church worship leader. He befriended the widow, and spent a great deal of time with her, which included numerous vacations together. The local townspeople noticed that she appeared to be missing, but the live-in friend claimed that she was sick, had checked-into a hospital in a large city under a pseudonym, and did not want to be bothered. Despite being estranged from relatives, several relatives attempted to maintain contact with the woman, but were unable to reach her for several months. Police investigation was hampered by the fact that there were no legal grounds by which they could investigate her home. Finally, a judge granted temporary guardianship to an adult son. A group of relatives and police officers entered her home, approximately nine months after she had last been known to be alive. The woman's body was found within a chest-type freezer in the home, with numerous frozen food items covering the body. The body was transported to the medical examiner's office for autopsy. Initial examination revealed the presence of four apparent gunshot wounds on the back. Following approximately 48 hours of body thawing at room and body cooler temperature, which was accompanied by rapidly developing decompositional changes of the skin, a complete autopsy confirmed the cause of death to be multiple gunshot wounds.

A 73-year-old woman's body was found in a chest-type freezer in the home that she shared with her live-in boyfriend, who confessed to killing her and placing her in the freezer two days prior to body discovery. Upon initial body examination, obvious sharp force injuries were identified. The body was allowed to thaw over the next four days, in a morgue refrigerator/cooler. Although noticeable external early decomposition was evident after thawing, its extent was not severe; however, partially frozen tissue was still evident on internal exam. The cause of death was multiple sharp force injuries.

Death investigators should be aware of the potential difficulties associated with performing autopsies on frozen bodies. Additional concerns arise if the frozen body represents a homicide. A detailed review of the two cases and the special concerns associated with frozen homicide cases will be presented.

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E32 Correlation of Bioelectric Impedance Metrics to Accumulated Degree Days Among Body Segments Using Gel Pad Electrodes

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After attending this presentation, attendees will understand the relationship between bioelectrical impedance metrics and Accumulated Degree Days (ADD).

This presentation will impact the forensic science community by providing an example of a new quantitative method for estimating Postmortem Interval (PMI).

Preliminary research conducted at Colorado Mesa University's Forensic Investigation Research Station (FIRS) demonstrated that Bioelectrical Impedance Analysis (BIA) metrics correlate with ADD. This research compared these correlations for different body segments on human cadavers using conductive gel pad electrodes.

Present methods for estimating PMI are limited by both subjectivity and the circumstances under which remains are found. The goal of this research was to develop an objective and quantitative technique that estimates PMI. BIA is a technique currently used to evaluate nutrition and body composition in humans and animals; however, it has also demonstrated the potential for forensic application. Forensic research using non-human study organisms also found relationships between BIA metrics and the PMI. For example, in rat abdominal walls, there was a relationship between resistance and PMI.[1] Similarly, in rat spleens, there was a statistically significant linear regression relationship between impedance (Z) and PMI.[2]

Between fall 2014 and winter 2015, BIA was conducted on human donors at the FIRS. For this experiment, conductive gel pad electrodes were attached to six human donors at anatomically defined landmarks. Gel pad electrode pairs were attached at a fixed distance of 10cm to measure four different body segments (hand-foot, hand-shoulder, shoulder-foot, and thigh-foot) on one side of the body. Single frequency BIA was used to measure the resistance (R, Ω) and reactance (X, Ω) of a 400μA and 50KHz Resistor Capacitor (RC) current passed through tissue daily throughout the decomposition event. Measurements ceased when the loss of tissue infrastructure was sufficient to result in failure to conduct a current. The BIA measurements were derived to mathematically convert each body segment into a cylinder. Circuit models in both series (s), and in parallel (p) were used for calculation of six BIA metrics: Resistance (R), Reactance (X), impedance (Z), phase angle (degrees), and R/X. Ambient temperatures were measured hourly using an on-site weather station. Mean daily temperatures were used to calculate ADD. Statistical analyses were conducted using Pearson product-moment correlation between ADD and each BIA metric. Separate correlation analyses were conducted for each individual human remains to correct for seasonality (statistical significance $\alpha = 0.05$).

The range of maximum PMIs yielding measurements within the sample for the hand-foot body segment was 10-45 days. The range of maximum ADD within the sample for the hand-foot body segment was 150°C-335°C. Because the hand-foot segment is the longest circuit — and therefore the most vulnerable to tissue breakdown and circuit disruption — this serves as a conservative estimate for the longitudinal efficacy of the method.

The correlation with ADD varied by BIA metric and by individual remains. The highest correlation for each body segment was hand-foot (15-07, $Xs = 0.93$), hand-shoulder (15-06, $Rs = 0.97$), shoulder-foot (15-02, $R/X = 0.97$), and thigh-foot (15-02, $Xs = 0.97$ and $Zp = 0.97$).

The percentage of statistically significant correlations between ADD and all BIA metrics varied more among body segments than BIA metric. For body segment, the percentage of significant correlations were hand-foot (67%), hand-shoulder (36%), shoulder-foot (24%), and thigh-foot (43%). For BIA metrics, the percentage of significant correlations was 43% for all metrics except Rs (48%) and Xp (33%).

Bioelectrical impedance analysis measurements significantly correlated with ADD for multiple body segments in human remains. This research highlights the difference among body segments, BIA metrics, and their relationships with ADD. Ultimately, multiple regression models will be developed to predict ADD as an indice of PMI.

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Forensic Science, Bioelectrical Impedance, Postmortem Interval

E33 Patterns of Violent Death in Clark County, Nevada: Homicide and Suicide Patterns During and Post-Recession

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After attending this presentation, attendees will better understand patterns of violent death within the metropolitan landscape and, specifically, patterns of homicide and suicide within Clark County, NV. This study examines violent death as it relates to recession and post-recession years (2008 and 2015, respectively) to see the impact that this tumultuous period may have had on those who experienced violent death.

This presentation will impact the forensic science community by: (1) providing information regarding the impact of recession periods on violent death within the metropolitan landscape by presenting a case study of homicide and suicide data from Clark County, NV; and, (2) increasing the attendees' awareness of how economic conditions and demographic factors may affect patterns of violent death.

Demographic information was collected for homicide and suicide deaths for 2008 and 2015 using the Clark County Coroner and Medical Examiner Officer records and analyzed for patterns of demographics, including age, sex, ethnicity, marital status, and zip code (as a proxy for socioeconomic status). These factors were then compared between the two years for both types of violent death to identify any differences in patterns and frequencies. For both homicides and suicides, the number of cases increased between 2008 and 2015. There were 153 homicides and 383 suicides in 2008 and 182 homicides and 416 suicides in 2015.

Homicides varied slightly between 2008 and 2015. Despite making up a smaller portion of the population in 2008, Black and Hispanic male homicides both outnumbered White homicides for the year, with Black males accounting for 39/153 homicides and Hispanic males accounting for 35/153, while White males only accounted for 32 homicide deaths for 2008. In 2015, White and Black males accounted for an equal number of homicide deaths (51/182 each), despite Black individuals continuing to make up a fraction of the population of Clark County. For both 2008 and 2015, Black individuals died younger than their White counterparts, with the majority dying before the age of 40 while White individuals were more likely to die between the ages of 30-60. In 2008, there was also an increase in the number of young male Hispanic deaths (21-30 years old) compared to 2015.

A comparison of suicide trends revealed little difference between the 2008 recession and 2015 post-recession years. The suicide rate was similar for both years at approximately 0.02% of the total population. Age, sex, and ethnicity trends were also similar with White males being by far the most commonly affected group in both years followed by White females. In 2008, White males accounted for 244 of the 383 total suicides and white females accounted for 71. In 2015, White males accounted for 232 of the 416 total suicides and White females accounted for 91 of the suicides. The number of suicides also increased by age in both years with generally the highest rates of suicides in the 41-50-year and 51-60-year age ranges.

Based on these data generated from the Clark County Coroner and Medical Examiner's Office records, there does not appear to be significant differences in suicide death demographic patterns between the recession and post-recession periods in Clark County, NV; however, there is a slight increase in the frequency of minority male homicides for 2008 in comparison to 2015, where they outnumbered White male deaths. This suggests that while the recession may have had little impact on the suicide patterns, it may have impacted the number of minority individuals who experienced homicide deaths.

Homicide, Suicide, Demography

E34 Gunshot Residue Documentation Using 3D Laser Scanning Methodology

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After attending this presentation, attendees will: (1) understand the use of 3D laser technology for the documentation of Gunshot Residue (GSR) evidence at crime scenes; and, (2) determine the limitations of 3D laser technology for the documentation of GSR.

This presentation will impact the forensic science community by illustrating how the use of 3D scanning technology for the documentation of crime scenes and physical evidence, while accepted in court, has little research background for its use as a documentation format. This study looked specifically at the use of 3D laser images for the documentation of GSR and its limitations.

This study focused on the limitations of the 3D laser scanner in relation to GSR and how minute traces, if any, can or cannot be detected by the laser scanner. If the scanner is able to detect minute traces, investigators can then use it at crime scenes to help reconstruct the events that transpired. The ability to determine if a suspect was standing relatively close to the victim who is shot in the back compared to shooting the victim from a distance can corroborate or disprove a story of self-defense.

Six distances of muzzle-to-target fabrics shot with a .40-caliber Smith & Wesson® were provided. Distances included contact, 3 inches, 6 inches, 12 inches, 18 inches, and 24 inches. First, the GSR targets were photographed using typical documentation techniques. Subsequently, they were documented using the 3D laser scanner. The Leica® C10 3D laser scanner was placed 17 feet away for two scans, and then 2 feet away for two scans. Medium resolution scans of the test room with the targets and highest resolution scans of the premade gunshot residue patterns were used to attempt to determine the muzzle to target distance of the shot. These long-distance and short-distance scans at different angles allowed for the software to have multiple angles and distances to stich together. The scans were then downloaded to a flash drive and to a computer to upload into the Cyclone software. After the scans were applied and registered, a TruView™ model was created in which the photographs taken of the GSR patterns were imbedded into the software.

A visual comparison of the GSR 3D laser scans to the traditional photographic images was performed to determine the resolution of the scans for their use and ability for range-of-fire determination. The 3D laser scans of the GSR patterns had a significant difference in the resolution compared to the photographs of the GSR patterns. The scans did allow for the user to see the burn pattern up to and including 12 inches. As the GSR pattern diminished with the further distances, the lesser quantity of powder became more difficult to see in the scans as compared to the traditional photographic images.

When working with the scanner, the closer the scanner is placed to the target, the denser the information points will be when one uploads the data onto the software. In this study, even when the scanner was placed two feet from the GSR patterns and at approximately the same height, with this optimal condition, the scans' quality was subpar to that of the traditional photographs. While the scanner does have the ability to resolve the shorter firing distance GSR burn patterns, there was difficulty determining the location of the bullet hole in the fabric in some of the patterns. As the scans were zoomed in, the resolution became increasingly poor. The scanner can be used for overall and larger pieces of evidence, but the smaller pattern evidence, such as GSR patterns or bloodstain patterns, should still be documented with photographs, then presented separately. Also, using the photos imbedded in the TruView™ scans could be an option.

3D Laser Scanning, GSR, Range of Fire

E35 National Missing and Unidentified Persons System (NamUs) Database Reconciliation: "No Body" Murder Trials and Missing Persons

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After attending this presentation, attendees will understand: (1) NamUs, its benefits, and shortcomings; (2) "No Body" murder trials; and, (3) the challenges in solving missing persons and unidentified decedent cases.

This presentation will impact the forensic science community by demonstrating a useful tool in an under-researched area and the investigation of missing and unidentified persons. Specifically, this presentation will provide the results of reconciling victims from "No Body" murder trials with the missing persons database.

There are more than 100,000 active missing persons cases on any given day, and more than 40,000 unidentified remains at medical examine/coroner offices throughout the country.[1] This number of unidentified remains will only continue to increase, as approximately 25% of the unidentified cases handled in medical examiner/coroner offices will remain such after one year.[2]

After a few years of strategizing and identifying challenges and tools available to investigate and solve missing persons and unidentified human decedent cases, NamUs was created. NamUs is a web-based database which can be used to "search cases in the missing persons database against cases in the unidentified decedents database in an effort to identify unidentified human remains and solve missing persons cases."[3]

NamUs is a repository of information on missing persons and unidentified remains and, also offers free DNA testing and anthropology and odontology information for cases across the country. The system automatically cross-matches comparisons for similarities when a new missing person or unidentified decedent case is entered and has aided in closing 12.19% of the missing persons cases entered and 33.14% of the unidentified decedent cases entered.[4]

As in any tool, this database is only as good as the information entered into the fields. There are many situations that preclude cases from being entered into NamUs (for instance, a person never reported missing by loved ones). Another such situation was found in "No Body" murder trials, which are trials for murder that occurred without a body ever being found. Since a perpetrator was tried for the murder and the victim was deceased in the eyes of the law, it was assumed that many of these cases were not entered into NamUs as missing persons. Therefore, should their remains be found, identification would be complicated at best.

Of the 444 "No Body" murder trials in the United States, Puerto Rico, and the Virgin Islands that occurred through June 2015, there were 464 victims.[5] Approximately 5% of the victims' bodies were since found and identified and approximately 38% of the victims were listed as missing in the NamUs missing persons database, however, slightly more than 57% of the victims were not listed in the missing persons database. This demonstrates a gaping hole in information available to aid investigators working to identify decedents; if the decedents are not listed as missing in the database, they will not be ruled in or out, nor will any DNA or other potential information be collected from family for potential identification.

The purpose of this presentation is to inform the forensic science community of the findings of the reconciliation of these "No Body" murder trial victims and the NamUs missing person database with the goal of increasing awareness of the system and ultimately improving outcomes for solving missing person and unidentified decedent cases. The study highlights a great tool, but also demonstrates how important the role of law enforcement officials, medical examiners and coroners, forensic scientists, attorneys, key policymakers, and victim advocates and families is in being diligent in using NamUs for missing person and unidentified decedent cases.

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Missing Persons, Unidentified Decedent, NamUs

E36 Crime Scene Investigations in Child Homicides

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After attending this presentation, attendees will better understand contextual and forensic characteristics of child homicide crime scenes as well as potential pitfalls associated with child homicide investigations. Data from an ongoing study will be presented.

This presentation will impact the forensic science community by increasing the overall knowledge of crime scene investigations in child homicides, which can contribute to an optimization of such investigations as well as reducing the risk of a "dark figure."

The study hypothesizes that there is little scientific guidance in whether crime scene investigations in unexpected child deaths should be conducted similarly or differently than unexpected adult deaths.

Child homicide cases pose a challenge for the criminal justice system and the professionals who are involved in such investigations.[1] Criminal investigations of unexpected deaths among children are emotionally burdensome and surrounded by potential "pitfalls" that may cause a homicide to be misclassified as being accidental or natural and elude detection. In addition, most cases occur in an intra-familial setting without the presence of potential witnesses, which further increases the complexity of the investigations.[2,3] Hence, child homicide investigations depend largely on the autopsy and the crime scene investigation as important sources of forensic evidence. Thus, a thorough examination of the scene together with a systematic search for signs evidential of criminal violence is necessary for the reconstruction of events leading up to the death of the child. The goal of this study was to elucidate which crime scene characteristics are associated with child homicide cases, a so far unexplored area of research.

In this ongoing population-based study of child homicides, police reports, including crime scene and autopsy protocols, of child homicides occurring from 1992 to 2012 in Sweden were collected; however due to the disposal of reports from crime scene investigations, only the years 1998 to 2012 were examined in this study.[3] Children between 15 and 18 years of age were excluded. In total, 53 cases with 61 victims (25 girls and 36 boys aged 0-14 years) were reviewed using a structured protocol involving epidemiological, forensic medical, and crime scene data. The data will be presented using descriptive statistics.

The annual frequency of child homicide offenses in Sweden was 4.0 cases (4.6 victims). A majority of the cases, 79%, were intra-familial in nature and most commonly committed in the parental home (*n*=40). The body was most frequently found lying on the floor in the parental bedroom. Obvious signs of violence were present in half of the crime scenes and a majority of victims displayed visual injuries (73%). The forensic medical examiner was called to the scene in one-third (31%) of the cases and 89% of the children (*n*=54) died immediately, most frequently from asphyxia and sharp violence (*n*=16 and *n*=15, respectively). Signs of neglect and abuse prior to death was present in 24% of the cases (*n*=10). Seven of 43 indoor crime scenes were cleaned by the perpetrator prior to the crime scene investigation (16%). There were active efforts by the perpetrator to mislead the investigation following 25 children's deaths (61%) and, in two cases (3%), the crime scene was staged to mimic another type of death other than homicide (accident or natural). In 31% of 59 cases (*n*=18), the victim's body was arranged (i.e., the body was covered, hidden, dressed, etc.).

The results of this study indicate that a death scene investigation in child homicide cases is vital not only for the detection of the willful killing of a child, but also for bringing justice to the victim; however, most of this study's findings cannot be related to previous research due to a world-wide lack in the literature. Which specific traits characterize child homicides needs to be further elucidated.

This study concludes that crime scene investigations are of importance in cases of a child's unexpected death, but also that there is little scientific guidance in how often a death is followed by a crime scene investigation and how the investigations may differ from crime scene investigations of the unexpected adult deaths.

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Child Homicide Investigation, Child Death Investigation, Forensic Evidence

E37 Analysis of Bullet Entry Hole Diameter by Varying Distance, Caliber, and Grain

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After attending this presentation, attendees will understand how shooting distance, bullet grain, and bullet caliber affects the bullet entry hole diameter in a solid target substrate (drywall). Gunshot Residue (GSR), bullet trajectory analysis, and firearm examination are also established methods used for crime scene reconstruction of shooting incidents. This research is an attempt at providing an alternative method for firearm identification (or exclusion) of a possible class of firearm when the weapon and ammunition are missing from the scene or the recovered weapon is unsuitable for testing.

This presentation will impact the forensic science community by providing a better understanding of the many components involved in a crime scene reconstruction when a shooting took place. It will be evident that conclusions cannot be based solely on one factor, but instead rely on careful scientific method-based analysis of several ballistic variables.

The following handguns were used in this study: Smith & Wesson® M7P9, 9mm caliber; Heckler & Koch® P2000, 0.40 caliber; and Smith & Wesson® M7P45, 0.45 caliber. Bullet entry holes were examined by firing multiple rounds at a drywall target. Three different ammunition calibers were used (9mm, 0.40 and 0.45). Each caliber was either a heavy or light grain type (9mm at 115 and 147 grain, 0.40 caliber at 155 and 180 grain and 0.45 caliber at 185 grain). All ammunition fired was Hornady® hollow point.

Additionally, there were three different firing distances depicting near contact, intermediate, and distant ranges, measured from the gun muzzle to the target at 4inches, 24inches, and 48 inches. Six rounds were fired for each caliber, grain, and distance for a total of 108 expended rounds. Each bullet hole was numbered and the diameter of the entrance holes bullet "wipe" was determined with an L-shaped Lynn Peavey metric ruler. Images of the ammunition before it was fired, as well as of each bullet entry hole, were taken with a Nikon® D3200 camera. The measurements were entered into an Excel® spread sheet and analyzed using the included Analysis of Variance (ANOVA) statistical test to determine significance (p value < 0.05).

Results of this study indicated that a larger bullet caliber does not necessarily produce a significantly larger entry hole diameter when compared to a smaller bullet caliber. Also, the same bullet caliber with different grain does not produce a larger or smaller entrance hole. Findings determined that firing the same caliber and different grain loads at different distances, and firing the same caliber with the same grain loads at different distances, did not affect the size of the bullet entry hole in dry wall.

These conclusions were based on the variables and drywall substrate used in this study and could vary when other types of weapons are fired. This type of scientific model approach to shooting incident reconstruction is needed to enhance expert testimony and admissibility in the courtroom that is not based on myths or anecdotal or erroneous beliefs.

Shooting Distance, Bullet Grain, Bullet Caliber

E38 Lack of Death Scene Investigations: The Potential Misdiagnosis of Sudden Infant Death Syndrome (SIDS), and Accidental Asphyxiation in Stockholm, Sweden

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After attending this presentation, attendees will better understand of the importance of Death Scene Investigation (DSI) in all infant deaths, not merely homicides, as well as an appreciation of how information gathered at death scenes must be taken into account when distinguishing between a diagnosis of SIDS and accidental asphyxiation.

This presentation will impact the forensic science by illustrating how a lack of DSI and poor information gathering regarding the infant's sleeping environment may skew data in favor of a borderline SIDS diagnosis, while failing to consider a diagnosis of accidental asphyxiation affects epidemiological studies and public health guidelines.

SIDS is a syndrome and, as such, is potentially the result of several pathologies. It is a diagnosis of exclusion and should only be applied when all other causes of death have been discarded. Further knowledge in the field of SIDS research led to a recent fall in the rate of SIDS diagnoses. This is partly due to an increasing reluctance among forensic pathologists to use the diagnosis in cases in which a possible unsafe sleeping environment may pose a risk for asphyxiation.[1] As per the San Diego Criteria introduced in 2004, a SIDS diagnosis should only be applied in cases of sudden infant deaths which remain "unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical histor."; however, in Stockholm over the past ten-year period, no cases of accidental asphyxiation as a primary cause of death were recorded with the National Board of Health and Welfare, Socialstyrelsen (the Board).[2]

All deaths in Sweden are registered with the National Board of Health and Welfare. In accordance with Swedish law, all infant deaths undergo a thorough review of clinical history as well as a forensic autopsy, including toxicology screening, histology, and neuropathology investigations and genetic analysis; however, there is currently no mandate or legislation enforcing mandatory DSI.

A review of all infant deaths under one year of age in Stockholm during 2005-2014 revealed a total of 111 cases, without any listing asphyxiation as a primary cause of death with the Board. All of the available information for each death, including death certificates, autopsy reports, police notes, and interviews with family members, were reviewed. Three cases were diagnosed as asphyxiation or covering of the airway, but only as a secondary diagnosis and thus were not registered with the Board. In total, out of the 111 cases, 33 were diagnosed as SIDS. A total of 20 cases were noted to exhibit aspects of potential asphyxiation when the additional information regarding the death scene and the sleeping environment were taken into account. Out of these, eight were recorded as SIDS/borderline SIDS, due to a lack of death scene investigations. These exhibited aspects of unsafe sleeping environments, such as being caught between the back of a sofa and a sleeping parent, sleeping between parents, or being found underneath a sleeping mother during breastfeeding. A further three cases were recorded as unknown cause of death, *causa mortis ignota*. Five cases were recorded as pneumonia and two as anoxic brain injury. Multiple organ failure secondary to anoxic brain injury and heat was recorded in two cases.

As is the case in Sweden, a lack of DSI precludes a true SIDS 1A diagnosis, according to the San Diego Criteria. This research also demonstrates that DSI is crucial in distinguishing between accidental asphyxiation and SIDS as it is a distinction which cannot be made based solely on autopsy findings and thus continues to pose a challenge for forensic pathologists.

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Accidental Asphyxiation, SIDS, Death Scene Investigation

E39 Gunshot Residue (GSR) Analysis by Single Particle Inductively Coupled Plasma Mass Spectrometry (spICP/MS)

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After attending this presentation, attendees will aware of a new method for analyzing GSR using spICP/MS.

This presentation will impact the forensic science community by demonstrating that spICP/MS is a fast, accurate, and promising method for GSR analysis that can identify and characterize hundreds of nanoparticles.

GSR contains micro and nanoparticles resulting from the rapid cooling of the discharge of gases and solid matter from firearms. GSR originates from the primer and propellant, as well as from the metallic components of the ammunition and firearm. The composition of GSR can vary mainly because of different primer compositions. The research presented is based on primers containing lead styphnate, barium nitrate, and antimony sulfide, which lead to a typical particle composition containing lead (Pb), barium (Ba), and antimony (Sb). The vast majority of modern ammunition uses this type of primer.[1]

After the discharge of a firearm, GSR particles can be deposited onto the shooter's hands, clothing, or other objects or people in the proximity. According to Locard's principle, GSR can also be transferred from one surface to another due to contact.[2] Thus, the analysis of GSR could provide some insight into the dynamics of a crime scene.

Scanning Electron Microscopy (SEM) with Energy-Dispersive X-ray (EDX) spectroscopy is the state of the art for GSR analysis; however, the setup and manual confirmation of results is tedious and time-consuming.[3, 4] The automated search of one blank stub can take two to six hours, depending on the instrument and chosen parameters. The duration of analysis could increase greatly if a sample contains a large number of detected particles.[5]

The research focus is on the use of spICP-MS to analyze GSR particles. With the availability of new analytical instruments with higher sensitivity and greater data processing capacity, it has been possible to identify and characterize GSR nanoparticles in a given sample in ten minutes. With the aid of an auto-sampler, the process can be fully automatized, allowing the analysis of more than 100 samples per day, independent of the number of particles in the sample. Another advantage of the technique is the possibility of fully automating post processing. The drawback of the method is that the use of quadrupole mass spectrometers only permits simultaneous analysis of two elements in each individual particle.

This presentation will discuss some of the results obtained with this new approach and open a new perspective for future research on GSR analysis. Swab samples collected from shooters' hands were sonicated in 10mL ofwater and analyzed with this technique, resulting in more than 600 particles per milliliter.

The low cost of analysis and less time-consuming sample preparation and analysis makes this new approach a promising procedure for GSR identification and characterization.

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GSR, Single Particle, ICP/MS

E40 "Digital Stringing" — The Practicality and Legitimacy of Determining the Area of Origin (AO) of an Impact Event From 3D Scans Recorded Via Structured Light Scanning

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After attending this presentation, attendees will be made aware of the possibility, practicality, and potential advantages of determining the AO through "digital stringing" from Blood Pattern Analysis (BPA) of a simulated impact event captured via structured light scanning.

This presentation will impact the forensic science community by providing an investigation of the possibility and capabilities of determining the AO of impact events using digitally captured 3D scans. Such a technique may prove useful in reconstructing impact events by providing a 3D representation of the AO, re-evaluating/verifying AO calculations, and/or providing the possibility of calculating the AO of a previously recorded BPA event that may not have been performed *in situ* during initial scene processing.

Multiple impact events were created by a third party using laboratory materials, including animal blood and various blunt instruments, in an enclosed space with white walls. The purpose of using such instruments was to simulate the blood spatter that may occur as a result of blunt force trauma, as opposed to sharp force trauma, high-velocity blood spatter (i.e., gunshots), and/or arterial spurts. After the impact events were simulated, a full 3D scan of the area was performed using a 4D Dynamics Real 3D Scanning Solutions PicoScan, structured light scanner with a Canon® EOS® 1000 D/Rebel XS camera equipped with an 18mm-55 mm zoom lens and an 800 x 600 resolution pico projector, to record the spatter. Following the scan, *in situ* stringing was performed and photographed by to record each impact event. Additionally, each individual impact spatter used for AO determination during the *in situ* stringing was documented. This was done in order to insure that the same spatter droplets were used while "digitally stringing" the AO of each event.

Inspired by the 'digital stringing' capabilities of the FARO® SCENE software, using 3D images captured via the Pico Scanner, attempts were made to string the events digitally.[1] Upon completion, the AO of the 3D scans of the impact events that were strung digitally were compared with the images taken of the AO that were strung *in situ*. Additionally, all impact angles calculated during the *in situ* stringing and "digital stringing" were compared.

The results were compared in order to determine the legitimacy and efficacy of using digital scans in order to accurately determine and represent the angle of origin of a series of impact events. While determining the AO of impact spatter is in itself an estimation, it is hypothesized that with accurate, highly detailed, 3D digital scans of a series of impact events, the AO of each event can be estimated digitally to the same standards as if it were done *in situ*. Furthermore, it is hypothesized that by providing a 3D representation of the AO to a jury, a better understanding of the impact events and the overall scene will be achieved.

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Digital Stringing, Blood Spatter, 3D Scan

E41 Estimation of Time Since Death Using Body Cooling Models of Pigs: A Pilot Study

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After attending this presentation, attendees will understand the correlation between postmortem cooling of the body, rectal temperature, ground surface temperature, and ambient temperature, which is essential for estimating time since death in the early stages of decomposition.

This presentation will impact the forensic science community by establishing a method to study algor mortis of pig carcasses and by presenting a formula for estimating time since death in the early stages of decomposition.

A facility for decomposition research using animal specimens was established at the Korean Police Investigation Academy (KPIA) in 2013. Various experiments are conducted to test the effects of different variables on the rate of decomposition. Currently, KPIA recommends that crime scene investigators employ the Henssge nomogram method at the death scene, which is the estimation of time since death using rectal and ambient temperature; however, there is a paucity of research on the effects of heat radiation and the Henssge nomogram. *Ondol* is a traditional floor-heating system in Korea, where the floor is heated from below and the heat radiates to warm the room. Bodies are commonly found indoors in Korea and the application of the Henssge nomogram is problematic due to the effects of the *ondol* heating system on the rate of postmortem heat loss in the body. The goal of this study is to test the effects of the Korean floor-heating system on heat loss using adult pig models and to create a novel formula for estimating time since death during the early stages of decomposition

Four 40Kg pigs (*Sus scrofa*) were killed by administering a euthanasia drug for each animal per the Soonchunhyang University Institutional Animal Care and Use Committee protocol. Three electric mattress pads were placed on the ground to maintain a constant temperature of the substrate similar to the *ondol* heating system. Each pig was placed on four different surfaces: low-heat, medium-heat, and high-heat electric mattress pads (experimental group), and the ground (control). Four temperature measuring probes were placed in each pig, inside of the rectum, on the body surface, between the body and the surface of mattress pad/ground, and on the mattress pad/ground. The probes were connected to a temperature data logger system, and a WatchDog® 2000 mini station was located at the research site to measure the ambient temperature. The temperature was recorded every minute and the pigs were monitored hourly for 48 hours by Closed-Circuit Television (CCTV). Statistical analysis was performed using the Statistical Analysis System (SAS) (version 9.3) program.

Spearman's Rank Correlation results demonstrate the magnitude of the relationship between four body temperatures and ambient temperature.

	Ambient temperature	Inside rectum	Between body and pad/ ground	Surface of body	Pad/ Ground
Ambient temperature	1				
Inside rectum	0.550	1			
Between body and pad/ ground	0.451	0.999	1		
Surface of body	0.842	0.956	0.945	1	
Pad/ Ground	0.208	-0.304	-0.332	0.048	1

The rectal temperature and the temperature between the body and the surface of the pad/ground are strongly correlated with postmortem cooling of the body rather than ambient temperature. The rate of cooling of the body is represented by a cube function of time rather than an exponential or bi-exponential function.

The results of this pilot study demonstrate that postmortem cooling is correlated with the rectal temperature and the temperature between the body and the surface where the body was laid. It indicates that postmortem cooling of the body is more influenced by ground surface temperature than by ambient temperature. Additionally, the study demonstrated that pigs can be good animal models that can substitute for human cadavers when studying the process of decomposition.

Time Since Death, Postmortem Cooling, Early Stages of Decomposition

E42 Preservation of Hair Stable Isotope Signatures During Freezing

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After attending this presentation, attendees will understand the suitability of law enforcement preservation protocols for hair Stable Isotope Analysis (SIA) in a range of typical casework samples.

This study will impact the forensic science community by scientifically validating current law enforcement practice. It will also serve to increase knowledge of the benefits of SIA to the law enforcement community.

Stable isotopes are an important tool in establishing the provenience of unknown human remains.[1,2] Studies demonstrate oxygen, hydrogen, carbon, and nitrogen isotopes in hair define an individual's travel and dietary history over months to years.[3,4] Studies validating SIA for provenience primarily analyze samples from living individuals or discarded salon waste, analyzed soon after collection and stored at room temperature. Although individual ancestry and cosmetic treatments of hair can influence measured drug concentrations and damage from Ultraviolet (UV) radiation, these factors have not been systematically studied in regard to stable isotopes. In contrast to validation studies, typical forensic case samples may include degraded material exposed for extended periods to dirt, rain, insect activity, and decomposition fluids.

Standard law enforcement collection techniques typically freeze evidentiary hair samples for DNA processing. Because samples may be frozen in contact with water or decompositional fluid, ice crystals may form during storage, with the potential both to physically damage the proteins of hair and allow isotopic exchange between samples and isotopically distinct local humidity. Although successful case reports suggest freezing is an appropriate storage method, a more systematic validation is needed.

Salon samples (n=14) were collected, including hair treated with relaxers and coloring agents. To evaluate preservation of degraded material, hair mats from the University of Tennessee's Anthropology Research Facility (n=2) and Texas State University at San Marcos' Forensic Anthropology Research Facility (n=4) were also analyzed, from decedants exposed on the ground surface outdoors for up to eight months. Each sample was separated into five aliquots: (1) control samples; (2) plastic clamshell for three weeks; 3) plastic clamshell for three months; (4) butcher paper for three weeks; and, (5) butcher paper for three months at -20°C. Each sample was packaged and sealed in accordance with the Mesa Police Department's Evidence Section guidelines.

To evaluate longer-term storage, paired samples from ten individuals at the University of Tennessee's Anthropology Research Facility were also analyzed. These were samples collected upon donor intake, with aliquots being stored at both room temperature and frozen. Periods of preservation ranged from 9 months to 4.1 years.

All samples were cleaned by standard methods and analyzed for carbon, nitrogen, oxygen and hydrogen isotopes by Isotope Ratio Mass Spectrometry (IRMS). There were no significant trends in $\delta^{18}O$, δD, $\delta^{13}C$, $\delta^{15}N$, or weight percent O, H, C or N in any of the samples for either sample storage period, or utilizing either packaging material. Freezing hair samples in typical law enforcement packaging is appropriate for forensic case work in stable isotopes.

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Stable Isotope Analysis, Forensic Provenience, Unidentified Human Remains

E43 An Examination of the Effects of *Salvia divinorum* Fortification on Stable Isotope Ratios

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After attending this presentation, attendees will understand the principles of stable isotope analysis and its impact on determining the geographic origin of commercially available samples of the plant species *Salvia divinorum.* Attendees will also understand the effect fortification has on stable isotope ratios and how this affects interpretation with regard to predicting the origin of cultivation.

This presentation will impact the forensic science community by revealing how the stable isotope ratios of fortified drug samples do not reflect those of unfortified drug samples. Interpretation of fortified sample stable isotopes results in an incorrect classification of the drug to a region of growth. This presentation will also contain the first known published data on stable isotope ratios of *Salvia divinorum* as it pertains to geographic areas of cultivation.

Salvia divinorum is a plant species found in Oaxaca, Mexico. The leaves of this plant contain the active compound Salvinorin A, which, when smoked, causes the user to experience hallucinogenic effects. Currently, *Salvia divinorum* is not listed as a scheduled drug under the United States' Controlled Substances Act, though some states such as Ohio and Texas have passed laws to prohibit its sale and/or use. Commercially available *Salvia divinorum* products are available in fortified extract concentrations claiming to contain up to 50 times the Salvinorin A concentrations naturally present in *Salvia divinorum*.

Stable isotope ratios of elements such as Carbon (C), Nitrogen (N), Oxygen (O), and Hydrogen (D) reflect the environmental conditions, such as atmospheric carbon dioxide and water stress, which are unique to a geographical region. These isotopes ratios are present in the local plant species of an area; thus, plants can inform researchers as to the elemental makeup of a region, as well as whether the plant was grown indoors or outdoors. In a previous study, Booth demonstrated the effectiveness of using stable isotope ratio data of marijuana for the determination of drug trafficking patterns.[1]

In this experiment, commercially purchased dried leaf samples of *Salvia divinorum* were finely pulverized and analyzed using isotope ratio mass spectrometers. Stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD) for *Salvia divinorum* were determined for the geographic regions of Oaxaca, Mexico, and Hawaii, USA. Samples with fortified extracts of 5x, 15x, 35x, and 50x were compared to standard organic leaves. It was determined that the stable isotope ratios were affected by increasing fortification. Analysis of variance of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ data demonstrated that fortified leaves resulted in a statistical difference from organic leaves, though this difference did not affect interpretive value. δD showed a statistically significant difference between organic leaves and fortified leaves. Hydrogen demonstrated the greatest variation with fortification and did not reflect its original geographic origin. Fortification displayed the ability to change stable isotope ratios such that the interpretive value of isotope data would no longer be accurate.

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Stable Isotopes, *Salvia divinorum*, Trafficking

E44 A Rare Case of Fatal Anaphylactic Reaction Following the Application of Gadobutrol, a Gadolinium-Based Contrast Agent, for Contrast-Enhanced Magnetic Resonance Imaging (MRI)

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The goal of this presentation is to sensitize forensic pathologists (and clinicians) to the fact that fatal anaphylactic reactions cannot only occur after application of a Computerized Tomography (CT) contrast agent which is generally well known, but also after application of an MRI contrast agent. For the forensic pathologist, it is a rare but important cause of death to be considered in forensic investigations.

This presentation will impact the forensic science community by expanding professional knowledge beyond the general known causes of anaphylactic reactions by discussing a rare trigger of a fatal anaphylactic reaction. Adverse drug reactions in gadobutrol in general have a very low incidence of 0.55%-3.5% and there are even fewer cases of fatal anaphylactic reactions; however, if an anaphylactic reaction occurs, it can present itself with a fulminant course within minutes. Even in a medical setting with highly trained professionals, the outcome can be fatal.

The rare case of a fatal anaphylactic reaction to gadobutrol, a MRI contrast agent, in a 42-year-old male is presented.

The patient underwent elective MRI for diagnostic clarification of a suspicious kidney tumor. He had undergone contrast-enhanced computed tomography previously without occurrence of any adverse effects. A few seconds after the application of the MRI contrast agent gadobutrol, the patient felt unwell and complained of nausea and dypnoea. With the suspected diagnosis of an allergic reaction, he was immediately administered an ampoule of the antihistamine clemastine, the H2-receptor-antagonist ranitidin and the glucocorticoid dexamethasone intravenously. Yet, within minutes, he went into cardiac arrest. Even though resuscitation measures were started immediately and successfully, he died two days after the event due to ischemic brain damage. In the blood samples obtained upon admission to the hospital, the enzyme tryptase was elevated several times higher than normal. Autopsy showed massive brain edema. Cause of death was paralysis of the respiratory system due to brain edema following the lack of oxygen resuscitation measurements as a consequence of anaphylactic reaction to the MRI contrast agent gadobutrol. There were no indications of medical or third-party negligence.

Anaphylactic reaction to an MRI contrast agent is very rare, but can take a relentless course within minutes of occurrence. Even in a medical setting with highly trained professionals, the outcome can be fatal.

Forensic pathologists have to keep in mind that fatal anaphylactic reactions can also occur after application of an MRI contrast agent. Clinicians should understand that even during MRI examinations, fatal accidents can occur and resuscitation equipment as well as a well-trained staff must be immediately available at all the times.

Fatal Anaphylactic Reaction, MRI Contrast Agent, Gadobutrol

E45 Evaluating Violent Crime Trends of Five Ohio Cities to Enhance Law Enforcement's Understanding of the Communities They Serve

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After attending this presentation, attendees will understand the importance of utilizing all available data, regardless of how simplistic, to continually analyze the effectiveness of law enforcement's role within the communities they serve. This will be emphasized by examining five northeast Ohio cities in detail.

This presentation will impact the forensic science community by demonstrating that a meaningful analysis of local, state, and federal law enforcement's impact on the communities they serve does not have to be complicated; rather, it can be achieved very easily. In many cases, a firm understanding of the dynamics of these communities and the trends of violent crimes occurring in each can suggest the success of a wide array of law enforcement initiatives and can push forensic professionals to implement the most up-to-date testing methods to accommodate the common evidence being submitted to area laboratories. Simplistic methods of analysis, such as the one examined here, are far from groundbreaking; however, this research stresses the importance of conclusions that can be drawn concerning the achievements or shortcomings of law enforcement agencies in a particular area, using very straightforward means. The conclusions achieved from these analyses may even serve as the motivation to continue with current practices or be catalyst to reform these approaches by law enforcement to better serve the needs of their communities.

In this research, the violent crime trends for five northeast Ohio cities were evaluated. Cleveland, Lorain, Salem, Warren, and Youngstown were assessed due to their proximity to each other. Each represents the largest city in their respective counties, and each falls within similar federal jurisdictions. Additionally, each of these cities reported violent crime data to the Federal Bureau of Investigation's (FBI) Uniform Crime Reports faithfully over the course of 20 years, from 1995 to 2014.

Each of these cities falls under the jurisdiction of the Cleveland Division of the FBI. Therefore, this was seen as a unifying factor of these cities. As a result, the data reported by the FBI was used to map the violent crime trends for each of these cities in order to maintain continuity. Thus, keeping true to these reports, murder/non-negligent manslaughter, forcible rape, burglary, and aggravated assault were considered for trend analysis.[1] Using a variety of statistical analyses, these local trends were then compared to the city's population to generate overall insight to the city's current crime rates and to determine if the trends were expressing the overall decrease over the 20 year period, with a slight increase in crime rates in recent years, as was expected. Once the local trends were compiled, they were compared to state trends and national trends. By drawing these broader conclusions, local trends were evaluated for similarities and differences to state and national data.

Through observation and after working with local and federal law enforcement, it became clear that efforts to combat crime took on a more proactive approach, rather than a reactive one. By implementing a variety of initiatives addressing specific components of crime, law enforcement began to target potential precursors to violent crimes instead of reacting to these crimes after they occur. One of the most threatening precursors was drug use. To observe the correlation of drug use and violent crime occurrence, data representing each was collected for the state of Ohio and analyzed to determine whether significant correlations could be drawn between the two, and how the use of drugs ultimately affects violent crime rates, if at all.

This presentation, although very simplistic in nature, enlightens individuals to the issues that plague five Ohio cities and clarifies how these struggles relate to other communities throughout Ohio and the nation. Through the straightforward analysis of these violent crime trends, law enforcement can access current practices to determine if the current needs of the community are being met. At the same time, forensics professionals can continue to improve their methods of analysis as they anticipate the cases and evidence that are most commonly worked. Finally, in addition to local success, analyses such as these can initiate conversation among different local law enforcement agencies from various cities in an attempt to continue to improve state and federal practices.

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Violent Crime, Crime Trends, Law Enforcement

E46 The Physiologic Effects of a TASER® Conducted Electrical Weapon (CEW) as a Function of Probe Spread

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The goal of this presentation is to understand how the spread between the probes of a TASER® CEW affects human physiology.

This presentation will impact the forensic science community by discussing how forensic investigators may have to assign relative weights to contributions of particular arrest variables in the cause of death. Most of the literature on CEWs involves large-spread exposures. This is a pilot study that shows the CEW probe spread needs to be considered in examining its relative effect on physiology.

Forensic Examiners (FE) may be presented with an Arrest-Related Death (ARD) case that involves many confounding variables, including suspect-and law enforcement -related variables. Legal proceedings may rely on the opinion of the FE on the relative contributions of the variables on the death. The purpose of this presentation is to review a recently performed pilot study examining the physiologic effects of a TASER® X26 CEW as a function of probe spread.

Previously published work has shown that the two most important variables in the effectiveness of the CEW are the region of the probes (muscle groups are stimulated) and the spread between the probes (number of motor nerves stimulated). Most of the published physiologic research utilized large-spread exposures (typical training exposures); however, in field use, exposures range from "drive stuns" (touching the metal contact points to the suspect) through a range of spreads. A New York Police Department study found an average deployment of 5.5 feet, resulting in a spread of about nine inches. In encounters in which multiple CEW deployments occur, it is not uncommon for these to be lower spread, less effective exposures. Recently, while a basic understanding of the physiology of CEWs could guide the FE in determining the relative contribution of the CEW exposures on the subject's physiology, there has been no research specifically examining the physiologic effects as a function of spread.

A pilot study examined the effect of a five-second TASER® X26 CEW on pH, lactate, catecholamines, and vital signs using back exposures with variable spreads: (1) a "drive stun"; (2) 1.5 inches; (3) 6 inches; and, (4) 20 inches. No significant changes in pH or vital signs were found with any of the exposures. With lactate, there was no change with the "drive stun" but small, statistically significant changes with the other spreads with a trend toward the change increasing with an increasing spread. There was no statistical difference between the groups in the change in catecholamines and no clear trend. Four subjects performed an additional 30-second exercise regimen. The pH and lactate for these subjects was significantly different from the five-second CEW exposures (median pH 7.28 v 7.34; median lactate 5.09 v 1.86). These subjects did not have catecholamines drawn with the exertion regimen. While the study is limited and only a pilot, there appears to be a greater effect on some physiologic measures as the spread increases. The effects of a 30-second exertion regimen are significantly greater than the five-second TASER® CEW exposure. This data is important for the FE to understand when evaluating an ARD case involving multiple variables.

CEW, TASER®, Physiologic Effects

E47 A Fatal Dive

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The goal of this presentation is to provide a suitable model for forensic pathologists to emulate in reporting dive fatalities; therefore, in divers' deaths, it is advisable to evaluate the concentration of Carbon Monoxide (CO)/ Carboxyhemoglobin (HbCO) in the blood.

This presentation will impact the forensic science community by emphasizing the rarity of diver fatalities by CO poisoning. In the international literature of the field, these deaths are rare. The vast majority of divers' deaths are due to drowning, air embolism (sudden decompression), pulmonary barotraumas, and natural causes.

One morning, after renting air cylinders from a diving center, a group of nine scuba divers went for a dive in the sea, reaching depths between 18 and 42 meters. After a few minutes in the water, all nine divers suddenly felt ill and attempted a fast ascent back to the surface. Once the divers arrived back at the boats, three of the divers' health conditions were so severe that, despite resuscitation efforts by other personnel present on the boat, they died at that time. The other four divers were transported to the nearest hospital for emergency attention and were given oxygen therapy in a hyperbaric chamber. An external examination conducted on the bodies of the three deceased divers confirmed the presence of conjunctival petechiae and diffuse and intense red cherry hypostasis. The postmortem examination found evidence of: integrity of the tympanic membrane, a hyper-expanded lung; the presence of epicardial and subpleuricpetechiae; and the presence of various air bubbles inside the cerebral vessels, with interruption of the blood flow. All the internal organs and the blood exhibited an intense red cherry color. Lab tests found evidence of high values of HbCO in the blood of the three deceased divers (Sub A=79.31%, Sub B=89.66%, Sub C=95.11%). Blood tests were also conducted 24 hours after the end of the dive on the four divers who had fallen ill and high values of HbCO were also present (Sub D=1.2%, Sub E=0.8%, Sub F=0.6%, Sub G=27%). Histological examinations of all organ samples (Hematoxylin-Eosin (H&E)) showed hypoxic brain and cerebral air microembolization, myocardial contraction bandnecrosis, endoalveolaroedema, small endoalveolar and sub pleural hemorrhages, and minimal air microembolization. A few diatoms were found in lung the samples but were absent in bone samples. Polyvisceral stasis was also present. Considering the autopsy, laboratory tests, and histological examinations, it was concluded that the death of the three divers and the illnesses of the other four were due to CO poisoning. Given these results, the cylinders used by the scuba divers during the dive were analyzed. Results confirmed the presence of CO in the cylinders in a very high concentration (Sub A= CO 2400ppmv; Sub B= CO 2015ppmv; Sub C= CO 1610 ppmv; normal CO levels in cylinders ≤10ppm); the investigation revealed that a dual compressor was used for loading the cylinders, and there were air pollution sources close to the compressor. As a result, the owner of the diving center was investigated.

Dive, Diving Cylinder, Carbon Monoxide

E48 A Rare Case of Late and Incidental Finding of a Hemimegalencephaly

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After attending this presentation, attendees will be informed regarding an atypical incomplete manifestation of a rare neurological pathology. This pathology was diagnosed at a late stage in a 47-year-old man, within a forensic context.

This presentation will impact the forensic science community by confirming the necessity of collaboration between pathologists, forensic pathologists, and toxicologists, and the importance of a systematic approach as well as expanding the range of autopsy indications, particularly for men less than 50 years of age who are victims of sudden death.

Hemimegalencephaly is a rare brain pathology with an overgrowth due to *de novo* mutations of germ lines or post-zygotic mutations during pregnancy. This is usually diagnosed in early childhood and a postmortem finding in a 47-year-old man is quite rare.

The forensic institute was contacted following the discovery of a warehouse employee found lying on the floor of his workplace.

The man had a previous medical history of cardiovascular disease, with ventricular extrasystoles and high blood pressure, which was treated using various medications including amlodipine. He also had a neurological disorder. At an early age, the man was diagnosed with epilepsy and treated from the age of three months to the age of 14 years, without any relapse after discontinuing the treatment. The patient was not followed-up for his neurological disease, but his wife reported signs that suggested a possible neurological injury: his schooling was described as "complicated," he had difficulties with physical coordination, and he had a tendency to lean his head to the right side when reading, which suggested a possible psychomotor impairment and a hemianopia. No major handicap or difficulties in job integration were reported.

At the external macroscopic examination, no cutaneous injuries were found, and only signs of an asphyxia syndrome were observed.

During the autopsy, cranial examination revealed a skull cap occipital asymmetry, an encephalon with normal weight (1,514g) but with a left hemispheric hypertrophy and a temporal lobe engagement. Macroscopic analysis of cerebral cross sections revealed a parieto-occipital architectural disorganization with marked distribution anomalies of white and gray matter, associated to a pachygyria. No other abnormality was found.

Toxicological analysis revealed an overdose of amlodipine, with a rate at least five times higher than the normal rate. Neuroanatomopathological examination confirmed the diagnosis of hemimegalencephaly.

The cause of death was an asphyxia syndrome most probably due to a seizure in a context of hemimegalencephaly associated with an overdose of amlodipine.

Hemimegalencephaly frequently remains an isolated form, but it can be in a syndromic form, associated with cutaneous vascular abnormalities (Epidermal nevus, Klippel-Trenauney-Weber syndrome, Neurofibromatosis Type 1, etc.) or a total form, which is very rare.

The classical clinical triad associates drug-resistant seizures evolving from the first months of life, contralateral hemiparesis, and severe psychomotor deficiency. A hemicorporal hypertrophy, a macrocephaly, or a colpocephaly were also observed. The clinical presentation may vary depending on the severity of the malformation.

In the literature, a history of early drug-resistant seizures or hemicorporal neurological injuries is routinely reported in adults with hemimegalencephaly, whose intellectual performance may be described as normal.

In the present case, the clinical triad mentioned above was suspected, but with a much lower degree of severity than usually observed. Furthermore, an overdose of calcium inhibitors can provoke seizures, which can potentiate the convulsing effects of this pathology.

Hemimegalencephaly, which is a rare congenital pathology with multiple clinical presentations, is usually diagnosed at an early stage. A late occurrence in the adult remains exceptional and frequently associated with disabling neurological manifestations. Nevertheless, more moderate forms can be observed. In France, routine forensic investigation is performed when a sudden death occurs at a workplace. In this case, it led to the fortuitous incidental and late finding of a cerebral pathology.

Hemimegalencephaly, Anatomopathology, Forensic Autopsy

E49 Forensic Archaeology, Ritual Crime, and Ethics: Assisting Law Enforcement While Maintaining Confidentiality as an Anthropologist

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After attending this presentation, attendees will understand of circumstances that contribute to an ethical dilemma when a crime scene suggests involvement of individuals from a descendant community in which the archaeologist also conducts research and relies on informants. How does one navigate around issues of confidentiality while assisting law enforcement without betraying professional trust on either side?

This presentation will impact the forensic science community by demonstrating the fine line of service and consultation an anthropologist provides to law enforcement that enables them to do their own analyses and create strategies to protect the interests of the public without jeopardizing the confidentiality of research informants.

Many forensic archaeologists are also engaged in archaeological excavation or research as academics. This presentation is an exploration of a case in which an anthropologist was called in as a forensic archaeologist and consultant to law enforcement who were investigating cemetery disturbances where recently interred graves were partially disinterred during nighttime clandestine activities. Undeniably, the evidence indicated ritual activity with supernatural overtones characteristic of some African religious practices, otherwise known as *hoodoo* and an expression of Vodun (voodoo) beliefs. Many rituals and religions from the African Congo and Gold Coast (West Africa) arrived with the enslaved Africans in the Southern states during colonization and the transatlantic slave trade of the late 17th and 18th centuries. Native Americans also comprised a percentage of the enslaved population in the Deep South for a time and Christian Baptist and Methodist ministers also left ideological impressions on enslaved peoples. The result was a co-evolution of new ethnic and magico-religious ideas unique to descendant populations today.

Contemporary incidences of cemetery disturbances and rituals are not as rare as one might think, although reporting of it is, as cemeteries are resistant to the information becoming public for fear of the harm it will do to their businesses. In the course of the investigation, the forensic archaeologist was confronted with the likelihood of a connection existing between the anthropologist's research populations, descendants of enslaved Africans from a historical plantation that was being excavated. Signature construction of "spell jars" and other paraphernalia were found at the crime scene that hinted at these origins.

Informants can reveal information in the course of ethno-historical interviews that, if shared, would be a breach of trust and ethics for an anthropologist/archaeologist and lead to serious ramifications within the community. Oftentimes magico-religious practices are taught or apprenticed generationally within families. The anthropologist, while providing forensic services and consultation to law enforcement, had to be cautious not to reveal informants' personal information.

Forensic Archaeology, Ethics, Voodoo

E50 Differential Taphonomic Effects in a Recently Discovered World War II Cemetery

Kristen N. Baker, MA, History Flight, 1890 California Avenue, Wahiawa, HI 96786; and Hillary R. Parsons, PhD*, History Flight Inc, 5409 Overseas Highway 101, Marathon, FL 33050*

After attending this presentation, attendees will better understand the unique postmortem taphonomic patterns of a coral atoll burial environment on human remains and material evidence from a recently discovered WWII cemetery (minimum number of individuals currently = 48).

This presentation will impact the forensic science community by identifying and describing unusual taphonomic effects on human remains and associated material evidence from a 72-year-old WWII context. Additionally, this presentation will contribute to the overall knowledge of the forensic community and current volume of data that documents and describes the effects of taphonomic variables on human remains and possible material evidence *in situ*, as well as its interpretation in a laboratory environment.

Specifically, attendees will view and learn about previously unseen and undocumented taphonomic effects of decomposing WWII military equipment (projectiles, military-issued equipment, personal gear, and military ordnance) in combination with environmental factors such as ground water fluctuations, sediment types, and salinity on human remains. The combined effects of materials interred with individuals and the surrounding environment has resulted in atypical effects on the human remains that might not be recognized by blind analysis alone.

Description of the site and sample: During the Second World War, the Battle of Tarawa was a costly victory for the United States Marine Corps. More than 1,200 United States Marines and sailors were killed in action during a three-day conflict. The remains were hastily buried in post-battle cemeteries and eventually lost to time and memory. As a result, approximately 500 of these Marines were deemed "unrecoverable." Seventy-two years later, one of the missing cemeteries was discovered and systematically excavated by a non-governmental organization called History Flight Inc. Efforts led to the repatriation of approximately 48 lost United States servicemen as of July 26, 2016.

The effects of the coral atoll environment contributed to differential preservation of human remains. In particular, the association of metals and materials found on military-issue equipment (such as "782 gear" which includes: snaps, fasteners, eyelets, canteens, helmets, rubberized canvas ponchos, and boots), ammunition and ordnance (M1 Garand clips, Browning Automatic Rifle (BAR) magazines, hand grenades, etc.), personal effects (rings, ID tags, religious medallions, cigarettes, lighters, pocket knives, coins, books), and miscellaneous objects such as post-battle trash produced a variety of previously undocumented microenvironments. Combined with natural environmental processes, such as a highly fluctuating water table, the absence of scavenger activity, and minimal postmortem disturbances, the burial context from this case presents unprecedented and valuable information for the forensic community.

Taphonomy, World War II, Battle of Tarawa

E51 Successful Planning for a Multidisciplinary Approach to Human Remains Recovery and Analysis in a Cold Case Homicide

Zachary R. Lysek, BA, Northampton County, Coroner's Office, 146 Country Club Road, Easton, PA 18045; Thomas A. Crist, PhD*, Utica College, 1600 Burrstone Road, Utica, NY 13502; and Dennis P. Asen, DDS, 201 N Irioquois Avenue, Margate City, NJ 08402*

After attending this presentation, attendees will understand the complexity of planning and budgeting for the recovery of clandestinely buried human remains, mobilizing an appropriate team of forensic scientists to identify the person and determine the cause and manner of death, and preserving the chain of possession of associated evidence for its effective presentation in court.

This presentation will impact the forensic science community by demonstrating how well-coordinated planning and execution of the multiple tasks involved in the recovery and analysis of skeletonized human remains over a period of 12 years resulted in the conviction of the perpetrator of a long-unsolved homicide.

In 2000, a 28-year-old man went missing from the house he shared with an older male friend and his family in rural Moore Township, Northampton County, PA. The friend continued to collect the missing man's disability checks through forgery for two years and then vacated the property in foreclosure. A search of the property in 2002 failed to locate human remains, but in 2004, construction excavations following demolition of the house unearthed a human tibia and three hand bones among dozens of animal bones. Upon identification of the bones as human by the consulting forensic anthropologist, aerial photography and Geographic Information Sysyetm (GIS) mapping were used to narrow the paramenters of the search location. A team of investigators from the Moore Township Police, the Pennsylvania State Police, and the Northampton County Coroner's Office then conducted systematic excavations following standard achaeological procedures. Sifting of more than 500 tons of soil over the next month necessitated the rental of a portable, industrial screener that was brought to the site. With the assistance of a cadaver dog, the field recovery team located previously disurbed, skeletonized remains of a human adult wrapped in a blue plastic tarp that had been buried more than five feet below the ground surface.

Analysis of the remains indicated that they represented a male of European descent who was 25-30 years old at death. A circular defect in the left temporal indicated a close-range gunshot wound that was almost certainly fatal. Radiography of the body mass prior to analysis revealed a .380 caliber projectile. Possible cut marks from a thin-bladed knife were present on at least one vertebra. Damage to the ribs and the left upper limb bones required distinguishing peri-mortem from postmortem insults. Analysis by the consulting forensic dentist of a mandibular fragment led to removal of a tooth for DNA analysis which in 2010 confirmed the identity of the remains as the missing young man. His friend was arrested and charged with first-degree homicide and disability check fraud. Based in part on the testimony of the coroner and the forensic anthropologist, he was convicted in 2012 and sentenced to life in prison without the possibility of parole.

This case study illustrates how the pre-planned availability of a multidisciplinary team of forensic specialists and crime scene investigators facilitated rapid responses to changing conditions at the recovery site, provided maximim flexibility in deploying the appropriate financial and technical resources, and fostered the resolution of a cold case homicide that had remained unsolved for more than a decade.

Crime Scene, Skeletal Remains, Cold Case Homicide

E52 A Death Investigator's Perspective: Lessons Learned in Response to the Crash of Flight 3407

Carraugh R. Nowak, MFS, Hilbert College, 5200 S Park Avenue, Hamburg, NY 14075*

After attending this presentation, attendees will understand: (1) the essential components of a Medical Examiner (ME)/coroner mass fatality plan; (2) the role of the ME/coroner/death investigator in a mass fatality event; (3) the multi-agency/multidisciplinary collaboration, from the scene through the final disposition of remains; and, (4) the lessons learned from this incident.

This presentation will impact the forensic science community by providing a death investigator's perspective of a mass fatality response: how the plan was written, implemented, and the after-action report used to adjust and improve the plan for similar events. This presentation will also add to current research in mass fatality planning/response and what can be expected with the multi-agency/multidiscipline response that is required when a mass fatality event of a certain magnitude arises.

Just after 10:00 p.m. February 12, 2009, Continental/Colgan Flight 3407 en route to Buffalo Niagara International Airport in Cheektowaga, NY, from Newark Liberty International Airport in Newark, NJ, crashed into a house in Clarence Center, NY. All 49 passengers/crew on board and one occupant of the house were reported dead.

As stated in the mass fatality protocol for the Erie County Medical Examiner's Office, more than 25 fatalities in one event was a mass fatality requiring the plan be implemented and additional resources contacted. In all, the Medical Examiner's Office was one of more than ten municipal, county, state, federal, and private organizations involved in the recovery and investigation.

Throughout the following 5 days, the crash debris and the remains and personal belongings of all 50 victims were recovered from the scene; all of this work was complicated by a long burning-fire, a snow storm, and freezing temperatures. Due to the impact of the crash and subsequent fire that had burned overnight, there was much fragmentation and commingling of the remains. In the weeks and months that followed, all victims were identified and released to the families for final interment. In November 2009, a special ceremony and interment of the commingled remains that could not be distinguished was held.

This presentation focuses on the challenges of a mass fatality event in on-site recovery, management, and collaboration, as well as how the mass fatality plan was implemented, both for continuing operations of daily case work and for the mass fatality response. Also, the lessons learned, involving external assistance, family communications, and multiple phases for identification of remains will be discussed.

Mass Fatality, Planning, Death Investigator

E53 Interpersonal Violence (IPV) — The Disaster After the Disaster

David A. Williams, DDS, Allegany Dental Care, 10809 Stansfield Road, Randallstown, MD 21133; and Joyce P. Williams, DNP*, 10809 Stansfield Road, Randallstown, MD 21133*

The goals of this presentation are to: (1) demonstrate the widespread prevalence of IPV following disasters; (2) describe the reasons IPV and sexual assault increase following a disaster; (3) discuss the impact of IPV citing exemplars from several disasters (Haiti, Hugo, Lo Prieta earthquake); and, (4) provide strategies to incorporate interventions that will improve safety and decrease violence after disasters/conflicts for populations.

This presentation will impact the forensic science community by acquainting the audience with the prevalence of IPV. Some of the causes, examples, and suggested interventions to prevent IPV will also be provided.

Thousands of disasters occur annually, and by the very definition are disruptive to communities. Vulnerable populations are even more susceptible when the structure of community is strained or collapses. No matter what the disaster, whether it is a natural event, a technological incident, or a combination of the two, there is the potential for an increase in IPV that must be recognized and addressed. Disasters are public health occurrences, at times life threatening, with a magnitude paralleling shortages of water, food, or vector-borne disease.

What happens following a disaster is a disruption of the normalcy of everyday life. The rate of gender-based violence (including sexual assault and domestic violence) in Mississippi rose from 4.6 per 100,000 per day, when Hurricane Katrina hit the state, to 16.3 per 100,000 per day a year later, when many women remained displaced from their homes and were living in temporary shelters and trailers. More recently, New Zealand police reported a 53% increase in callouts to domestic violence incidents over the weekend of the Canterbury earthquake in April 2010.

Factors contributing to sexual violence that can be resource deficient or of a cultural nature include: male perpetrator dominance over female victims, psychological and emotional abuse in refugee camps, absence of support systems for protection, and alcohol and drug abuse. Homelessness and unemployment are characteristic during the recovery period. Displaced populations also suffer from politically motivated violence against refugees because of a lack of physical protection; safety in these situations is absent. Disasters cause impoverishment, which can induce some people to adopt negative coping strategies, including transactional sex. There may be an increase in child/early marriages and trafficking in disasters. One agency, the United Nations Refugee Agency (UNHCR), is proactively working to mitigate violence by developing guidelines to respond to sexual violence.

Social determinants may be categorized as individual, intimate relationship, community, and social. Victims seeking assistance encounter resistance by the reversal of the problem to the victim. Reports found the following: Is it right to deny or forgive men's violence? Resources to diffuse violence can be family, who may be ignored, accused of over-reacting, and blamed for not caring well enough for their men; and lack of adequate response from health professionals and appropriate referrals. Poor awareness of trauma-informed care and the prevalence of IPV by responders hinders victim interventions and the development of mechanisms for collecting data, training staff, and developing policies.

Several notable references were documented in which marginalized communities were the sites of widespread violence. The occurrence of violence is a global problem. There was nearly triple the number of child abuse allegations in the first three months following Hurricane Hugo in 1989; after Hurricane Katrina in 2005, the rate of intimate partner violence increased to three times the national average. One year following the earthquake in Haiti, every one of the women and girls interviewed had been directly involved or had witnessed transactional sex (i.e., providing sex for food, security, or other necessities). Following the Black Saturday bushfires in Australia in 2009, reports of domestic violence were directly related to the bushfires. New Zealand police reported a 53% rise in domestic violence after the Canterbury earthquake.

Humanitarian aid and civil protection are priorities following any Mass Casualty Incident (MCI); disasters can become focal points to leverage upgrades within populations. Effective planning must ensure processes to prevent IPV, to identify and provide safety for everyone, and sufficient resources. Sustainable development is possible if leadership establishes mechanisms for communication between the community and the government. The outcome is to have an environment without violence.

E54 An Analysis of Forensic Scientists' Job Stress and Satisfaction

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After attending this presentation, attendees will understand the results of an extensive, nation-wide survey of the factors that influence forensic scientists' job satisfaction and levels of stress.

This presentation will impact the forensic science community by identifying both human and workplace factors that affect job satisfaction for forensic scientists and by providing recommendations for supervisors so positive factors can be accentuated while negative factors may be overcome.

Numerous studies of workplace performance and job satisfaction among criminal justice system professionals were performed, with a goal of identifying factors that positively or negatively influence employee interactions, levels of stress, job retention, personal fulfillment, and overall satisfaction with one's employment. The findings from such studies can be valuable to administrators and supervisors and were used to improve work conditions, resulting in increased performance; however, job satisfaction and stressors were not examined among forensic scientists, in spite of the fact that these scientists are an integral part of the criminal justice system, and regularly interact with other members of that community. Further, forensic scientists may face many of the same hurdles encountered by others in the criminal justice community, such as long hours, strict deadlines, limited resources, and outcomes of their work that can have far-reaching impacts on both individuals and society.

In the National Institute of Justice (NIJ) supported study presented here, 899 forensic scientists at the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD-LAB)-accredited local, state, and federal public laboratories, along with accredited private laboratories, were surveyed in 2012 using an electronic format sent to all accredited laboratories, using a paper format in 2013 to increase representation. Demographic factors such as sworn or civilian, sex, and forensic area of expertise were considered, as were workplace factors such as organizational makeup, work environment, job expectations, and time demands.

In general, forensic scientists' levels of job satisfaction were moderate to high, in keeping with other professionals in the criminal justice system. Satisfaction levels did not differ significantly based on being sworn or civilian, or by forensic subdiscipline. Linear regression models showed significant relationships between a lack of managerial support and increased levels of stress, as did negative relationships with prosecutors, longer work hours, and ambiguity about job expectations. Individuals in different forensic subdisciplines identified different factors that resulted in increased stress and, in general, females reported more stress than did males. Outside of work, a variety of different methods were used to cope with stress, which in general were not considered negative (such as increased smoking or drinking).

The overall results of this study indicate that while forensic scientists generally enjoy and find satisfaction with their jobs, there are a number of steps their supervisors could take to improve workplace conditions and reduce stress for the scientists.

This project was supported by a grant awarded by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. Points of view are those of the authors and do not necessarily represent the official position or policies of the United States Department of Justice.

Forensic Scientists Occupation, Crime Laboratories, Job Stress and Satisfaction

E55 Identification of Originator Attributes From Fingerprints Via Chemical Assays

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After attending this presentation, attendees will understand that fingerprints can be used as more than just a picture for comparison and can be used for identifying certain attributes of the fingerprint originator based solely on the composition of the fingerprint content. Attendees will also learn that there are various methods that can be used for this type of analysis.

This presentation will impact the forensic science community by providing new methods for the analysis of fingerprints in order to generate essential information about suspected individuals directly at a crime scene. This concept will provide a simple yes/no response within minutes to confirm originator attributes. In addition, these systems can potentially be incorporated into field-deployable devices (similar to glucometers) or connected to hand-held smartdevices that will allow rapid analyses that can be used and interpreted by operators with no scientific training.

Fingerprinting was introduced to the field of forensic science more than a century ago and has since become common practice for identification purposes; however, this area has seen minimal improvements since its establishment; it has stalled at simple visual comparison and matching, even though fingerprints — as samples of biological origin analogous to blood — have the potential to provide much more information. Currently, only the shape, size, and unique patterns associated with fingerprints are compared using various computational programs, which continues to be a time-consuming process that requires an expert's opinion. While this method is fairly well established, it is not applicable for all situations. For instance, when only partial or smudged fingerprints are collected, a match is unlikely to be found.

In addition to the situations mentioned above, one of the greatest setbacks in fingerprint analysis is that if a matching fingerprint is not saved in a database or if the person of interest is not physically present for comparison, the print is reduced to merely exclusionary evidence, despite being stored in a separate database for future use with newly obtained fingerprints. The same can be said about DNA. Even though DNA can provide the most significant information about the fingerprint originator, DNA analysis can take weeks or months. Additionally, only a few nanograms of DNA at most can be recovered from a fingerprint as the majority is lost during collection and extraction. Ultimately, even if DNA was collected, it is possible that a matching profile may not exist.

The purpose of the proposed approach is to address the issue of a fingerprint being partial or smudged as well as the issue of not having an immediate matching image or DNA profile. It has recently been demonstrated, using bioaffinity-based enzyme cascades and chemical assays, that the amino acid content in fingerprints can be used to differentiate between male and female fingerprints. The research displayed here further investigates the use of straightforward chemical assays instead of the more complex biochemical assays. Chemical tests are fairly well known, especially in the field of forensic science where there are field kits that are used for the on-site analysis of drug samples. The most common tests for illicit substances are Marquis, Simon's, and Chen's tests.

As with any multi-analyte system (enzymatic or chemical), it is possible for multiple amino acids to correspond to the same attribute, which can, therefore, compromise the overall results. To eliminate this possibility, it is pertinent that there be systems developed that are restricted to one analyte (amino acid) or a specific combination of analytes that are correlated to the desired originator characteristics. To insure that the methods presented here are practical and can be used on samples left on more than one particular surface, research demonstrating the performance of the system on samples collected from various surfaces is also provided.

The developed chemical assays also have the potential to be coupled with a portable apparatus for use directly onsite where the assay can subsequently be performed and the results interpreted by non-scientific personnel. This

can be conducted in a manner that is similar to water test kits and the VOCkit system, which is a small strip that has a grid of several dozen indicator chemicals imprinted on it that is used by the Army for the detection of threat agents, such as anthrax, sarin and mustard gas.

Fingerprints, Identification, Forensics

E56 Death Scene Investigation: Limitations and Potentials of a Logical Investigative Process

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After attending this presentation, attendees will understand the logic and statistics regarding mental prejudices and scientific reasoning which may interact, negatively influencing the results of a death scene investigation during the phases of observation, collection, and interpretation of data.

This presentation will impact the forensic science community by proposing a revision of the logic and scientific procedure used to identify manner of death (natural, accidental, suicide, or homicide).

Investigators (police officers, pathologists, anthropologists, etc.) who visit the scene must ascertain the facts, according to individual expertize. Although the professionality of forensic teams is high, an error of evaluation is always possible (e.g., misinterpretation of natural death as homicide or vice versa suicide as homicide).

More or less abstractedly, in the bodies or on the death scene, investigators search for pathognomonic evidence of manners of death: a lesion on the body, its suspect position, something anomalous in the surrounding environment, etc.

In this operative context, investigators' mental orientations, partly due to the conditions under which they operate, may lead to a kind of bypassing of usual reasoning or, in particular, neglecting national statistics regarding the cause of death. Investigators must be acquainted with this kind of contextualization, as an objective datum.

In this study, the United States and Italy were compared. In both countries, the number of deaths due to natural causes is far greater than that of homicides, which in turn is far higher than that of accidental deaths. For example, in 2013 in the United States, there were 41,149 suicides and 12,253 homicides[1, 2]. In Italy, natural causes of death totaled 613,520, suicides 4,258, and homicides 464.[3]

Although the current practice is to refer to a suspicious death as if it were a case of homicide, statistics require various systems of reference in cases of investigative reasoning. First, the possibility that the death is not due to natural causes must be considered, followed by that of suicide, homicide and, lastly, accidental death.

In addition, testified causes of death (e.g., in hospital) must be distinguished from untestified causes. This new distinction, not always included in Italian systems of classification, could reverse statistics regarding the first two causes of death, natural death and suicide, so that the first cause of death to be verified in a death scene would be suicide.

Nevertheless, forensic requirements must be remembered: any crime scene investigators must know how to work on a death scene without the risk of compromising future investigations. This means integrating logistic-statistic requirements with those of criminal investigation.

When ascertaining the cause of death, reasoning must always be carried out step by step, assessing aspects regarding the type of death, starting from the most significant statistical categories.

In order to facilitate a logical and scientific examination, a criterion of classification, integrating investigative needs (timing, evidence of contamination, etc.) and limiting possible errors due to excessive use of intuition is proposed.

In conclusion, this study focuses on the need to prioritize analysis of the manner of death according to its statistical frequency within the reference context. In addition, this study proposes a checklist of points to be analyzed in every case, to sustain the logical procedure of analysis and assessment. This checklist evaluates several variables by a scoring system to improve the reconstruction of the events: evidence on/of the body, external examination of the corpse, information regarding the victim (any hospitalizations, drugs taken, diseases), weapons or dangerous instruments near the body, witnesses statements, autopsy, and laboratory tests on biological samples.

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E57 Morphometrics of the Aging Process of Latent Fingerprints

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After attending this presentation, attendees will have an inside view of the latest research on latent fingerprint degradation processes based on measurable visual parameters.

This presentation will impact the forensic science community by demonstrating the feasibility of the technique used for determining aging patterns.

For many years, scientists focused on understanding the nature of latent fingerprints as well as improving and developing visualization techniques and reagents on different surfaces following exposure to a range of environmental conditions. In the 21st century, a suspect not only needs to be placed at a scene, but the timeframe in which this valuable evidence was deposited needs to be determined.

The reliability of latent fingerprints frequently arises in court when the fingerprints cannot be directly correlated to the moment a crime was committed. A methodology that could determine, as precisely as possible, the time a latent fingerprint was deposited would be of great value to law enforcement and the courts. Such a methodology would provide the ability to more accurately place a suspect within the timeframe of an alleged crime beyond any subjective witness or victim statements. Timing is crucially important in penal cases to further strengthen the probative value of any evidence in either exonerating or incriminating a suspect. Intensive research is being conducted globally to determine the age of latent fingerprint depositions on different surfaces. Unfortunately, many approaches usually involve expensive equipment and complex techniques that are time consuming and require great expertise by highly skilled researchers.

The present study summarizes the conclusions of a five-year proof-of-concept project that set the foundation for an empirical, inexpensive, and quantitative method to establish the aging process of latent fingerprints. This method is based on the observation of certain morphometric changes (degradation parameters) caused by monitored indoor environmental factors. This approach consists of determining, as precisely as possible, a fingerprint's age in which the sole requirement is a common powder developer used in the field (i.e., titanium dioxide). The proposed method is easy to apply, inexpensive, and does not require sophisticated forensic techniques or scientific expertise.

This study revealed visual patterns of degradation which are highly dependent on the environmental conditions monitored. Factors considered include temperature, relative humidity, air currents, type of fingerprint depositions (sebaceous-rich and eccrine-rich), various exposures to natural daylight (direct, penumbra, and darkness), and type of substrate (glass and plastic) over a continuous period of six months. The morphometric approach generates statistical data from the quantifiable analysis of four visual parameters of degradation commonly observed in the progression of a fingerprint aging process: (1) width of ridges; (2) color contrast between ridges and furrows; (3) number of ridge discontinuities; and, (4) number of viable minutiae. Preliminary data allow the discrimination of the aging processes of fingerprints exposed to different environmental conditions with unique aging patterns for each fingerprint.

The most significant results to date can be summarized as: (1) the number of minutiae remains unchanged over time for fingerprints exposed to direct light but varies for other conditions; (2) color contrast is a good estimate of degradation but requires observing the color histogram profile together with the Mean/Standard Deviation (SD) for a correct interpretation; (3) the width of ridges remains relatively stable over time, increasing or decreasing depending on the combination of factors; and, (4) the number of ridge discontinuities alone is not informative and requires normalization by combining with another parameter, specifically, the level of visual quality (clarity) of ridge features. Generally, exposure with direct light has not shown a significant effect on the four parameters for sebaceous samples on glass. Contrary to common belief, this supports the concept that fingerprints are not always better preserved in the dark. Further, the highest variability in minutiae count is observed on plastic substrates.

The age of fingerprints could be included as a new step in the examination process to increase the robustness and reliability of evidence; however, this method needs to be further developed by exploring aging patterns from different powder developers and donors as a function of time. In the near future, the proposed scientific method could strengthen the probative value of criminal evidence while saving laboratory costs, reduce the number of suspects wrongly convicted or exonerated, and minimize annoyances to people inadvertently associated with a crime scene.

Latent Fingerprints, Aging, Parameters

E58 A Follow-Up Study: Recovery of "Touch" DNA From Selected Firearms Using the Single 4N6FLOQSwabs™ Method

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After attending this presentation, attendees will be familiar with collecting DNA samples from firearms and the feasibility of detecting "touch" DNA using a single-swab method.

This presentation will impact the forensic science community by demonstrating that the single-swab method is effective in collecting "touch" DNA samples from individual areas on rifles and handguns, a process that can help preserve evidence and minimize the generation of artificial and useless DNA mixtures.

The recovery of biological evidence in the form of "touch" DNA samples from firearms is of tremendous value to forensic investigations and the criminal justice system. As with any DNA sample presumably left on a surface through contact with the epidermis, the quantity and quality of DNA recovered from firearms can vary greatly due to factors such as the physiology of the handler/shooter, the frequency of handling and cleaning the surfaces of the firearm, the type of firearm, the number of contributors and sample collection methods. For several years, the double-swab method has been utilized by many laboratories for collecting DNA evidence from a firearm. Typically, a wet swab is first used to hydrate and collect a portion of the biological material, followed by a dry swab to collect as much of the remaining sample as possible. This swabbing technique will often be coupled with swabbing multiple surfaces from the firearm followed by combining the wet and dry swabs in a single extraction, maximizing the amount of DNA sample available for typing. While these approaches can maximize the quantity of total DNA collected from a firearm, the primary disadvantage is the creation of swabbing-mediated mixtures of DNA samples from multiple surfaces of the firearm. Such mixtures can obscure single-contributor profiles that can be present on certain surfaces of the firearm but not others, thereby rendering any DNA data from that firearm inconclusive or useless for any comparison, even for the legitimate exclusion of true non-contributors. A previous study, demonstrated that useful DNA profile data can be obtained by single-swabbing certain parts of a pistol and a set of ammunition. This study evaluates the single-swab method for the recovery of "touch" DNA samples from individual areas on four different rifles (.45 cal. Commando Mark III, .223 cal. Colt® AR-15, 7.62×39mm AK-47, and .223 cal. Ruger® M-14), one shotgun (12-gauge Remington® Mod. 870), and eight different handguns (.40 caliber Smith &Wesson® Mod. 4006, 9mm Glock® Mod. 17, 9mm Beretta® Mod. 92-F, 9mm Browning® Hi Power, .50 cal. Smith &Wesson® Mod. 500 revolver, .45 cal. Smith & Wesson® revolver Mod. 625, .357 cal. Smith & Wesson® revolver Mod. 66, and .22 cal. Smith & Wesson® Mod. 617 revolver). All firearms, belonging to the same right-handed owner/shooter, were swabbed for DNA using the COPAN® crime scene 4N6FLOQSwabs™ that were pre-wetted with 15uL of sterile water. Individual swabs were extracted using the COPAN® Nucleic Acids Optimizers (NAO), a semi-permeable basket that retains fluid until centrifuged with the PrepFiler Express™ on the AutoMate Express™ DNA Extraction System by Thermo Fisher. DNA was quantitated using the Quantifiler® Human DNA Quantification Kit by Thermo Fisher. The AmpFℓSTR® Identifiler® Plus PCR Amplification Kit by Thermo Fisher was used for DNA amplification. The amplified fragments were separated on the Applied Biosystems® 3130 Genetic Analyzer by Thermo Fisher and the data analysis was performed with GeneMapper® ID-X v1.4. DNA profiles attributable to the owner/shooter were obtained from all the firearms tested. The data reflect the distribution of biological material found on specific areas of four rifles, one shotgun, and eight handguns tested. Furthermore, localized area swabbing of all firearms revealed, at least in part, single or predominant contributor DNA profiles that could have been otherwise obscured through multi-area swabbing. Therefore, in order to maximize biological evidence preservation and the chances of recovering valuable fingerprint evidence from the same firearm, practitioners are urged to consider using the single 4N6FLOQSwab™ technique for collecting "touch" DNA evidence from specific areas on firearms as an alternative method to the multi-area double-swabbing method.

Touch DNA, Firearm Swabbing, DNA From Firearms

E59 Fentanyl Analogue Despropionylfentanyl Deaths Invade Virginia

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After attending this presentation, attendees will understand the spread of a new fentanyl analogue, despropionylfentanyl, throughout Virginia. Demographics of those impacted are also discussed.

This presentation will impact the forensic science community by: (1) showing how quickly new drugs and new analogues of drugs are spread; and, (2) discussing the impact the new analogue has within the state of Virginia.

In 2014, there were a total of 47,055 drug overdose deaths in the United States, a 6.5% increase compared to 2013. Comparatively, the rates of fatal opioid overdose increased by 14.0% from 2013 to 2014. The number of fentanyl-related deaths in the United States is also growing. According to law enforcement reports, this also coincides with increased availability of illicitly manufactured fentanyl.[1] Fentanyl is a very potent synthetic opioid. It can be used as a way to manage chronic and acute pain of those suffering from cancer and is often used for patients undergoing heart surgery. Fentanyl is a schedule II substance under the Controlled Substance Act.[2]

The potency of fentanyl is about 50 times that of pure heroin and about 100 times that of morphine. The popularity of fentanyl as a recreational drug has dramatically increased and because of its potency, when used improperly or mixed with heroin or other drugs, the chance of death due to overdose is very high.[3]

This increase in deaths has been reported in many areas of the United States, including New Hampshire, New Jersey, Rhode Island, Pennsylvania, the St. Louis metro area, and New York.[4] In Virginia data, deaths due to fentanyl overdose abruptly began to increase dramatically in 2013. Between the years of 2007 and 2012, the yearly deaths from fentanyl ranged from 48-68. In 2013, the number nearly doubled to 102 and it steadily began to grow: 134 deaths in 2014, 224 deaths in 2015, and 136 deaths in the first quarter of 2016. The projected number of deaths from fentanyl in 2016 is 375.

In addition to fentanyl, many fentanyl analogues are beginning to appear from many jurisdictions around Virginia, most recently including acetylfentanyl and furanylfentanyl. Beginning February 2016, Virginia noticed a rise in deaths due to despropionylfentanyl, a seemingly new fentanyl analogue. From February to May 31, deaths were attributed to despropionylfentanyl. At the time of this writing, the event locations were mostly in the northern region of Virginia, spreading down to the eastern region. In this study, the event location will be used, not the death location, to track the spread of despropionylfentanyl.

The northern Virginia cases appear to be clustered around the District of Columbia area and in the border counties/cities with Maryland. Loudon County and Fairfax County, both of which border Maryland and the District of Columbia metro area, experienced the most despropionylfentanyl deaths in the northern region to date. In the eastern region of Virginia, Norfolk and Virginia Beach encountered the majority of cases.

The central and western regions of Virginia have not undergone any cases related to despropionylfentanyl yet. Usually illicit drugs spread throughout Virginia following interstate highways and would come into the central and western regions of Virginia. The currently observed pattern suggests that this drug is entering Virginia from two points: the District of Columbia metro area to northern Virginia and the southeast coast of Virginia to Norfolk and Virginia Beach. From these two points, Virginia may expect a further spread of deaths due to despropionylfentanyl.

Comparatively, the demographics of cases between the two regions represented are very similar: males and Whites are dying more frequently than their respective counterparts; however, people of all ages are dying of despropionylfentanyl.

The most common drugs being detected in the postmortem toxicology are: fentanyl, Benzolecgonine (BE), morphine, ethanol, cocaine, and methamphetamines. The only other fentanyl analogue being detected is furanylfentanyl. Currently, Virginia has no method to quantify the concentration of either analog and therefore both are reported as "present." The Virginia Office of the Chief Medical Examiner plans to continue tracking the spread of despropionylfentanyl across the state.

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2. *Fentanyl*. Drug Enforcement Administration-Office of Diversion Control. March 2015.
3. *Fentanyl Overdose Data* from the Centers for Disease Control and Prevention Injury Center. www.cdc.gov/drugoverdose/data/fentanyl.html.
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Despropionylfentanyl, Fentanyl Analogues, Overdoses

E60 Forensic Investigation in Suspected Cases of Infanticide — Two Case Reports in Portugal

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After attending this presentation, attendees will have: (1) learned about two infanticide cases that occurred in Portugal; (2) reviewed the *legis artis* regarding the forensic investigation of this particular type of homicide; and, (3) become aware of how the judicial system deals with this particular crime, hence contributing to its further characterization.

This presentation will impact the forensic science community by exposing the legal definition and forensic investigation procedures in cases of suspected infanticide in Portugal. The complex role of the forensic pathologist in such cases, in its multiple aspects, will be emphasized, namely the search for irrefutable evidence of a separate existence between mother and child, estimation of biological maturity, and differential diagnosis between death due to natural causes or to any act of omission or commission. The importance of a thorough forensic investigation, including death scene examination, analysis of circumstantial information concerning socioeconomic/clinical background of the suspected mother, and the need for ancillary histological, genetic, and toxicological analysis will be highlighted.

Two cases of suspected infanticide are reported, both referring to newborn infants, one male and the other female, whose births were apparently concealed by their mothers. One infant was found dead and disposed of in a plastic bag in the back of the supposed mother's car and the other infant was found in a residence's septic tank. Extensive forensic autopsies were performed, further complemented by forensic clinical evaluations of the suspected mothers. Full X-ray screenings were conducted prior to autopsy and all available organs were collected for ancillary histological examination. Blood samples, as well as liver, kidney, and stomach specimens, were obtained for toxicological analysis. Dried blood spot specimens were collected on filter paper for genetic investigation. In both cases, the autopsies revealed signs of decomposition, the presence of the umbilical cord, external morphological measurements and organ development compatible with full-term gestation fetuses, and the absence of congenital abnormalities.

The female fetus was found in a septic tank next to the alleged mother's residence, in a putrefied state, after an anonymous judicial complaint reporting a concealment of birth was made. Confronted by the accusation, the mother (a single young mother of low socioeconomic status) said the fetus was a stillbirth and confessed that she had tried to conceal the child in the septic tank. During autopsy, both macroscopic and histological appearances of the lungs exhibited no signs of a live and separated existence.

The male baby was found in the trunk of the supposed mother's car, a 40-year-old teacher. The mother confessed that she had been in labor in the bathroom and that the fetus was born in a cephalic presentation, falling into the toilet along with the placenta. The umbilical cord was allegedly cut with scissors. The woman also said that the fetus was stillborn, she did not ask for help, and she placed the baby inside a plastic bag in the trunk of her car. During autopsy, both macroscopic and histological appearances of the lungs demonstraited signs of a live and separated existence. Forensic evaluation detected recent delivery signs on the mother, who was convicted.

These casereports emphasize the importance of a thorough forensic investigation in cases of suspected infanticide, including a complete autopsy and, if possible, a physical examination of the alleged mother to seek signs of a recent pregnancy or childbirth, in order to help the legal system in the complex task of dealing with this particular crime.

Infanticide, Stillborn, Forensic Investigation

E61 A Unique Medicolegal Investigation Following a Suicidal Gunshot Wound of the Head

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After attending this presentation, attendees will be able to recognize that atypical scenes involving firearms can lead to erroneous assumptions regarding the manner of death. Attendees will also understand how this type of case necessitates the utilization of a multidisciplinary medicolegal investigative approach in order to arrive at an accurate manner of death.

This presentation will impact the forensic science community by illustrating how inaccurate conclusions can lead to additional expenditures of resources and potential ill will with surviving family members.

The presentation and positioning of firearms in suicidal gunshot wounds, while not predictable, often follow familiar patterns. Deaths caused by the use of semiautomatic handguns can result in multiple differing presentations of the firearm, which may include: the weapon ejecting the spent casing and automatically chambering an additional round, the opened breach that is often observed when the magazine is exhausted, or "stove piping" or other jamming of the spent shell casing. Conversely, revolvers typically demonstrate the familiar pattern of the spent casing being found in the cylinder position, directly under the hammer.

This study presents an unusual case of a suicidal gunshot wound resulting in a unique death scene investigation due to presentation of the weapon and ammunition. This atypical death scene generated concerns by investigators, who began to question the assumed manner of death.

A 76-year-old man was discovered dead in the pump house of his residence. The decedent suffered from psychiatric illnesses that included auditory hallucinations and paranoia with suspicion of an imagined impending arrest. The initial investigation of the crime scene did not lead to any suspicions of foul play due to his history of mental illness and a note identified within the home.

The body of the decedent was discovered leaning against the back wall of the non-air conditioned building. Examination of the fully clad body revealed full rigor and blanching-dependent lividity that was consistent with the found position of the body. A contact gunshot wound was identified on the right side of the head. A corresponding defect was noted on the scalp on the left side of the head. Blood was observed on the head, right arm, and clothing of the decedent. Liquid, clotted, and dried blood had pooled on the floor around the decedent. Blood spatter was identified on the wall of the building to the right of the decedent.

During the investigation a black, .38 caliber, Rossi® revolver was found gripped in the decedent's right hand. The cylinder was open and all of the live rounds and the spent shell casing were emptied or nearly emptied from the weapon. The subsequent shells were located on both sides of the decedent's right leg and underneath the leg.

This unusual finding required a multidisciplinary medicolegal investigation that revealed the death to be consistent with a suicidal gunshot wound of the head, despite the atypical presentation. This case, yet again, illustrates the importance of utilizing a multidisciplinary medicolegal approach in arriving at a cause and manner of death.

Multidisciplinary, Suicide, Firearm

E62 Those in Their Current Role the Longest Are Less Likely to Report Hindrance in the Use of Joint Investigations Practices: Data From the 2013-2015 CDC/FBI Joint Criminal-Epidemiological Investigations Workshop Course Evaluations

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After attending this presentation, attendees will be able to identify the hindrances reported by the Centers for Disease Control and Prevention (CDC)/Federal Bureau of Investigation (FBI) Joint Criminal-Epidemiological Investigations Workshop participants and to understand their associations with the role of participant, number of years in role, and workshop location. As a result, attendees will better understand the barriers and perceptions that prevent successful collaboration between public health and law enforcement for the identification and response to biological threats.

This presentation will impact the forensic science community by illustrating that the longer an individual serves in a role, the less likely he/she is to report hindrance in the use of joint investigation practices. This presentation is relevant to law enforcement, epidemiologists, emergency preparedness managers, laboratorians, community health specialists, public health nurses and physicians, and others who contribute to the identification and/or response during a biological threat incident.

The CDC and the FBI conducted Joint Criminal-Epidemiological Investigations Workshops to develop relationships and promote inter-agency collaboration between public health, law enforcement, and other agencies prior to and during the response to incidents involving biological threats. To help the CDC and the FBI in allocating their training resources more efficiently and in identifying a target audience for future workshops, this study explored the association between the presence or absence of hindrance in applying joint investigations practices on the job with the number of years in reported roles, the role of the participant, and the workshop location.

Data were collected from the 2013-2015 workshop participants who completed course evaluations. Four variables were extracted: (1) number of years in the role; (2) the role of (law enforcement, public health, emergency responders, and other); (3) the hindrance in applying joint investigations practices (lack of opportunity, lack of management support, incomplete understanding, other, and no hindrance reported); and, (4) workshop location (13 locations). Descriptive statistics were calculated, and differences in the number of years in a role across different factors were evaluated by the Kruskal-Wallis test. Logistic regression analysis was performed to evaluate the association between presence or absence of hindrance and the number of years in the role, the role of participant, and the workshop location. Statistical analyses were performed using Statistical AnalysisSYstem (SAS) 9.3 and Statistical Package for the Social Sciences (SPSS) 21.

Of the 975 workshop participants, 72.61% completed course evaluations. Further data management and analysis indicated that for numbers of years in a role, the overall mean was 12.41 (Standard Deviation (SD) = 9.87), median was 10.00, and range was 1-44. More than half of the participants were in public health roles (53.67%). A little more than half of the participants reported a hindrance in applying joint investigations practices (51.83%). Kruskal-Wallis tests demonstraited that significant differences in the distribution of the number of years in a role were observed between roles ($p<0.001$), presence/absence of hindrances in applying joint investigations practices ($p=0.009$), and workshop locations ($p=0.004$). Logistic regression analysis showed that of the three predictors, number of years in a role is the only factor that is significantly associated with hindrance in applying joint investigations practices ($p=.016$), suggesting that for every year that the participant has stayed in the reported role, the participant is less likely to report a hindrance (Odds Ratio (OR)=0.976, 95% CI 0.958 0.995).

Findings in this study suggest that the longer the participant stays in his/her role, the less likely it is for him/her to report a hindrance regarding the application or use of joint investigations practices. This may be attributed to the knowledge from the real-world experience accumulated over time in a role and/or authority gained to independently perform duties associated with the role. Based on these findings, it is suggested that the workshop be targeted to those junior personnel with lower-level experience or fewer number of years in a particular role. Secondly, encourage more personnel from law enforcement and other government agencies involved in emergency preparedness and

response are encouraged to attend the workshop. In this way, the targeted audience may be more likely to overcome barriers and perceptions that prevent collaboration between public health and law enforcement for the identification and response to biological threats.

Number of Years, Role, Hindrance

E63 Navigating Toward a Blind Proficiency Testing and Verification Program

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After attending this presentation, attendees will understand the difference between blind testing and verification, know the challenges faced by quality assurance personnel when attempting to administer blind proficiency testing to laboratory staff, and be equipped with a variety of options for resolving problems that may accompany blind test preparation.

This presentation will impact the forensic science community by discussing the feasibility of successfully incorporating blind proficiency testing and verification into the quality assurance programs of crime laboratories, thereby suggesting ways to increase the confidence in overall laboratory performance.

Proficiency testing is a regular, planned activity of every accredited crime laboratory as a means to demonstrate the continued competence of personnel, as well as the effectiveness of analytical operations. While some laboratories developed proficiency testing programs that are more stringent than the minimum standards set forth by their accrediting bodies (e.g., testing frequency, number of personnel involved, and areas of operations tested), open proficiency testing is more common than blind proficiency testing. The absence of blind proficiency testing in the forensic field is not due to a lack of understanding its necessity; rather, it is because of the difficulty in implementing it successfully.

An open proficiency test, a practical test given to an analyst who is aware of being tested, carries several disadvantages. First, most test samples purchased through external providers are relatively easy to work, when compared to normal casework. This is partly due to the fact that laboratories have different protocols, and external test providers must utilize standard samples that can be successfully analyzed by personnel from different laboratories. Secondly, a test sample does not generally appear like a typical casework sample; therefore, laboratory personnel are likely to be focused on the fact that they are taking a test. Moreover, even though they are instructed to treat the test samples in the same manner as case samples, analysts are not always able to do so. Some analysts may be inclined to anticipate the results they believe they should obtain. This is contradictory to the intent of proficiency testing. In contrast, a blind proficiency test is a practical test taken by an examiner who is unaware that it is a test. Blind proficiency testing, when executed successfully, can offset most of the issues that arise with open proficiency testing.

Verification occurs when results and conclusions are confirmed as being acceptable by a second analyst prior to reporting. Blind verification can augment the robustness of a laboratory's proficiency testing program, as it is intended to determine if two analysts can independently arrive at the same conclusion without prior knowledge of each other's work. Both blind testing and blind verification remove the element of confirmation bias produced by the nature of the exam. Both techniques assess the uniformity of operations more thoroughly and, therefore, elevate the level of assuredness a laboratory can provide regarding the reliability of its personnel and operations.

In 2015, the Harris County Institute of Forensic Sciences (HCIFS) Quality Management Division developed and optimized a blind proficiency testing and verification program for the crime laboratory. Quality Management drew from several sources to ensure the effectiveness of each test, including purchased specimens from external providers, and collaborated with law enforcement personnel who regularly submit evidence and the laboratory's evidence receiving staff. The end result was the successful creation of custom-made, typically packaged evidence for each laboratory discipline that was able to be shuttled through the system as normal casework.

Not surprisingly, a different set of challenges emerged when this new program was launched. Concealing the planning efforts so the laboratory staff would remain uninformed proved to be the biggest challenge, both logistically and from an ethical standpoint. Ensuring the laboratory information management system would assist and not hinder the process also proved to be difficult. Additionally, decisions needed to be made regarding the disclosure of test results to management and test participants. These concerns and the various proposals raised to address them are discussed in this presentation.

Blind Proficiency, Verification, Quality Assurance

E64 Understanding Violence Against Law Enforcement by Youth Street Gangs

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After attending this presentation, attendees will understand: (1) why, within the last year, youth gangs are more violent toward law enforcement; and, (2) various solutions to help keep officers safe while investigating gang-related violence.

This presentation will impact the forensic science community by informing and educating attendees on a new trend in the streets involving youth gang members that confront law enforcement officers with violence by using the same tactical skills that military or law enforcement officers use. This is a recent phenomenon involving youth gangs that will have a major impact on the safety of those associated with forensic science and law enforcement communities.

In recent months, violence toward law enforcement increased by 80%, according to the Department of Justice. In some metropolitan areas of the country, this figure was nearly 110%. One cannot turn on the television or radio without hearing about another officer-involved shooting. In Los Angeles County, CA alone, the Los Angeles Police Department (LAPD) and the Los Angeles County Sheriff's Department (LASD) increased helicopter patrols and 911 screenings in the wake of the recent police ambushes. Youth street gangs throughout the United States still continue to terrorize the neighborhoods they claim as their own, causing the citizens in these gang-infested neighborhoods to live in constant fear of their lives every single day; however, a new trend out on the streets is making a fake 911 call, then ambushing law enforcement when they respond to these fictitious calls for help. As law enforcement responds to the location of the scene, youth gangs are now using urban-style tactical warfare learned from the military and using that training against law enforcement as they respond to the scene, seriously injuring or killing officers. Whereby in the past, youth gangs would retreat when confronted by law enforcement, now they are advancing toward law enforcement while shooting, using the same tactics as the officers themselves use, such as, "slicing the pie" or "button hook." There are other various forms of urban tactical warfare learned in the military and the police academy that the gang members are learning on a daily basis and using against the police. Last year in Los Angeles County, CA, there were a total of 385 shots fired at police in 2015. From January 1, 2016, to July 1, 2016, there were a total of 368 shots fired at police. In 2016, 88% of shots fired were "gang related;" in 2015, only 74% were "gang related." This is a serious "officer safety" concern for law enforcement who respond to these gang related violence calls on a daily basis.

Why are these gang members shooting at police? Interviews of 125 Los Angeles gang members out on the streets and in the jails were conducted between January 2015 and July 2016 as to why they would decide to shoot at law enforcement. This study identified ten distinct manifestations of these shootings against police and 12 solutions to help keep officers safe while out on the streets investigating these gang-related shootings. A sample of the findings include: distinct cultural differences between African American, Latino, and Asian American gangs as to why they engage violently with the police; state of mind (motivation) of the various gangs; disrespect felt toward police while being questioned, detained, or arrested; covert and overt racism experienced by the gang members; A "getting even" mentality; and being male or female in the gang. These findings culminate into the recent influx of violence against law enforcement by gang members. In Los Angeles County alone, there are 1,351 documented gangs with a gang membership of more than 750,000. Similar results were seen across the country, according to the National Gang Crime Research Center in Peotone, IL. There are more than 28,800 gangs in the United States, with a total gang membership of 950,000; 90% are male and 10% are female. The ethnic composition nationwide includes: 47% Latino, 31% African-American, 13% Caucasian, 7% Asian, and 2% "mixed race," according to the Office of Juvenile Justice and Delinquency Prevention of the United States Department of Justice.

Youth Street Gangs, Officer Safety, Youth Violence

E65 Homicide in a Horse Barn

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After attending this presentation, attendees will: (1) understand the importance of thorough body examination in the evaluation of suspected homicides; and, (2) recognize that not all cases presenting as suspected homicides represent true homicides.

This presentation will impact the forensic science community by reiterating the fact that certain accidental deaths can mimic homicides.

While no case should be considered "routine," many homicides are relatively "straight-forward." These cases require diligence and professionalism, but do not require extraordinary investigative methods or deductive skills. In contrast, certain homicides require not only a great deal of time and effort, but also superb investigative skills. Among these so-called "difficult" cases, one of the most challenging for investigators is the sexual-assault homicide, particularly when the alleged victim is an elderly person, and there are no obvious suspects upon initial investigation.

This study presents the case of a moderately obese elderly woman who lived alone whose partially nude, beaten body was found face-down in her horse barn on a warm August evening. Her grandson came to her farm looking for her after family members were unable to make contact with her. Police responded and initiated a homicide investigation. The woman's daily routine was to attend to her horses both in the morning and evening. Initial evaluation of the body suggested that the woman had suffered blunt force injuries. Portions of her clothing, including her bra and shirt, were adjacent to the body; her pants were partially lowered. A water hose had been left running, muddying the dirt floor on which she was lying. Also, the gates to the pasture were open, thus allowing the horses free access to the barn and pasture. No obvious weapon was identified. The scene was secured by police, and the body was sent for forensic autopsy.

At autopsy, trace and sexual activity evidence was collected. Early decomposition was noted. The presence of blunt force injuries of the head and torso was confirmed on external examination. On internal examination, although subscapular hemorrhage was evident, there was no intracranial trauma to explain death. Examination of the cardiovascular system suggested a possible contributing explanation for death, as the heart was enlarged (640 grams), with associated coronary artery atherosclerosis, thus predisposing to a fatal cardiac dysrhythmia. Upon examination of the gastrointestinal tract, the presence of numerous gastric Wishnewsky ulcers suggested a prolonged "down-time" with a final mechanism of death related to hypothermia. Without an adequate explanation for the blunt force injuries, the case was still considered a possible homicidal assault, with contributing factors of heart disease and eventual hypothermia; however, careful evaluation of the trunk injuries revealed a horseshoe-shaped postmortem abrasion overlying a contusion, indicating that the horses had produced the postmortem injuries. Re-evaluation of the entire body disclosed an apparently shortened and outward-turning left lower extremity. X-ray exam and sectioning of the soft tissues of the hip confirmed the presence of an acute antemortem left femoral neck fracture.

Based on these findings, it was concluded that the woman had apparently gone to the barn to attend to her horses, having opened the gates and turned the water on to fill the water troughs. She apparently broke her hip and collapsed to the ground. Unable to move herself due to her weight and old age, she remained on the barn floor, which eventually became saturated with water. As a result, despite the relatively warm summer ambient temperatures, she succumbed to fatal hypothermia. The horses eventually became hungry and attempted to rouse her with their front hooves, thus explaining the postmortem injuries. The absence of clothing may have resulted from the horses pulling the clothing off with their mouths or as a result of paradoxical undressing associated with hypothermia.

The case illustrates the importance of cooperation between investigating agencies when facing a possible homicide. A series of accidental deaths that were originally considered possible homicides were recently reviewed for this study.[1] Failure to recognize accidental deaths, and instead consider them homicides, can result in wasted time and resources, false allegations, and possible life-altering consequences.

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Homicide, Accident, Death

E66 Markers for Genuine and Fake Suicide Notes

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After attending this presentation, attendees will better understand the benefits and limitations of the most current research regarding distinguishing identifiers for genuine and fraudulent suicide notes.

This presentation will impact the forensic science community by illustrating the need to be aware of specific characteristics of suicide notes that suggest a staged scene.

If an estimated 20%-25% of suicidal people leave notes, the ability to accurately evaluate note authenticity during early investigative stages affects 8,000-10,000 cases each year in the United States.[1] Some notes were part of homicides staged as suicides and some ambiguous messages were erroneously accepted as suicide notes. Due to assumptions and errors, the percentage of fraudulent notes is unknown. Those not trained in note analysis tend to rely on cultural myths, which leads to improper decisions.[2] A standardized checklist for death investigators and first responders based on research concerning suicide notes used in staging can benefit the investigative community.

A survey of methods used for differentiating genuine from non-genuine suicide notes will be presented, including protocol statements from prior content analysis of suicide notes, computer software programs, and research on homicides staged as suicides.[3-5] Collectively, these research areas provided constructs for developing the Suicide Note Authenticity Checklist (SNAC). Although the SNAC cannot prove that a suicide note is part of a staged scene, those notes that show a sufficient number of characteristics of known fake notes can alert investigators to the need for further investigation, such as expert note analysis. Case analysis will demonstrate the SNAC's utility in the field, and genuine notes will be compared against notes used in homicides staged to look like suicides.

The research items were genuine and non-genuine suicide notes, written by adults in the United States, 21 years and older, analyzed for specific language and behavior. "Genuine notes" are authenticated notes written by people who committed suicide. "Non-genuine notes" include those that were: (1) written by matched subjects in control groups; (2) computer generated as control or practice documents; or, (3) written by someone other than the decedent to stage a death as a suicide.

The SNAC is based on categories organized according to a frequency distribution ratio for items found in confirmed genuine and fraudulent notes, such as relationship status, emotional state, cognitive state, and personality issues. Within each category are differentiating elements for genuine versus fraudulent notes, which are useful in developing cues for authenticity. The SNAC was tested on a series of genuine notes, fraudulent notes, and control documents.

This presentation describes how the SNAC was developed, demonstrates how to use it, and makes suggestions for validation testing and for training, as well as for comparison with other populations, notably adolescents and those from other cultures.

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Suicide, Suicide Notes, Staged Suicide

E67 Investigation of a Mummification-Type Bondage Death

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After attending this presentation, attendees will understand an unusual case of bondage that presented as a possible homicide.

This presentation will impact the forensic science community by providing insight into a type of bondage known as mummification.

A 27-year-old White male was found dead on the floor of his apartment bedroom. The decedent was bound in duct tape from the mouth to the ankles. The apartment door and all the windows were locked. Duct tape was applied in organized rows, and several of the ends of the tape were placed at the decedent's back, which led to the preliminary conclusion that the tape could not have been self-applied. The tape was wrapped from just below the nose to the neck. A second application of tape started at the shoulders and continued to the hips. The final application of tape started at the upper thighs and ended at the ankles. The ankles were also bound under the tape by a red rope. The tape around the hip area had been torn slightly in a few places, prompting the initial theory of a second person's involvement. Assessment of rigor mortis and livor mortis at the scene was not possible due to the extensive coverage of the body by the duct tape.

At autopsy, the tape was carefully cut away to reveal a black nylon body suit and a black spandex undergarment, to which the duct tape adhered. There was no direct contact of tape to skin. Tape application was performed in a meticulous, organized pattern. The decedent was also wearing a diaper that was full of feces. Boxes of adult diapers were found in a closet in the decedent's apartment. Duct tape covered the decedent's mouth. A ball gag was found in the decedent's mouth and behind that was a bandana, tied in knots. The bandana was saturated with saliva which caused it to expand in size.

Postmortem toxicology of blood and urine was negative for alcohol and drugs. Cause of death was certified as "asphyxia due to obstruction of the upper airway and restriction of chest wall movement." Manner of death was left pending until the police concluded their investigation, which included checking credit card purchases, viewing video from store surveillance cameras, and interviewing the boyfriend of the decedent, who lived out of state.

A review of internet sites identified duct tape bondage as mummification and described various ways in which it can be achieved. Most sites gave warnings about the danger of asphyxiating and purported to give advice on how it can be accomplished safely. Several writers shared near death experiences when engaging in this type of activity.

Mummification, Bondage, Duct Tape

E68 Medicolegal Death Investigations After Disasters: Newly Developed Tools to Improve Data Collection

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After attending this presentation, attendees will understand activities undertaken by the Centers for Disease Control and Prevention (CDC) to develop data collection tools for increasing the accuracy of identifying disaster-related deaths and associated risk factors during the death scene investigations after a natural disaster. In addition, attendees will understand how death scene data can improve public health emergency preparedness and response.

This presentation will impact the forensic science community by providing an overview of the work CDC has conducted thus far on developing a suite of supplemental disaster death scene investigation tools and by offering the forensic science community the opportunity to review and provide feedback on these new resources.

Data collected at the death scene are the foundation for identifying the cause and manner of any death; however, because of the complexities of a disaster, collecting information beyond what is routinely required for determining cause and manner of death may be needed to ensure that the death is appropriately attributed to a disaster. In recent disaster events, considerable disparities arose between the number of disaster-related deaths reported and recorded by state-based vital statistics departments and those reported by other agencies, including local, state, and federal state-based Emergency Operations Center(s), the National Oceanic and Atmospheric Administration-National Weather Service Storm Database, and the American Red Cross. Enhancing the data collected during disaster-related death investigation could improve the accuracy of reporting disaster-related deaths. To this end, CDC funded NORC at the University of Chicago to examine current practices in disaster-related medicolegal death investigation and convene a workgroup, comprised of medical examiners and coroners, forensic pathologists, death scene investigators, forensic anthropologists, and epidemiologists to collaboratively develop tools for investigators to use in a disaster-related scene investigation. Similar to the Sudden Unexplained Infant Death Investigation (SUIDI) guidelines, this project provides tools for death scene investigators and death certifiers to collect disaster-specific data, ultimately allowing for more accurate and consistent attribution of deaths to disasters.

The proposed disaster-related data collection tools will not replace routine data collection tools but will aid the death scene investigator in consistently collecting disaster-specific information. Supplemental tools were developed for frequently occurring disasters, such as hurricanes, tornados, and extreme heat and cold exposures. Examples include information about the scene (e.g., presence of basement or tornado shelter in home); information about the decedent (e.g., engagement in activities related to disaster preparation or clean up); and information about the disaster (e.g., weather conditions or ongoing alerts). In addition, a user guide was developed to assist investigators in using these tools.

Enhanced data collection practices at disaster-related death scenes will improve the public health sector's ability to identify risk and preventive factors associated with disaster-related deaths. Death scene investigation data in medical examiners' and coroners' reports can assist state and local public health officials to better target response and recovery efforts by identifying people at high risk of mortality and refine strategies to prepare, respond, and recover from future disaster events.

Disaster, Investigation, Mass Fatality

E69 Investigations of Healthcare Serial Killers (HSKs) and Implications for Health Data Analytics

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After attending this presentation, attendees will be able to appreciate the difficulties in correctly identifying HSKs and those who may have such tendencies.

This presentation will impact the forensic science community by propositioning for potential data analytics for the detection of HSKs.

There were several cases of healthcare professionals deliberately harming or killing patients under the guise of bona fide treatment, in some cases under a delusional belief that this was a correct action. In the United Kingdom, a family physician, Harold Shipman, was responsible for more than 200 deaths of patients under his care, most of them female. There were several missed opportunities to curtail this killing spree, and the subsequent public enquiry only increased the pressure on the actors in the criminal justice system to not miss any potential HSKs.

Subsequently, there were several highly publicized investigations of apparently excessive deaths suspected of being murders, which turned out to be false alarms. There were many studies of excess mortality related to poor healthcare and incompetence, rather than deliberate criminal acts. Several measures were developed to detect poorly performing hospitals. In some cases, the causes are a paucity of qualified staff and overworked doctors and nurses. There are major difficulties with hospital-level mortality statistics.[1,2] Dr Foster®, which uses the Hospital Standardized Mortality Rate (HSMR) methodology, has a threshold set by calculating the risk of a false alarm of 0.1% which when combined with expert review, still resulted in forty-two mortality alerts in one year.[3] The United Kingdom Department of Health has likened the HSMR to a "smoke alarm," but even this very modest claim for its sensitivity and specificity was called into question.[4] The problems with hospital-level statistics apply all the more to ward-level statistics.

Data analytics could improve on human monitoring. Several systems are in development for big data analytics in healthcare, which use routinely gathered data. Previous studies found that clinicians identified with dramatically higher mortality had plausible reasons for such differences with no concerns about their practice on further examination. There is considerable heterogeneity of clinical populations that requires accommodation in any statistical modeling.

Studies of statistical approaches combined with analyses of the traits of both serial killers and specifically HSKs reveal possible approaches to detect healthcare serial killers. As Gill noted in his analysis of flawed statistical evidence in the Lucia de Berk case, there are certain characteristics that are not helpful.[5] Due to individual habits in selecting particular shifts, temporal patterns will not necessarily be helpful in identifying HSKs. Victim characteristics may or may not be helpful; for example, it is obvious that certain wards or practitioners will only treat female patients, older patients, or those with terminal conditions. Identification of factors relevant to the psychopathology associated with serial killers not associated with particular healthcare settings would be a plausible means for detecting HSKs. Further study is required to assess whether data analytics will prove useful.

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Forensic Psychology, Data Analytics, Healthcare Serial Killers

E70 The Forensic Implications of Current Practices in Emergency Services

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After attending this presentation, attendees will be able to identify the factors leading to the destruction of evidence by fire fighters and Emergency Medical Services (EMS) personnel during an emergency response and how current practices contribute to spoliation.

This presentation will impact the forensic science community by underscoring the incompatibility of current practices in emergency and tactical response with best-practice forensic guidelines for evidence collection, and by discussing practical solutions for scene preservation in unsuitable conditions or an ongoing emergency response. This presentation is also of interest to private-sector investigators who determine responsibility in civil liability cases.

Emergency services professionals and volunteers (local, state, and federal) are required to complete National Incident Management System (NIMS) training developed by the Federal Emergency Management Agency (FEMA) in order to receive government funding. Forensics and attribution fall under the core capabilities of FEMA National Preparedness in that the Department of Homeland Security's strategic goals include forensic analysis to "attribute terrorist acts (including the means and methods of terrorism) to their source"; however, the primary mission of an emergency responder is life-safety. Whether a mass-casualty incident or a rural brush fire, firefighters, paramedics, and other rescue personnel often perform their function with little regard for the subsequent investigation. Arson for the purposes of crime concealment can destroy evidence by fire damage, but also by allowing evidence to be washed away or damaged by water during extinguishment activities. On an active fire scene, multiple emergency responders from a municipality, as well as those from other participating agencies through mutual aid agreements, can be present at any given time. The introduction of trace materials from other locations and biological transfer from various individuals will corrupt the subsequent crime scene, making it difficult to identify meaningful evidence.

The first officer on scene for a non-emergency call has more latitude and opportunity to limit entrance and erect barriers to preserve the crime scene. Conversely, law enforcement has little control in rescue operations performed by emergency personnel. For this reason, private-sector investigators can be prepared with challenging circumstances, arriving on scene sometimes days after an emergency incident has been terminated. Continuing education of firefighters and other emergency responders is the first line of prevention in regard to the destruction of forensic evidence, but a secondary investigator can avoid misinterpretation of evidence at the fire scene by studying the local fire marshal's incident summary, photo-documentation, and investigative report. The fire marshal responds concurrently with emergency services and receives the same dispatch notifications. The fire marshal can provide a first-hand narrative of the incident from the initiation of emergency response to the termination of the incident at headquarters and a science-based fire investigation report.

Fire Investigation, First Responder, Fire Marshal

E71 The Use of Heterospecific Development Data for Time-Of-Colonization (TOC) Estimation

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After attending this presentation, attendees will understand the limits of using closely related Calliphoridae species growth data to determine TOC estimations when using species that have not been studied.

This presentation will impact the forensic science community by determining if this suggested practice is acceptable in forensic entomology casework, where errors in the estimation of TOC and Postmortem Interval (PMI) can have important implications for reports and testimony of forensic entomologists.

A historical analysis of forensic entomology reveals several centuries of practical use of entomology at the crime scene. Modern research advanced forensic entomological knowledge in recent decades, and it is now commonly known that insect and arthropod evidence can assist in the estimation of the PMI via the TOC of insects on the remains. This estimate is based on how long insect larvae have been feeding on the decedent (assuming colonization occurred shortly after death) and is determined through the use of known development rates for the insect species attracted to the body at known temperatures. The most commonly utilized arthropod for this analysis is the blow fly (Diptera: Calliphoridae), as these flies are often the first to colonize both human and animal remains.

This method of analysis relies upon intimate knowledge of growth rates of the insect species observed on decomposing bodies, which is highly useful when this knowledge is available but problematic when these data are unknown. Since blow flies are poikilothermic, they are significantly affected by fluctuating ambient temperatures. Each species has a required amount of heat above a minimum threshold necessary to complete development and each species has its own requirements for completing the developmental milestones necessary to complete development. This knowledge is gained through intensive study of each species and is, therefore, only available for a select few blow flies in the country.

When unstudied Calliphoridae are encountered at a scene, a problem may arise. It has been suggested that development data from closely related flies (i.e., those within the same taxonomic tribe) may be used to approximate development rates and therefore TOC; however, there is, little independent data to support this suggestion. This study attempted to test this hypothesis.

In this study, Calliphoridae larvae were collected from human remains at the Forensic Anthropology Research Facility at Texas State University. TOC estimates were calculated using development data from heterospecific organisms within the same tribe and compared to known placement rates for the cadavers. Statistical comparisons between the use of specific and within-tribe development data and the actual time of cadaver placement were used to determine if the development data of closely related species may be confidently used to determine an accurate TOC estimation.

PMI Estimation, Forensic Entomology, Calliphoridae

E72 Authentication of Museum-Curated Tsantsas (Shrunken Heads) Utilizing Next Generation Sequencing Technology

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After attending this presentation, attendees will understand the difference between authentic and inauthentic tsantsas, the proper methods for the extraction and amplification of processed and degraded samples, and the capabilities of next generation sequencing in generating genetic profiles for geographic profiling and genetic linkage.

This presentation will impact the forensic science community by showcasing next generation sequencing technology and its usage potential for mass disasters, mummified remains, ancestry identification, cases involving missing persons, and the sale of antiquities on the black market. Next generation sequencing allows for a highly sensitive and discriminatory analysis, even with expected low template and/or highly processed DNA seen in the tsantsas samples. The results of this research will also allow museums to confidently identify the tsantsas within their possession as authentic or inauthentic, supported by morphological characteristics and genetic analysis.

Authentic shrunken heads, referred to as *tsantsas*, were prepared in a precise, ritualistic manner, by the Shuar tribe of Northern Peru and Southern Ecuador, resulting in key morphological characteristics. *Tsantsas* serve as trophies following a death in battle between subtribes of the Shuar. To prepare the *tsantsa,* the skin of the entire head is removed from the skull and boiled in water, concentrated with natural tannins for 30 minutes, resulting in a head that is one-fourth its original size. Hot rocks and plant extracts are applied to the skin to further preserve the shrunken head and to prevent degradation. Forgeries include primates and inauthentic human preparations, which lack morphological characteristics observed in authentic *tsantsas.* Some forgeries were prepared by members of the tribe in a non-traditional manner, while others were prepared by individuals outside of the tribe. Inauthentic shrunken heads were created for tourists and private collectors to capitalize on and profit from European beliefs regarding New World savages. Some of the notable morphological characteristics are also observed in inauthentic shrunken heads, suggesting that DNA analysis is also required for discrimination. Authentic *tsantsas* are of South American descent and inauthentic preparations are often European; therefore, next generation sequencing was implemented to determine the biogeographical ancestry of more than 40 heads, sourced from various museums.

Genetic markers such as Short Tandem Repeats (STRs), Y-chromosome Short Tandem Repeats (Y-STRs), Single Nucleotide Polymorphisms (SNPS), and Ancestry Informative Single Nucleotide Polymorphisms (AISNPS) were identified through data analysis of next generation sequencing results in order to determine the provenience of the shrunken heads, as a means of authentication beyond morphological characteristics. Species identification was also conducted to classify the shrunken heads as human, sloth, or primate, since non-human preparations are also common within museum collections. Y-STRs and mitochondrial DNA were analyzed to determine the potential relatedness amongst authentic *tsantsas* via paternal and/or maternal lineages, respectively, due to polygamous relationships and the mixture of gene pools within the subtribes of the Shuar.

Next generation sequencing of genetic markers allows shrunken heads to be identified as authentic or inauthentic on the basis of biogeographical ancestry, in addition to morphological assessments, which may be inconclusive. This method of analysis can also be applied to prosecutions involving the sale of antiquities on the black market, threatening cultural preservation. On a broader scale, next generation sequencing of STRs and SNPs permits the identification of individuals following mass disasters and in cases with missing persons or unidentified human remains. Next generation sequencing is advantageous and more efficient in comparison to more traditional methods, due to its high throughput capabilities and degree of success with degraded DNA samples, demonstrated with the complex shrunken head samples analyzed.

Shrunken Heads, Next Generation Sequencing, DNA Analysis

E73 Rapid Detection of Saliva Presence Using the Phadebas Press Test: A Comparative Study of Three Common Bra Materials and Sealed Envelopes

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After attending this presentation, attendees will be provided with a summary of practical difficulties encountered and tips acquired for the presumptive screening of saliva presence for subsequent DNA analysis.

This presentation will impact the forensic science community by drawing attention to a time-and cost-saving technique that is simple enough to be used in-field (or to be included in sexual assault kits) and has the ability to at least exclude evidence that crime scene technicians should not submit for DNA analysis.

Sexual assaults in the media are not uncommon headline news. Prior to the current era of DNA profiling, forensic science often, as in the prosecution of the infamous serial rapist Ted Bundy, relied upon the now heavily criticized discipline of bitemark analysis; however, even today, quite often the criticism expressed is the lag time or delay in the processing of DNA evidence as it is assumed there is an abundant presence of suitable semen and/or saliva samples in sexual assaults. This is often exasperated by the "CSI effect" or the layperson's expectation of DNA being able to quickly solve any crime just like on television.

Several serious challenges to DNA still remain unanswered: (1) the victim feels so violated she first takes a long shower to cleanse herself and washes away suitable DNA evidence from her body before contacting police; (2) unlike semen, even if saliva is deposited on the victim's clothing during biting or drooling, actually finding the saliva using varied light sources is not only notorious for a high frequency of false positives (which lack the epithelial cells needed for generating DNA profiles), but also adds to the workload and delays both the lab analyst as well as the victim and society; and, (3) a need exists for validating a faster, more accurate presumptive test for saliva deposition in the context of real-world evidence, such as on a bra after a rapist bites a victim's breasts or on a sealed envelope after a white collar criminal mails a letter. In this empirical study, the use of blue dye-linked starch paper (Phadebas Press Test) to detect the presence of amylase activity as the result of saliva deposition after biting was tested on worn bras containing three common types of carrier materials: 90% polyester/10% spandex (main body); 100% polyurethane (foam padding); and 100% polyester (backing). The paper's ability to detect saliva deposited on gum arabic (envelope glue) from sealed envelopes, a common type of evidence encountered in blackmail and white collar-based crimes, was tested.

With both the bras as well as envelopes, the approximate locations of all saliva deposited were documented prior to later testing for the determination of false negatives. Likewise, the areas that were known to be saliva-free served as a control to safeguard against false positives. To account for the possibility of false positives specifically as it relates to the practice of sealing envelopes with water instead of saliva, envelopes sealed with water were tested as well. For added realism due to the inherent gap between saliva deposition and evidence collection, all experimental evidence was left to sit at 25˚C (room temperature) for a period of three days. Since the "press-and-spray water" test was successful in providing the white-to-blue color change indicative of the approximate saliva spot in all instances, it was concluded that the above conditions are suitable for amylase activity to be observed.

Subsequent Polymerase Chain Reaction (PCR) reactions yielded complete DNA profiles from the original samples in the majority of detected stains. Of the original samples that failed to generate complete DNA profiles, nearly all generated profiles when the corresponding Phadebas paper was used for PCR. Preliminary findings suggest the amylase-starch-dye reaction has little to no effect on PCR chemistry. Thus, dye-linked starch paper press tests are useful as a time-and cost-saving measure since this presumptive and potentially simple-enough-to-be-used-in-field (or to be included in sexual assault kits) test has the ability to at least exclude evidence that crime scene technicians should not submit for DNA analysis. Finally, a summary of practical difficulties encountered and tips acquired during these experiments and suggestions for a more detailed protocol for future use will be provided.

Sexual Assault, DNA, Presumptive Screening

E74 Analysis of Bullet Wipes by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS)

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The goal of this presentation is to inform the forensic community that the Gunshot Residue (GSR) coating applied to a bullet when fired and then deposited on target (bullet wipe) can have evidentiary value in some shooting cases.

This presentation will impact the forensic science community by explaining how the analysis of bullet wipes by SEM/EDS can potentially identify the source firearm, depending on the ammunition, both current and historical, by comparing the GSRs of the bullet wipe as well as within the firearm bore.

Coatings of GSR on discharged bullets have been known for many years. As a bullet travels down the bore of a firearm, GSR particle adherence to the bullet's surface occurs, the particles then being deposited on the target upon its strike. Indeed, the identity of a bullet entrance defect in the target frequently depends on the bullet's wipe on that surface and the wipe is often identified by a chemical test.[1] Sloughing off of GSR particles from the bullet's surface also likely occurs during its travel from the muzzle to target.[2]

Burnett described an antistatic organic compound shown to be effective for viewing fabric samples in the SEM.[3] There is little to no charging in the SEM under high vacuum, but it is stable under a focused electron beam rastering for elemental analysis by EDS.

For most caliber bullets with perforated fabric targets, the fabric with the entire bullet wipe area can be excised and mounted on a 13mm D SEM stub. Three drops of the antistatic compound are dripped onto the sample, followed by a light dabbing with filter paper to remove the excess. There is an insignificant GSR transfer to the filter paper. Pressing a sticky GSR sampler on the bullet wipe will pick up GSR, but this is not a good sample representation as excising the entire, or part of the, defect area with the bullet wipe and mounting on an SEM stub. Bullet-wipe GSR tenaciously adheres to fabric fibers. When the defect area is covered with dried blood, the blood can be removed with little apparent disruption (either of GSR removal or chemical change) of the bullet transfer area by a bleach solution of sodium/calcium hypochlorite.[3]

During the discharge of a bullet through the firearm bore, the primer GSR mixes with previous primer deposits from the bore surface.[4] Many of the muzzle GSR particles generated by the current shot are a mixture of GSRs from previous shots.

Many ammunition manufacturers produce lead-free primers of different inorganic compositions.[5] Examination by SEM/EDS of both the bullet wipe and bore GSR could identify bullet source in some cases if a leadless primered ammunition was used with the current shot or previous shots. Repeated shots with leadless primered 38 caliber Remington® UMC Leadless cartridges increases the contribution of potassium with each shot of the resultant bullet wipe GSR.

One fabric sample of bullet wipe adjoining areas revealed different compositions (Lead, Antimony, Barium (PbSbBa) and Pb) of the GSR. The Winchester® bullet in this test had an exposed lead base. This suggests there is a stable component of gas flow behind the bullet as it travels down the bore of the firearm. The volatized lead from the base of the bullet preferentially flowed, likely through an eddy, through a groove in the bore rifling at the side of the bullet.

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Gunshot Residue, Bullet Wipe, Memory Effect

E75 A Comparison of Accidental Self-Inflicted Gunshot Wounds Occurring in Rural vs. Urban Settings

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After attending this presentation, attendees will be more familiar with trends related to fatalities resulting from accidental self-inflicted gunshot wounds occurring in an urban setting as compared to those occurring in a rural setting. More specifically, this presentation will primarily compare data related to decedent demographics, types and calibers of firearms involved, number and location of wounds, and how the injuries were sustained.

This presentation will impact the forensic science community by providing a better understanding of the differences and similarities between deaths resulting from accidental self-inflicted gunshot wounds occurring within rural and urban settings, respectively. Recognizing these trends on scene examination and during autopsy may assist when determining the manner of death.

Data was extrapolated from cases examined at the Cook County Medical Examiner's Office, Chicago, IL, and the Travis County Medical Examiner's Office, Austin, TX. For the purposes of this study, cases from Cook County represent the urban setting, while cases from Travis County represent the rural setting.

Information was retrospectively collected from cases examined at the Cook County and Travis County offices between 1986 and 2016. To identify suitable cases for inclusion, a search was conducted for cases in which the manner of death was accidental and the cause of death was related to gunshot wound(s). Cases in which there was any doubt regarding the accidental circumstances of the shooting, undetermined cases, and cases in which the wounds were not self-inflicted were excluded. A total of 49 cases, 25 from Cook County and 24 from Travis County, satisfied the study's criteria.

All decedents in the study were male and each exhibited only a single gunshot wound. In the rural sample, the average age of the decedent was 28.8 years. Only 20.8% of decedents in the rural sample were under 18 years of age. The average age was only 16.88 years in the urban sample with 64% of the sample represented by decedents under 18 years. In the rural sample, 87.5% of decedents were Caucasian. Black individuals comprised the remaining 12.5%.

In the urban sample, there were no significant difference between the percentages of Caucasians (48%) and Blacks (52%).

In both samples, a handgun was more likely to be involved than a long gun. Additionally, medium caliber weapons were most likely to be used, comprising 50% of the rural sample and 48% of the urban sample. The most common wound location in both samples was the head/neck region, comprising 79.2% of the rural sample and 96% of the urban sample. Decedents in the rural sample were more likely to sustain wounds to the chest and upper extremities than their urban counterparts. Decedents in the rural sample were more likely to sustain their injuries outdoors (54.2%), while in the urban sample, the shooting was more likely to occur within a residence (76%). Interestingly, 88% of decedents in the urban sample received either contact or close range wounds, while 58.3% of decedents in the rural sample were shot at an indeterminate range with no evidence of contact or close range fire.

The circumstances under which the injuries occurred represented the most significant difference between the two samples. In the rural sample, 45.8% of decedents received their injuries while handling the firearm, but in the urban sample, only 12% of decedents injured themselves in this manner. In contrast, 64% of decedents in the urban sample received their injuries while playing with the firearm, but only 25% of decedents in the rural sample.

This suggests that to be effective in reducing firearm fatalities, preventive/educational strategies need to take into consideration geographic differences among the at-risk population.

Gunshot Wound, Urban vs. Rural, Accidental

E76 Comprehensive Forensic Analysis of 3D-Printed Firearms

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3D-printed firearms are expected to become more accessible to criminals as technology improves and costs decrease. This may result in 3D-printed firearms making their way into crime laboratories as physical evidence related to a crime. After attending this presentation, attendees will have a better understanding of how 3D firearms are printed, the possible risks to public safety when this technology is used to manufacture plastic firearms, and the success of current forensic analytical techniques when applied to 3D-printed firearm evidence.

This presentation will impact the forensic science community by enhancing its awareness of these types of firearms and demonstrating the ways in which current, routine analytical techniques in the forensic laboratory can gather useful information from them. The types of analysis that may provide more discriminating and conclusive results will also be indicated.

In May 2013, the Department of Homeland Security issued a bulletin warning of the ease in which 3D-printed guns are being manufactured, and their inability to be detected at security checkpoints.[1] The bulletin noted that "advancements in technology and decreasing 3D printer costs will likely mean even more sophisticated printed guns will become easier to acquire," and that the nature of these firearms will make detection impossible, barring a pat-down of every individual at every checkpoint. The bulletin also emphasized the challenges and difficulty associated with testing firearms made without serial numbers or unique identifiers. Under these circumstances, the Colorado Bureau of Investigation (CBI) was contacted to perform routine forensic analysis on pre- and post-fired 3D-printed firearms created under real-life conditions in order to evaluate the ability of current forensic techniques to meet this upcoming challenge.

CBI scientists performed Gunshot Residue (GSR), polymer, DNA, latent print, and firearms analysis as is standard when a conventional firearm has been submitted by a law enforcement agency, and worked closely with engineers during the selection, printing, and firing of the 3D-printed guns. This enabled forensic scientists to record information regarding the firearms' construction, handling, and firing success before forensic analysis was performed. This unique opportunity to witness firsthand exactly what goes into firing a 3D-printed gun — from the way the weapon is handled prior to firing to the way the weapon itself reacts to being fired — allowed scientists to understand potential challenges to conventional analysis. All components of the weapons were submitted to the laboratory for typical processing methods and were evaluated for useful forensic data. Scientists then determined whether typical analysis would meet the needs of law enforcement and public safety if 3D-printed firearms become as prevalent as predicted.

The research discussed in this presentation includes analysis from the forensic science disciplines of firearms, biological sciences, trace evidence, and latent print analysis, and examines traditional forensic processing of firearm evidence and how those methods can be used or modified for optimal results when handling the components of 3D-printed, polymer-based guns.

The purpose of this presentation is to highlight the success and limitations of conventional forensic analytical techniques when applied to 3D printed firearms.

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1. DHS: It is impossible to stop 3D plastic guns from getting past security checkpoints, Homeland Security News Wire, http://www.homelandsecuritynewswire.com/dr20130524-dhs-it-is-impossible-to-stop-3d-plastic-guns-from-getting-past-security-checkpoints (July 28, 2016)

3D-Printed, Firearm, Plastic

E77 Benefit or Burden?: Forensic Science K-12 Outreach

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The goal of this presentation is to acquaint attendees with forensic science outreach efforts in the K-12 population. This presentation will emphasize that forensic science outreach in the K-12 population is not only a benefit but is also essential for planting the seeds of success for the future of forensic science. Not only does forensic science outreach educate the general community as a whole, but for those hoping to pursue forensic science, it will also result in higher-education students who are more knowledgeable and prepared for their studies in forensic science and ultimately in their careers.

This presentation will impact the forensic science community by highlighting the benefits of operational forensic science facilities participating in K-12 outreach. To aid in alleviating the difficulties many facilities face when attempting to implement outreach activities, attendees will be offered numerous outreach activity examples that can be used at their own places of employment.

Additionally, as a result of this spike in interest, forensic agencies, laboratories, and educational institutions are being inundated with calls and email requests to shadow forensic scientists and/or visit their location for demonstrations and presentations. Unfortunately, with staffing limitations and workload demands facing many operational forensic facilities, it can be difficult to grant these requests. With limited time to plan, implement, and execute these requests, they can be quite burdensome.

The George Mason University College of Science created a novel program, the Science, Technology, Engineering, and Math (STEM) Accelerator, which among many responsibilities addresses the outreach needs of our community. The STEM Accelerator program was created in 2011 with a focus on the success of students in STEM at all levels from K-16. In particular, the program is tasked with four major goals of increasing the number of STEM majors: (1) improving retention rates of STEM students; (2) reducing their time to graduation; (3) helping them join the STEM workforce; or, (4) continue their education upon completion of their Bachelor's degree in STEM disciplines. Created as an interdisciplinary unit, this division consists of faculty members from multiple departments who have special responsibilities besides teaching that includes coordinating and promoting STEM activities that help achieve the four primary goals. Currently, the program includes faculty from mathematical sciences, chemistry, biology, physics, astronomy, computational sciences, atmospheric ocean and earth sciences, and forensic science.

To help fulfill the goal of increasing the number of STEM majors, the faculty of the STEM Accelerator program are actively involved in K-12 outreach. Due to the high level of interest in the community, most STEM Accelerator outreach events include a forensic science component. Activities were created for events such as Cub Scout and Girl Scout badges, the STEM Mania camp for third to fifth graders, the Females of Color and those underrepresented in STEM (FOCUS) camp for middle school girls, the Envision Law and Crime Scene Investigation (CSI) conference for high school students, various STEM days (all levels), elementary forensic science after-school clubs, and several others.

Outreach, STEM Education, CSI Effect

E78 The Changing Landscape of Forensic Science Education: How a Historically Black University Is Addressing the Need for Underrepresented Minorities

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After attending this presentation, attendees will better understand of an undergraduate forensic science program at a historically Black university and the importance of increasing the numbers of underrepresented minority forensic science graduates.

This presentation will impact the forensic science community by demonstrating the benefits to forensic science and the criminal justice system of a more diverse forensic scientist population.

Forensic science educational programs have flourished since *CSI* first aired on television in October of 2000. In fact, more than 15 years later, there are more than 139 campuses in the United States that provide some type of forensic science program with either a BS, MS, associate's degree, or a certificate provided upon completion. Indeed, forensic science continues to be one of the high-need subject areas in Science, Technology, Engineering, and Math (STEM) fields at the national level. The United States Bureau of Labor Statistics (BLS) projects that forensic science technicians will grow by 31% over the 2006–16 decade, faster than the average for all occupations, with job opportunities best for those who have a BA in forensic science.

Locally and nationwide, the number of forensic science graduates among minorities is very low. Currently, African Americans make up only 8% of forensic science program graduates. Thus, there is a strong need for the development of undergraduate forensic science programs and research for underserved minority populations. In 2013, Southern University at New Orleans (SUNO) successfully implemented the Forensic Science Bachelor of Science degree program. At the present time, SUNO is the only university in Louisiana, and one of only four Historically Black Colleges and Universities (HBCUs) in the nation, offering this degree.

SUNO's forensic science degree program is committed to producing technically knowledgeable and skilled graduates equipped with the basic foundational science and laboratory problem-solving skills necessary for success in the crime laboratory. Upon completion of the Forensic Science program, graduates will be prepared to function as forensic scientists or for advanced study in areas such as forensic science, biomedical research, medicine, and law. This presentation will reflect the challenges faced when establishing a forensic science program at an HBCU.

Ultimately, this program seeks to increase the number of African Americans in the field of forensic science in order to bridge the ever-widening gap in the criminal justice system in the United States. The broader impacts of this program include: (1) increased recruitment and full retention of underrepresented students with a strong commitment for academics, STEM fields, and the forensic science profession; (2) a significant advancement of the forensic science program at SUNO; (3) establishment of partnerships with K-12 schools and STEM teachers and students; and, (4) an improved critical transition from K-12 to undergraduate study. Other impacts include expansion and diversification of the forensic science workforce as well as serving as a sustainable model for supporting diverse, academically talented minority students in the forensic sciences.

Forensic Science Education, Underrepresented Minorities, STEM

E79 Visualizations for Introducing Database Concepts in Forensic Science

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After attending this presentation, attendees will be familiar with the attempts to expand the knowledge base within forensic science education by implementing exercises in database operations and querying via customizable animations.

This presentation will impact the forensic science community by illustrating the need for forensic science educators to fill a gap in computer science education within the forensic science discipline.

Databases are used by everyone in society today, from the home computer user organizing his or her household budget information or family images to the high-level scientist organizing his or her data from their research. The caching and querying of data from databases is also used in the forensic sciences in the form of Laboratory Information Management System (LIMS) systems in crime labs; discipline-specific, searchable databases such as Automated Fingerprint Identification System (AFIS) and Combined DNA Index System (CODIS); and by the criminal justice system as a whole. While databases are used extensively by many, it is fair to presume that not all of the users know how the database operates. By understanding how a database operates, a user may be able to take greater advantage of the power that databases can have.

College courses in database design and operation are available, but they are more readily available to upper-division students in the computer science majors. Students with a major in some type of natural science degree, such as biology, chemistry, or forensic science, do not have exposure to database design and operations unless they choose to take additional courses over and above their already stringent degree program.

The animations are the result of a collaborative National Science Foundation grant at Arizona State University and Villanova University to develop customizable visualizations to introduce the fundamentals of relational databases to students of many majors. The animations are available at the project website (http://databasesmanymajors.faculty.asu.edu/). One of the foci of the grant is to demonstrate to students outside of the computer sciences how relational databases operate and how to query such a database. The demonstration is achieved by animations which depict, step by step, how databases and database queries operate. By viewing these animations, the student gains a working knowledge of the use and development of databases being used in educational and professional settings. In gaining this knowledge, the student is better prepared to take advantage of the power that databases and database queries have to offer, and forensic science students, in particular, have yet another skill set to use in real-world applications.

Grant co-Principal Investigators (PIs)/volunteers from the faculty at Arizona State University in the areas of statistics, forensic science, and ecology were instructed to complete the database animation customizations and document the time it took for the customization completion as well as any other suggestions and comments. While attempting to perform the customizations for the database animations specific to forensic science examples, there were a number of challenges that arose in using a forensic science database as an exemplar. These challenges allowed both the forensic science faculty customization volunteer and the designer to explore the gaps in understanding between what may be considered "typical" databases that regularly have data deleted and overwritten versus a forensic science database that needs to maintain and archive any "mistaken entries" or "deleted" information due to the involvement of forensic science in the criminal justice system.

By exploring these differences that arise in forensic science regarding the use of databases, educators and students are better prepared to handle the unique issues within forensic science that may occur using this and other methods of information collection, filing, and storage, as well as information retrieval and querying.

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Database, Education, Forensic Science

E80 Fatal Dog Attacks: An Eight-Year Study From 2009 to 2016 in Italy

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After attending this presentation, attendees will better understand the dog-bite phenomenon that is becoming increasingly important in regard to both political and public health issues with implications for human and animal welfare. This presentation will also evaluate the rise in occurrences of human fatalities caused by dogs in Italy.

This presentation will impact the forensic science community by providing scientific data from a well-controlled experiment, offering increased information for forensic investigation. An appropriate forensic approach should include an exhaustive analysis of the scene, the victim, and the dog.

Domesticated animals, particularly dogs, have provided companionship, labor, recreation, and entertainment for humans; however, as a result of the most diverse causes, this interaction has not been free of conflicts. The alarming statistics reported around the world reveal that dog attacks today represent a health hazard in which prevention strategies were not always successful.[1, 2] Most of the dogs involved in these events are known to the victim or belong to him/her.

Research identified a striking co-occurrence of multiple, controllable factors: (1) no able-bodied person being present to intervene; (2) the victim having no familiar relationship with the dogs; (3) the dogs owner failing to neuter/spay the dog; (4) a victim's compromised ability, whether based on age or physical condition, to manage their interactions with the dog; (5) the owner keeping dogs as resident dogs, rather than as family pets; (6) the owner's prior mismanagement of the dogs; and, (7) the owner's abuse or neglect of dogs. Four or more of these factors were present in 80.5% of cases; breed was not one of those factors.

This Dog Bite-Related Fatality (DBRF) study was based primarily on media reports that identified the number of fatal dog attacks, victims' sex and age, and the breed of the dogs in Italy.

This study found 19 DBRFs in Italy from 2009 to 2016 (2.37 cases per year); these data increase sharply in relation to a previous study that describes 32 DBRFs between 1984 to 2009, with a frequency of 1.28 cases per year.[3] The study found no significant difference based on victim's sex (8 women and 11 men). As previously described, the study confirms that dogs are known, or belong, to the victim (14 cases); in 4 of the other cases in which the victim did not know the dog, the aggression was conducted by mongrel dogs. As previously described, the victims are often more than 65 years of age (47.4%) or less than 5 years of age (42.1%). Finally, the study found no specific breed was more likely to injure a human over another breed.

Aggression causes are poorly known (for example, if the death is caused by the human aggressor to the dog or if it is an unexplained event). Certainly, when DBRF occurs, there are emotional implications in public opinion. This should promote the use of targeted studies, which are still lacking both internationally and, to a greater extent, nationally.

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Fatal Dog Attacks, Italian Cases, Dog

E81 The Hand-Drawn Composite — Old School Still Works With New Tech

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After attending this presentation, attendees will understand that new is not always best. Often, an older procedure or approach will stand the test of time and technology. This is most certainly the case with the discipline of forensic art. The use of desktop publishing, Adobe® Photoshop®, and scanning images have assisted Forensic Artists (FAs) in their work; however, computer facial software programs have not eliminated this discipline. Strong, detailed images that can elicit a public response or corroborate or eliminate known facts are still needed in criminal investigations. The failure of most software programs to produce strong images and be flexible to use in the field has contributed to insuring the FA's workload. Across the nation, the majority of these images are hand drawn and should not be discounted.

This presentation will impact the forensic science community by highlighting a complex case and the flexibility used with an old-school approach — the hand-drawn sketch. Presented here is a Los Angeles County Sheriff's Department (LASD) case of battery, kidnap, and rape, from the South Los Angeles station. This case will demonstrate the use of two forensic composites that worked as corroborative evidence once the suspects, three years later, were identified through DNA.

These forensic composites were drawn in 2011 at the emergency room of Harbor General Hospital, in Carson, CA. The victim was badly beaten; her jaw was broken into many pieces, among other injuries. Her speech was arduous and very limited. The LASD FA responded to these conditions by using an unorthodox method. She asked the victim if she could draw and, when she agreed, gave the victim the paper and pencil. The victim drew very basic images of each suspect that highlighted the features she remembered. Her images were strong and had character. The FA used these visual images as descriptors, just as if the victim had verbally described them to her. The FA worked from these drawings to develop initial images that the victim then modified and these were adjusted accordingly. The hectic and busy Emergency Room (ER) was not the ideal place for an interview or for completing a drawing, but drawings were finalized. The drawings by the victim were also preserved as evidence, treated like the composites that were created by the FA.

This case did not move along quickly as no one responded to the composites nor did the DNA from the victim assist at that time. The suspects, as it turned out, were ages 17 and 18 and had no prior offenses that incurred the documentation of DNA. Three years later, at ages 20 and 21, they committed crimes for which they were arrested and DNA samples were taken. At this point, South Los Angeles Station Detectives were notified that their unsolved case was viable and had new information.

This case was adjudicated in 2014. The victim and the FA testified, among others. The jury appreciated the victim's sincerity and that the composites looked like the defendants. The composites worked and supported the case. Both suspects received heavy sentences due to the high number of offenses involved, including the use of a gun.

Forensic Art, Hand Drawn Composite, Forensic Art Communication

E82 Using Drones for 3D On-Site Body Documentation — Experiments Inspired by Real-Life Forensic Cases

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After attending this presentation, attendees will: (1) understand the benefits and costs of employing unmanned aerial drones for crime scene photography and innovative 3D on-site documentation techniques; and, (2) comprehend technological and methodological advances that allow documentation of forensic scenes and physical evidence in 3D in a rapid, affordable, yet photorealistic and accurate manner.

This presentation will impact the forensic science community by presenting results that advance currently available techniques employed in the course of crime scene documentation and reconstruction.

Following substantial progress in digital imaging technologies, several projects invested in establishing image-guided postmortem examination while utilizing medical imaging techniques, optical surface scanning, or photogrammetry. It has been well demonstrated that these techniques provide numerous benefits in decision-making conducted inside autopsy rooms or forensic laboratories. Yet, as far as outdoor crime scene and on-site deceased remains documentation is concerned, experts rely almost exclusively on traditional digital photography, the gold standard in forensic documentation. This limits the ability to interlink autopsy findings with relevant contextual information, as it provides ground views to the scene and discards surface depth – information highly valuable in terms of spatial assessment.

This presentation paper was inspired by two recent forensic cases admitted for postmortem examination at the Department of Forensic Medicine, Masaryk University, Czech Republic. Both cases involved young females who fell off a cliff at Brno-Hády, an abandoned limestone quarry located at the northwest end of the Brno city limits. Due to its level overview of the city, the quarry is a popular, albeit treacherous, gathering and viewing site.

In both incidents, the dead bodies were discovered at the lower level of the quarry, at sites located approximately 100 meters apart. While the first incident was ruled an accidental fall due to alcohol intoxication, in the second case, foul play was initially suspected. Based on case reports, the crime scene was reconstructed using a fully clothed dummy and crime scene equipment at the place that generally coincided with the original location of the second body.

This two-fold project sought to propose a drone-based protocol for outdoor forensic scene photography and to test the applicability of drone-based photography for 3D scenery and on-site body documentation. A commercial drone DJI Phantom 2 equipped with a GoPro® HERO4 digital camera was employed. The system was operated remotely by an experienced operator, although not one specifically trained for the task. Given the cascade-like character of the landscape, two sets of photographs were taken with a different operator's position (i.e., different starting position for the drone); one set was taken on the upper level of the quarry and the other was approximately 20 meters from the reconstructed scene. Once the air documentation was conducted, the scene was captured in detail from the ground using a Nikon® 7000 camera equipped with an 18-105mm lens.

From the two operator positions, nearly 2,000 images were taken (4K resolution, 4,000 x 3,000 pixels), corresponding to 25 minutes of capturing time. In addition, 100 photographs were acquired to capture the scene on the ground. Of 500 drone-based pre-selected images, a final set of 250 images was further processed with Agisoft PhotoScan® program in order to generate 3D textured digital models. The generated point cloud of approximately two million vertices was appended by texture files (JPEG, 4,096 x 4,096 pixels) before being scaled using the meter scale placed by the dummy and marking cones enclosing the forensic scene.

Employing the drone enabled the documentation of small-to-large areas of the forensic scene in a relatively short timespan. Although dependent on a rather time-consuming image post-processing phase, the 3D photogrammetry approach produced high-resolution 3D images, comparable in quality to any professional digital camera. It also

provided photorealistic high-quality 3D surface records of the dummy as well as the marking equipment. Comparing image-based measurements with real distances revealed the accuracy and reliability of the achieved results. This suggests that the images can eventually be admitted as evidence in court. Altogether, the acquired results lay down the foundation for future work employing drone-based techniques, which most likely will have to respect safety requirements on the operation of drones, as new rules are developed in Europe as well as in the United States.

Drones, Scene Documentation, 3D Digital Models

E83 Implementing a Drone Program for the Houston Forensic Science Center Crime Scene Unit

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After attending this presentation, attendees will understand how to use an Unmanned Aircraft System (UAS), also known as a "drone," for aerial photography at crime scenes as well as how the Federal Aviation Administration (FAA) regulations regarding commercial drone usage allow for practical implementation made difficult by previous regulations. The new FAA regulations became effective in August 2016.

This presentation will impact the forensic science community by making it clear that new FAA regulations make it possible to operate a drone program that allows for aerial photography of crime scenes. The development of Standard Operating Procedures (SOPs) and policies for drone usage will be discussed.

In an attempt to stay at the forefront of forensic science, the Houston Forensic Science Center purchased a DJI quad-copter UAS in 2015 and began exploring how its unique photo and video capabilities could be used at crime scenes. At that time, government agencies were subject to cumbersome FAA regulations requiring a licensed pilot to fly the drone or an extensive multi-year process that would have allowed the regulated use of the aircraft. Later that year, the FAA announced less cumbersome rules for hobbyists; however, they did not include government or commercial use of the aircrafts. The FAA indicated more feasible regulations were on the horizon for the regulated commercial use of drones. In the meantime, SOPs, policies, and training regimens were able to be developed so that a UAS could be utilized at crime scenes once the regulations were published. In the interim, under a general public operator's license, the UAS was utilized for training and familiarization purposes. This developed a base of knowledge of training material and the extensive experience needed to safely operate the drone in Houston, TX.

In June 2016, the FAA released the rules for UAS commercial operations. Licensing was available in the fall of 2016. The new regulations are far less cumbersome than the previous rules, which required an FAA-licensed pilot to be present when operating the UAS. Most of the regulations pertain to rules and documentation, but operators are also required to pass an FAA aeronautical safety exam at an FAA facility. After personnel complete these requirements in the fall of 2016, air operations will begin shortly thereafter.

The UAS will be utilized for large, outdoor scenes during daylight hours, primarily in areas where a unique aerial perspective could help clarify, how an incident evolved or reveal different positions of interest. Houston has a high number of drownings in local bayous, rivers, and other bodies of water. Often the body can only be seen and retrieved from a boat prior to an investigator's arrival. A UAS will allow investigators to view bodies from above prior to recovery, eliminating some of the obstacles that can obstruct the view from the banks. In the future, the use of the drone can be expanded to fatal car crashes where a direct overhead view of skid marks, debris, and other visual references could assist an investigation. Previous to this, agencies had to use a police helicopter for aerial photography and video; however, helicopters are expensive to fly and do not have the low-altitude, hover, and camera capabilities of a UAS. For smaller agencies with fewer financial resources, a helicopter is often not an option. But, with an investment of approximately $600, an agency can obtain the hardware needed to launch a UAS program. This presentation will share with attendees the challenges and experiences in implementing a UAS program. It also delves into the differences between the old and new FAA regulations and the exemptions required to implement a flight program. The criteria used to select a UAS, as well as the technical and legal aspects of developing SOPs, policies, and a training regimen for such a program are also presented.

Drone, Photography, Crime Scene

E84 Learning to Spot Aquatic Crimes Against Children (ACAC): An Examination of Situational Context Identifying Six Categories

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After attending this presentation, attendees will be able to better recognize and investigate signs of foul play specific to six categories of ACAC. This presentation will present the situational context of both fatal and non-fatal ACAC committed by caregivers, which are then staged as drowning accidents, land-based deaths, illness, or confessed homicidal drowning.

This presentation will impact the forensic science community by using the categorization of past case information to impact the medical, forensic, and child welfare communities by providing them with an increased awareness and early recognition of ACAC, relevant questions to be added to child death investigative forms, suggested evidence to document and collect on scene, specific investigative procedures, tactics for attorneys to obtain justified judicial outcomes, and stimulate further areas of research.

According to the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics (NCHS), drowning was the most common *unintentional* cause of death for children ages 1-4 years in the United States, and was within the top five causes for older children. This study explains how cases of ACAC can be easily misdiagnosed as unintentional deaths. The manner of death in several of the studied case histories was originally diagnosed as accident, and later changed to homicide only after the cases were re-opened and homicide convictions resulted.[1, 2] It is likely that other ACAC are missed and falsely diagnosed as unintentional, because law enforcement, death investigators, medical and child welfare personnel, and prosecutors typically receive little or no training specific to ACAC.[3]

A compounding problem is that body-found-in-water investigations often initiate with assumptions of drowning and accident for cause and manner of death.[4] One of several reasons for these initial assumptions is that law enforcement and medical personnel often respond in rescue mode to incidents of children reported as found in water.[5] The minds of rescue-mode responders are usually occupied with patient care and grieving caregivers, rather than on questions such as, does it make sense that the child was in the water, are there red flags regarding the child's physical presentation, and why did the child not survive immersion?[6] Another important area of concern is that investigative tools, such as the CDC Sudden Unexpected Infant Death (SUID) form, lack questions on aquatic variables, such as last time of immersion and bathing techniques.[7]

Crimes against children are often perpetrated by caregivers who have primary or temporary responsibility of their victims. Thus, this study focused on ACAC involving caregiver offenders.[8] After reviewing autopsy, toxicology, and investigative reports, six categories of ACAC were created: (1) punishment; (2) homicide; (3) birthing incidents; (4) Munchausen-by-proxy; (5) aquatic sexual sadism; and, (6) pedophilia. Some categories are divided into subcategories. For example, the punishment category includes 1a (fatal) and 1b (non-fatal). The homicide category includes 2a (terrestrial homicides staged as accidental drowning deaths); 2b (aquatic homicides staged as terrestrial deaths); 2c (murder-suicides); and 2d (homicidal drowning committed by clinically mentally-ill offenders who confess).

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Drowning, Homicide, Child Abuse

E85 A Non-Contact Passive Approach for the Effective Collection of Target Cadaveric Volatile Organic Compounds (VOCs) for Canine Training Aid Development

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The use of authentic biological material from a human corpse for canine training is illegal in several countries. Moreover, in the countries where the use of human remains is legal, the limited availability of these materials for practice may affect the reliable training of canine teams. After attending this presentation, attendees will better understand of how the development of a training aid suitable for daily operations is useful in providing safe and effective human cadaveric remains detection training for enhanced detection capabilities. This study presents a non-contact passive approach for the collection of target VOCs emanating from human biologic cadaveric materials to be used as useful canine training aids.

This presentation will impact the forensic science community by demonstrating that the creation of training aids using a static non-contact passive collection method could be used to capture all VOCs of biological material from human corpses onto adsorbent material and, in turn, be used for excellent canine detection training in all countries where the use of authentic human cadaveric samples for dog training is prohibited.

In recent years, many studies on the odor signature of canine detection of human remains and blood traces have determined that reliably trained detection dogs do not alert to generic scent; rather, they alert to cadaveric decomposing human tissues traces, maximizing the location of human remains that were deliberately buried to escape detection in an efficient, cost- and time-effective manner.

The investigative, scientific, and medical evaluation of the crime scene requires a relatively short response time to avoid contamination and, therefore, a well-trained dog allows very large areas of research to be covered more quickly, preserving any and all possible evidence, and can assist in locating clandestine burials and human remains deposited or scattered on the surface.

Many studies determined that the compounds of human decomposition are similar to those of pigs; but a dog trained on pigs is simply a dog trained to find dead pigs. Studies also suggest that only a few VOCs, evolved from a biological specimen of human decomposition, can stimulate canine olfactive alerts in every cross-matching condition.

This research was performed based on previously identified human cadaveric VOCs, such as dimethyl disulfide, carbon disulfide, heptanal, nonanal, ethanol, and acetaldehyde. This study was performed using pieces of organs (skin, muscle, fat, brain, heart, lung, spleen, liver, and kidney) from four traffic-accident fatalities (two men and two women, excluding cases of intoxication). The samples were stored in separate 18 ounce glass jars. The glass jars were covered by a film with holes in it, above which were arranged several VOC-free 5″x5″ cotton gauze pads. The jars were then closed by a film cover and maintained at room temperature. The gauze pads were placed approximately ten inches above the samples and later used in part for the chemical analysis and in part for dog training procedures.

Gas Chromatography/Mass Spectrometry (GC/MS) was used to detect the VOCs released from biological specimens of human decomposition and from gauze pads. The headspace extractions were repeated every 15 days in time intervals ranging from 0 to 120 days for each glass jar. The National Institute of Standards and Technology (NIST) mass spectral library and extracted ion chromatograms were used to identify the compounds.

The results demonstrated that collection of human cadaveric VOC's is the same for authentic human cadaveric samples and for "exposed to samples" gauze pads, at each analysis time. At the same time, the gauze pads were used, in many field trials, to test the canine's ability to detect and alert the VOCs released from human cadaveric bodies onto gauze pads via a non-contact passive method. Other field trials were performed to test the canine's

ability to detect and alert the VOCs release from authentic human cadaveric samples. For both rows of testing, the dogs demonstrated an excellent sensitivity, with recovery rates ranging between 99.72% and 100%.

K-9 Cadaver Searches, Non-Contact Passive Collection, Volatile Organic Compounds

E86 Fatal Asphyxia by Bolus in Elders With Mental Illness: The Role of Oral Health and Antimuscarinic Action of Drug Treatment

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After attending this presentation, attendees will be able to describe the impact of oral health in death by bolus.

This presentation will impact the forensic science community by demonstrating the role of an optimal level of oral hygiene with the control of xerostomia in these psychiatric patients.

Asphyxia may be caused by a suicide, homicide, or accident. This presentation explains the mechanism of the so-called death by bolus, due to obstruction of the upper airway. In this form of accidental suffocation, the foreign body is made of food, which penetrates and occludes completely the larynx or the bronchial branches. The victims are usually subjects in a state of acute intoxication by alcohol or drugs, partially or totally edentulous, with neurological or psychiatric disorders. The voracity in the act of swallowing food may be a predisposing factor. The partially edentulous and reduced periodontal support of the teeth, associated with dry mouth as a consequence of psychiatric therapy, causes difficulty in chewing, swallowing, and in the formation of food bolus. In particular, the use of atypical antipsychotic chloric drugs, such as olanzapine, clozapine, and tricyclic antidepressants, causes dry mouth for specific antimuscarinic action on the cholinergic system.

This study reports a case of an elderly woman found dead in her home. There was a deep purplish-red hypostasis on the posterior of the cadaver that was still fluid, and imprints could be created with the pressure from a finger. The inspection of the oral cavity identified the presence of material similar to the texture of bread (material that was also found on the floor). An account presented by her family during the judicial inspection revealed the medical history of the victim, who appeared to be suffering from paranoid schizophrenia, the most common subtype. The victim's psychiatrist confirmed this medical history. A toxicological analysis of the victim's body fluids was performed. The toxicoloical postmortem test results revealed the presence of tricyclic antidepressants in the body fluids and confirmed the existence of drug treatment at the moment of death. The dental consultation clearly revealed severe periodontal disease and partial edentulous. The analysis of the respiratory tract was performed through the withdrawal of the heart and lungs. At the opening of the trachea, the oral cavity revealed material that was also found in the carinal and bronchial angles: its pultaceous consistency had occluded the airway.

The combination of data collected during the judicial inspection and the results from the autopsy determined that the elderly woman's death was caused by acute respiratory failure as a result of an obstruction of the airway by a food bolus (bread). In fact, patients with psychiatric illnesses are subject to increased risk for fatal events of asphyxia. The causes can be attributed to the pathological condition which affects the swallowing reflex and the use of tricyclic antidepressants and antipsychotics, especially atypical chloric types, such as olanzapine and clozapine, which cause dry mouth for specific antimuscarinic action on the cholinergic system. Since the correct formation of the food bolus derives from both effective chewing and proper salivation, in psychiatric patients with diseases of the stomatognathic system, the formation of the bolus and its consequently correct passage from the oral cavity to esophagus is more complicated. These results point out a possible correlation between the genesis of events of asphyxia caused by the obstruction of the airway by a food bolus and oral health in patients with psychiatric disorders who are in drug treatment. The prevention of accidental asphyxial episodes in patients affected by these diseases could be accomplished through appropriate treatment and the observance of an optimal level of oral hygiene in combination with the control of xerostomia by means of appropriate protection or any alternative pharmacological therapies.

Forensic Sciences, Oral Health, Asphyxia

E87 The Essential Role of Forensic Medicine in the Investigation and Documentation of Torture and Ill Treatment

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After attending this presentation, attendees will be informed of several steps and procedures that need to be followed by forensic medical experts in the investigation and documentation of torture as well as the difficulties and pitfalls, which are based on actual cases.

This presentation will impact the forensic science community by dealing with topics that are relevant worldwide: torture, ill-treatment, and detention in terrible conditions.

In 1948, humanity marked an important milestone with the adoption of the Universal Declaration of Human Rights. One of its 30 articles (Article 5) stipulates that no one shall be subjected to torture or other cruel, inhuman, or degrading treatment or punishment.

Since then, there have been a number of other international regulations that reinforced the legal obligation of states to prevent, prohibit, criminalize, and investigate alleged cases of torture or cruel, inhuman, and degrading treatment, as well as the obligation to ensure that all perpetrators are forced to answer for their actions and that victims receive appropriate reparation; however, 65 years later, there continues to be a marked discrepancy between the law and reality. Torture, ill-treatment, and detention in terrible conditions continue to occur all over the world, including in countries generally considered as paragons of virtue in the sphere of human rights.

This regrettable situation reinforces the need for a more thorough and systematic investigation and documentation of these practices in all countries. Such an investigation and documentation is essential if the world is to eradicate ongoing abuses and prevent new cases and even possible deaths. But thorough investigation and documentation is also necessary to achieve other objectives, such as ensuring that perpetrators are brought to justice, that victims received proper reparation (compensation, and other forms of rehabilitation to which they are entitled), and that official bodies and the general public are made aware of such practices in order to prohibit them completely or encourage reform.

The investigation of torture and cruel, inhuman, and degrading treatment or punishment is not, however, an easy task. Forensic medicine has, in this domain, a fundamental role and valuable guidelines about how to proceed in the forensic investigation and documentation of such situations have been developed. The Istanbul Protocol in one of the examples. This role will: (1) involve the assessment of possible lesions and signs of abuse, even in the absence of specific complaints or accusations; (2) include the documentation of signs of possible physical or psychological abuse; (3) interpret evidence and deduce possible causes; (4) proffer an opinion as to the extent to which the medical evidence correlates with the specific allegations made by the examinee and/or agents potentially responsible; (5) make effective use of the information obtained in order to thoroughly document and disclose torture practices; and, (6) ensure that the legal and governmental authorities and the local and international community are fully informed of the physical and psychological consequences of the kind of torture used. This will also involve an assessment of the detention conditions that, in some cases, can amount to cruel, inhuman, or degrading treatment or punishment.

To investigate and document such cases with the thoroughness they deserve requires regular expert practice and a continuous effort to remain abreast of new developments through ongoing training, study, and reflection. This presentation provides information on new torture situations and their physical and psychological consequences, transmits knowledge of new means of diagnosis and their potential, generates reflection on experiences arising from interventions in the field, and divulges new standards and guidelines.

Torture, Ill-Treatment, Human Rights

E88 Explosive Force During a Wheel Blowout Induces Death

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The goals of this presentation are to present the power of a tire explosion and the effects a tire explosion has on a body.

This presentation will impact the forensic science community by illustrating the destructive potential of a pneumatic tire toward a body during an explosion in a confined space.

Explosive force during a wheel blowout occurring close to people approaches 20% mortality rates. Wheel blowouts, destroying the tire and rim, can cause bodily harm or even death when individuals are close to the explosion. Tire blowout causes vary, but common cases are punctures from sharp objects and structure failures due to wear or temperature fluctuations. Ruptured tires have been reported to displace air at speeds up to 1,000km/h and are powerful enough to move and cause damage to a nearby individual. In addition, the shock wave that is created can produce sudden changes of overpressures from 6.89 to 10.34 bars (100 to 150lb/in^2); however, there is a mortality of 1% for overpressure near 50 bars (725lb/in^2). Explosions occurring during heavy braking or when the rim is substantially heated (direct soldering on the rim) can cause shock waves with overpressures near 70 bars (1,000lb/ in^2). This leads to higher mortality rates of up to 50%. In comparison, the explosion from an 81mm mortar shell causes a shock wave of overpressure around 0.5 bars (7.25lb/in^2).

Tire explosions can result in primary, secondary, and tertiary lesions that may additionally cause fractures and amputation. The distribution of lesions depends on the position of the victim at the time of the accident. A higher proportion of skull, upper torso, and limbs are the areas more commonly affected (20%-48%) than other regions of the body.

A 55-year-old man died due to injuries acquired during the explosion of an agricultural pneumatic tire in a confined area. The individual was propelled against fixed elements, as this tire is capable of causing a shockwave overpressure up to 70 bar (1,000 lb/in^2). The tertiary lesions produced at the time of the accident are detailed.

The location of cranial thoracic lesions is consistent with the literature for this type of accident and is distinguished by the presence of seven immediate lethal lesions, first and foremost decapitation and the externalization of brain material. Multiple amputations of the upper limbs, an opening of the rib cage with externalization of the left lung, and a tear in the heart and aorta as well as the pulmonary veins were also observed. Diaphyseal amputation of the upper limbs can be related to the primary lesions. Indeed, a blast primarily causes diaphyseal fractures of articular fractures; however, the cranioencephalic trauma was attributed to tertiary lesions, which resulted in brain material deposited on the staircase behind the victim. The severity of this type of tertiary lesion is unreported in the literature.

Proximity to the explosion is proportional with more lesions and higher damage to the body. The victim's proximity to the explosion was extremely close, as observed from the welding marks, and the proximity caused serious bodily damage and dilapidation from the blast energy. Moreover, the explosion in a closed space accentuates different lesion features due to the blast reflecting waves against a solid surface. Mortality rates are higher (77 against 8%, $p<0.001$) when an explosion occurs in a confined space. Here, the accident occurred in a hangar with walls less than five meters away from the body. Shock waves reflecting against the solid structures heightened the lethal character of the explosion.

In this accident, multiple factors led to the individual's death. The explosion, and not the bursting of the pneumatic tire, occurred in a confined space in close contact with the victim, thus not allowing the energy (overpressure) of the accident to dissipate into an open area. The gravity of the tertiary lesions was not the goal of our study, but rather to present an evaluation of the destructive potential at a short distance of the energy released during an explosion from a pneumatic tire in a finite space.

Tire, Blast, Injuries

E89 Survey on Deaths and Causes of Death in a University Hospital: The Calabrian Experience

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After attending this presentation, attendees will be informed regarding the mortality rate in a southern Italian university hospital and better understand data related to the patients, the causes of hospitalization, and the discharge units. This presentation also describes the Italian National Institute of Statistics (ISTAT) death forms, a data collection system for deceased individuals.

This presentation will impact the forensic science community by increasing knowledge of statistical information regarding hospital deaths that allows the investigation of the existence of modifiable risk factors (such as low nurse staffing levels, medical errors, or nosocomial infections) as well as updating prevention and risk management programs.

Despite improvements in patient safety during the past years, inpatient mortality remains a current problem.[1] As one incentive for hospitals to focus on the goal of reducing mortality, publicly available data comparing hospital quality use mortality as an essential component; however, although several hospitals sought to reduce inpatient mortality, there is a scarcity of literature on effective methods to achieve this goal.[2, 3]

In Italy, a major role for the statistical management of deaths is provided by the ISTAT. A survey tool, the "ISTAT death form," is an official document based on an international standard recommended by the World Health Organization (WHO). In Italy, the death form is composed of two parts: (1) part A, "medical", completed by the doctor or coroner with the information regarding the causes that led to death; and, (2) part B, compiled by the register office, with demographic and social information regarding the deceased. The selection of the hospitalization and death causes follow the criteria and rules established by the International statistical Classification of Diseases and related health problems (ICD-10), ensuring the possibility of obtaining standardized data and making comparisons over time. This retrospective study reviews ISTAT death forms, from 2012 to 2015, to characterize mortality in a southern Italy university hospital. All death forms were reviewed in detail, considering the age and sex of patients, investigating the causes of admission and death, and the hospital discharge units.

The sample was comprised of 353 inpatients who died in the hospital from 2012-2015. The proportion of men (63%) exceeded that of women (37%) and 72% of the subjects were aged 65 and older. The average age of inpatients who died was 71 years throughout the period from 2012 to 2015. The sample was divided according to pathological causes that determined hospitalization. The cardiovascular diseases were clearly the most numerous (70%), followed by respiratory diseases (9%). The study found that death was due in 55.12% of the cases to cardiac dysfunctions and 31.16% to Multiple Organ Failure Syndrome (MOFS). The medical units were divided into three areas: medical care, surgical care and intensive care. Most mortality occurred in the intensive care area (62.4%) due to cardiogenic shock, pulseless electrical activity, MOFS or complicated sepsis.

This study enabled the detection of information for the deaths and causes of death that occurred in a small university hospital. This type of search should be extended, over time, to study the trends of mortality and determine if there are modifiable risk factors (such as nosocomial infections). Ultimately, it would be interesting to know how many of these deaths were due to potentially preventable medical errors.

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Hospital Mortality, Epidemiology, Statistic Tool

E90 Killing Methods in Sicilian Mafia Families

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After attending this presentation, attendees will understand various principles of Mafia murders, especially regarding the characteristics of both injuries and of weapons used.

This presentation will impact the forensic science community by contributing to the primary objective of legal medicine, by clarifying the method of murder, and verifying the reliability of police informants.

The recent conclusion of the operation "M. N." that dealt a heavy blow to the Mafia clans in the Sicilian province allows representation of the activity conducted as part of the judicial forensic process.

In the late 1980s in the area of Nebrodi, a mountain range located in the Tyrrhenian coast of the province of Messina, Sicily, a Mafia war of saw dozens of murders, disappearances of the bosses, drug dealers, and brutal executions in a style similar to what happened during those same years in Central America. The fighting of Barcellonesi gangs against the Tortoriciane gangs, families against families, occurred in order to manage the drug market and the market of landfills and waste, as well as to obtain contractors and subcontractors of a new railway line.

This study examined 41 cadavers autopsied between 1987 and 1992. Some of the bodies were found at the murder scene and others far from the site of the crime, in an area that is difficult to access. Nine bodies were found inside their own cars (two of which were burned) and the others were in public places (abandoned farm houses, agricultural land, streets, and public areas). The condition of the bodies varied in relation to the date and the manner of the murder: there were 33 ''fresh'' bodies, 3 burned bodies, 3 skeletonized bodies, and 2 putrefied bodies. All were Caucasian (with the exception of a Moroccan); there were 40 males and 1 woman. All were between 17 and 60 years of age. The bodies were mostly members of the "cosche mafiose" and only rarely were they unwitting victims. There was one mutilated victim. Some of the bodies were found after a period of more than two years from the official notification to the police regarding the missing person and were discovered thanks to the help and collaboration of ''repented'' Mafia members. In these cases, anthropometric analyses were necessary. Crime scene investigations were therefore required and conducted by medicolegal experts cooperating with police officers.

Ballistic experts examined the weapons, bullets, and cartridges found at the crime scenes and with the bodies of the victims. Firearms were used in all the murders; in some cases, a modified shortened rifle with sawed off barrels (called the "lupara") was used that has a high lacerating power at short distances. The head shot lesions produced by firearms were mostly localized in the occipital region, typical of ''executions,'' while, in the remaining cases, there were primarily multiple lesions distributed over the chest, abdomen, and limbs.

In conclusion, the forensic analysis depicted the "modus operandi" of Mafia clans of the 1980s and 1990s.

Sicilian Mafia, Criminal Organization, Forensic Investigation

E91 A New Type of Theft in the Government's Courier Cargo Truck Service in Brazil

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After attending this presentation, attendees will understand a new type of theft in the government's courier cargo truck service in Brazil. Knowing the dynamics of this crime makes it possible to extract the elements needed for an adequate crime scene analysis and to understand the factors that make it possible to happen. It also makes it possible to discern unusual types of evidence found in attacked vehicles, as well as to understand the difficulty for drivers in identifying precisely when and where the crime happened.

This presentation will impact the forensic science community by discussing a crime scene involving a vehicle in motion. The victim often does not realize that the cargo was stolen until arriving at the final destination. This lack of information hampers or makes it impossible to locate the exact spot where the crime happened and, additionally, makes the crime scene processing a more complex task.

In Brazil, the majority of the courier cargo is transported by trucks. The vehicles are responsible for transporting orders from one state to another. They are the main targets of a type of crime that occurs while the vehicle is in motion and without the driver's knowledge. This type of crime ended up being popularly called "Spiderman theft." This crime is usually committed by several individuals properly appareled (clothes and proper tools). In moments of reduced truck speed, they climb onto the rear bumper of the vehicle or on areas of the truck´s chassis. With the vehicle still in motion, and taking advantage of bumpers, the criminals forcefully open the door of the cargo bay by breaking locks and seals. Then one or more criminals enter the cargo bay and plunder the orders, choosing whatever they want. The stolen load is then thrown along the road and collected by a support vehicle or (in the case of more fragile goods) is put inside the criminals' clothes. When the truck again reduces speed, the criminals loosely close the door of the cargo bay and leave the truck. In this way, the driver only realizes that the cargo was stolen when the vehicle arrives at the destination or when a visual inspection of the truck is conducted at an intermediate stop. The driver, when questioned, does not know exactly when or where the cargo was stolen.

This type of crime often leaves few traces that assist in the determination of identification. Criminals often use garments that cover the entire body, including parts that are padded to improve their own protection in the event of any accidents. They generally use hard gloves as well. It is extremely difficult to obtain biological traces such as a papillary impression or material for DNA analysis that reliably relates to the crime scene perpetrators.

If no other forensic evidence that could lead to the culprits is found (such as blood from cutting themselves forcefully opening the cargo bay door), the forensic analyses of this crime scene is then based on the vehicle's tachograph to validate the driver's testimony. Such analyses can indicate whether or not the truck stopped to unload goods. This is complemented by several other analytic sources, such as data from the satellite's tracking system, the presence of marks in unusual locations (dirt or scrapes on the door's lockers or in higher spots of the door, etc.), the presence of objects that prevent the cargo bay door from fully opening (strings, thin ropes), and deformation/damage to the door and its locks.

Courier, Theft in Motion, Climb

E92 Association of Foot With Footwear: A Study of the Characteristic Features of Rubber Slipper Insoles

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After attending this presentation, attendees will understand the usefulness and methodology of associating footwear with the perpetrators in crime scene investigations and of conducting further research in this area.

This presentation will impact the forensic science community by presenting standards to identify the weight-bearing marks created and darkened by pressure, heat, and perspiration that may be left by the sole on the insole of the rubber slippers, which is helpful in matching the footwear evidence encountered at the crime scene.

Foot impressions are found as evidence and collected from almost all types of crime scenes and link the crime and the perpetrator. Comparison of the shape, size, or morphology of a foot impression has been successfully used in criminal investigations and in the administration of justice in the past. Due to the uniqueness of an individual's footprint, the impressions left on the insoles of the footwear can also be individualistic to that person. The impression on the insoles of the footwear depends on the pressure distribution under feet. This can be further correlated with the shape, size, angulation, interspaces of the toes, humps, creases, overall length of the foot, the contour of the ball, the shape and placement of the toe pads, the contour of the arch, accidental characteristics, deformities, corns, instep region, inner and outer margins of the foot, a particular habit of the person, and the body weight of the person. Due to the highly individualistic nature of the gait and foot morphology of a person, it can also be observed that the combination of gait and foot morphology should make the forces and areas of contact beneath the foot correspondingly highly individualistic.

Limited studies are available on the characteristic impressions of the sole of the foot on the insoles of the footwear. In the absence of any set standards of characteristic features of the foot inside the footwear, the identification of the criminal becomes difficult. This problem has prompted the present research. The main objective of the investigation was to identify the weight-bearing marks created and darkened by pressure, heat, and perspiration that may be left by the sole of the foot, including the toe pads on the insole of the rubber slipper. The study also compares the pattern on the insoles of the rubber slippers with the footprints of the person for identification purposes. The study is based on a random sample of 200 subjects and their rubber slippers (100 males, 100 females). The study has been conducted as a part of a major research project funded by the University Grants Commission, New Delhi, India. The target group was identified and a pair of rubber slippers was given to each subject for five months in the summer season. The subjects were instructed regarding the proper use of the slippers and certain precautions. After five months, the rubber slippers were collected from the subjects who were studied for various individual insole characteristics. Standing footprints were also taken from each participant, using the inking method for comparison purposes. Upon examination, in most cases, the ball and the heel regions displayed maximum weight-bearing areas of the foot and, consequently, a clear mark was left on the insole of the slipper. In many cases, the big toe and the instep region also revealed a variety of weight-bearing marks that may be well correlated with the foot impression of the person. Overall, the study concluded that the weight-bearing marks on the insole of the footwear may be helpful to a great extent in associating the footwear found at the crime scene with the perpetrator; however, while conducting this study, certain precautions were followed in the controlled conditions that will be discussed in this presentation.

Crime Scene Investigation, Footwear, Identification

E93 A Case Report of a Migrating Bullet: An Unusual Cause of Postmortem Confusion

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The goal of this presentation is to share an unusual case of an unusual phenomenon involving retained intracranial projectiles.

This presentation will impact the forensic science community by discussing how single gunshot wounds pose challenges to medical examiners when projectiles do not end up where expected (e.g., internal ricochets and embolisms).

Migrating bullets of the central nervous system are rare sequelae of penetrating gunshot wounds. Typically, such cases have been described in the neurosurgical literature because they can produce complications in the management of patients, such as decline in neurologic status, delays in rehabilitation, and difficulties in bullet removal. In contrast, few postmortem reports have described this phenomenon.

A 24-year-old male sustained a single gunshot wound of the head and was taken to a nearby trauma center where he survived for two days before expiring from his wound. The body was brought to the Dutchess County (DC) Medical Examiners Office (MEO) and an autopsy was performed the next morning with a postmortem interval of approximately 18 hours. External examination revealed a wound located above the left ear and a wound over the right eyebrow; both had been previously sutured in the hospital. After removing the sutures, it was found that the wound above his left ear was round with a circumferentially abraded margin consistent with an entrance gunshot wound. The wound above the right eyebrow was a crescent-shaped vertically oriented wound with sharp edges, no abrasions on the adjacent skin, and was more consistent with an exit wound; however, examination of the postmortem Anterior –Posterior (AP) X-ray of the head revealed a radiopaque object near the inside of the left side of the calvarium (i.e., next to the entrance defect). A few tiny radiopaque fragments were also noted within the brain along the wound track extending from left temporal to right frontal, including a tiny fragment outside of the cranial cavity in the soft tissue of the right frontal scalp near the exit wound. Upon opening the cranial cavity, a large fragment of a small caliber, deformed, non-jacketed bullet was recovered from the left side of the skull near the entrance wound. Examination of the bullet suggested that part of it may have exited out of the right frontal scalp. The right frontal bone beneath the exit wound revealed outward beveling of the skull consistent with a bullet directed left to right.

Postmortem AP and lateral postmortem radiographs of the head were taken prior to commencement of the autopsy in order to localize the projectile. Premortem hospital Computed Tomography (CT) scans and radiographs were not available for review prior to the start of the autopsy but were subsequently examined after the postmortem examination. Subsequent review of the premortem CT scans of the head confirmed that the bullet recovered at autopsy had shifted from the right frontal area to the left temporal area prior to the patient's death. The admission CT scan revealed a radiopaque object near the right supraorbital area with hardening artifact noted

Even with the advantage of postmortem radiography, bullets embedded within dense bone may prove difficult to identify and recover. Another situation in which medical examiners may be similarly challenged occurs when encountering a migrating bullet. A migrating bullet is one that shifts its position inside the body after initially coming to a complete rest. Clinical documentation of this is typically performed by radiography, in the past, by serial X-ray studies, and today, by follow-up CT scans. Such cases have been reported in penetrating wounds to various body parts, including the head and spine. Medical examiners need to be aware of this unusual phenomenon of retained intracranial projectiles.

Migrating Bullet, Gunshot Wounds, Intracranial Projectiles

E94 Who Do the Voodoo? A Rapid Field Technique to Identify the Provenance of Unknown Human Skeletal Remains in "Ritual" Cases

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After attending this presentation, attendees will better understand of the importance of cross-disciplinary research when investigating the influence that the burial environment has on human skeletal remains.

This presentation will impact the forensic science community by providing a rapid screening technique that can take place at the scene to provide information on the provenance of questionable human remains.

Many cases in the media have reported the discovery of human skeletal remains discovered through the investigation of "ritual" cases linked to the practice of witchcraft or Santeria, otherwise known as voodoo.[1, 2] Although, in many cases there were indications that the skeletal remains recovered were used in ritual, there is little indication as to where the bones were acquired. It is important to establish whether the skeletal remains were from a cemetery, medical/anatomical collection, or a possible murder victim in order to determine whether a crime was committed and the extent of that crime.

The Field-Portable X-Ray Fluorescence (FPXRF) is commonly used in the geology-based field to test various materials, including soil, rock, minerals, and metals, to determine the chemical composition of the sample in question. Bone may also be tested with this equipment to attain an indication of the mineral composition without loss of evidence, due to the non-destructive nature of the testing.

Fifty adult human skeletons with known burial status from Gloucester, United Kingdom were selected for the initial phase in this study. The first rib from either the left or right side was selected to limit variability in the results. The flat portion of the rib shaft was scanned three times and the resultant chemical composition averaged. Each scan took 60 seconds, with the resultant output given as a percentage of the overall mineral composition of the sample-scanned area.

The results indicated significantly lower levels of calcium and strontium in coffin burials ($p<0.001$), with significantly increased levels of iron, titanium, indium, niobium, and lead ($p<0.05$). The accumulation of heavy metals present in the coffin burials are most likely due to the material composition of the burial chamber. Both coffin and crypt/vault burials were tested, with similar values present for both. There was only one coffin burial that did not fit the trend exhibited by the other burials. Skeleton 148 was an individual identified as having suffered from phosphorous poisoning due to working in a 19th-century match factory in Gloucester. In this case, the increased levels of phosphorous present in this skeleton probably prevented the calcium degradation normally seen in coffin inhumations. Further investigation into this individual is underway to determine the effect this ailment had on the taphonomic degradation of the skeleton.

Presented here is a pilot study that, when combined with anthropological investigative techniques, could provide a rapid on-scene assessment to indicate the potential provenance of human skeletal remains. Further human skeletal collections are being tested from a range of time periods and backgrounds to determine the limitations of this technique. Further work to test other elements of the skeleton are also underway, as well as the calibrations required for subadult remains.

This study was a preliminary investigation into the difference between coffin inhumations and those placed directly into the ground (including shrouded burials). Using this portable equipment in a unique way to assess human remains, it was possible to examine remains at the scene quickly, non-destructively, and without disturbing potential evidence prior to collection. This enables the law enforcement officers and officials to determine the severity of a suspected crime, with results available immediately on any questionable remains in "ritual" cases.

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2. Rodrigue A. Science to assist police in ritual remains case. *WWL CBS New Orleans.* 2016. Accessed August 1st 2016.

Field Portable XRF, Burial Types, Bone Mineral Composition

E95 Novel Bioaffinity-Based Cascades for the Determination of Biological Sex From Fingerprints

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After attending this presentation, attendees will recognize a new bioaffinity-based method for fingerprint analysis and that fingerprint samples can be used for more than pictorial comparison. These cascade-based assays can be applied in multiple areas so other researchers could apply this methodology to their own research.

This presentation will impact the forensic science community by establishing that fingerprints can be used as a biological sample, similar to blood and sweat. This presentation will also introduce a more rapid method of fingerprint analysis that can one day be applicable for on-site usage. This new method will also encourage other researchers to put more effort into creating techniques that can be used by personnel without scientific training.

In the past century, fingerprinting became a universally accepted and reliable method for identification; however, fingerprint analysis still consists only of pictorial/visual comparisons and ignores the importance of sweat/sebum residue left with the latent prints. There has been no effort to overcome the discipline's dependence on the existence of a prerecorded matching fingerprint for comparison. Due to this limitation, an information-rich latent print may not be used to its full potential or may even be deemed unusable. A bioaffinity-based cascade for the determination of biological sexes from the chemical composition of the sweat/sebum left as the latent prints has been developed.

The research presented addresses the current limitations in fingerprint analysis by using a bioassay system that focuses on the components of fingerprints. Bioaffinity-based assays were developed for the determination of biological sexes from those components. In one assay, L-amino acid oxidase was used to target the amino acids present in the sebum and sweat left on latent fingerprints. A statistical analysis was first performed on 50 mimicked samples to determine the feasibility of this method. Further analysis was then performed on real fingerprint samples collected from volunteers. The contents of those fingerprint samples were extracted from the fatty content of the fingerprint using a newly developed method for subsequent analysis. The assay proved a viable method for differentiating between male and female fingerprints.

Further research led to the testing of authentic fingerprint samples collected from various surfaces as well as the development of other bioaffinity-based assays capable of differentiating between biological sexes via less complex systems. Other bioaffinity-based assays will also be developed in the future for the determination of other physical attributes, such as age group and ethnicity. While these assays will not be able to clearly identify a person of interest, they will be useful for quickly narrowing suspect pools when identification is not possible. The assays will also be useful in cases in which there is not enough time for the process of identification (possibly via DNA) to be completed. The assays that were developed and are currently in development have the potential to become a portable method that can be used for on-site analysis. Also, due to how easily the assay can be performed and interpreted, specialized training for the execution of the analysis is unnecessary, unlike most currently available techniques. These assays could become a very powerful tool for forensics.

Bioaffinity, Fingerprint, Cascade

E96 Preliminary Studies Focusing on How Forensic Science and Law Students Understand Evidence Interpretation and the Importance of Empirical Evidence

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After attending this presentation, attendees will be informed of the results of an empirical study that sought to identify the perspectives of the United Kingdom's forensic science and law undergraduate students with regard to the complexity of the interpretation of forensic evidence and the need to perform experimental studies to establish an evidence base upon which inferences can be made.

This presentation will impact the forensic science community by increasing awareness of the United Kingdom's undergraduate-level courses curriculum and the degree to which the complexity of the interpretation of forensic evidence has been incorporated into the curriculum. This study was designed to identify the perspectives and to increase the understanding of the United Kingdom's forensic science and law undergraduate students who took part.

The 2009 National Academy of Science (NAS) Report (United States) and the 2011 Law Commission Report (United Kingdom) highlighted concerns within forensic science and presented the need for evidence bases to underpin the collection, analysis, interpretation, and presentation of forensic evidence.[1,2] Such evidence bases are critical for forensic science to be able to demonstrate the basis upon which the significance of evidence is inferred. There is recognition that it is important for professionals in the forensic science and legal field to incorporate an understanding of both the law and the principles of forensic science to enable more effective dealing with evidence and more robust approaches to identifying, analyzing, and interpreting evidence. There is also a need for students to be aware of the issues these reports highlighted because they are likely to be going into the field of crime investigation, law or a forensic service provision. In order to assess the current perspective and understanding among undergraduate students studying law and forensic science, the goal of this United Kingdom-based study was to identify the perspective of these students and determine to what degree a discussion-based seminar would increase their understanding of the need for experimental studies that produce the data for evidence bases that can be utilised to increase the robustness of forensic interpretation in court.

To address this goal, pre- and post-seminar surveys were conducted to assess the understanding of undergraduate students reading forensic science or law concerning issues around effective interpretation of evidence before and after a seminar was delivered. The degree to which the understanding of the students changed after participating in a discussion-based seminar was then examined. Fifty percent of the courses the student participants were enrolled in incorporated the interpretation of evidence into the course curriculum.

The findings demonstrated that the extent to which students have an appreciation of the issue of empirical evidence bases and their role in trace evidence interpretation varied. Law students appeared to judge that these issues lacked relevance to their field and, as such, did not need to have an increased awareness of the current landscape of forensic science. It was shown that the students who are studying programs that include interpretation of evidence in the curriculum have a much better understanding of the need to establish an evidence base by performing experimental studies and the complexity of interpreting evidence than the students in courses that did not cover it. This finding supports the need to incorporate forensic interpretation into the undergraduate curriculum so that those entering the forensic science and legal field are better equipped to handle forensic evidence and to motivate them to increase their research to begin addressing the scientific underpinning of different forensic fields. Additionally, by addressing this study to both law and forensic science students, it provides the opportunity to highlight how the need to establish an evidence base in forensic science affects both the science and the legal sphere, demonstrating the need to communicate and collaborate with each other when performing further experimental studies.

Reference(s):

1. The National Research Council: Committee on Science, Technology, and Law. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies of Science.
2. Law Commission (2011) *Expert evidence in criminal proceedings in England and Wales*. Law Com No. 325. London, The Stationary House.

Evidence Interpretation, Education, Current Issues

E97 The Trials and Errors of Transitioning to a Hybrid Learning Platform

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After attending this presentation, attendees will have gained insight into the process of transitioning a traditional on-ground or Face-To-Face (F2F) classroom to a hybrid, or blended, classroom in order to fully embrace the learning styles of all students and to take advantage of technology. Additionally, attendees will understand what worked and what did not work for two different educators and pick up tips on how to successfully implement hybrid learning into their own curriculum.

This presentation will impact the forensic science community by providing information on transitioning to a hybrid learning platform and how to overcome the barriers that come with change. Attendees will also learn, step-by-step, how a criminal justice program transitioned from fully on-ground to hybrid, as well as the problems encountered, what worked best, and what needed to be revised in order to achieve the goals of a successful hybrid classroom.

Effective hybrid-course instructional design blends on-ground and online classroom methodology and is based on student-directed instruction.[1] Finding a balance between the two types of instruction is the goal of hybrid learning; however, this is not achieved overnight or without trial and error. Students and educators alike may be resistant to change and effort is required from both to successfully implement change.[2,3] Hybrid learning offers a variety of advantages that have earned hybrid learning the name "education for the future."

Many individuals believe that when transitioning from a traditional learning environment to an online program, the learning is diminished and student engagement is lost.[4,5] With hybrid learning, many of the obstacles associated with both online and traditional brick-and-mortar learning can be overcome.[6] Hybrid learning also allows for maximum student engagement, which is one of the biggest challenges in any educational setting.

Over the past few years, hybrid learning has grown in popularity. Ideally, hybrid classes combine the best attributes of both F2F and online formats into a course delivery method that is both flexible and accessible, while providing students an interpersonal experience with instructors and a physical connection to campus.[3] Hybrid learning caters to different types of students from Baby Boomers to Gen Xers, bringing together paths of learning that allow students to learn from each other as well as forensic educators.[7]

Through continued growth, trial, and error, the field of education will continue to change by capitalizing on new techniques that maximize the student's education experience and skills.

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1. Rosenshine B., Stevens R. (1992). The use of scaffolds for teaching less structured academic tasks. Paper presented at the annual meeting of the American Educational Research Association, San Francisco.
2. Reynard R. (2003). Internet-based ESL for distance adult students – A framework for dynamic language learning. *Canadian Modern Language Review.* 60(2). University of Toronto Press. Toronto, Canada.
3. Reynard, R. (2007). Hybrid learning: Challenges for teachers. *The Journal: Transforming Education Through Technology*. Retrieved from: https://thejournal.com/Articles/2007/05/17/Hybrid-Learning-Challenges-for teachers.aspx?Page=1.
4. Moore M.G. (1993). Theory of transactional distance. In *Theoretical Principles of Distance Education*. 22-38. New York: Routledge.
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6. Dwight J. Garrison J. (2003). A manifesto for instructional technology: Hyperpedagogy.*Teachers College Record.* 103(3), 699-728. Retrieved from: http://www.tcrecord.org/.
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Education, Hybrid Learning, Delivery Modality

E98 Creating an Ethical Reasoning Curriculum for Forensic Science Majors

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After attending this presentation, attendees will understand how and why an ethical reasoning module was created for forensic science students.

This presentation will impact the forensic science community by highlighting the importance of educating forensic science students on the use of abductive reasoning skills in order to promote ethical behavior.

The presentation will describe the development of each of the modules that are currently contained in the ethical reasoning curriculum. Additionally, preliminary results regarding the effectiveness of the modules to teach students about reasoning patterns and the connection between forensic science and ethical conduct will be presented.

The field of forensics uses evidence from a crime scene to develop scenarios regarding the how, what, why, and who of a criminal action. The way in which the forensic scientists or forensic investigators interact with this evidence or data can lead to ethical issues. Forensic science practitioners and investigators have a moral obligation to act in an ethical manner that promotes the common good through justice. The criminal justice system punishes those who commit crimes while protecting the innocent. Forensic scientists and investigators function as a facet of the government tasked with providing justice by solving crimes. Training individuals on best practices based on proper abductive reasoning patterns upholds ethical behavior that promotes/protects the common good and fosters justice for all.

Previous research focused on the reasoning of detectives involved in sexual assault and homicide cases. The information gathered from this research led to the development of an ethical reasoning curriculum. This information is presented in five online modules that can be shared across universities and organizations. Each unit outlines specific learning objectives, contains the educational information with examples, and provides practice.

The first unit focuses on the identification of the three different types of reasoning models: (1) abductive; (2) deductive; and, (3) inductive reasoning. The second module focuses on different learning models typically discussed in the forensic science program (e.g., behaviorism, cognitive information processing, cognitive bias). The next module connects the three types of reasoning with the applications in forensic science and forensic investigation. Activities in this module include transcripts from cases identified in the earlier research as well as materials and video clips from shows such as *Forensic Files* and *Dateline*. The modules progress from simple to complex case examples. The use of real-life case examples is imperative for students to understand the impact of the forensic practitioner's actions.

After the connection to forensic science, the next unit focuses on the ethical principles of common good and justice. The content and activities allow students to understand the role of various members within the criminal justice field (i.e., investigator, forensic scientist, lawyer, judge). The connection between proper practices and ethical behavior are emphasized. Students will understand the ethical obligation to uphold justice and promote the common good. The fifth module further discusses abductive reasoning and the six modes.[1] The module integrates the previous materials into full case studies completed by individuals or groups. These full cases present information at different times in the analysis in order to simulate how information is obtained in a case.

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1. Shank, G., Cunningham D.J. (1996, April). *Modeling the six modes of Peircean abduction for educational purposes.* Paper presented at the annual meeting of the Midwest AI and Cognitive Science Conference, Bloomington, IN.

Ethics, Reasoning, Education

E99 The Arizona Forensic Science Academy: A Model Program for Delivering Forensic Science Education to Criminal Justice Practitioners

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After attending this presentation, attendees will better understand a national model for delivering a training and education program designed to inform criminal justice practitioners about forensic science, including foundational and introductory topics for officers of the court and advanced technical lectures for scientific experts. Attendees will also be provided with a model that can be easily implemented within their own jurisdictions to advance the practice of forensic science by all criminal justice practitioners.

This presentation will impact the forensic science community by demonstrating a successful program that has consistently delivered training and education about forensic science to the criminal justice community in the state of Arizona since 2011. As acknowledged by the National Forensic Science Commission, training related to the technical aspects of forensic science in both criminal and civil courts is necessary and an essential component of advancing forensic science.[1] Sharing lessons learned and model curricula enables others to adopt similar programs and establish best practices for forensic science training and education programs for all criminal justice practitioners.

The Arizona Forensic Science Academy was established by the Forensic Science Advisory Committee of the Arizona Attorney General's Office in response to a recommendation in *Forensic Science in the United States: A Path Forward*, a 2009 National Research Council Report, to provide forensic science training to legal professionals.[2] The Academy was designed to increase criminal justice practitioners' understanding of forensic principles, scientific methodologies, and evidentiary concerns. Courses in the Academy are taught by scientific and medical experts and provide practical and technical training to prosecutors, defense attorneys, and judges. The experts who have facilitated training in the Academy span the spectrum from both locally and nationally recognized experts from academia, crime labs, medical professionals, law enforcement, defense experts, private industry, judges, and attorneys.

In addition to the classes delivered to officers of the court, the Academy also provides specialized training for forensic scientists and other technical experts. Four primary training programs have been developed for criminal justice practitioners: (1) a Basic Academy; (2) an Advanced Academy; (3) a "3-D" (Domestic Violence, Drugs, and DUI) Academy; and, (4) a Forensic Science Lecture Series. Since the Academy's inception in 2011, more than 1,000 criminal justice professionals received more than 10,000 hours of continuing education. The newest program to be delivered is a dedicated two-day symposium for judges from the limited, general, and appellate jurisdictions. Topics identified for inclusion in the symposium include evidentiary issues, crime lab policies and practices, forensic biology/DNA, DrivingUnder the Influence (DUI) issues and forensics, medical-legal death investigation, controlled substances, data recovery from cell phones, Global Positioning System (GPS) evidence, digital photography/video, forensic neuroscience issues, accident reconstruction, toxicology, child physical and sexual abuse, and pattern evidence.

A unique aspect of this program is the collaboration between various criminal justice stakeholders to deliver training programs to practitioners — regardless of their role in the criminal justice system. In the past, this type of training was offered exclusively to prosecutors or defense attorneys and it was rare for prosecutors and defense attorneys to attend the same training event. The collaborative nature of the Arizona Forensic Science Academy's training programs resulted in improved relationships between the various stakeholders, a greater understanding of forensic science principles in the Arizona criminal justice community, and increased access by trial attorneys to the resources available to them in the crime labs and from technical experts in the state of Arizona.

Reference(s):

1. National Commission on Forensic Science. (2015, December 8). *Work Products Adopted by the Commission.* Retrieved from The U.S. Department of Justice: National Commission on Forensic Science: https://www.justice.gov/ncfs/file/818206/download.
2. The National Research Council: Committee on Science, Technology, and Law. (2009). *Strengthening Forensic Science in the United States: A Path Forward.* Washington, DC: The National Academies of Science.

E100 Transforming Forensic Science Education With Service and Experiential Learning

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The goal of this presentation is to provide attendees with a framework for utilizing service-learning projects in the forensic science classroom.

This presentation will impact the forensic science community by demonstrating how forensic science discipline knowledge can be integrated with practical applications in the community.

The National Service-Learning Clearinghouse (2011) defines service learning as a teaching and learning strategy that integrates meaningful community service with instruction and reflection to enrich the learning experience, teach civic responsibility, and strengthen communities.[1] Service learning represents a potentially powerful form of pedagogy because it provides a means of linking the academic with the practical. There is growing evidence that when students apply theoretical material learned in the traditional classroom in a "real-world" setting, there is a positive effect on student learning and interest in the subject matter[2]. Service-learning projects also benefit the community by providing new perspectives into the work of the university and strengthening community relationships with faculty and students.

This presentation will provide a framework for incorporating service learning into course curricula. Service-learning projects completed by the University of Central Oklahoma (UCO) Forensic Science Institute students that integrate forensic science discipline knowledge with practical applications in the community will be used to emphasize the efficacy of this model as a tool for transformative education. Projects that will be discussed include the application of digital forensics to assist Oklahoma tornado victims, electronic recycling, participation in the Oklahoma Innocence Project, cold case reviews, and active involvement in the federal outreach program GEAR-UP.

Service learning at the Forensic Science Institute offers a transformative learning experience to students by developing "beyond-disciplinary skills," broadening students' perspectives in relation to themselves and their community, integrating meaningful community service, and placing students at the "center of their own active and reflective learning experience."[3]

Presentation attendees will be introduced to the concept of service learning as a transformative learning tool. They will be provided with examples of how this concept has been successfully implemented at the Forensic Science Institute and will also have the opportunity to listen to student and community reflections on how service learning at the Forensic Science Institute was transformational for them.

Reference(s):

1. National Service-Learning Clearinghouse (2011). Retrieved from: http://www.service learning.org /what service-learning.
2. Astin A.W., Vogelgesan L.J., Ikeda E.K.,Yee J.A. (2000). *How Service Learning Affect Students*. Higher Education Research Institute, University of California, Los Angeles.
3. Center for Excellence in Transformative Teaching and Learning (CETTL): The University of Central Oklahoma. (2015). *Transformative Learning*. Retrieved from http://www.uco.edu/central/.

Forensic Science Education, Service Learning, Transformative Learning

E101 Forensic Science Curriculum for High School Students

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WITHDRAWN

E102 Examination of Genetic Drift in Mitochondrial DNA (mtDNA) Heteroplasmy in Hair Samples Using Massively Parallel Sequencing (MPS) Approach

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After attending this presentation, attendees will understand the manner in which mtDNA heteroplasmy drifts in human hair shafts when employing an MPS approach.

The use of an MPS approach to forensic mtDNA analysis enhances the discrimination potential of the testing method. This presentation will impact the forensic science community by providing information on how the ratio of heteroplasmic sequence variants drifts between hair samples when interpreting MPS data.

MtDNA is commonly analyzed in forensic hair samples due to its high copy number, as the amount of nuclear nDNA is typically below what is needed for Short Tandem Repeat (STR) analysis. While mtDNA analysis is not as discriminating as nDNA testing, the ability to resolve heteroplasmic sequences (the presence of more than one mtDNA variant in a cell) can increase the weight of the mtDNA analysis. Differences in the ratio and presence of heteroplasmic variants exist between maternally related family members, allowing for more discrimination between relatives. Variations can also exist within a single individual. For example, the cells that make up hair follicles form in a clonal manner, creating a bottleneck effect during fetal development, allowing for genetic drift among hair follicles within an individual. The differences in variant ratios within an individual may pose a challenge when interpreting heteroplasmy in forensic cases. Rates of heteroplasmy in a population and at each nucleotide position are required to properly generate statistical weight estimates in support of a match between evidence and a reference. A better understanding of the patterns of drift in the heteroplasmic ratios within an individual are needed to determine their impact on interpretation criteria.

This study focuses on characterizing the drift in variant ratios among hair samples from different regions of the scalp from single individuals with varying levels of heteroplasmy. Sequencing has historically been performed with the Sanger method (Sanger-Type Sequencing (STS)). This method sequences multiple mtDNA strands at a time, reporting the predominant sequence, or haplotype, for mtDNA analysis. If a heteroplasmic variant is present in at least 10% of the mtDNA strands, it can be detected in STS data; however, the individual variants cannot be resolved. To detect lower levels of heteroplasmy and resolve the variants, MPS has the ability to identify variants as low as 1%.

Hair samples were collected from human subjects exhibiting known levels of heteroplasmy. Two groups of subjects were studied: (1) five donors who exhibited low levels of heteroplasmy, (2%-5% in their blood and buccal swabs); and, (2) five donors who exhibited higher levels of heteroplasmy (>10%). Five hair samples were collected from each of the ten donors from different regions of the scalp, including the forehead, neck, left temple, right temple, and crown. The DNA from each hair shaft was extracted using an optimal lysis and magnetic bead purification method, amplified using the Promega® PowerSeq™ Mito System protocol/kit (a 10-plex amplification method for the entire mtDNA control region), library preparation was conducted using the TruSeq® method from Illumina®, and the library was run through MPS analysis on the MiSeq® from Illumina®. The level and nature of genetic drift was assessed for the hairs collected from each set of donors. In particular, drift was assessed in relation to donors with low or high amounts of known heteroplasmy, determined from reference data of blood and/or buccal cells. In addition, the observation of unexpected variants was cataloged and evaluated.

Mitochondria, Heteroplasmy, Hair

E103 Visualization of Fingerprints on Post-Blast Improvised Explosive Device (IED) Fragments and Debris

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After attending this presentation, attendees will understand more about the ability to detect fingerprint ridge detail on post-blast fragments, including from the IED itself and the surrounding objects in the immediate vicinity of the blast. Attendees will learn the consequences of gathering such information in terms of identifying those associated with conducting IED blasts.

This presentation will impact the forensic science community by evaluating a new, novel, non-destructive technique for post-blast fingerprint detection and visualization. It will also highlight a method by which DNA and fingerprint ridge detail have the potential to both be collected from post-blast materials from a single fingerprint deposited.

Recovering fingerprints after an explosion is a difficult process, with potential identification possible if fingerprints can be visualized post-blast. IEDs, such as pipe bombs, make use of whatever material is available. Copper, steel, and sometimes brass are materials commonly used to assemble a pipe bomb or IED. With the current international climate regarding IEDs and terrorism, it is essential that research is conducted into the post-blast identification of a bomber or bomb maker.

Detection of fingerprints on unexploded devices is possible using regular techniques targeting physical or chemical reactions with the fingerprint deposit; however, post-blast, the constituents of fingerprints are often obliterated, water evaporated, and the print potentially disintegrated. A novel approach to detecting the ridge patterns is therefore needed.

Vehicle-bound IED, mailbox bomb IED, and pipe bomb IED experiments were conducted. Both sebaceous and eccrine fingerprints were deposited on brass, copper, and steel discs, directly onto brass, copper, and steel pipe bombs, as well as on a United States mailbox. Gunpowder and C4 were the explosives used in the different scenarios. Post-blast, the discs and fragments from all scenarios were analyzed using an in-house-built, multi-spectral imaging system. Some were swabbed for DNA analysis prior to fingerprint analysis.

A varying level of ridge detail remained depending on the position of the discs in relation to the IED and blast epicenter, as well as the type of metal used for the pipe bombs. Fingerprint ridge detail was also visualized from the metal clasp on the front of the mailbox. Depending on whether the fingerprints deposited were eccrine or sebaceous also influenced the visualization capability. One major finding from the DNA swabbed fingerprints was that ridge detail was still visualized on a number of occasions even after swabbing for DNA first, highlighting the ability for both DNA and fingerprint ridge detail to be potentially collected from a single deposited print. Development of the technique, and possibly using an array of various other imaging tools, may allow for increased post-blast visualization. Being able to visualize any level of ridge detail for any of the scenarios discussed here is noteworthy.

Improvised Explosive Devices, Fingerprints, Post-Blast Identification

E104 Preparation of "Gummy" Quality Control (QC) Material and the Analysis of Medible "Gummy" Products

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After attending this presentation, attendees will become familiar with the preparation of Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in edible/medible "gummy" product QC materials and the detection and quantification of THC and CBD by Ultra-High Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC-MS/MS). These products include "gummy" candies such as "medible gummy bears" and "medible gummy worms."

This presentation will become the forensic science community by providing a method for the preparation and analysis of matrix-matched QC material that is necessary for the analysis of "gummy" products.

The purpose of this study was to prepare QC material containing THC and CBD in edible/medible "gummy" products and to validate an LC-MS/MS method for the analysis of medible "gummy" products.

The legalization of marijuana in the United States for both medicinal and recreational use increased in the past few years. Currently, 24 states have legalized marijuana for medicinal use. The United States Drug Enforcement Administration classified marijuana as a Schedule 1 substance. The Food and Drug Administration (FDA) does not regulate formulations or products that contain marijuana/or marijuana extracts in states that "legalized" marijuana. THC and CBD are the two most common cannabinoids found in these formulations or products. Marijuana edibles or "medibles" are typically candies and baked goods labeled with THC and/or CBD and the cannabinoids come from marijuana or marijuana extracts. THC is the major psychoactive compound of marijuana. CBD found in marijuana is reported to have medical properties, including analgesic, anticonvulsant, and anti-psychotic activity.

Presented is a method for the preparation of QC materials containing THC and CBD for use in the analysis of medible "gummy" products and an LC-MS/MS method for the analysis of these products. "Gummy" QC samples were prepared at two different THC and CBD concentrations, 5mg/40g and 10mg/40g. The method calibration range for THC and CBD was 0.8µg/g – 80µg/g. Fortified QC samples were prepared at 0µg/g, 2.4 µg/g, 25 µg/g, and 60µg/g THC and CBD, and were analyzed with each analysis batch. The Limit Of Detection (LOD) and Limit Of Qquantitation (LOQ) were administratively set at 0.8µg/g. All samples were prepared by adding 8.0µg/g Internal Standard (ISTD) to a 25mg aliquot of either the calibrator, QC, or samples. Samples were dissolved in 0.5mL deionized water, incubated at 56ºC for five minutes, and extracted with 0.5mL deionized water using a UCT Clean Screen FASt™ column.

Extracted samples were analyzed using an LC-MS/MS operated in positive ionization mode. Chromagraphic separation was performed on a reverse phase C18 rapid resolution 4.6x75mm column. The mobile phase was 20mM ammonium formate in water (A) and 20mM ammonium formate in methanol (B) isocratic at 10:90 for six minutes, followed by a gradient to 0:100 over 1min. The column flow rate was 0.5 mL/min. The acquisition mode was Multiple Reaction Monitoring (MRM). The following transition ions (m/z) were monitored in MRM mode with their corresponding collection energies (eV) in parentheses: CBD: 315>43 (35) and 315>93 (23) THC: 315>43 (35) and 315>122 (29); and THC-d3: 318>93 (25) and 318>123 (35). The total run time for the analytical method was 8.0min.

No carryover was observed in the LC-MS/MS method. Precision and accuracy were determined at three fortified QC concentrations (n=3) on five separate days. The bias was <20% for each concentration with a <20%CV. No matrix effect was determined in ten different commercially available gummy bear brands/types. Prepared QC material was determined to be stable for at least one month.

This work was supported by a National Institute of Drug Abuse (NIDA) of the National Institutes of Health (NIH) grant.

UHPLC-MS/MS, THC, Medible

E105 A Comparison of Latent Print Proficiency Tests With Latent Prints Obtained in Routine Casework Using Automated and Objective Quality Metrics

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After attending this presentation, attendees will better understand of the differences in quality between latent print samples from proficiency tests and casework and will be able to facilitate discussions among laboratory management regarding the appropriate use of proficiency tests and potential paths forward.

This presentation will impact the forensic science community by evaluating how well the quality of latent print proficiency test samples represent those encountered during routine casework and by starting a conversation on what the data *really* means.

Accreditation bodies require that forensic practitioners remain proficient in the skills needed for their type of examinations. In order to fulfill this requirement, examiners complete a sanctioned proficiency test from an International Organization for Standardization /International Electrotechnical Commission (ISO/IEC) 17043 accredited proficiency test service provider, where possible. These tests are designed to comply with accreditation requirements by monitoring the performance of the laboratory, from when the examiner receives the test to when results are released. A typical latent print examination proficiency test contains approximately 12 latent prints of varying levels of difficulty that were created in ground truth settings. These latent print impressions are assumed to mimic the quality of impressions received during routine casework; however, there is a dearth of research to actually verify this. The experience of the latent print community is generally that proficiency tests offer higher quality and less complex examinations than those typically examined in normal casework. To objectively measure the general quality of latent prints, automated latent print-quality software, Latent Quality Metrics (LQMetrics®), included within the Universal Latent Workstation (ULW) software, was utilized. The LQmetrics® is designed to evaluate several parameters of latent print quality which latent print examiners utilize to estimate the quality of the latent and generate a score (bounded between 0 and 100 — higher scores indicate higher quality impressions). In this experiment, the LQMetrics® scores are used as objective and reproducible measures of latent print quality. Latent prints were obtained from the previous eight proficiency tests, totaling more than 120 fingerprints, dating back to 2010. A random sample of latent prints from routine casework determined to be "of value for identification" were collected. Quality scores were generated using LQMetrics® and the derived score from each dataset were compared. This presentation will illustrate how well the quality of latent prints from proficiency tests represent the quality of latent prints derived from casework. The intent is to elicit conversations from management regarding the appropriate uses of latent print proficiency tests and potential paths forward.

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

Proficiency, Latent Prints, Quality Metrics

E106 Analyzing Bare Footprints in Criminal Cases — Interpreting a Large-Scale Footprint Study in India

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After attending this presentation, attendees will understand the value of distinctive characteristics found in bare footprints and will help attendees comprehend the basics of analysis of bare footprints in criminal investigations in regard to linking (or unlinking) a footprint to a suspect.

This presentation will impact the forensic science community by presenting the detailed analysis of the bare footprints in terms of distinctive (and, at times, unique) characteristics studied on a large North Indian sample.

Bare footprints found at crime scenes may be impressed in various types of substrates and surfaces, such as rain-covered surfaces, newly carpeted and cemented floors, oil, mud, blood, dust, paint, sand, etc. These footprints may provide useful clues in the investigation of a crime. Previous studies have shown that footprints have distinctive characteristics that allow them to be matched to an individual and may have unique characteristics.[1] This distinctiveness is based on various, and, at times, unique, features in bare footprints.[2] This presentation is an outcome of studies conducted on adult male Gujjar populations inhabiting the Sub-Himalayan region of north India and on the students of Panjab University, Chandigarh, India, as part of major research project funded by University Grants Commission, New Delhi, India. The main objective of the research was to study the bare footprints of adults and describe the importance and utility of distinctive features of bare footprints in personal identification.

The study was conducted on 700 adults (500 males, 200 females) ranging in age from 18 to 30 years old. Various features of the bare footprints were analyzed, such as distinctive features of toes, humps in the toeline, phalange marks, flatfoot condition, pits, crease marks, cracks, corns, deformity, etc. The study also evaluated relative lengths of the toes, which provide valuable information on their morphology and divides bare footprints into different categories based on the relative morphological lengths of the toes: when T1 > T2, when T2 > T1, and when T1 = T2 > T3. The data indicates that the majority have type T1 > T2 (males: 55.0% on the left side and 51.4% on the right side; females: 57.0% on the left side and 60.0% on the right side), the next most common type is T2 > T1 (males: 39.4% on the eft side and 38.8% on the right side; females: 34.5% on the left side and 33.5% on the right side); and the least common type is T1 = T2 > T3 (males: 5.6% on theleft side and 9.8% on the right side; females: 8.5% on the left side and 6.5% on the right side). The study concludes that these characteristic features of bare footprints and relative lengths of toes can provide useful clues to help establish personal identity when complete or partial footprints are recovered at crime scenes and may assist with including or excluding the presence of an individual at the scene of the crime.

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Forensic Podiatry, Bare Footprints, Personal Identification

E107 A Study of Drug Trafficking on Cryptomarkets: Structure, Organization, and Comparison With the Traditional Market Through a Canadian Perspective

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After attending this presentation, attendees will improve their knowledge regarding drug trafficking on cryptomarkets.

This presentation will impact the forensic science community by encouraging cooperation between criminologists and forensic scientists to better assess and tackle the problem of drug trafficking on cryptomarkets.

Cryptomarkets are hosted on the dark web and are mainly dedicated to selling drugs of abuse.[1] They combine tools to ensure anonymity (as the virtually untraceable cryptocurrency bitcoin or Pretty Good Privacy (PGP) encryption), warranty a trustful delivery, quantify satisfaction to create customer loyalty, and deliver products through postal means. This also reduces substantially the capacities of detection and action of law enforcement agencies.[2-4] Selling drugs on cryptomarkets disrupts the structure and organization of trafficking, (for instance, by putting an end to physical interactions between sellers and buyers).[5] It even appears as a revolution and a criminal evolution in the general drug trafficking scheme.[6,7]

This study is a quantitative and qualitative analysis of data collected on eight cryptomarkets listing 3,685 proposals and 198 vendor profiles, which seeks to upgrade knowledge on this trend, and is also followed by local, national, and international Canadian providers. The study discusses types of drugs offered (cannabis in various forms, ecstasy, psychedelics, and stimulants) and the destination countries of Canadian sellers. Moreover, through the identity-verification tool of PGP that may confirm the membership of similar usernames and reveal the existence of relationships between different ones, this research gives a glimpse into the structure of these distribution online networks and the involvement of traders at various geographical levels. Additionally, information about membership, identity duplication, and sellers' behaviors between different markets, indicating prolific traders who can be targeted first, is provided. Such an analysis details their organizations and especially informs on the targeted customer base (user vs. reseller). Finally, this study assesses whether cryptomarkets are only an extension of the traditional market or, on the contrary, are completely independent with few, if any, relationships with the former.[8-10]

This study determines that the analysis of online data is able to provide knowledge on illicit activities, despite the fact the activities occur on markets fostering anonymity of users. Such knowledge is mandatory to develop efficient surveying or controlling policies. Nonetheless, dark net trafficking remains difficult to assess through only digital data, as it is virtual and uncertain, making the study of this criminal evolution difficult.

This situation invites alternative strategies that presently consist of removing the webmaster of a single cryptomarket, the efficiency of which could be questioned, to disrupt trafficking on dark net markets. For instance, from a law enforcement perspective, interventions could better target main actors operating on various sites below detection levels. A more holistic approach to investigate this trend that would rely on a combined set of digital and physical data within a single collaborative intelligence model is suggested.[10-12]

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Darknet, Intelligence, Drug of Abuse

E108 Maxi Trial "Iscaro-Saburo": The Judicial Birth of the Fifth Italian Mafia

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After attending this presentation, attendees will understand the background of the Mafia in Apulia Italy through criminological analysis and judicial ambiguity.

This presentation will impact the forensic science community by explaining that both scientists and jurists are needed for close interdisciplinary cooperation in the identification of an event that has great political and socio-economic impacts on society.

Italy has historically suffered from a high rate of criminality, organized violence, and the influence of political and economic life from organized crime groups such as the Mafia, in particular the so-called "Cosa Nostra," in Sicily, the "Camorra" in Campania, the "'Ndrangheta" in Calabria, and the "Sacra Corona Unita" in the Apulia region.

The Mafia is a criminal phenomenon with roots in the Sicilian landowner society of the 19th century when bureaucracy was unable to control some very important areas, such as construction industries, public tenders, public services, and credit management. Although the existence of criminal organizations has ancient origins, only in the 1960s did the Italian State begin to face this phenomenon with concrete acts. Gradually, the Mafia developed, with equal influences, in other Italian regions, creating the Mafia, 'Ndrangheta, Camorra and Sacra Corona Unita.

Law enforcement authorities became aware of the existence of the Gargano Mafia in the 1970s with the murder of Lorenzo Ricucci, shot by Pasquale e Francesco Li Bergolis. Ricucci was a longtime friend of the powerful "Primosa" family. From this criminal act a violent clash originated between two factions: The Lombardi and Miucci family vs. the Primosa family flanked by the Alfieri and Basta family. This feud led to dozens of deaths, with the ultimate goal of not only controling the entire area, but also imposeing their rule on the territory.

Although the 3rd clause of the 416-bis article of the Italian Penal Code defined the "Mafia–like criminal organization" and constitutive elements of crimes, the judicial history of the Gargano Mafia was characterized by the acquittals of all accused subjects from 1979 to 1999. On July 11, 2001, the Court of Assizes of Appeal of Bari explained these acquittals as being linked to a feud, since the executions were not committed in the typical Mafia mannor. Simultaneously, the acquittals guaranteed prominent members greater strength and more respect.

Due to the synergy between detective work and forensic sciences, the phenomenon of "Gargano Mafia" finally had its official recognition with the judgment of the Court of Assizes of Foggia on March 7, 2009. This organization used to express itself with a Mafia-Like behavior based on the power of intimidation and the code of silence (the so-called "omertà"). Furthermore, this Mafia family, as well as others was characterized by a rigid hierarchical structure, strong leaders, and, above all, had a strong grip on the territory in which it operated: the Foggia province. This judgment, expressed itself during the maxi-process referred to as "Iscaro-Saburo," in which 107 defendants were accused of 22 murders, 4 attempted murders, extortion, robbery, port influences, and illegal possession of weapons, as well as criminal association and drug trafficking. Currently, after four years, 133 hearings, 700 witnesses, and thousands of pages of transcripts of wiretaps, the judgment concluded with 14 acquittals, 7 convictions varying from 4 to 27 years of imprisonment (for a total of 140 years in prison), and three life sentences.

Mafia, Apulia Region, Trial

E109 The Face of the Laboratory: Creating and Implementing a Client Services and Case Management Division

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After attending this presentation, attendees will understand support roles in a laboratory setting, the impact those roles have within the laboratory, and the meaning of providing quality customer service to internal and external clients.

This presentation will impact the forensic science community by demonstrating how to create and manage a cohesive laboratory support system that effectively incorporates case management and customer service.

Most laboratories run by law enforcement agencies are supported by evidence technicians, custodians of records, administrative assistants, and laboratory assistants. Traditionally, these support roles were dispersed throughout the laboratory, each working independently rather than cohesively. For example, there may be one evidence technician per individual section (i.e., biology, firearms, controlled substances, etc.), while smaller law enforcement agencies may only have one evidence technician or property clerk responsible for retrieving and storing evidence. The Houston Forensic Science Center (HFSC) had to create its own support system once the laboratory transitioned away from law enforcement management.

As the crime laboratory evolved, so has the Client Services and Case Management (CS/CM) Division created by HFSC. CS/CM sets itself apart by incorporating all laboratory support roles into a unified, structured division. The division plays an integral role in internal case management, but a significant portion of the group is dedicated to external support, popularly referred to as customer service. Currently, the CS/CM division's responsibilities include, but are not limited to: administrative support, laboratory support, customer service, record management, and receiving and supply management. Many of the division's specialists juggle multiple job functions, unlike more traditional laboratory units. The goal is to have a cohesive division with interchangeable experience and responsibility by diversifying the staffs' knowledge and widening its support capabilities.

As an independent local government corporation, HFSC seeks to improve its relationships with clients. This includes their main client, the Houston Police Department, as well as internal personnel, other law enforcement agencies, defense attorneys, and the district attorney's office. Furthermore, HFSC is determined to gain the public's trust and squash the perception that forensic laboratories favor the prosecution. CS/CM assists HFSC in achieving this goal by ensuring transparency, providing all clients documents and records, and working to reduce, and hopefully eliminate, cognitive bias. CS/CM acts as the liaison between the outside world and the laboratory, shielding analysts from unnecessary information that will not assist in the analysis of evidence. The division also relays pertinent information to the external client in an efficient manner.

By creating a division similar to CS/CM, a laboratory's administrative functions, basic duties, and day-to-day tasks can be removed from the analysts and given to a managed group of individuals. The greatest impact of this is creating more time for the analysts to perform laboratory functions and focus on evidence analysis. Attendees of the presentation will learn how valuable an asset a division similar to CS/CM can be to a laboratory's daily functioning. CS/CM assists in producing quicker responses and more efficient processes. This, in turn, leads to quicker turnaround times and smaller backlogs.

Customer Service, Client, Support

E110 Bioelectrical Impedance Analysis as a Technique for Estimating the Postmortem Interval (PMI) in Human Remains

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After attending this presentation, attendees will understand the concept of Bioelectrical Impedance Analysis (BIA), how this relationship has the potential to inform the estimation of PMI, and the relationship between bioelectrical impedance metrics and Accumulated Degree Days (ADD).

This presentation will impact the forensic science community by introducing a new quantitative method for estimating PMI.

Current analytical models used in the estimation of PMI are subjective in nature and therefore limited by individual experience and potential error. In forensic investigation, these variables are complicated by the microenvironment in which the decomposition event occurs. The most widely accepted method for quantitatively estimating PMI utilizes Total Body Score (TBS) to estimate ADD; however, TBS ultimately relies upon qualitative categories of linear change which significantly reduces the objectivity of the method.[1] This problem is amplified in arid environments where rapid desiccation is followed by prolonged periods of stasis, slowing TBS while PMI continues to progress. In response to these issues, the potential for BIA as a tool for estimating PMI was investigated at Colorado Mesa University's Forensic Investigation Research Station (FIRS) in the arid region of western Colorado.

BIA technology was originally developed as a means for quantifying the proximate body composition (water, lipid, and lipid-free dry masses) of living humans. Quantifying fat and water masses remains the primary application, but the technique has been expanded to organisms other than humans, such as fish and mammals. Additionally, the technique is used to both determine health status in living humans (in industries ranging from health care, athletics, and health and beauty) and to determine the status of food products. Because BIA was developed for use on the human model and is broadly employed in an array of disciplines, it is easily adopted to forensic application, widely accessible, affordable, minimally to non-invasive, and user friendly.

BIA is uniquely suited to decomposition studies because, by design, it measures the resistance (R; Ω) and capacitive reactance (Xc; Ω) of an electrical current passed through biological tissue. The dielectric properties of tissue functions as a Resistor–Capacitor (RC) circuit with minimal equipment or invasion. Within this circuit, there are components which cause resistance to electrical currents and components that serve as capacitors (units that hold an electrical charge). In biological tissue, the extracellular fluid and the intracellular fluid are the resistors, whereas the cell membranes act as a capacitor. During decomposition, as desiccation occurs and the volume of extracellular fluid decreases, the resistance of the tissue increases. Conversely, as autolysis decreases the volume of intact cell membranes, the reactance of the tissue decreases. The potential for BIA as an investigative tool tested positively in several studies utilizing rats as a human proxy.[2-4] It is therefore hypothesized that changes in the structure and composition of biological tissues inherent to decomposition can be quantified using BIA with the ultimate goal of developing models for estimating PMI.

A pilot study was launched at FIRS in the fall of 2014 to assess the efficacy of BIA within a human sample. The initial stage of inquiry focused on model development with an emphasis on comparing BIA equipment and measurement techniques, including: electrode type, electrode distance, and body segment. Two electrode types were tested on human remains: (1) conductive gel pads; and, (2) hypodermic needles (0.711mm x 38mm aluminum hub) inserted subcutaneously between skeletal structures. Two approaches for electrode distances were tested: (1) one based on anatomical landmarks that vary with stature; and, (2) fixed-distance electrodes where electrodes remain at a fixed distance of 8cm, regardless of stature. Finally, multiple body segments were measured to identify segments that best correlate with ADD. These body segments include: hand to foot, hand to shoulder, shoulder to foot, thigh-foot, transverse abdomen, and thorax. Significant correlations between BIA and ADD were identified within all groups tested. These results are presented, as well as future directions.

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Forensic Science, Bioelectrical Impedance, Postmortem Interval

E111 Measuring Desiccation Using Qualitative Changes

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After attending this presentation, attendees will better understand desiccated remains as a major confounding factor in the estimation of the Postmortem Interval (PMI). A novel scoring system tailored to the gross and qualitative changes observed in desiccated remains is proposed as a method for refining extant predictive models.

This presentation will impact the forensic science community by providing results from a controlled experiment that addresses a gap in data concerned with the estimation of PMI as it pertains to desiccated remains. This presentation augments the body of research concerned with forensic taphonomy by demonstrating the analytical power of regionally specific decomposition studies.

Standard methods for estimating the PMI in anthropological perspective rely on gross morphological changes presented along the continuum of decomposition. This process is widely regarded as linear, with an emphasis placed on active decomposition (the brief period of dynamic change that typifies early decay). Prolonged periods of stasis, such as desiccation, are de-emphasized or regarded as too homogeneous to carry probative value. Total Body Score (TBS) shows a high correlation with PMI, but this correlation falters in advanced decay when the microenvironment causes desiccation.[1,2] Once remains progressed to this stage, a period of prolonged stasis, defined by a TBS of 23-24, may persist for months and into years.

Biological stasis is a confounding factor when attempting to use predictive models to estimate PMI. In a longitudinal study in Texas, Suckling et al. demonstrated that the method was not accurate when Megyesi's equation was used for TBS values >22.[3] Connor and France, and Baigent et al. presented similar issues in western and central Colorado, respectively.[4, 5]

Nonetheless, throughout periods of "stasis," remains continue to interact with the microenvironment, suggesting that this period of suspended activity is analytically, as opposed to biologically, defined. Noted changes in the literature include dehydration of outer tissues and reduction of desiccated tissues.[6] While less dynamic than early stages of decomposition, desiccation progresses through significant, definable stages of change, beginning with the drying of digits observed in early decomposition and progressing toward dehydrated tissue overlying bone that precludes skeletonization. Changes in color, tissue quality, the release and integration of moisture, and the thickness of the tissue layer are some specifics noted to date. It was hypothesized that these changes correlate to ranges of ADD and, therefore, may be used to refine methods for estimating the PMI beyond a TBS of 22.

Using longitudinal observations accrued across three years of study at Colorado Mesa University's Forensic Investigation Research Station (FIRS), a new scoring system termed Total Body Desiccated Score (TBDS) was developed to increase the resolution of changes presented by desiccated remains. A pilot experiment produced a scoring scale of 1-100, composed of five categories (color, bloat, moisture, desiccation, and skeletonization). These categories were augmented by the addition of defined qualitative changes to further increase resolution.

Photographic packets were compiled to test the new model. To represent a range of seasons and PMI, a sequential data pool of 40 donors was sampled and every fifth donor was selected (n=8). Each donor was represented by monthly data points commencing on the date of deposition and ending either at the time of recovery or at the terminus of the study (May 2016). Body numbers and dates were replaced by randomly generated numbers. This method produced 112 data points; each data point represented one body and one date. Each packet was independently scored by observers variably acquainted with the FIRS sample. Scores assigned by a senior study researcher were eliminated to avoid hindsight bias.

The preliminary results showed that correlations between TBDS and ADD and TBS and ADD were approximately equal for a TBS below approximately 20, but the TBDS did correlate better with ADD at higher TBS scores. Thus, gross changes presented throughout the trajectory of desiccation do carry analytical weight and may be used to refine methods for estimating the PMI using predictive models.

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Taphonomy, Desiccation, Arid Environment

E112 Comparative Surface Decomposition of Humans and Pigs (*Sus scrofa*): Soil Processes

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WITHDRAWN

E113 What Forensic Archaeology and Forensic Taphonomy Can Offer to Medicolegal Death Investigation

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The goal of this presentation is to familiarize attendees with the principles, practices, and methods of forensic taphonomy and forensic archaeology, in particular the main advantages and types of information offered by the application of these disciplines at a variety of outdoor and fatal fire scenes.

Although relevant to all forensic specialists, the information and advantages discussed are particularly relevant to those professionals conducting postmortem examinations of human remains, such as forensic pathologists, since their tasks in determining cause and Manner Of Death (MOD) in these complex scenarios are compounded by the additional alteration and layers of information added by the postmortem exposure of the remains to an array of environmental processes and agents. This presentation will impact the forensic science community by illustrating how the correct application of forensic archaeology and the careful consideration of forensic taphonomy can help to improve coordination and information flow between scene and laboratory analysts.

Medicolegal death investigation is a complex multidisciplinary endeavor involving law enforcement, forensic pathologists, and other forensic scientists. The information required by each of these specialists to clarify the death event is obtained from evidence and contextual observations gathered from the body at autopsy, at the crime scene, and from police investigation of circumstances surrounding the event. MOD is presented after carefully piecing together all the information and evidence assembled by the diverse medicolegal investigation teams. The contextual and evidentiary information derived from the scene of the crime always provides one of the key pieces of the puzzle. Every question that relates to both cause and MOD requires some understanding of the physical context of the remains; thus, all members of the investigative team are justified in demanding that best practices be applied to gather this information at the crime scene. At the indoor scene, law enforcement collects and analyzes evidence through comprehensive and well-developed criminalistics protocols that produce robust, detailed descriptions of context, and hypotheses of whether and how evidence is associated to the death event. The goal is to reconstruct the events surrounding death, including who was involved and how long ago it happened; however, scenes such as outdoor ones, burials, or fire scenes pose complex challenges, as a longer or more intense exposure to natural processes and agents adds several layers of alteration and new information, which must be identified in order to strip it from the truly forensically significant layer. This complexity, and the difficulty of identifying and recording all of the relevant parameters at these scenes, often leads to lowered expectations regarding the amount and utility of evidence and information that can be collected at these scenarios, as compared to indoor scenes. This presentation argues and illustrates how the application of forensic archaeological principles, methods, and practices in the processing of these scenes, in conjunction with the guiding principles and framework provided by the discipline and approach of forensic taphonomy, serves to solve these problems, resulting in event reconstructions as comprehensive, reliable, and defendable in court as those from indoor scenes.

Forensic taphonomy has been defined as the study of what happens to a human body after death. Since most of what happens to the body (and evidence) at an outdoor scene is the result of alteration or modification by natural agents, the recognition and documentation of the specific role played by each of these agents is critical to understanding and explaining evidence displacement, loss, or alteration, and ultimately providing hypotheses of the role humans play in altering a scene or evidence after the death event, representing one of two major assessments provided by forensic taphonomic investigations. The best way to collect this evidence is through the implementation of forensic archaeological practices that require exhaustive descriptions of context (location and immediate scene environmental, climatic and biotic characteristics), careful exposure of the remains while noting stratigraphic relationships, and detailed notation of the spatial distribution of the evidence through a variety of cartographic means, which, in turn, allows for robust hypotheses of association of the evidence to each other and to a particular event. The other primary assessment that a proper forensic taphonomic analysis provides is estimation of Postmortem Interval (PMI) and how long the body has been at this location. These estimates are obtained

through careful analysis of context and consideration of biological tissues preservation, as well as temperature, climatic, and other exogenous factors, such as insect and animal predation and alteration. This presentation explains how information for these forensic taphonomic assessments are best acquired, analyzed, and interpreted at the outdoor scene.

Forensic Taphonomy, Forensic Archaeology, Outdooor Scene Recovery

E114 Trauma and/or Taphonomy? Analysis of Peri-Mortem Trauma Observed in a Post-Battle World War II Cemetery and the Complications of Taphonomy in Discerning Trauma

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After attending this presentation, attendees will better understand a variety of WWII combat traumas viewed in a recently discovered United States WWII cemetery (minimum number of individuals currently = 48). Attendees will also learn about an unusual type of taphonomy that mimics peri-mortem trauma and possible microenvironments contributing to these postmortem changes.

This presentation will impact the forensic science community by providing an unprecedented sample of WWII combat-related traumas that includes a first look at distinct and unfamiliar taphonomic factors and their effects on the preservation of human remains.

This taphonomic process is undocumented (according to research) and could be valuable to the forensic community as it represents a previously unknown condition that resembles the effects of peri-mortem thermal alteration. Attendees will learn of the modern battlefield peri-mortem traumas documented during the analysis as well as how to recognize differential taphonomy that may obscure or mimic trauma through the use of skillful field techniques.

Description of the site and sample: During the Second World War, the Battle of Tarawa was a costly victory for the United States Marine Corps. More than 1,200 United States. Marines and sailors were killed in action, hastily buried in post-battle cemeteries, and lost to time. As a result, approximately 500 of these Marines were deemed "unrecoverable." Seventy-two years later, one of the missing cemeteries was discovered and systematically excavated by a non-governmental organization called History Flight Inc. Their efforts led to the repatriation of 48 lost United States servicemen from one locality as of July 26, 2016. It is from this locality (often referred to as Cemetery 27 in various documentation) that the information was gathered.

A variety of combat traumas were observed in the recovered burial population including, but not limited to, peri-mortem gunshot wounds, sharp force trauma, peri-mortem thermal alteration, blunt force trauma, and a variety of peri-mortem fractures. Atypical traumas, such as that resulting from bomb fragmentation (aka "shrapnel"), were observed as well, but do not necessarily fall into any of the above categories. While the majority of the skeletal trauma is clear and reasonably distinct, a newly documented taphonomic effect was found to mimic peri-mortem thermal alteration. This taphonomic effect is the result of direct contact between skeletal materials and the military-issue rubberized canvas poncho with which many individuals in this cemetery were interred. In addition, this unusual taphonomic effect created by contact with the poncho was detrimental to the perseveration of human remains. Skeletal elements contained within, covered with, or placed on top of the rubberized canvas ponchos are heavily fragmented, friable (often disintegrating with the slightest touch), and discolored, compared to skeletal remains without contact with this material.

Trauma, WWII, Battle of Tarawa

F1 Carotid Sinus Hypersensitivity, Psychogenic and Vasovagal Syncope, and Homicide: The Importance of Negative Results of Biologic Evidence in Court

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After attending this presentation, attendees will understand the juridical, jurisprudential, and investigative problems related to sudden death after a physical assault without evidence of trauma on the victim.

This presentation will impact the forensic science community by proposing an interdisciplinary review among jurists, forensic pathologist, judges, and prosecutors to examine discriminating or determining factors in cases of sudden death without positive (biological) scientific evidence.

The problem of a sudden death without physical evidence of trauma or natural pathological condition (e.g., myocardial infarct, brain hemorrhage) is not well known and an extensive case history was reported in 1964.[1]

This presentation proposes a review of these cases in recognition of the modern approach to suspicious death scenes. Recent scientific acquisitions of specialized disciplines are able to interpret the absence of elements as a positive finding. The absence of any positive biological evidence can be considered as evidence itself.

One example of such a case involved a husband who discovered his wife in bed with another man. The lover escaped through the window, and the wife tried to follow him when her husband seized her "by the throat." She collapsed and died. The autopsy did not reveal any evidence of trauma to the neck.

The only certain element of the case was circumstantial: that of the pressure on the woman's "throat" by her husband. The author, concluded that the case "could only be explained" by a mechanism of death by inhibition (vasovagal syncope).[2]

The review of the case revealed two critical points: (1) a specific circumstantial element (the pressure "on the throat"); and, (2) the lack of any physical trauma to the victim's external or internal organs.

In similar cases, it is crucial to understand if the vasovagal syncope provoked by the aggressor is intentional or due to an accident. Different elements need to be considered: predisposing pathologies, lack of postmortem findings, the victim's age, the reliability of witness statements, and the absence of "functional" causes of death.[2,3] In addition, psychological elements of the crime (such as the motive and deliberate actions of the perpetrator), the scenario in which the death took place, and the degree of the aggressor's knowledge of human anatomy and physiology. Furthermore, it is important to investigate sudden deaths related to jobs where pressure on the neck can be administered (e.g., physiotherapist, masseur), increasing the risk of vasovagal syncope.

In conclusion, this study suggests that sudden death with negative postmortem findings needs a review not only of the scientific investigation but also of the juridical principles and jurisprudence.

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Carotid Sinus Hypersensitivity, Vasovagal Syncope, Negative Biological Evidence

F2 Improving the Results of Forensic Science: Two Successful Solutions Developed in Brazil

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After attending this presentation, attendees will understand how some low-cost solutions can result in immediate responses for ordinary problems faced by forensic science. Two solutions have been particularly successful, workshops and postgraduate programs, to share knowledge.

This presentation will impact the forensic science community by demonstrating how low-cost solutions can result in immediate responses for ordinary problems faced by forensic science. These solutions can be replicated in other countries, including countries with restricted budgets.

The papresentation seeks to present relevant solutions developed during the process of improving the results in forensic science in Brazil.

It is well known that investing in infrastructure is one of the simplest ways of improving the results of forensic sciences. Expanding equipment laboritories and workstations of forensic scientists return higher production and quality of analyses and reports. Another key aspect is increasing the number of scientists and the distribution of human resources by specialization. Another important issue is the need for ongoing training for these professionals. All these aspects are part of good criminalistics management practices and involve financial and human resources; however, this type of investment usually comes with high costs, and some countries cannot afford to increase spending on infrastructure to improve forensic sciences. Brazil, as an emergent country, has to develop solutions to cope with this situation to achieve better results without excessive costs. Workshops and post-graduate programs to share knowledge are two solutions that have been particularly successful.

In the first solution, workshops were organized in different Brazilian cities and substantial analyses results were presented. The data regarding crimes that were judged between 2011 and 2012 were collected from three Brazilian states. The results were presented in a separate paper and concluded that 77% of the information in the forensic reports were explicitly mentioned by the judges in their decisions. Preliminary data seem to confirm that there exists a positive and strong correlation between the knowledge shared in workshops and the optimized use of the forensic experts' reports.

In the second solution, the involvement, articulation, and coordination among government institutions presented evidence of significant improvement of forensic results.

After the beginning of a postgraduate program involving servants from several public bodies, in 2016 there were 27 special operations coordinated by Federal Police of Brazil, federal prosecutors, and auditors of the Ministry of Transparency, Monitoring, and Control. This postgraduate program involves sharing experiences in investigations and a discussion forum about experts' examinations and audits that result in identification of corruption crimes in Brazil. The detailed statistical results have been consolidated to demonstrate the correlation between this institutional action and the identification of overbilling, corruption, and fraud in different ways.

Sharing experiences, understanding the limits of action of forensic science, and working together can improve the experts' work, intensify confidence, and make the institutions stronger. It is a good example that has been working well in Brazil for the last decade, and it can be observed in sucessful cases, such as the so-called "mensalão" and "lava jato."

Sharing Knowledge, Corruption, Forensic Experts Reports

F3 Assessing the Strength of Fingerprint Conclusions

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After attending this presentation, attendees will understand the importance of assessing the complexity of a comparison and the demonstrable level of association found between two impressions. Assessing these factors allows for the strength of any pattern evidence conclusion to be more accurately discerned.

This presentation will impact the forensic science community by protecting against over-interpretation and ensuring conclusions are as accurate as humanly possible.

False convictions have pushed the topic of forensic errors into the national spotlight. Fingerprint conclusions are very accurate, but errors have occurred. The strength of any conclusion needs to be measurable since criminal proceedings rely heavily on this type of information. Attempts to articulate the strength of fingerprint conclusions have persisted for decades (e.g., counting points, Scientific Working Group on Friction Ridge Analysis, Study, and Technology (SWGFAST) Sufficiency graph, statistical modeling, etc.). This presentation will discuss past methods for determining the strength of conclusions, discuss their limitations, and present an alternative approach that is both easy and effective.

Historically, fingerprint conclusions have been reported in a categorical fashion, such as "the impression has been identified to John Doe." Reporting conclusions in this manner has made conclusions sound conclusive, when in reality they may be strongly supported with visual data, marginally supported with visual data, or lack visual data that can be successfully demonstrated to others (i.e., simply the beliefs of the practitioners stating the conclusion). In order to determine the strength of the conclusion, the basis behind the conclusion needs to be assessed. Conclusions have been reported categorically as a means of simplifying a very intricate process that was based on a large number of non-quantifiable variables. No statistical model has been able to express the strength of conclusions despite ongoing and previous efforts dating back to the late 1800s.

The lack of a clearly defined criterion for arriving at conclusions makes it difficult to evaluate a practitioner's conclusions. Without a standard, there is no means of judging correctness. This is extremely concerning when people's liberties and lives are on the line. Currently, the only way to assess a conclusion is to ask for another practitioner's opinion, which is mistakenly viewed as a measure of accuracy. Repeating a conclusion is simply measuring whether or not the conclusion is acceptable to another practitioner; it is not establishing absolute truth.

Instead of oversimplifying conclusions as categorical variables (identification or exclusion), it is more appropriate to present decisions on a continuum that expresses the complexity of a comparison (e.g., Basic, Advanced, Complex) and the demonstrable level of association (such as overwhelming, compelling, persuasive, considerable, marginal, none or none found). The complexity of a comparison is important because it determines the extent of testing required to ensure the interpretation and amount of data are sustained under a critical review. The results of the testing establish the acceptable level of association, which indicates the strength of a conclusion (e.g., a complex comparison does not indicate that a conclusion is weak; it indicates that additional quality assurance measures are required to establish a strong conclusion).

Conclusions based on specific criterion and vetted against rigorous scrutiny will preempt errors and make conclusions more trustworthy than conclusions based on personal thresholds and confidence levels. Clear thresholds also make it possible to judge the acceptable level of association used to support a conclusion, which helps assess the risk of error for each conclusion. Measuring acceptance or rejection based on a criterion is a far more informative approach than judging conclusions based on the beliefs of other individuals. Ultimately, utilizing the following method will provide stronger conclusions and allow others to assess the strength of conclusions.

This method can also be beneficial to re-assess conclusions arrived at using a different method. The level of complexity, the degree of testing performed, and the level of association will establish the strength behind any conclusion.

Fingerprints, Level of Association, Conclusions

F4 Fingerprint Science

Joseph B. Kadane, PhD, Baker Hall 232L, Pittsburgh, PA 15213*

After attending this presentation, attendees will have a more skeptical attitude toward testimony concerning fingerprints.

This presentation will impact the forensic science community by bringing more science into fingerprint analysis and testimony.

To what extent are statements and testimony about fingerprint evidence supportable scientifically? This study presents a statistician's view to answer this question.

With respect to whether each of the fingers on each person's hands are distinguishable from each other's, there is good evidence that fingerprints are partially, but not wholly, determined genetically; however, there are not good models of the joint genetic-environmental process leading to fingerprints that might permit one to address the problem. With respect to the empirical claim that identical fingerprints have not yet been found on distinct people, this presentation illustrates shows that it would require approximately 500 comparisons per second since the Big Bang to compare the fingerprints of every pair of people now alive.

Recent work does reveal that fingerprints are quite stable over time, barring severe accidents or other maiming.

Generally, the Automated Fingerprint Information System (AFIS) is used to winnow a database of possible donors down to a handful that the analysist studies; however, the AFIS is proprietary, so the analyst does not know what similarity measure is used, and hence cannot testify about its properties. Also, there are several different AFIS entities, which can and do provide different results. Consequently, the analyst cannot be confident that the handful of "most similar" fingerprints to the mark from a crime scene is actually the most similar. The database to which AFIS is applied may or may not contain the actual donor. The training of fingerprint analysts does not include training as a criminal sociologist, so a fingerprint analyst is poorly placed to opine on the probability that the source's fingerprint is in the database on which AFIS is run.

A fingerprint analyst is well-advised to study the mark first, and to list the minutiae and other facts about the mark to be looked for in the handful of prints from AFIS. Suppose one of them coincides with the mark on most or all of those indicia. The general literature encourages the analyst to then claim near certainty that the mark was made by the donor of the similar ten-print. Certainly the coincidence of marks allows the analyst to claim that the set of possible makers of the mark is reduced, perhaps drastically; however, there is no scientific principle that allows the analyst to estimate the size of the remaining group, and, in particular, no criteria supporting the reduction of that group to a single person.

While these findings do not support fingerprint evidence as it is now testified to in court, there is still valuable circumstantial evidence from fingerprints in the exclusion of many possible donors. Hence, the hope is that fingerprint analysts will testify more modestly in the future.

Fingerprint Uniqueness, AFIS, Fingerprint Testimony

F5 Lay Understanding of "Identification": How Jurors Interpret Forensic Identification Testimony

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After attending this presentation, attendees will have a greater understanding of how laypersons interpret the word "identification" in the context of forensic testimony, with specific emphasis on fingerprint evidence. Attendees will also be better informed when discussing the use of appropriate terminology when standardizing scientific reporting practices throughout the forensic community.

This presentation will impact the forensic science community by generating essential dialogue regarding how expert forensic testimony should be expressed to legal practitioners and fact-finders through evaluating potential lay jurors' interpretation of the word "identification" in the context of forensic testimony.

For the past several decades, several forensic disciplines have utilized the term "identification" to express the highest level of association between an evidence sample and a known source. Recently, the terminology and language used in forensic technical reports and expert testimony have come under scrutiny from the legal and academic communities, who are seeking to ensure the language conveys the appropriate strength of the evidence. Beginning in 1998, at the recommendation of the Technical Working Group on Friction Ridge Analysis, Study, and Technology (TWGFAST), the fingerprint community espoused testimony in terms of single-source attribution and "to the exclusion of all others" to convey the expert opinion that two fingerprint impressions were made by the same individual. During the next two decades, the fingerprint community considered the terms "identification" and "individualization" synonymous, yet toggled between them to ensure their testimony conveyed the intended meaning that "the two impressions were made by the same source" to laypersons, despite several criticisms regarding the scientific validity of such claims. Then, following the landmark Report by the National Research Council in 2009, the International Association for Identification (IAI) cautioned its members against "stating their conclusions in absolute terms when dealing with population issues." As a result, in the same year, the (now referred to as "Scientific") Working Group for Friction Skin Ridge, Study, and Technology (SWGFAST) eliminated the phrase "to the exclusion of all others" from their recommended language for expert testimony, but maintained the term "individualization" or "identification" and its related definition, "the conclusion that corresponding impressions originated from the same source." Although the fingerprint community now recognizes that available scientific literature does not provide sufficient support for claims of "to the exclusion of all others," the terms "identification" and "individualization" continue to be recommended by professional bodies and espoused by forensic practitioners across several domains. When considering appropriate terminology for use by forensic experts, policy makers must ensure that the language utilized is both in accordance with appropriate scientific principles and properly understood by laypersons in accordance with the intended meaning. The broader question, then, becomes whether the terms "identification" or "individualization" continue to be interpreted as "to the exclusion of all others" despite the elimination of such a phrase. This question was explored through a survey of lay potential jurors throughout the United States. This presentation will explore the results of that survey and discuss implications for policy and practice to ensure the forensic sciences utilize optimal terminology and language to maximize juror understanding of the forensic evidence.

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

Identification, Individualization, Fingerprint

F6 Roadside Saliva: Are Oral Fluid Collection (OFC) Devices the Proper Tool to Help a Non-Physician Make a Diagnosis at a Roadside Traffic Stop (RTS)?

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After attending this presentation, attendees will think critically about the efficacy of hand-held devices that are intended for use by police officers to make arrest decisions regarding whether a driver is impaired by controlled substances.

This presentation will impact the forensic science community by increasing knowledge of the protocol used by law enforcement in attempting to diagnose an impaired driver. Attendees will also better understand enforcement efforts in two states that are part of a national trend. Attendees will appreciate the need for improvement in the current methods and the need for caution in a diagnosis by a police officer of drug-induced impairment of a driver.

The State of California is in the midst of a multi-county experiment using officers trained as Drug Recognition Evaluators (DREs) and Roadside Saliva analysis using a portable device called Oral Fluid Collection (OFC). The OFC is able to react chemically with enzymes produced by saliva to detect the presence of different categories of drugs. The State of Michigan is about to implement an experimental program in five counties, under which officers who are specially trained as DREs will use the protocol to attempt to detect impairment as well as the RTS to support an arrest decision.

An OFC is a device an officer can carry with him or her in the field. The device is on the forefront of law enforcement efforts to gain approval and acceptance of a tool that purportedly allows for a probable cause determination of drug-induced impaired driving. The devices are used in conjunction with the 12-step DRE protocol of what is essentially a "diagnosis." The device uses enzymatic immunoassay methodology to detect the presence of certain controlled substances, both prescribed and illegal, per se, to consume.

The concern regarding a high rate of false positives is significant. The data obtained by constituent groups in Michigan led to widespread rejection of legislation to enable the use of RTS in 2014. Follow-up legislation, what is now MCL 257.625r, 625s, and 625t, established a one-year pilot project in five Michigan counties.

Oral, Fluid, Device

F7 Retrograde Extrapolation as a Part of Accident Reconstruction: A Case Study

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After attending this presentation, attendees will better understand of the different factors influencing the retrograde of ethanol concentration based on breath or blood ethanol reports.

This presentation will impact the forensic science community by utilizing and comparing the multiple equations used to obtain a retrograde range of ethanol concentration, their limitations, and their uses.

One of the goals of accident reconstruction is to recreate the circumstances at the time of a collision in order to determine the factors that contributed to or caused the collision to occur. Driver impairment by alcohol is one of the factors that can contribute to a collision and needs to be condidered in accident reconstruction. Because of injury or other factors involved in crash investigation, it is not uncommon that a traditional Driving Under the Influence (DUI) investigation, consisting of investigatory questions, observations of physical symptoms and behaviors, field sobriety testing, and field breath testing are not possible. Also, there may be a significant delay in obtaining the blood alcohol sample. The determination of the extent of alcohol intoxication at the time of the collision may then rest on a very limited amount of information.

Retrograde extrapolation is the technique used to estimate the blood alcohol level at an earlier time based on a later chemical test value. The accuracy of retrograde extrapolation is contingent on the elimination rate of alcohol and the phase of alcohol metabolism of the subject at the time of the collision. Because these factors are typically unknown, retrograde extrapolation in such cases has been criticized. Rather than presenting a single calculation based on uncertain assumptions, another approach would be to use the reasonable range of each variable and present several calculations that comprise the reasonable range of possible blood alcohol concentration at the time of the collision. The reasonable range of factors would include the measurement uncertainty of the blood alcohol test result, the typical range of ethanol elimination, the reasonable time to peak alcohol level, and the reasonable range of Widmark factors. Calculations can then be performed for an average value, a highest and lowest range of what might be expected.

This method of retrograde extrapolation was used in the case of a farmer who collided with a motorcyclist, resulting in the death of the motorcyclist. The farmer's blood alcohol level was tested at 0.03g/100ml a little more than three hours after the collision. This method of retrograde extrapolation was used to demonstrate the wide range of possible blood alcohol levels at the time of the collision, both assuming and not assuming that the subject was past the peak alcohol level at the time of the collision.

Retrograde Extrapolation, Accident Reconstruction, Crash Blood Alcohol Content

F8 The Use of Standardized Field Sobriety Tests for Drug Impairment: An Evaluation of the Research, Ethical Implications, and Legal Issues

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After attending this presentation, attendees will understand the challenges associated with the use of Standardized Field Sobriety Tests (SFSTs) assessing impairment in cases involving Driving Under the Influence of Drugs (DUID), the ethical implications, and legal issues which arise (e.g., *Daubert* challenges/due process questions).

This presentation will impact the forensic science community by providing an in-depth exploration of the legal and ethical challenges associated with using SFSTs in cases involving DUID.

SFSTs, which were designed to assess impairment associated with alcohol intoxication, have been used for more than four decades.[1] With the implementation of Drug Recognition Evaluations, cases involving suspected drug use may undergo more extensive evaluation by a trained expert. (the validation and training of drug recognition is beyond the scope of this presentation); however, not all individuals are subjected to this more comprehensive examination and are only evaluated using SFSTs: the Horizontal Gaze Nystagmus (HGN), the Walk and Turn (WAT), and the One-Leg Stand (OLS).[1-3] Research has been conducted on individuals under the influenced of compounds such as amphetamine, dl-3,4-methylenedioxymethamphetamine and methamphetamine, and cannabis using SFTS, with results demonstrating that the use of these tests may not be adequate to assess impairment with specific compounds within a class of drugs or at varying drug concentrations.[4,5] Although validated for accurate impairment with alcohol intoxication, SFSTs have not been evaluated and validated with all possible drug classes that may be encountered during a roadside stop. Therefore, this study asks the questions, "Should testimony about the performance on SFSTs by a driver suspected of impairment because of the ingestion of controlled substances be admitted at trial: (1) at all; (2) as statements of perceived fact without any conclusion or opinion attached; (3) as statements of perceived fact from which the trier of fact may draw inferences and form opinions; (4) as statements of perceived fact from which the testifying witness may draw inferences or form opinions about the operator's state of sobriety; and/or, (5) as statements of perceived fact from which the testifying witness may draw conclusions about the identity of the substance causing the operator's behavior."

The ethical implications arise from the interplay between the expert and the lawyer who presents the expert's testimony. Attendees will learn about the differences between the ethical rules that govern prosecutors and defense counsel. Attendees will also learn about the American Bar Association's Criminal Justice Section Standards, Prosecution Function, Standard 3-3.3, "Relations With Expert Witnesses," and Standard 3-1.2(c), "The Function of the Prosecutor." The presentation will also explore the American Bar Association's Criminal Justice Section Standards, Defense Function, Standard 4-4.4, "Relations With Expert Witnesses." In addition, the American Bar Association's Model Rules of Professional Conduct, Rule 3.3, "Candor Toward the Tribunal," Rule 3.4, "Fairness To Opposing Party and Counsel," and Rule 3.8, "Special Responsibilities of the Prosecutor" will be presented. The question, "Is it ethical to offer the testimony concerning a defendant's performance on the SFSTs without offering evidence of validation, sources of error, and error rate?" will be discussed.

Attendees will learn about and discuss legal issues that arise from a proffer of an expert opinion regarding a driver's performance on the SFSTs as an indicator of drug-induced impairment. This presentation will include a discussion of some of the factors enumerated in the Federal Rules of Evidence, 702: helpfulness to the trier of fact, an adequate basis in fact, the necessity of showing both the reliability of the methodology, and the reliability of the application of the methodology to the facts of the case. *Daubert v. Merrell-Dow Pharmaceuticals, Inc.*, 509 US 579, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993) and its progeny.[6]

Reference(s):

1. Burns M., Moskowitz H. (1977) *Psychophysical tests for DWI arrest*. U.S. Department of Transportation, National Highway Traffic Safety Administration. Final report, Publication No. DOT-HS-5-01242.
2. O'Keefe M. (2001) Drugs driving—standardized field sobriety tests: a survey of police surgeons in Strathclyde. *J Clin Forensic Med*. 8(2):57–65.

3. Luke A. Downey, Amie C. Hayley, Amy J. Porath-Waller, Martin Boorman, Con Stough. The Standardized Field Sobriety Tests (SFST) and Measures of Cognitive Functioning. *Accident Analysis & Prevention*. 86 (2016): 90-98.
4. Beata Y. Silber, Katherine Papafotiou, Rodney J. Croft, Con K. K. Stough. An Evaluation of the Sensitivity of the Standardised Field Sobriety Tests to Detect the Presence of Amphetamine. *Psychopharmacology*. 182.1 (2005): 153-59.
5. Luke A. Downey, Rebecca King, Katherine Papafotiou, Phillip Swann, Edward Ogden, Con Tough. Examining the Effect of Dl-3,4-methylenedioxymethamphetamine (MDMA) and Methamphetamine on the Standardized Field Sobriety Tests. *Forensic Science International*. 220 (2012): E33-36. National Center for Biotechnology Information. U.S. National Library of Medicine. Web. 29 July 2016.
6. *Daubert v. Merrell-Dow Pharmaceuticals, Inc.*, 509 US 579, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993).

SFSTs, DUID, ABA CJ Standards

F9 Social Media and the Justice System

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After attending this session, attendees will better understand of the impacts that the use of social media is having on our justice system, particularly by law enforcement, lawyers, litigants, and jurors. Attendees will see examples of the consequences of that use and of the various techniques that can be utilized to cope with those impacts.

This presentation will impact the forensic science community by providing a better understanding of the way in which modern technology is having a forensic impact on the quality of our justice system.

The use of social media has become a huge part of our culture. The justice system is directly impacted by this technological phenomenon. This presentation focuses on some of the impacts that social media is having on the American justice system, including its usage by police and lawyers, and especially the juror misconduct problems it presents.

Law Enforcement: According to 2014 research conducted by Lexis Nexis, 81% of law enforcement professionals actively use social media as a tool in investigations, 26% use social media to investigate daily (a 16% increase since 2012), and 73% believe that using social media can help solve crimes more quickly. Users of social media post incriminating comments, photographs and videos. Gang members boast about crimes or post photos or videos holding firearms or drugs. Many agencies have covert monitoring programs for such media, often using fictitious names. When a user posts information to the public, it is generally not protected by the Fourth Amendment. "Government officials may use public information to justify an arrest or conviction, and without Fourth Amendment protection, users may be subject to criminal liability based on personal photographs, location check-ins, or status updates posted on social networking websites."

Lawyers: Criminal defense lawyers have an ethical and legal duty to investigate the alleged crime and witnesses, and many believe that includes a duty to conduct surreptitious online investigation of social media postings; however, there are several unresolved ethical issues, especially when the investigation may constitute improper contact with parties or witnesses. Attorneys also face significant issues regarding online investigation of jurors.

Jurors: Jurors accessing or communicating on the internet, whether through social networking or using the internet to research the case or witnesses, can undermine basic concepts of due process. If judgments are based on information that the defendant and the prosecution are not able to see, this contradicts elementary judicial principles. The internet has replaced both newspapers and television as primary sources of information. The beauty, or some would say the terror, of the internet is that it comes without any filter of authenticity or accuracy. But jurors want and expect to use these same online resources they use to address any other question they have.

Judges "instruct" jurors not to do what they want to do. Most jurisdictions recommend that the instruction be specific, telling jurors, for example, not to use Google® or Bing®, not to post to Facebook® or Twitter®, or blog about the trial. Some recommend that jurors be specifically told that violations of the instruction will result in them being found in contempt of court and facing criminal sanctions. But judges need to tell jurors why it is so important. A video of a sample instruction will be shown.

There are those who advocate that we do nothing. They argue that the world has changed and that the courts need to simply accept and accommodate that change. They would allow jurors free access to the online sources they use in the rest of their lives and then trust the so-called "democracy of the internet" and the free exchange of ideas among the jurors to reach a just result. To do so would abrogate our basic commitment to justice that is not based on bias or prejudice.

Social Media, Jurors, Internet

F10 Analyses of Multiple Mobile Devices and Services in Distracted Driving Cases: Data Synchronization and Defeating Unscientific Applications of Those Data

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After attending this presentation, attendees will better understand: (1) the value of seeking all potentially relevant mobile devices and service records associated with a distracted driving case; and, (2) the value of synchronizing mobile device and service data — including text messages, Global Positioning System (GPS) track points, service provider business records, and e-911 center records — into an integrated case timeline and incident map.

This presentation will impact the forensic science community by alerting digital forensic scientists and trial lawyers to the importance of: (1) thorough investigation and examination of all mobile devices and service records associated with a distracted driving case; (2) synchronizing mobile devices and service data into an integrated case timeline and incident map in such a case; and, (3) recognizing, and reacting to, any misuse of such data.

For the defense in a criminal case to have first-in-time access to digital devices that contain potentially dispositive evidence is likely rare. Yet that is what happened when law enforcement responders from state, county, and municipal agencies had the opportunity to examine those devices first following a serious traffic accident in rural northwestern Minnesota but neglected to do so. This presentation addresses the synchronization of three sets of mobile device data to determine exactly when and where the operator of a motor vehicle was when she sent and received text messages and exactly where and when the motor vehicle accident occurred.

The criminal case that gave rise to the digital forensic investigation pertinent to this presentation was charged as criminal vehicular homicide and criminal vehicular injury. The State alleged that the defendant had made a left turn onto a two-lane highway just in front of two motorcyclists. Both motorcyclists were seriously injured in the accident; one of the motorcyclists died six days later. The State's case was essentially that the defendant was texting at or just before the moment of impact, and thus was grossly negligent.

At the accident scene, law enforcement did not seize the defendant's cell phone (an old feature phone, not a "smart phone" in any meaningful sense), nor did they seize the defendant's stand-alone GPS unit. More than four months passed before the State actually charged the defendant. Once the defendant had submitted her phone to the mobile device forensic scientist, all data relevant to the accident had been overwritten by her subsequent daily use of the device; however, on forensic examination, the GPS unit's data were found to be completely intact. Because mobile device, cell carrier, and GPS timing are based on Coordinated Universal Time (UTC) with sub-second accuracy and limited latency periods, a mobile device forensic scientist possessing multiple data sets can synchronize them and determine within a documented, small margin of error exactly where and when any given text message was sent or received during a GPS-tracked trip. In this case, the forensic scientist determined that the defendant received her last text message more than a full minute before the accident, and that, at that moment, she was in a completely different location from the accident that subsequently ensued. The jury acquitted the defendant on all felony charges.

The State also disclosed a "Google® flyover video" that it intended to introduce as a demonstrative exhibit at trial. The flyover video used the GPS track points to create a short animated video of the defendant driving her vehicle the last several miles of the trip, culminating in the accident. Because of data and process weaknesses, including the margin of error inherent in civilian GPS devices and arbitrary movie-making choices by the "producer" the Google® flyover video playback made the defendant's driving appear deranged and was therefore profoundly prejudicial. The digital forensic scientist and lawyer worked closely together to build a motion *in limine* and supporting affidavit to successfully keep this exhibit from being seen by the jury.

Mobile Device Forensics, GPS/SMS Text Synchronization, Google® Flyover Exhibits

F11 When DNA Alone Is Not Enough: Exoneration by Computer Interpretation

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After attending this presentation, attendees will understand why merely testing DNA is not enough for criminal justice. Even with DNA data, accurate and informative interpretation of that data is needed for accurate identification information.

This presentation will impact the forensic science and legal communities by providing a case example of how sophisticated interpretation of DNA data was needed to exonerate an innocence man, long after the DNA had been tested.

On a freezing December night in 1989, five men savagely gang-raped a motorist after bumping her car on Indiana highway I-65. Darryl Pinkins and two other innocent men were misidentified as her attackers through clothing stolen from their car and left in the victim's car. Pinkins was convicted of rape and criminal deviate conduct in 1991 and sentenced to 65 years in prison. Despite his incarceration, the bump-and-rape crimes continued.

In 1995, Darryl Pinkins sought the assistance of the Innocence Project, then a law clinic at Cardozo Law School in New York. In turn, in 1999, the Innocence Project contacted Frances Watson, clinical professor at the Wrongful Conviction Clinic, IU McKinney School of Law. Professor Watson and her students represented Darryl Pinkins and codefendant Roosevelt Glenn through decades of unsuccessful state post-conviction and federal *habeas corpus* proceedings.

In these proceedings, it was shown that the State used false science to convict the men. There was faulty hair comparison testimony and meaningless blood typing inclusion evidence, yet the courts considered this flawed evidence harmless.

A 2001 DNA analysis of semen on the victim's jacket and sweater showed mixtures of two or more people. Each mixture had a clear 80%–90% major contributor that did not match the accused. But this limited DNA analysis was not enough to exonerate.

In 2007, Greg Hampikian of the Idaho Innocence Project began working with Watson. They demonstrated that the blood typing evidence was incorrectly presented during trial and not relevant in light of DNA exclusions, including new post-conviction DNA evidence. But the court ruled that the two unidentified major DNA genotypes in the semen, plus the three accused, equaled the five perpetrators — so post-conviction relief was denied.

In 2014, Dr. Hampikian recruited Dr. Mark Perlin of Cybergenetics for *pro bono* assistance. The TrueAllele® computer system provided the statistical science needed to establish innocence beyond doubt. More complete analysis of existing DNA data revealed the genotypes of all five perpetrators. This new finding persuaded the State that the wrong man had been convicted.

Genotyping modeling compared evidence with evidence to calculate exclusionary match statistics. The computer discovered new genotypes from 5%–10% minor contributors by jointly analyzing DNA mixture data. Kinship analysis revealed that three of the perpetrators were brothers. These capabilities found the victim and five unidentified genotypes in the semen and hair evidence. The defendants were not linked to the crime.

Acceding to exculpatory DNA evidence found by science, Lake County Prosecutor Bernard Carter vacated Pinkins' conviction. Instead of holding a hearing on newly analyzed DNA evidence, that morning the court released him from prison. Pinkins had spent 24 years in an Indiana prison for a crime he did not commit. Computer reanalysis of old DNA data proved that Pinkins and Glenn were innocent.

The DNA evidence was available 15 years ago, but limited interpretation methods could not extract its exculpatory information. The interpretation failure in Pinkins' case was ultimately rectified by accurate and objective data analysis. Absent such advanced computer modeling, in thousands of cases, DNA evidence is routinely misinterpreted or wrongly considered to be inconclusive.

Failed DNA interpretation cost Darryl Pinkins 15 extra years in prison. Other innocent people may be wrongfully imprisoned by inadequate DNA interpretation. Accurate and automated computer interpretation can revisit cases

with “inconclusive” DNA, examining old forensic data for new exculpatory evidence.

This presentation is a case study in which better interpretation of DNA evidence exonerated an innocent man.

DNA Evidence, Mixture Interpretation, Actual Innocence

F12 Utilization of Infrared Photography to Detect and Document Gunshot Powder Residue on Clothing: A Comparative Study

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After attending this presentation, attendees will understand the benefits of using a digital (infrared conversion) camera compared to a standard digital camera to detect and document Gunshot Residue (GSR) patterns on dark and multicolored clothing as well as other parameters essential for a close-range, shooting incident reconstruction.

This presentation will impact the forensic science community by indicating the best non-destructive test for examining GSR and for accurately documenting photographically the bullet entry hole in dark, multi-colored clothing. This is difficult to accomplish with a standard digital camera sensitive to the spectrum of light ordinarily "visible" to the sensor (Complimentary Metal-Oxide Semiconductor (CMOS) or Charge Coupled Device (CCD)) in digital cameras. This will be the first time quantitative data was obtained for Infrared (IR) detection of GSR particles on dark, multicolored cloth fabric substrates and close-contact muzzle-target distances.

The purpose of this study was to compare the effectiveness of a Nikon® D7000 IR camera in detecting and documenting GSR to that of a standard Nikon® D7100 Digital Single-Lens Reflex (DSLR) camera on dark or multicolored clothing. This study will examine three variables for comparison between the images produced by the two cameras: (1) number of particles counted; (2) diameter (mm) of the GSR pattern; and, (3) the bullet entrance hole diameter (mm) on the clothing. Ten different types of fabrics (substrates) will be used as well as one control substrate (white cotton shirt). A GSR pattern was created on three samples from each substrate, at the distances of 6″, 9″, and 12″ in order to produce shooting incident reconstruction standards. Results were analyzed by an independent-sample *t*-test to determine if the IR camera provided a statistically significant difference (p value< 0.05) in the three ranges analyzed compared to the DSLR camera.

This study indicated there were no significant differences observed in the number of particles visualized/counted between the IR camera and the DSLR camera images; however, there were significantly larger diameters of the entrance holes and the GSR residue fields using the IR camera compared to the standard DSLR camera.

Effective documentation of GSR patterns can lead to the exploration and possible determination of muzzle-to-target distances involved in a crime scene shooting incident reconstruction. Considerations should be made to utilize an IR camera whenever possible for possible expert firearm examiner testimony in the courtroom, especially in close-range shooting incidents.

Firearms Evidence, Gunshot Residue (GSR), Infrared (IR) Photography

F13 Off Label: A Hope or a Liability? An Analysis of Cases in Oncology

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After attending this presentation, attendees will be aware of distributive justice concerns and their consequences on medical liability.

This presentation will impact the forensic science community by illustrating how off-label use provides a valuable treatment alternative (with scientific support) for cancer patients by reducing the time related to the duration of the trial and the final approval of the Ministry of health.

Off-label refers to the use in clinical practice of drugs already registered but used in situations that, owing to dosage, routes of administration, disease, or population are prescribed differently than the approved practice by the Ministry of Health. Very often these medicines are widely known, but new scientific evidence suggests their being used even in clinical situations not provided for in the Ministerial information sheet.

In Italy, the use of medicinal products for indications other than those authorized is regulated by specific laws.[1] One of the areas in which the off-label use of drugs is most prevalent is cancer; the proper use of off-label therapies can represent a real hope for cancer patients for both the prognosis and their quality of life.

A mapping of the Oncology Departments in Puglia, Italy, and Centralized Handling Units for the preparation of cytotoxic chemotherapy was conducted. In addition, the medical oncologists and the hospital pharmacists were provided with a specially formulated questionnaire. The responses were then analyzed according to statistical and descriptive canons.

The study revealed only a partial knowledge of the topic of off-label; therefore, an in-depth exploration of the field of study was conducted by analyzing the current situation at the Oncology Institute of Bari (IRCCS) in order to assess the diseases for which they are in greater demand, the reasons for which they are used, the authorization process adopted, the procedures put in place in order to safeguard patient health, and, at the same time, the responsibilities of the physicians and the hospital.

The requests for off-label therapies that had arrived at the pharmacy between 2011 and 2015 (85 cases) were examined. This study selected the two diseases for which such treatment was implemented (Hodgkin's lymphoma and small-cell lung cancer), and examined the medical records. Having identified the "formal" shortcomings, a specific procedure for the use of off-label drugs within the Oncology Institute of Bari was implemented.

Given a partial formal shortcoming (there was an adequate informed consent form in only 33% of the cases) that has currently been compensated for, the results obtained revealed very good use of off-label drugs; the therapy had positive effects either on the prognosis or on the quality of life. The sector with the most reassuring results was Hematology. All patients with Hodgkin's lymphoma treated in the last year with brentuximab vedotin + bendamustine achieved a complete remission of the disease.

In Italy, the topic off-label is still controversial. On the one hand, it can promote clinical trials by creating valid therapeutic alternatives for patients, often at lower cost and in a shorter time, and, for that reason, it would be implemented specially in research institutes (like the Oncology Institute of Bari); on the other hand, it must be carefully controlled to prevent a disproportionate use which may lead to complications, which are as important for patient health as for the liability of the physician and the hospital.

Reference(s):

1. Law no. 94/1998 and Law no. 296/2007, Art. 1, paragraph 796, letter Z – Financial law 2007.

Off-Label Drugs, Hodgkin's Lymphoma, Liability

F14 Three Coins in a Fountain: How the Florida Arrestee Database Solved a New York John Doe Rape Case 20 Years After the Fact

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After attending this presentation, participants will learn how a John Doe indictment led to a rape conviction 21 years after the crime.

This presentation will impact the forensic science community by illustrating: (1) how to preserve the legal viability of a backlogged rape case with a John Doe indictment; (2) how an arrestee database led to a Combined DNA Index Sysem (CODIS) hit; and, (3) how best to present trial evidence in an old, cold sexual assault.

Like many newcomers to New York hoping to make a living in the arts, Wendy Webster (not her real name) worked in a restaurant. Late on a snowy January evening in 1995, business was slow, so the kitchen closed early, and Wendy ducked into a neighborhood movie theater in Greenwich Village to catch a late show; ironically, a courtroom drama called "Murder in the First." Walking home after the movie, Wendy was accosted by a man who asked her the time, then put his arm around her shoulders and held a knife to her side. He frog-marched her to the vestibule of a nearby brownstone, robbed her, and forcibly raped and sodomized her. Rifling through her wallet and reading her driver's license, he warned her that he knew where she lived, and, if she reported the assault, he would come back to kill her.

Undeterred, Wendy ran the few blocks to St. Vincent's Hospital, where an Emergency Room (ER) doctor took samples for a rape evidence kit and police took her report. The investigation hit a dead end, after early Registration Fragment Length Polymorphism (RFLP) testing failed to tie the attack to a serial rape case happening in Manhattan. The rape kit remained in New York Police Department (NYPD) custody, where it sat, untested, as part of a rape kit backlog.

With the advent of CODIS, New York law enforcement officials saw the wisdom of testing that backlog, and Wendy's case was one of the 17,000 sent for Short Tandem Repeat (STR) DNA testing. That testing generated a profile but no match in CODIS. Prosecutors decided to indict that DNA profile in order to stop the clock on a 10-year statute of limitations for rape. In 2003, Wendy testified before a grand jury, with no assurance her attacker would ever be found.

Fast forward to 2015, when prosecutors were notified of a DNA match to that John Doe indictment. Joseph Giardala was arrested after he scavenged coins from a Palm Beach shopping mall fountain, then bit the security guard who arrested him. That bite got Giardala swabbed for Florida's "arrestee" database, and a few weeks after that swab was taken, a CODIS hit identified Giardala as Wendy's attacker.

Prosecutors were able to find Wendy through the phone number of a friend listed in her medical records. A Google® search found the ER doctor, still practicing in New York City. The Crime Scene Unit detective, nearing retirement, was still with the NYPD. A new swab taken from the defendant and analyzed by the Office of the Chief Medical Examiner (OCME) allowed a criminalist to testify about the match between the defendant and the rape kit evidence. The jury deliberated for fewer than two hours and convicted Giardala in 2016 for the crime he committed 21 years earlier.

John Doe Indictment, Arrestee Database, Cold Case

F15 Presenting Probabilistic Genotyping Evidence in Court

Robert F. Hedges, JD, Muskegon County Prosecutor's Office, 990 Terrace Street, Muskegon, MI 49442*

After attending this presentation, attendees will better understand how to approach the presentation of probabilistic genotyping evidence in court at both *Daubert/Frye* admissibility hearings and at eventual trials.

This presentation will impact the forensic science community by addressing the unique concerns in presenting probabilistic genotyping evidence in court, at both *Daubert/Frye* hearings and trials, so that the evidence is understandable, persuasive, and sufficiently comprehensive.

Many crime labs throughout the United States are now using probabilistic genotyping software to assess DNA profiles, especially in DNA mixture cases or cases with compromised samples. The two most common products being used are STRMix™, from the Institute of Environmental Science and Research (ESR), New Zealand, and TrueAllele®, from Cybergenetics of Pittsburg, PA. Both products have now been admitted in several cases in the United States.

The case of *People v Muhammad*, 14th Circuit Court, Muskegon, MI, which was the first civilian case in the United States to offer STRMix™ evidence.[1] The case involved an armed robbery of a gas station in which the victim could not identify the robber because he was wearing a mask; however, during the robbery, the victim managed to pull out his own gun and started shooting at the robber. As the robber fled the victim's gunfire, he literally ran out of his left shoe. The entire episode was captured on video and the video will be available as part of this presentation.

The shoe was confiscated as evidence and analyzed at the Michigan State Police Crime Lab. The laboratory found a DNA mixture of four or more persons that was too complex for further analysis, using then available (2014) analytical tools. The shoe was subsequently sent to a private DNA laboratory for further testing and a profile was developed from a previously untested part of the shoe (toe area of the sole) where a mixture of two persons was found; however, that sample was too compromised to permit a conclusion using traditional DNA statistical analysis. The DNA profile developed at the private laboratory was then forwarded to Dr. John Buckleton of ESR whose team was able to use the STRMix™ software to conclude that the suspect, Mr. Muhammad, was one of the two donors, with a likelihood ratio of one trillion to one.

There will be a discussion of materials available to educate the attorney on the science of probabilistic genotyping. Attention will then be directed to the types of evidence available for *Daubert* or *Frye* admissibility hearings. Subjects will include expert testimony, validation studies, peer-reviewed literature, presentations, and approvals by various government boards, commissions, and other authoritative bodies. The exhibits used in the Michigan case will be presented and made available for copy online or by email.

There will be a discussion regarding preparing for the opposing expert witness. In the Michigan case, the defense called a well-qualified DNA expert, but he was not particularly well-versed on the nuances of probabilistic genotyping.

Finally, focus will be on how to best present probabilistic genotyping evidence to the jury; how to convince jurors that the science is well-tested and reliable; how to communicate complicated issues without losing substance; and strategies for presenting the evidence for the most persuasive impact.

Reference(s):

1. *People v Muhammad,* Case No. 14-65263 FC, 14th Cir Ct (Mich) (12-15-15).

Probabilistic Genotyping, DNA Mixtures, STRmix

F16 Wrongful Convictions and DNA Exonerations: Understanding the Role of Forensic Science as a Contributing Factor

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The goals of this presentation are to: (1) examine more closely wrongful conviction cases that include forensic science as a contributing factor; and, (2) identify what can be learned and how to ameliorate erroneous convictions when forensic scientists perform testing, interpret results, and render conclusions.

This presentation will impact the forensic science community by providing a greater understanding of the extent to which forensic science can be strengthened based on an evaluation of wrongful convictions.

There is no greater travesty in the criminal justice system than the conviction and punishment of a person for a crime he or she did not commit. According to the Innocence Project (IP), a national litigation and public policy organization dedicated to exonerating wrongfully convicted individuals, 342 individuals have been exonerated as a result of DNA analysis. Erroneous convictions, like most catastrophic mistakes in the criminal justice system, are rarely caused by a single identifiable act or weakness; instead, multiple failures in the process can lead to a negative outcome. The IP lists six "contributing causes" for wrongful convictions: (1) eyewitness misidentification; (2) false confessions or admissions; (3) government misconduct; (4) inadequate defense; (5) informants; and, (6) unvalidated or improper forensic science; however, Gould et al cautions that "without a comparison or control group of cases, researchers risk labeling these factors as 'causes' of erroneous convictions when they may be merely correlates."[1] Gould et al designed a unique experimental strategy to study factors leading to rightful acquittals or dismissal of charges against an innocent defendant (near misses) that were not present in cases that led to the conviction of an innocent person. After identifying a set of erroneous conviction and near miss cases, then analyzing the cases using bivariate and logistic regression techniques, the researchers identified ten factors that led to a wrongful conviction of an innocent defendant instead of a dismissal or acquittal. The ten factors are: (1) younger defendant; (2) criminal history; (3) weak prosecution case; (4) prosecution withheld evidence; (5) lying by a non-eyewitness; (6) unintentional witness misidentification; (7) misinterpreting forensic evidence at trial; (8) weak defense; (9) defendant offered a family witness; and, (10) "punitive" state culture.

In reviewing the 342 cases cited on the IP website, 157 cases (46%) included a reference to "Unvalidated or Improper Forensic Science." While cross-referencing the same cases listed on The National Registry of Exonerations (NRE) website, some inconsistencies were identified, making it challenging to reconcile the data. The NRE does use six categories of "contributing factors" (not referenced as causes), which are similar to those listed on the IP website, and are as follows: (1) mistaken witness identification; (2) perjury or false accusation, (3) false confession, (4) official misconduct; (5) inadequate legal defense; and, (6) false or misleading forensic evidence. Although neither the IP nor the NRE websites use the ten factors identified by Gould et al, the categorical descriptions used by the NRE website are more aligned with the academic literature and were therefore used for this study.

Advances in DNA technology and forensic DNA analysis have improved how cases are investigated, how forensic evidence is interpreted, and our understanding of erroneous convictions in the criminal justice system like no other single investigative or scientific discovery. With this invaluable resource, the criminal justice system has come to learn more from past mistakes, and forensic science is commonly linked with wrongful convictions in the media, legal reviews, and academic research. Forensic science, when incorrectly perceived as a single discipline, causes observers to conflate matters and acquire their own misperceptions about all forensic science disciplines. Even more pervasive, many references to wrongful convictions in the popular media do not cite scholarly articles and often rely on other media articles and unverified sources.

The forensic disciplines most frequently cited in wrongful conviction cases are serology (ABO blood typing), microscopic hair analysis, and bitemarks; however, the last case involving any of these three disciplines was in the mid-1990s. Very few (less than 1%) of the 157 exonerations involved latent fingerprints, firearms, bloodstain pattern analysis, footwear and tire tread analysis, and handwriting. Finally, 98% of the DNA exonerations that are associated with "false or misleading forensic evidence" also involved two to five additional contributing factors. The focus of this study is to examine more closely wrongful conviction cases that include forensic science as a

contributing factor to identify what can be learned and how to ameliorate erroneous convictions when forensic scientists perform testing, interpret results, and render conclusions. In many cases, interpretation of results from scientifically sound disciplines (e.g., forensic biology) and potentially ambiguous testimony could likely be the focus for improvement.

Reference(s):

1. Gould J.B., Carrano J., Leo R., Young J. *Predicting Erroneous Convictions: A Social Science Approach to Miscarriages of Justice*. National Institute of Justice Final Technical Report, National Criminal Justice Reference Service Document No. 241839, Washington, DC, 2013. Available at: https://www.ncjrs.gov/pdffiles1/nij/grants/241389.pdf.

Exonerations, Wrongful Convictions, Forensic Science

F17 The Yara Gambirasio Case

Michele Vaira, JD, V. le I Maggio 27, Foggia 71122, ITALY; and Luciano Garofano, PhD*, Accademia Italiana di Scienze Forensic, Via G. D'Annunzio n.9, Parma 43100, ITALY*

After attending this presentation, attendees will be aware of the scientific and judicial issues that have characterized the Italian criminal trial of Massimo Bossetti, who was accused of the murder of Yara Gambirasio.

This presentation will impact the forensic science community by exposing the complex investigative techniques that led to the killer.

The Case in Brief: The case refers to the murder of Yara Gambirasio, a 13-year-old girl, that occurred on November 26, 2010, in the small town of Brembate di Sopra, Italy.

Three months after Yara's disappearance, on February 25, 2011, her partly decomposed, frozen corpse was found. Despite having a dozen knife lacerations to her throat and back, Yara died of exposure.

After Yara's death, the police launched a massive screening program which involved not just combing DNA data banks, but administering 15,000 voluntary DNA tests on woman and men living in the area. Nothing so large had previously been attempted anywhere in the world. For the first time, the genetic screening of an entire territory was implemented.

Among those asked to undergo the voluntary DNA tests was Damiano Guerinoni, whose DNA was very similar to that of the killer (nicknamed "Ignoto Uno"). This young man was unquestionably a close relative of the killer, so the police began to investigate his family. His father was one of 11 brothers and sisters. One of Damiano Guerinoni's uncles, Giuseppe Guerinoni, had died 11 years before, so the police decided to go to his widow's house in order to sample traces of DNA. On a sheet of paper a small part of DNA was found that was perfectly compatible with DNA found on the girl.

Giuseppe Guerinoni and his wife had three children, and all three were quickly excluded as suspects. It was then clear that Giuseppe Guerinoni must also have had a male child out of wedlock. The police now had to track down the illegitimate child of Giuseppe Guerinoni.

The police located one of Giuseppe Guerinoni's former colleagues, Vincenzo Bigoni, who said that Giuseppe Guerinoni was a ladies' man. The police identified 532 women still living whom Giuseppe Guerinoni had known in his life and with whom he could have had sexual relations.

These women included Ester Arzuffi, who consented to a DNA test without protest. Her DNA was a perfect match for that found on the dead girl — perfect in the sense that she had the female part of the DNA of "Ignoto Uno," as would the mother of the killer. Arzuffi had been married to the same man, Giovanni Bossetti, since 1967. The couple had three children, two twins (a boy, Massimo, and a girl, Letizia) and a younger son. Everyone had always assumed the father of all three of the children was her husband, but DNA tests revealed that the father of her twins was Giuseppe Guerinoni.

One Sunday evening in June 2014, the police set up a roadblock near Massimo Giuseppe Bossetti's home in Mapello and flagged down his car. They breathalyzed him and he passed, so they let him proceed; however, they now had his DNA on the breathalyzer tube which they swiftly sent to the laboratory for analysis. The match between the DNA on the breathalyzer tube and the DNA on the dead girl's clothing was clear. Massimo Bossetti was the killer.

The Process: On February 28, 2015, the investigation was closed, and Bossetti remained the only suspect. On April 27, 2015, the trial of Yara Gambirasio versus Bossetti began. The charge was murder with the aggravating circumstance of cruelty. On July 1, 2016, the Court of Assise of Bergamo sentenced Bossetti to life imprisonment for the murder.

The Critical Aspects of the Process: The first critical aspect is the abnormality found by the results of mitochondrial DNA and nuclear DNA in one of the samples analyzed. All biological traces sampled from the slip and the leggings of the victim referred to the same male profile that matched the defendant's DNA, except for one sample that revealed a different mitochondrial DNA. In that specific sample, the mitochondrial DNA was different from the defendant's!

The second critical aspect is from a procedural point of view. The DNA was analyzed within the investigation phase, during a period of time in which the defendant was not suspected of the crime and without any guaranteed representation by the presence of his consultants. Furthermore, the analysis cannot be repeated because the DNA has been fully utilized.

DNA, Murder, Yara Gambirasio

F18 Trouble With Y: Tribal Populations Cannot Be Pooled

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After attending this presentation, attendees will have learned that with current population data, a reasonable statistical evaluation of Y haplotype matching evidence in a Native American context is impossible. Therefore, its use at trial is precluded.

This presentation will impact the forensic science community by alerting attendees to appreciate: (1) the inadequacy of pooled Y haplotype population data in place of tribe-relevant data for tribal populations such Native Americans; and, (2) the confused and therefore confusing nature of a formula that Scientific Working Group on DNA Analysis Methods (SWGDAM) recommends.

Native Americans account for a large share of individuals prosecuted by the federal government for sexual assault. Y haplotype DNA is often vital evidence in sex cases; however, because Native Americans constitute a small segment of the United States population, too little attention has been given to the vital and distinguishing fact of tribal population structure, though the work of Hammer is important and helpful.[1]

In several recent Arizona sexual assaults, a Y haplotype match was featured as evidence. No population data was available for Y haplotypes of the relevant tribe. Instead, the prosecution offered a statistic based on pooled data from Native Americans in general. In one case, after a *Daubert* hearing, the court correctly understood that pooling Native Americans as a substitute for data from the relevant tribe "manufactures diversity" and therefore excluded the Y haplotype evidence.

Y haplotypes are patrilineally transmitted DNA data, copied as a unit from father to son, accurate except for the occasional (once per twenty generations) mutation. The Y picture is very different from the more typical autosomal forensic DNA (which comes from the 22 chromosome pairs excluding Y or X) primarily (though not only) because Y mutates much faster. (That's because the relevant sense of mutation here is any change in the transmitted unit, which is a single locus for autosomal but a block of about 17 loci for Y. Hence, the relevant mutation rate for Y is 17-fold faster.) Still, Y mutations are infrequent enough that a tribe arising from a small group 500 years ago will not have accumulated very much diversity. Hence, the chance for an innocent tribe member to be inculpated by a coincidental match is large. On the other hand, mutation is fast enough that over the 14,000 years Native Americans have inhabited the Americas, great diversity, mostly *between* tribes, has evolved. Therefore, a pooled database covering more than 100 tribes with common pairwise ancestors often 5,000 or more years in the past exhibits great diversity. The consequence, when it is used as it was in the Arizona cases, is to artificially exaggerate the strength of the evidence against the suspect. This is true even though the method of calculation of the matching statistic intends to be quite conservative by making statistical allowances.[2] But statistical allowances may not and do not compensate for the fundamental statistical sin of using population data that is not approximately representative of the relevant population.

The matching chance claimed from the pooled North American data was 1/35 — one chance in 35 that a random innocent man, if that's what the suspect was, would match. The above theoretical discussion explains why the procedure is wrong, but is it a genuine practical concern that the very modest-seeming prosecution 1/35 claim really overestimated the chance of a random match? Research from Finland says yes.[3] It reports a remarkable lack of diversity among men in some regions of Finland. For example, among the men in a region a few hours drive from Helsinki, two random men have one chance in ten to match — considerably larger than the supposedly generous estimate of one in 35. The Finns are not even an obviously tribal people. At a minimum, it is appropriate to regard evidence assessment based on the overall Native American database as merely random numbers.

Reference(s):

1. Hammer M., Redd A.J. (Jan 2006) *Forensic Applications of Y Chromosome STRs and SNPs*. Technical research report prepared US DOJ grant and submitted to the DOJ.
2. SWGDAM Y-chromosome Short Tandem Repeat (Y-STR) Interpretation Guidelines. http://www.fbi.gov/.../fsc/oct2009/standards/2009_01_standards01.htm/.

3. Palo *et al* (2007) High degree of Y-chromosomal divergence within Finland – forensic aspects. *Forensic Science International: Genetics*.(2007)1:120-124.

Y Haplotypes, Native American, Forensic Mathematics

F19 Moving Toward New Requirements for the Admissibility of Evidence

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After attending this presentation, attendees will better understand how forensic science expert witnesses can offer their expert opinions meeting *Daubert* standards in subjects in which there are presently little or no statistics available upon which to base their conclusions, such as in cases involving pattern evidence.

This presentation will impact the forensic science community by providing lawyers and judges an understanding of the difficulties experts have in framing their expert opinions in cases in which they cannot rely on significant amounts of research with academic institutions or on probabilities to express the degree of certitude they are attempting to convey to the triers of fact.

Nearly 70 years after the *Frye* rule, the so-called general acceptance test, *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, affirmed Rule 702, Federal Rules of Evidence, the *Frye* rule, and modified the admissibility requirements for scientific evidence.[1] In 1999, the Court published the *Kumho Tire Co. v Carmichael* case and extended the rule to include both scientific and technical expert testimony.[2]

In *Kumho Tire*, the courts considered expert testimony in a case concerning tire manufacture and the resulting tire failure. The expert in *Kumho Tire* relied upon his experience in tire failure. The court noted that his examination did not meet the *Daubert* obligation. It went on to opine that all expert opinions, whether based on scientific and/or technical subject matter, are required to meet the same standards. (The expert grounded his opinion on observations made on the tire in question and testified that his opinion was based on his experience but did not give scientific or technical information to back up that opinion.) The court concluded that an expert's testimony had to demonstrate that the techniques used in the examination and subsequent testimony were reliable and met the same standards laid out in the *Daubert* case.

Thus, expert opinion testimony, whether scientific or technical, is subject to the same requirement as outlined in the Federal Rules of Evidence, Rule 702: (1) that the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (2) that the testimony is based on sufficient facts or data; (3) that the testimony is the product of reliable principles and methods; and, (4) that the expert has reliably applied the principles and methods to the facts of the case.

Embedded within the court's requirement to ensure the dependability of the forensic science laboratory's work and the expert's testimony is the notion of reliability. In *Daubert*, the court suggests that the following factors may be considered as a means to determine the admissibility of scientific evidence: (1) Has the technique been tested in actual field conditions (and not just in a laboratory)?; (2) Has the technique been subject to peer review and publication?; (3) What is the known or potential rate of error?; (4) Do standards exist for the control of the technique's operation?; (5) Has the technique been generally accepted within the relevant scientific community?; and, (6) Expert witnesses need to give consideration on how they might respond to such questions.

One particular subject for consideration is how experts express levels of certainty — how sure are they about their opinion? At one time, experts could state, with virtual certainty, that two items of evidence were unique and came from a common source. It was not uncommon for a fingerprint expert to state that two prints came from the same person, to the exclusion of anyone else. Today, a statement expressing that level of certainty is likely to be challenged. Probabilistic statements are viewed as the appropriate way to express levels of certitude.

Here is a complication: how can we express the likelihood that two items came from a common source when their probabilities of occurrences are unknown? Given a shoe print with wear patterns and gouges in the heal and sole, we do not have a numeric probability value to suggest the uniqueness of those marks. It is not possible to testify when probabilities of occurrence are unknown. How are experts to express their opinions about the likelihood that two items came from a common source when statistics are not available and perhaps may never be available?

Indeed, opinions concerning the admissibility of scientific and technical expert evidence have a ways to go before these issues are fully dealt with. Until then, we may be faced with *ipse dixit* testimony (e.g., "based on my training and experience, it's my opinion that the items are consistent with, or a match for, a common source!") Will that be adequate?

Reference(s):

1. *Daubert v. Merrell Dow Pharmaceuticals, Inc.,* 509 U.S. 579, 113 S.Ct. 2786.
2. *Kumho Tire Co. v Carmichael,* 526 U.S. 137

Admissibility, *Daubert*, Probability

F20 Science and Law: Ships Passing in the Night

Donald E. Shelton, JD, PhD, University of Michigan-Dearborn, Criminal Justice Program, 4901 Evergreen Road, Dearborn, MI 48128-2406*

After attending this presentation, attendees will have a better understanding of the ongoing reluctance of the judicial system to accept scientific conclusions about faulty forensic science evidence and appreciate the basic conflict between the scientific search for truth and the legal reliance on precedent and *stare decisis*.

This presentation will impact the forensic science community by demonstrating how the legal system functions so differently than the scientific community and by informing how and why the courts are so resistant to change.

Nearly eight years ago, the National Academy of Sciences (NAS) found that, with the exception of DNA, the scientific basis for most forms of forensic science evidence had never been established. Nevertheless, most criminal courts continue to routinely admit almost all prosecution expert testimony. For the most part, judges, trial or appellate, do not weigh the scientific validity of evidence, as *Daubert* mandates. Most judges simply rationalize admissibility based on the prior admission of such evidence by other judges. The typical analysis is *stare decisis*, rather than the scientific inquiry required by *Daubert*. Why this disconnect between science and the law? Why do judges ignore scientific conclusions about faulty evidence that can, and does, lead to the conviction of innocent people?

One explanation may be the basic pedagogical differences between legal training and scientific study. The scientific method is a method of research in which a problem is identified, relevant data are gathered, a hypothesis is formulated from these data, and the hypothesis is empirically tested. It is designed to detect error and to rule out theories that are not true. Science seeks to explain reality on the basis of objective verification rather than subjective opinions, then to repeatedly verify that explanation.

On the other hand, American legal training is based on the concept of precedent and *stare decisis*, that the answer to present questions can always be found by looking to the past. For every legal issue, lawyers are taught not to seek the answer objectively based on the available information, but rather to simply ask how judges have answered the question in the past. When lawyers become judges, they utilize that same method that they were taught and that they have honed as practicing attorneys. The law is a search for certainty while science is a search for truth.

One hundred fifty years ago, Tocqueville observed that "Americans have retained the law of precedents; that is to say, they continue to found their legal opinions and the decisions of their courts upon the opinions and decisions of their predecessors. In the mind of an English or American lawyer a taste and a reverence for what is old is almost always united with a love of regular and lawful".[1] Oliver Wendell Holmes said: "It is revolting to have no better reason for a rule of law than that it was laid down in the time of Henry IV. It is still more revolting if the grounds upon which it was laid down have vanished long since, and the rule simply persists from blind imitation of the past".[2]

There are reasons for the law's reliance on precedent. It fosters stability and predictability. But, it also reflects a cognitive bias originating in our human tendency to prefer the status quo and to develop simple explanations for observed problems.[3]

These conflicting approaches to discovering truth and error come into stark conflict in the latest scientific revelations about forensic science. *Daubert* places the gatekeeping responsibility for the admission or barring of allegedly scientific evidence on judges who are not trained, or inclined, to look beyond what other judges have done in the past. Relying on opinions by judges yesterday who did not have the information that is available today, most of the judiciary continues to admit whatever has been admitted in the past. In some cases, courts even take "judicial notice" of the reliability and admissibility of forensic science evidence because many prior judges have admitted it.

With the mounting wrongful convictions attributed to faulty forensic science evidence, the law's relationship to science and technology is critical. Small changes are being made, mostly from the legislative and administrative sectors. Those piecemeal changes are not fundamentally affecting court admissibility decisions in any meaningful way nor does the adversarial system seem capable of convincing judges to even question the reliability of government evidence.

Reference(s):

1. Alexis de Tocqueville. *Democracy in America*. Ch. 16 (1840).
2. Oliver Wendell Holmes, Jr. T*he Path of the Law*. 10 Harvard Law Review 457 (1897).
3. Goutam U. Jois. *Stare Decisis is Cognitive Error*. 75 Brooklyn L. Rev. 63 (2009).

Science and Law, Admissibility, Legal Precedent

F21 Dealing With Len Bias Laws and *Burrage v. United States*: Best Practices to Avoid Potential Pitfalls for Attorneys and Medical Examiners

Stephanie Domitrovich, JD, PhD, Sixth Judicial District of PA, Erie County Court House, 140 W 6th Street, Rm 223, Erie, PA 16501; and Jeffrey M. Jentzen, MD*, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109*

After attending this presentation, attendees will understand the medicolegal aspects of the current heroin epidemic and the potential pitfalls in the successful courtroom presentation of drug-related deaths.

This presentation will impact the forensic science community by illustrating the need for pre-trial coordination between attorneys, law enforcement, first responders, and forensic experts in the successful adjudication of drug-related deaths.

The opiate crisis in the United States fueled an expansion of numerous drug delivery case prosecutions known as "Len Bias" cases. Len Bias, a stellar University of Maryland basketball player, died in 1986 allegedly of cocaine toxicity two days after becoming the number-two draft pick of the Boston Celtics. Bias's death was the tipping point for instituting the 1986 Anti-Drug Abuse Act by Speaker of the House Tip O'Neil, a Boston native. This legislation increased incarcerations as a result of mandatory 20-year federal prison sentences for drug possession and delivery. Not until recently, on May 19, 2016, did Maryland eliminate its mandatory drug sentences; however, such laws still exist in other state and federal jurisdictions.

An increasing number of states have recently criminalized drug delivery causing death, charging homicide. While some jurisdictions charge heavily, others, such as Wayne County (Detroit), rarely invoke these charges, claiming that "police have not brought us any cases where we have been able to charge."[1] Successful prosecution of drug-related deaths requires coordination between attorneys, law enforcement, and first responders.

Recently, the United States Supreme Court recently in *Burrage v United States* 571 U.S. ___ (2014) unanimously reversed and remanded a 20-year mandatory minimum sentence of an alleged heroin dealer and held "at least where use of a drug distributed by the defendant is not an independently sufficient cause of the victim's death or serious bodily injury," a defendant cannot be liable under the penalty enhancement provision of the Federal Controlled Substances Act, unless such use is a "but-for" cause of the death or injury.[2] The Unted States Supreme Court rejected the government's "contributing cause" test. Their rationale will be discussed in detail in this presentation.

The metabolism and toxicity of heroin, a synthetic narcotic of morphine that is rapidly metabolized and rarely detected in the body, will be discussed. Heroin is first metabolized to 6-Monoacetylmorphine (6-MAM) and later to morphine. Presence of 6-MAM is diagnostic of heroin use. Codeine is frequently a contaminant of the manufacturing process and is commonly detected in heroin users. Some toxicologist/pathologists use a morphine/codeine ratio of greater than ten to be indicative of heroin use.

Although the legal requirement for causality requires an application of a "but for" standard, medical literature reports between 25% to 40% of postmortem examinations reveal a major pathological finding not identified prior to death. Decedents may have co-morbidities, such as victims having severe coronary artery disease as well as the use of cocaine, raising questions of potential competing causes of death.

Many medical examiners may forego autopsy examinations, electing to rely solely on the detection of drugs in the body. Drug concentrations alone are not a reliable cause and manner of death. Patients could develop a tolerance to medications (opiate). Few definitive lethal levels of drugs, such as cocaine and opiate tolerance, exist.

Medical examiners must work under increasing economic and logistical pressures to make diagnoses in the most cost-effective and efficient manner, often resulting in a rationalization of these deaths without actually demonstrating the drugs involved, which may influence the ability to proceed. For example, Washtenaw County, Ann Arbor, MI (pop. 350,000) typically records 40 to 50 heroin deaths per year, of which 15.9% to 22.5% of cases are negative for 6-MAM in the blood and urine, detected only upon further testing of vitreous fluid.

Victims may be under the influence of multiple drugs identified in the body. The bodies of victims of drug toxicity will usually contain multiple drugs from various providers. Half of heroin deaths solely contain heroin, while the remaining cases contain a mixture with other potentially fatal drugs.

Physiological factors inherent to the victim, such as postmortem drug redistribution and pharmacogenomics, may influence postmortem concentrations of these drugs, making medical examiners' interpretations relatively impossible to determine.

The American College of Medical Toxicology and the National Association of Medical Examiners convened an expert panel of pathologists and toxicologists to determine best practices in these cases.[3] Their findings included obtaining complete autopsies with toxicology results as well as considering the context of circumstances surrounding death, medical history, and scene findings; completing scene investigations and obtaining prescription information and pill counts; retaining blood, urine, and vitreous humor; conducting toxicological panels for opioid and benzodiazepine analyses, as well as other potent depressant, stimulant, and anti-depressant medications; interpreting postmortem opioid concentrations in correlation with medical history, scene investigation, and autopsy findings; and other best practices, which will be discussed.

Reference(s):

1. Eric Litke. More States Push Homicide Charges in Heroin Overdoses. *USA Today* 25 July 2014.
2. *Burrage v. United States*, 571 U.S. ___ (2014).
3. Gregory Davis and the National Association of Medical Examiners and American College of Medical Toxicology Expert Panel on Evaluating and Reporting Opioid Deaths. Recommendations for the Investigation, Diagnosis, and Certification of Deaths Related to Opioid Drugs. *Acad Forensic Pathol.* 3,1 (2013): 62-76.

Drug-Delivery Deaths, Heroin Deaths, Len Bias Law

F22 "You Have the Right to Remain Silent": Autopsy Reports and a Defendant's Sixth Amendment Confrontation Rights

Stephanie Domitrovich, JD, PhD, Sixth Judicial District of PA, Erie County Court House, 140 W 6th Street, Rm 223, Erie, PA 16501; and Jeffrey M. Jentzen, MD*, University of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109*

After attending this presentation, attendees will understand the limitations of out-of-state subpoenas, the requirements of the Confrontation and Compulsory Process Clauses, and the process expert witnesses may wish to follow in the event of responding to subpoenas.

This presentation will impact the forensic science community by educating expert witnesses on the availability of legal measures to respond to out-of-state judicial decrees.

A forensic pathologist, who has practiced for 30 years, receives a subpoena from another state requesting court appearance with five days' notice on a 20-year-old homicide case in the location of his/her previous employment. The forensic pathologist declines to appear. In another instance, a prosecutor orders an exhumation on a 30-year-old homicide case to re-examine a gunshot wound on the skeletonized body due to the fact that the original pathologist who performed the autopsy has died.

Over the course of a career, forensic pathologists perform thousands of autopsies. Forensic pathologists serve as expert witnesses and regularly receive subpoenas to testify before fact finders. In some instances in which the original pathologist, who performed the autopsy and authored the report, is unavailable due to death or distance, another forensic pathologist will frequently testify before the court. Is the autopsy report considered a business record and can be admitted without testimony of the author, or is the autopsy report to be considered simply as a testimonial, which triggers a defendant's Sixth Amendment Confrontation Clause rights and provides the defendant in a criminal case the right to confront the witnesses testifying against the defendant? Does the forensic pathologist possess rights within the Compulsory Process Clause? Can the pathologist be compelled to testify in an out-of-state trial?

In *Crawford v Washington*, the United States Supreme Court held that admission of out-of-court statements of witnesses who do not appear at trial is prohibited by the Confrontation Clause if the statements are testimonial unless witnesses are unavailable and the defendant has had the prior opportunity to cross-examine the witnesses.[1] The United States Supreme Court did not define the word "testimonial" but stated in general terms that the primary class of statements implicated by the Confrontation Clause includes statements made under circumstances leading an objective witness reasonably to believe that statement would be available for use at a later trial.[2]

In *Williams v Illinois*, a majority of United States Supreme Court justices held expert witness testimony on a DNA profile produced by an outside laboratory from a rape victim's vaginal swabs matching the defendant's DNA profile produced by a state police laboratory from the defendant's blood sample did not violate a defendant's right to confrontation.[3,4] The DNA report itself was not admitted into evidence and was not shown to the fact finder. The expert witness also did not quote or read from the report or identify the report as the source of any of her opinions. Four of the five justices reasoned that the statements in the DNA report were non-testimonial because, first, the out-of-court statements were related by the expert solely for the purpose of explaining the assumptions on which the expert's opinion relied and were not offered for their truth. Secondly, even if the DNA report had been admitted into evidence, it was not a testimonial document because it was not prepared for "the primary purpose of accusing a targeted individual," which distinguished this report from the forensic reports in cases of *Melendez-Diaz* and *Bullcoming*. The United States Supreme Court delayed "for another day any effort to spell out a comprehensive definition of 'testimonial." States are divided on this issue.

The Rhode Island Supreme Court and other state courts have adopted the Federal Rules of Evidence FRE 803(6) that identifies the autopsy report as having special attributes and qualifies as a business record rather than simply a testimonial. This allows the autopsy report to be received in evidence regardless of the absence of the pathologist who performed the autopsy and authored the original report.[5]

In addition, most states provide legislation directing subpoenas and other out-of-state judicial requests to be initially submitted to the district or county court of the expert's residence.[6] In this way, the expert has the ability to

file a motion to quash and to act upon the request through a local court. The expert has no requirement to recognize or respond to a decree rendered outside of his or her state and without following the required procedure.

Expert witnesses are mobile, frangible, and in limited supply. Laws and rules of court should facilitate the admission of evidence and expert testimony while balancing the need to comply with the Confrontation Clause and respecting the availability of expert witnesses.[7]

Reference(s):

1. *Crawford v. Washington*, 541 U.S. 36 (2004).
2. Sarah Clifton. Confronting the Coroner: The Admissibility of Autopsy Reports Post-Melendez-Diaz. *Federal Criminal Defense Journal*, Vol.III (2010); 1-26.
3. *Williams v. Illinois*, 132 S. Ct. 2221 (2012).
4. Ronald Coleman, Paul Rothstein. "Williams v. Illinois and the Confrontation Clause: does Testimony by a Surrogate Witness Violate the Confrontation Clause?" *PublicSquare.net.* (Dec. 6, 2011*).* http://publicsquare.net/williams-v-illinois-and-the-confrontation clause-part-I.
5. *Federal Rules of Evidence FRE 803(6).*
6. *Michigan Complied Laws Act 236 of 1961 Section 600.1852(2).*
7. Daniel Capra and Joseph Tartakovsky, " Autopsy Reports and the Confrontation Clause: A Presumption of Admissibility. 2 V.C.J.L. 62 (2014).

Confrontation Clause, Compulsory Process Clause, Sixth Amendment

F23 Lonesome Dove: The Solitary Life of a Forensic Laboratory Legal Advisor

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After attending this presentation, attendees will gain an understanding of the complexities involved with providing legal counsel to a large forensic analysis laboratory that is comprised of traditional forensics, military operational forensics, and research.

This presentation will impact the forensic science community by illustrating the challenges presented when serving as an intermediary between forensic scientists, research scientists, and the legal community. This includes identifying the customer and understanding their proposed end state.

The Defense Forensic Science Center (DFSC), of which the United States Army Criminal Investigation Laboratory (USACIL) is a part, provides full-service forensic laboratory support to the Department of Defense (DOD) criminal investigative organizations and other DOD customers. In addition to forensic services relating to DNA identification, digital evidence, drug chemistry, firearms and toolmarks, forensic documents, latent prints and trace evidence, the USACIL provides training to DOD special agents, investigators, prosecuting attorneys, and defense attorneys. There are two attorneys assigned to the organization serving more than 300 employees, including 186 examiners. In support of the forensic examiners, attorneys assist with discovery preparation, mock trials, and trial preparation.

Being a part of a very small subset of attorneys advising forensic laboratories, laboratory attorneys must be self-sufficient learners, educators, and mediators. Attorneys must remain proficient in court procedures and core legal disciplines. Likewise, they must stay abreast of scientific terminology and methodologies, understand the scientific method as it relates to research and validation studies, and still find innovative ways to make this information relevant and understandable to attorneys in the field. They must understand the jurisdictional standards for the admissibility of scientific expert testimony and be able to explain these terms to scientists.

When laboratory attorneys encounter issues regarding legislative issues, *Brady* notifications, and *Daubert* challenges to forensic disciplines, to whom do they turn? What resources are available? Moreover, what happens when the forensic laboratory becomes the test-bed for novel means of reporting results? How does one explain that what may be scientifically responsible may not be readily acceptable by the legal community? Often, what is considered scientifically relevant and legally relevant may diverge as the word "relevant" has a different meaning for lawyers than it does for scientists. What happens when an examination is requested that the scientists do not believe is scientifically relevant or capable of answering the ultimate question?

For the laboratory attorney ever seeking to bridge the gap between science and the court room, everyone's opinion must be considered and addressed.

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

Judicial, Education, Science

F24 The New Standard: The New York State Experience Post-Sean John

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After attending this presentation, attendees will learn who is an appropriate DNA expert witness to call at trial after a *Crawford* challenge and what information to elicit on direct examination.

This presentation will impact the forensic science community by illustrating the challenges in preparing a DNA expert for trial and presenting the evidence without violating the basic tenets of the Sixth Amendment.

In April 2016, the New York State Court of Appeals handed down a four-to-three decision in *People v Sean John*. In this case, the defendant was involved in a fight outside his home during which it was alleged he pointed a firearm at a neighbor. When the police arrived to the scene, another neighbor informed the police that they had observed the defendant enter the common area of the basement with something in his hand. The officers entered the basement and found a box which contained a loaded 9-millimeter handgun. The victim in the case later identified that firearm as the same one that the defendant had pointed at him earlier in the day. That firearm was swabbed by the New York Police Department (NYPD) Evidence Collection Team, and the buccal swabs were submitted for DNA testing.

During the trial, the People called the assigned criminalist who signed the DNA reports to testify to the DNA testing and the match to the defendant's DNA profile. The Court of Appeals held that such practice was a Sixth Amendment Confrontation Clause violation because the People introduced the DNA reports, which implicated the defendant into evidence using one expert who was permitted to "parrot" the recorded findings that were derived from other criminalists' casework. The Court held that an analyst who witnessed, performed, or supervised the generation of a defendant's DNA profile, or who used his or her independent analysis of the raw data, but not merely a testifying analyst functioning as a conduit for the conclusions of others, must be available to testify at trial. The Court did clarify that not every analyst who has contact with the evidence must testify at trial, rather just one individual who has analyzed the raw data and electropherogram edits and comes to his or her own, independent conclusion as to the results. A DNA laboratory that uses a multiple-analyst model may now need to modify their standard operating procedures so that a single analyst is qualified to testify as to the DNA profile testing. For example, an analyst who generated the DNA profile from one sample may also observe the final stage of testing or retesting involved in the generation of the other profile or comparison.

The decision in *People v Sean John* reflects a sharp deviation in standard practice for prosecutors who commonly call the assigned criminalist to testify at trial. The holding poses significant challenges for lawyers and criminalists alike as it calls into question who exactly is the appropriate expert to call at trial and what level of detail must be elicited on direct examination. This decision will require experts to dedicate more time in preparing for direct examination by extensively reviewing the raw data, bench notes and edit sheets, as well as the post-edit electropherograms created by other criminalists before testifying at trial.

Reference(s):

1. *The People of the State of New York, Respondent, v Sean John, Appellant*. 26N.Y.3d 1101 (2016).

Litigation, Expert, Sixth Amendment

F25 The Academy Standards Board (ASB) for Firearms and Toolmarks: Legal Issues

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After attending this presentation, attendees will better understand the progress of the ASB on Firearms and Toolmarks toward developing consensus on forensic standards. In particular, issues that relate to lawyers and courts will be emphasized in this presentation so the Jurisprudence Section members and others in attendance can participate in developing consensus on those legal issues.

This presentation will impact the forensic science community by providing information about the ASB's work on standards for forensics and tool marks, and feedback from the participants will impact the work of the ASB itself.

The Annual Meeting of the American Academy of Forensic Sciences (AASF) in New Orleans, LA will include reports and information regarding the work of the ASBs. The Chair of the ASB for Firearms and Toolmarks, who is a member of that ASB representing issues of jurisprudence as an academic and practicing lawyer, will seek to update the Jurisprudence Section regarding legal issues arising in the ASB and to obtain a sense of the thinking of the Section to return to the ASB. While the Firearms and Toolmarks ASB is concerned with all aspects of the forensic disciplines involved, the Jurisprudence Section will be particularly able to help elucidate aspects related to how these disciplines interact with lawyers and the courts.

As most know, the AAFS has been authorized by American National Standards Institute (ANSI) to create consensus Standards Boards in several of the Forensic disciplines. ANSI, the leading private industry standards group in America, oversees the creation, promulgation, and use of norms and guidelines that affect commerce in this country. As acknowledged by Past President Dr. Victor Weedn, it is both an honor and an opportunity for AAFS to be involved in this process.

In the past 20 years, with increased news of wrongful convictions, there has been a national effort to create standards for the forensic science disciplines, beginning with industry groups and continuing with the Scientific Working Groups (SWGs) created by the Federal Bureau of Investigation (FBI). Following the 2009 National Academy of Sciences (NAS) Report, there was an effort to move standards creation away from the FBI and from the domination of law enforcement in general. AAFS was supportive of this and has participated with the National Institute of Standards and Technology (NIST) and the Organization of Scientific Area Committees (OSACs).

While NIST is within the Department of Commerce, it has been partnered with the Department of Justice (DOJ) in the National Commission on Forensic Science (NCFS). The NCFS, created in 2013, develops policy recommendations for the Attorney General regarding forensic science. The commission is co-chaired by Deputy Attorney General Sally Q. Yates and Under Secretary of Commerce for Standards and Technology and NIST Director Willie E. May. It has been noted that the NAS Report had recommended an independent federal agency be entrusted with this role. The reporting of the OSACs to NIST and to the NCFS for recommendation to the Attorney General has now been characterized by some as keeping forensic standards under law enforcement control.

On the other hand, the DOJ itself, independent of NIST and the OSACs, promulgated its own standards for forensic disciplines during the summer of 2016. The DOJ also has actively participated directly with AAFS through the attendance and participation of Deputy Attorney General Yates and the support of the Attorney General herself. Thus, the DOJ has demonstrated its sincerity in improving forensic standards on its own, through AAFS and in its participation with NIST in the OSAC process. Nevertheless, ANSI and the AAFS have concluded that consensus among all of the stakeholders, reached in a non-governmental setting, would be a valuable contribution to the overall process.

As of this writing, the Firearms and Toolmarks ASB (F&T ASB) had an organizational meeting in July 2016 and has scheduled its first full working video conference meeting in September 2016. By the time of this presentation in February 2017, the F&T ASB should have completed other video conferences and an in-person meeting at the AAFS Annual Meeting itself. Given the strength of the other members of this ASB, February 2017 should be a good time to report to the Jurisprudence Section and other interested AAFS members on the legal issues being addressed by the group. It is hoped that those in attendance at the Annual Meeting will be able to provide constructive feedback for delivery to the F&T ASB.

Forensics and Toolmarks, Academy Standards Board, Legal

F26 *Frye*, *Daubert*, or None of the Above: What Rules Govern Admissibility of Scientific Evidence in Court?

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The goals of this presentation are to: (1) familiarize attendees with the criteria courts use in state and federal jurisdictions for admissibility of scientific evidence; (2) explore the differences between *Frye*, *Daubert*, and hybrid tests for admissibility; and, (3) explore whether the tests for admissibility are truly different — or if they should be.

Lawyers are frequently faced with the need to present scientific evidence to courts and to juries. Judges are charged with deciding whether such evidence is sufficiently helpful and reliable to be admissible in court. This presentation will impact the forensic science community by helping lawyers, judges, and science professionals to understand the standards various courts use in deciding whether scientific evidence can be presented in court.

After attending this presentation, attendees will understand the criteria that federal courts, as well as courts in the 50 states, apply when deciding whether scientific or technical evidence can be presented in court. Some courts apply the so-called "*Frye"* standard, while the federal courts and many state courts apply the so-called "*Daubert*" standard. Still other jurisdictions apply a hybrid standard. This presentation will explore what these tests for admissibility are, how they differ, and how lawyers and scientists can present information as effectively as possible to maximize the likelihood that the scientific and technical evidence they wish to present in court will be allowed.

The *Frye* test derives from a 1921 case out of the District of Columbia Circuit, where the court refused to admit evidence of polygraph test results, because the court found that the science and reliability of polygraph testing "has not yet gained such standing and scientific recognition among physiological and psychological authorities as would justify the courts in admitting expert testimony deduced from the discovery, development, and experiments thus far made."[1] The line of legal authorities descended from *Frye* have required that the scientific theory underlying the evidence and the technique or methodology used to implement it, must be generally accepted in the scientific community for the evidence to be admissible in court. Some jurisdictions require only the first factor for admissibility; others require both factors to be shown before scientific evidence may be presented in court

In *Daubert v Merrell Dow Pharmaceuticals, Inc.*, the United States Supreme Court announced a new standard for admissibility of scientific evidence in federal courts.[2] The test requires a trial judge to analyze several factors before allowing expert testimony on scientific or technical matters: (1) whether a theory or technique can be tested; (2) whether it has been subjected to peer review and publication; (3) the known or potential error rate of the theory or technique; and, (4) whether the theory or technique enjoys general acceptance within the relevant scientific community.

Some jurisdictions have tacked on additional requirements before scientific or technical evidence can be allowed in court.

Hard as it may be to imagine, there is disagreement among courts and among legal commentators as to whether *Frye* or *Daubert* is the more rigorous test for the admissibility of scientific or technical evidence. Regardless, it is important for lawyers and forensic scientists to be familiar with the criteria for admissibility and to be prepared to meet those criteria.

This presentation will include a paper containing a survey of the criteria that courts from all 50 states and the federal courts use in deciding whether scientific and technical testimony will be allowed into evidence. This presentation will also include a discussion concerning how these various criteria can be reconciled or harmonized.

Reference(s):

1. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).
2. *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993).

Admissibility, *Daubert, Frye*

F27 Lawyer-Scientist: Issues of Competency, Validation, and Ethics

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After attending this presentation, attendees will understand the competency, validation, and ethical problems inherent in the term "Lawyer-Scientist.'

The presentation will impact the forensic science community by discussing improprieties of using the self-proclaimed moniker "Lawyer-Scientist."

The Ozian Option: "I can't give brains, but I can give you a diploma" is a similar scenario to the "Lawyer-Scientist" situation.

The practice of law is a business in a very competitive market. Attorneys attempt to distinguish themselves through marketing their accomplishments or specialization. Any licensed attorney can purchase their promotional "Lawyer-Scientist" moniker by completing three separate one-week courses sponsored by the American Chemical Society's (ACS's) Law Division. No science background is required. The fee-based introductory forensic gas chromotography course is predominantly for attorneys who represent clients charged with drug- and alcohol-related traffic offenses. Purported accreditation and certification is through participation in a private laboratory, not a governmental licensing authority, academic institution of higher learning, or attorney state licensing authority. Continuing education and training are not offered or required.

Upon completion of the course, the attorney may possess something akin to the initial knowldege of a technican; however, the scientist, generally conducts research and experiments, designs and creates instrumentation and applied techniques, and publishes and advances knowledge in their field.

Programs and courses are offered for attorneys to provide them with intense training for a better understanding of the scientific methods and analyses in their field. Attendees are susceptible to the false impression that investing a few weeks of time qualifies them to make refined and complicated scientific judgments on matters involving areas of forensic science.[1] Knowledge of gas chromatography cannot be acquired through a short course. Completion of the ACS short course does not transform the attorney into a scientist.

ACS sanctioned the establishment of "The ACS Forensic Lawyer-Scientist Designation as recognized by the Chemistry and Law (ACS-CHAL) Division of the ACS."[2] The carefully worded designation was shortened to "The ACS-CHAL Forensic Lawyer-Scientist Designation" or just "ACS-CHAL Forensic Lawyer-Scientist."; however, attorneys simply use "Lawyer-Scientist" in their promotional advertising.[2] Both terms are readily subject to misrepresentation and misinterpretation. The program was apparently approved by the ACS without realizing the ulterior marketing motives nor being properly apprised of legal, moral, and ethical implications. Additional course information is at the "Lawyer-Scientist | ACS Hands-On Forensic Chromatography Course" website.[2]

The courses are conducted by Axion Laboratories, Inc., which is a private commercial business in Chicago, IL. The courses are not certified by any agency or authority.[3] Certification establishes trust in the protocols, quality control, training, instructor qualifications, accreditation, certification examinations, and independent review.[3]

Is the "Lawyer-Scientist" engaging in intellectual dishonesty, deceptive self-promotion, or unregulated ethics for a competitive financial position? An attorney's integrity is paramount. Attorneys are bound by legal standards and a code of ethics to foster integrity, honesty, competency, and public trust. Every state has strict codes of ethics for lawyers derived from the American Bar Association (ABA) Model Code of Professional Responsibility.[4] Under Canon 1, DR 1-102, a lawyer "shall not engage in conduct involving dishonesty, fraud, deceit, or misrepresentation."[4] Canon 2, EC 2-9, lists examples of deceptive advertising, including: "misstatements of fact, suggestions that the ingenuity or prior record of a lawyer rather than the justice of the claim are the principal factors likely to determine the result, inclusion of information irrelevant to selecting a lawyer, and representations concerning the quality of service which cannot be measured or verified."[4] DR 2-101 states "a lawyer shall not use any false, fraudulent, misleading, deceptive, self-laudatory, or unfair statement or claim."[4] Under Canon 9, "a lawyer should avoid even the appearance of professional impropriety."[4]

The term "Lawyer-Scientist" is arguably misleading under DR 1-102 Promotional use of "Lawyer-Scientist" is

probably deceptive under EC 2-9, and might well violate DR 2-101. Attorneys advertising themselves as "Lawyer-Scientist" invite professional sanctions.

The ACS course provides useful training in familiarity with technical terms and procedures in gas chromotagraphy. Unfortunately, it is inappropriately used as a marketing tool to create misplaced client reliance on questionable trustworthiness and qualifications of the attorney. "Lawyer-Scientist" incorrectly insinuates scientific competence that is most probably misleading. Professional oversight and great scrutiny should be used. There are no degrees of honesty.

Reference(s):

1. Haack, Susan. *Evidence Matters: Science, Proof, and Truth In The Law*. Cambridge University Press, 2014.
2. Lawyer-Scientist | ACS Hands-On Forensic Chromatography Course, http://www.forensicchromatography.com/lawyer-scientist/, last visited August 1, 2016.
3. Axion Laboratories, Inc. website, http://axionlabs.com, last visited August 1, 2016; The importance of validation, See, Haber L., Haber R.N., 2009. *Challenges to Fingerprints*. Lawyers & Judges Publishing Company.
4. American Bar Association, 1983. *Model Rules of Professional Responsibility 2010*. American Bar Association.

Lawyer-Scientist, Scientific Competence, Professional Responsibility

F28 Constitutional Requirement to Litigate Scientific Evidence

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After attending this presentation, attendees will understand the United States Constitution's requirement to litigate scientific evidence.

This presentation will impact the forensic science community by analyzing constitutional standards of competency concerning the use of forensic science in the courtroom.

Criminal law is based upon constitutional law. Law enforcement agents extensively rely upon scientific principles and technology in criminal prosecutions. All cases involving criminal charges generally entail some aspect of scientific evidence and forensic science. Forensic science is used to convict the guilty and to protect and exonerate the innocent. It is the most persuasive evidence. The Due Process Clause, Confrontation Clause, and the Sixth and Fourteenth Amendments of the United States Constitution require attorneys to adequately understand scientific principles for litigation of forensic science issues. The Sixth Amendment states, "[i]n criminal prosecutions the accused a person shall . . . have the Assistance of Counsel for his defense." The Supreme Court revised the standards for admissibility of scientific evidence and expert witness testimony through the seminal cases of *Daubert*, *Joiner*, and *Kumho Tire*.[1-3] The controversial issues of reliability, peer review, error and uncertainty rates, and standardization still adversely affect competent use of forensic science.

The reliance on forensic sciences in criminal cases has increased substantially in recent years through advancing technology, thereby fostering oversight of the scientific evidence used in criminal cases. A nationwide movement has emerged advocating investigation, research, and improvement of scientific methods in forensics. This sentiment is perpetuated by the discovery of flawed forensics, high-profile crime laboratory scandals, fraud, and wrongful convictions, as well as the exposure of junk sciences and issuance of the National Academy of Sciences Report in 2009 (NAS Report) condemning problems endemic in forensic science disciplines. The NAS Report poignantly discussed the legal profession's failings concerning scientific evidence.[4]

The Sixth Amendment and Due Process Clause are emerging as sources of regulation to increase the reliability and validity of scientific evidence and competency of counsel. The courts have sought to create workable standards to assist litigators in admitting and using forensic sciences during trial. A constitutional difference exists between admitting the expert's opinion and using the expert to introduce the underlying report from a third party as a basis to form an opinion.[5-8] Furthermore, use of false evidence, debunked sciences, or repudiated expert witness opinions is a basis for challenging a conviction through a writ of habeas corpus and new trial.[9,10] Rules governing expert witness qualifications lack specificity and discernable standards despite the courts' attempt to stay current with the rapid advancements in forensic science.

Developments in forensic science have prompted the Supreme Court to issue decisions increasing counsel's duty to competently litigate forensic science evidence. The standard for effective attorney representation is whether the performance was deficient and errors existed depriving a person of fair trial (e.g., but for the attorney's conduct, there would be a different result).[11] This obligation requires a working knowledge of forensic science. Attorneys still lack a fundamental understanding of scientific issues, which impedes effective and competent representation. The inability of counsel to adequately vet scientific evidence through cross-examination has led courts to place considerable dependence on sound laboratory techniques, careful litigation, complete disclosure of scientific procedures, scientific methodologies, and the limitations of forensic evidence. Most of these decisions are made at the trial court level on a case-by-case basis. Unfortunately, the "courts continue to rely on forensic evidence without fully understanding and addressing the limitations of different forensic science disciplines."[12]

Scientific developments, societal sophistication, and court decisions have strengthened the obligation of counsel to litigate forensic science evidence. Attorneys must improve their understanding of forensic science to competently represent their clients in accordance with constitutionally mandated principles of due process and confrontation.

Reference(s):

1. *Daubert v. Merrell Dow Pharmaceuticals, Inc.,* 509 U.S. 579 (1993).
2. *General Electric v. Joiner*, 522 U.S. 136 (1997).
3. *Kumho Tire Co, Ltd. v. Carmichael*, 526 U.S. 137 (1999).
4. Committee on Identifying the Needs of the Forensic Sciences Community, National Research Council. *Strengthening Forensic Science in the United States: A Path Forward.* 110 (2009).
5. *Crawford v. Washington*, 541 U.S. 36 (2004).
6. *Melendez-Diaz v. Massachusetts*, 129 S.Ct. 2527 (2009).
7. *Bullcoming v. New Mexico*, 131 S. Ct. 2705, 2710 (2011).
8. *Williams v. Illinois*, 132 S.Ct. 2221 (2012).
9. Calif. Penal Code, Title 12, Chpt. 1, Sect. 1473 Writ of Habeas Corpus, eff. Jan. 1, 2015 (2016).
10. Texas Code of Criminal Procedure, Chpt. 11, Art. 11.073, eff. Sept. 1, 2013 (2016) Habeas - Procedures related to certain scientific evidence (The Junk Science Writ).
11. *Strickland v. Washington*, 466 U.S. 668 (1994).
12. *Strengthening Forensic Science in the United States: A Path Forward*. supra, 53.

U.S. Constitution, Litigation, Scientific Evidence

F29 On The Threshold of Injustice: Manipulating DNA Evidence

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After attending this presentation, attendees will understand how DNA evidence can be manipulated by adjusting thresholds and other subjective data interventions.

This presentation will impact the forensic science and criminal justice communities by illustrating how DNA data can be subtly adjusted to achieve a biased result. In the provided case example, exclusionary evidence was used to produce an inclusionary match statistic.

Fingernail DNA evidence was obtained from a homicide victim. Short Randem Report (STR) laboratory analysis revealed a possible DNA mixture containing the victim's DNA, and perhaps a second person's. Working for the prosecution, an operator used his probabilistic genotyping software program to compare this possible mixture with that of a defendant.

Reliability Rule 702 of the Federal Rules of Evidence (FRE) for the admissibility of expert testimony requires sufficient data, a reliable method, and that the method be reliably applied to the data.

Sufficient Data: The fingernail data contained potentially exculpatory DNA peaks between 30rfu and 50rfu that did not come from the defendant. According to the software, the mixture ratio was approximately 250:1.

Reliable Method: The software's validation studies have been published using a data threshold of 30rfu. None of these studies used a 50rfu threshold that discards more DNA peaks.

The software's validation studies have decreased to a mixture ratio of 25:1. No published studies have examined ratios as low as 250:1, the level of a few cells.

At a ratio of 250:1, the minor contributor may be too low to detect. Studies by the developer determine that the software is unreliable at this level. The program cannot accurately distinguish between true and false matches. It can give a positive association, whether or not a person's DNA is actually present in the mixture.

Reliably Applying Method to Data: The expert operator found a 250:1 mixture ratio. Such a tiny purported minor component may not even be from a real person. The software was only validated for 25:1 mixtures, not for a 250:1 ratio. Applying an unreliable method to insufficient data is not reliable.

The operator chose a threshold of 50rfu, but the fingernail evidence contained potentially exculpatory evidence between 30rfu and 50rfu and the software was validated for using more peaks at 30rfu, not fewer at the higher 50rfu level. Applying an unreliable method to insufficient data is not reliable. In fact, running the software at a validated 30rfu threshold would exclude the defendant. The fingernail evidence was exculpatory. The software proves that the defendant's DNA was not present.

A software operator can subjectively choose his/her data to obtain the answer he/she wants. Running the validated software program on all the relevant data excludes the defendant, and helps prove his innocence. The operator's invalid data choices provide a scientifically baseless answer that could lead prosecutors to a wrongful conviction.

Relevance Rule 403 balances the probative value of evidence against its prejudicial nature.

Probative: Ignoring crucial exculpatory evidence (i.e., data peaks between 30rfu and 50rfu) does not prove anything. Running an unreliable computer program (i.e., unvalidated at 50rfu or for 250:1 mixture ratios) does not prove anything. The prosecution's expert ran an unreliable method on insufficient data to report a non-scientific result. When there is no science behind an expert's opinion, it has no probative value.

Prejudicial: DNA is highly prejudicial evidence. Telling a juror that DNA connects important evidence to a defendant strongly conveys guilt. If that DNA connection is wrong, no amount of good science to the contrary can "unring" the bell. The jury should not hear flawed DNA evidence that can unfairly harm a defendant and irreversibly taint a trial.

Prosecutors can present DNA to jurors as infallible evidence, but expertly manipulating the data can alter its interpretation and provide subjective or inaccurate results. Most statistical DNA software allows the user to choose their data. This case study illustrates the danger of such practices.

DNA Evidence, Biased Statistics, Admissibility

F30 Cross-Disciplinary Communication: Understanding Commonly Used Terms in Statistics

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The goal of this presentation is to explain the meaning of commonly used statistical terms relating to error, specifically, measurement error; in addition, the ways in which these definitions may differ from those for these same terms used in different contexts or by different professions will be highlighted. Key concepts and the relations between these concepts that will be examined include: (1) random error; (2) systematic (non-random) error or bias; (3) reliability; (4) validity; (5) accuracy; and, (6) precision.

This presentation will impact the forensic science community by increasing the criminal justice system stakeholders' understanding of important statistical concepts and terminology and by improving communication among people with different backgrounds and training. As the work of forensic science expands beyond forensic practitioners, judges, and attorneys to include statisticians and researchers with expertise in adjacent fields, in can be expected that conflicts of terminology will arise more often. Thus, this presentation seeks to facilitate understanding and to better integrate these different communities as they focus on the shared goal of improving forensic science.

There is growing recognition that terminology between forensic science disciplines needs to be standardized as disciplines often develop specialized terminology for their field or specific meanings for words with more general usage. The way terms are understood across groups must also be improved. Criminal justice system stakeholders may associate meanings with terms that people outside their discipline or expertise may interpret in very different ways. These differences can lead to miscommunication, particularly with statistical terminology relating to measurement error, as the specific meanings of some commonly used terms are often misinterpreted. Adding to the difficulty in communication is that different terms can be used for the same concept, and that concepts can be both distinct and interrelated.

One important concept is the difference between random error and systematic error. Both of these types of error must be considered for any kind of measurement, including categorical classification outcomes (i.e., "included" or "excluded") and continuous variables. Random error is "directionless," that is, equally likely to result in over- and under-estimations of the true value. Random error influences the precision and reliability of a measure; reliability refers to the repeatability or consistency of a measure, or how much variation one would see in a measure or test that was conducted multiple times.

Systematic or non-random error refers to a consistent inaccuracy that leads to a "biased" result. Bias in this context refers to an error that occurs in a particular direction (i.e., an error that leads to an over-estimation, or an error that leads to an under-estimation). Note that "bias" as used in this statistical context does not convey the connotation that this term can imply in more general usage (i.e., prejudiced or unfair). Systematic error will influence the accuracy and validity of a measure; validity refers to how well a measure reflects the "true" value of a test or measure used in a specific population and conditions.

In general usage, the terms reliability and validity are sometimes used interchangeably, and it is sometimes said that reliability ensures validity; however, in statistical usage these terms refer to different concepts, and it is important to note that a test that is reliable may or may not be valid. A common analogy used to describe these terms uses darts that are thrown onto a dartboard. If all of the darts cluster closely around the center of the dartboard, the "test" (the person throwing the darts) is both precise (reliable), with little random error, and accurate (valid), with little systematic error. But if the darts cluster in the lower right-hand corner of the dartboard, the "test" would be reliable (precise) but inaccurate (biased or invalid); there is little variation in the results, but the results are off the mark.

Reliability, Validity, Bias

F31 Logical Reasoning: Old Solutions for Current Issues

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After attending this presentation, attendees will understand the difference between posterior probabilities and likelihood ratios and appreciate that the logical approach to evidence interpretation is nothing new in the evaluation of forensic science results.

This presentation will impact the forensic science community by bringing awareness to the error of the transposed conditional and by drawing attention to publications explaining the logical approach to evidence interpretation dating back to the Dreyfus case at the end of the 19th century and beginning of the 20th century.[1]

A current discussion topic in forensic science is the scale of conclusions a forensic scientist uses to report the evaluation results of the examination of scientific evidence. Some conclusion scales used in forensic science today consist of verbal equivalents of posterior probabilities (i.e., probabilities that take into account all of the evidence) of propositions of interest in a case.[2,3] For example, "There is strong probability that the John Doe of the known material wrote the questioned material."[3] From a logical perspective, one can only arrive at such a conclusion if one has the following two ingredients: (1) the odds or the probability that the John Doe of the known material wrote the questioned material before examining the known material and the questioned material; and, (2) how much more probable (or less probable) the observations on the questioned material are if John Doe of the known material wrote the questioned material than if someone else wrote the questioned material.

This is nothing new. More than a hundred years ago, French mathematicians Jean Gaston Darboux, Paul Emile Appell and Henri Poincaré explained this reasoning process in the opinion they gave in the notorious Dreyfus case.[4] Not knowing the prior odds or prior probability described in point (1) above, they argued that they could only report a conclusion of the type "the odds of the proposition of interest after examining the evidentiary material become X times greater than what they were before this examination." Further, this logical reasoning process was not specific to France and Europe: in the 1930s American forensic scientist Wilmer Souder of the National Bureau of Standards (now the National Institute of Standards and Technology) published similar ideas for the evaluation of observations made on typewritten documents.[5]

The study of these historical ideas on the interpretation of scientific evidence illustrates a way forward for reporting conclusions for examinations conducted in casework and highlights the importance of transparency regarding the assumptions made by the forensic scientist that lead to the scientist's conclusions.

Reference(s):

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Interpretation, Likelihood Ratio, Conclusion

F32 Brazilian DNA Database on Trial

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After attending this presentation, attendees will better understand the acceptance of the DNA database by the Brazilian Supreme Court.

This presentation will impact the forensic science community by discussing how an international justice system understands what the limits are for using one of the most important forensic tools.

After establishing a DNA database network using the Federal Bureau of Investigation's (FBI's) Combined DNA Index System (CODIS), Brazil passed a bill in 2012 to collect DNA from those convicted of heinous crimes as well as from suspects, under the conditions of a court order; however, four years after the law took effect the mandatory DNA collection of convicted offenders is still not a common procedure. As an example, the last DNA database report pointed out that less than 1,000 individuals are present in the Brazilian DNA national database. In a country where violent offenses are relatively frequent and known for its large prison population, this data corresponds to less than 1.5% of convicted offenders that should be in the database. One of the main reasons for this extreme delay is the resistance from some policy makers and lawyers who think the DNA collection from convicted offenders, without their consent, is unconstitutional.

Despite these difficulties, the network, composed by 19 DNA laboratories (18 states plus the federal police) linked to the Ministry of Justice, has been growing in number, especially from crime scene samples, and has currently helped more than 200 investigations, including an innocence case.

Recently, a new fact emerged that can help change and leverage the law and, consequently, the database: the Supreme Court has received the Petition nº 937 837 in which the Public Defender of Minas Gerais State questions the constitutionality of offenders' DNA collections. The Supreme Court considered that the constitutional issue has legal and social relevance, manifesting in recognition of the existence of general repercussions of the matter. The decision was unanimous. With the recognition of the general repercussion, the substantive decision of the Supreme Court in the appeal will have a binding effect and will be applied to all similar cases in the Brazilian Judiciary.

As the issue is very new to the Supreme Court, a group of forensic scientists has been called to assist the judges with the technical and scientific fundamentals as *amicus curiae*. Presently, the group is writing a brief to be used and included in the judgment.

The effort should also help the epidemic of sexual assault in Brazil. A recent survey by the government calculated nearly 500,000 sexual assault cases occurring per year in addition to isolated collective rapes cases, such as the one reported in Rio this year prior to Olympic Games.

In a country with more than 50% of prisoners awaiting judgment, the correct use of the DNA database can be extremely positive to avoid testimony-only-based arrests and the absence of physical evidence in trials.

DNA Database, Brazil, Supreme Court

F33 Women and Violence: Politics, Science Education, and Judicial Integrity

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After attending this presentation, attendees will understand how events over the past two generations have resulted in political and social pressures on law school curricula that have compromised the understanding and use of science in analyzing and interpreting facts regarding intimate violence.

This presentation will impact the forensic science community by providing an understanding of case strategies and presentations in our courtrooms that promulgate misconceptions about science and law regarding human interactions.

This presentation will focus on a succession of case cohorts related to sexual politics, including the 1990's "recovered memories" allegations that led to changes in the law of more than the states in the nation, the "Day Care" abuse cases that resulted in extensive false convictions, and in particular, an emerging mass of criminal cases premised on an assertion that, due to one's particular gender, such persons are not capable of committing violence. For years, law professors in law schools have avoided including intimate violence law in their curricula, thereby preventing their law students from learning about scientific evidence regarding intimate violence. Law schools, therefore, continued to foster traditional misconceptions about the word "violence" being attributed to "males" since violence is allegedly committed only by males.

Such erroneous perceptions, in turn, have left law school graduates either under-informed or critically misinformed about the scientific evidence in this area. This lack of scientific knowledge has resulted in practicing attorneys unable or unwilling to advance criminal cases that include intimate violence.

This presentation will provide a summary of the history and literature in this and related areas and examine the extensive body of scientific evidence related to intimate violence. The relationship between sex, violent actions, and variations in the styles of those actions were considered well established and undisputed until the 1960s. This slowly began to bring about a "sea change" in subsequent decades through abandonment of *Frye* generally acceptable theories and instead the application of Federal Rules of Evidence (FRE) 702 with the flexible factors under *Daubert* testing the reliability of the methodology of experts in this field. This was only altered through "novel" case congregations such as "recovered memory," but even then was punctuated by periodic studies and scholarly texts warning of emerging problems in legal education and high-profile factual reviews by high-profile attorneys such as Alan Dershowitz in the 1990s.

In the interim, scientific literature steadily evolved. This literature usually directly contradicted the feminist narrative, including findings such as the availability of weapons tending to equalize violence between the sexes. Studies revealed women were more at risk of violent attack by another female than by a male intimate partner. At times, clashes occurred between the radical feminist response and the scientific researchers who were often themselves female.

This is not to say that males and females do not differ in expressions of violence, and these important differences will be reviewed; however, over the past decade the stalemate between sexual politics and science has led to new tactics. As the media has repeatedly demonstrated, political forces at odds with science now assert their right to their own beliefs, that all beliefs have equal validity, and that students in particular should be shielded from contrary perspectives, including scientific findings. This led first to requirements for "trigger warnings" before unpopular information could be presented in the classroom, then "safe spaces," and now to "civil rights" protections.

Today, despite knowledgeable forensic experts being available to testify as to sex differences in violence, law students are effectively blocked from learning about scientific underpinnings due to notions of being politically unfashionable. Such shielding is such a disservice to the profession of law for it not only handicaps law professionals but also prevents the judiciary from undertaking its constitutional mandates. By not discussing the underlying scientific evidence regarding intimate violence in our law school classrooms, educators have turned our long held traditions about justice and truth upside down, thereby flat-lining the curve of knowledge about intimate violence.

Scientific Education, Intimate Violence, Judicial Integrity

F34 What's the Law Got to Do With It? Forensics in a Pediatric Health Setting: A Case Report

Maria Susana Ciruzzi, PhD, Hospital Nacional de Pediatría Prof. Dr. Garrahan, Combate de los Pozos 1881, Buenos Aires 1045, ARGENTINA*

After attending this presentation, attendees will understand the hard work that is performed at a pediatric health setting, especially in the Emergency Room (ER) unit, when a crime victim arrives or is referred for medical treatment because of her injuries. Attendees will learn how forensic principles are followed by properly trained clinicians and nurses.

This presentation will impact the forensic science and social communities by discussing key aspects of the important role physicians and nurses play in assisting crime victims in their recovery, in the collection and preservation of crime evidence, as primary witnesses of the criminal *modus operandi*, of the victim's direct and immediate statement, and of the health consequences the perpetrator's criminal behavior has on the victim and her family. It is often taken for granted that judges, prosecutors, and defence attorneys should have some training in and knowledge of criminology, criminalistics, legal medicine and crime scene investigation. But, what happens when a gunshot wound victim arrives at the ER or when a drug addict rushes into the ER because of an overdose or when a sexual abuse victim seeks help at a health setting? Are physicians and nurses prepared not only to assist the victim from their own medical and nursing expertise but also to act and assist courts and police forces in gathering and preserving evidence so that the bad guy could be apprehended, brought to trail, prosecuted and sentenced?

Presently, clinical forensics, forensic epidemiology and forensic documentation are an important part of clinicians and nurses' skills in order to assist the victim, to collect and to preserve the evidence, and to act as one of the best reliable witness' court prosecutors, and defense attorneys can find. Physicians and nurses are the very first people a victim arriving at a health setting meets. They can tell firsthand the victim's attitude, fears, and needs. They not only collect physical evidence, but also document the victim's words on the chart. They are trusted people for the victim and her family.

The importance of training health professionals in forensics will be illustrated by a reported sexual abuse case that happened in Buenos Aires Province, Argentina, and that was referred to a pediatric hospital in the City of Buenos Aires. The critical role played by these professionals in collecting and preserving the evidence and as witnesses before the court will be discussed, as will the importance of the clinical chart as documentary evidence. Finally, physicians' and nurses' roles in preventing revictimization, in assuring victim's rights, and in protecting the victim from the stress and guilt that sexual abuse often creates will be discussed. All of this is illustrated in the context of an actual case that provides an example of the practical application of forensic principles and training, as well as the type of legal outcomes and sentences this type of case yields.

Finally, the concept of "Public Health Legal Preparedness" and its application at a pediatric health setting will be discussed.

Forensics, Pediatric Health Setting, Sexual Abuse Victim

F35 Reconciling Differences Between Lawyers and Forensic Scientists in a Law School Setting Toward Advancement of the Professions

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After attending this presentation, attendees will have ideas and an interest in exploring simulation-based educational opportunities focusing on the intersection of law and forensic science. Through the use of simulations, interdisciplinary courses focusing on law and forensic science are able to explore matters of skill, judgment, and ethics in the use of expert testimony in civil and criminal litigation. The key objectives of this presentation are to share tested methods for teaching at the intersection of law and forensic science and to underscore the importance of using accurate, data-supported simulations in the training of skills, judgment, and ethics.

This presentation will impact the forensic science community by advancing the quality of training tied to the use of expert testimony in civil and criminal litigation. Key goals of the course described in the presentation include: (1) understanding the distinct roles of judges, prosecutors, defense attorneys, and experts when forensic science is offered as evidence in criminal or civil proceedings; (2) recognizing the potential for wrongful conviction when lawyers are not prepared and experts make errors, intentional or not, or stretch to assist in the win; and, (3) introducing and simulating the skills and judgment needed to be competent and prevent errors. Interdisciplinary training for lawyers and forensic scientists advances the pursuit of excellence for future professionals in law and forensic science.

Proposition: Law school learning opportunities advance the pursuit of competence at the intersection of law and forensic science. Interdisciplinary training of future lawyers and forensic professionals with well-crafted simulations provides opportunities to explore roles and reconcile differences while developing needed skills and judgment.

Method: Indiana University (IU) Robert H. McKinney School of Law offers a two-week, two-credit, intensive summer course reconciling law and forensic science using clinical classroom methodology. In its tenth year, the course also is a capstone for students obtaining a masters of forensic science degree at Indiana University-Purdue University Indianapolis (IUPUI). Law and forensic science students are assigned teams and given specific roles in simulation exercises based on forensic evidence analysis undertaken by the forensic science students and outside experts.

For example, a plastic pipe bomb covered in electrical tape is found in an unattended bag at the airport. A forensic analysis, including production of raw data and a lab report, is completed. The expert seeks to give an opinion that the tape from the device is consistent with the tape in the suspect's home.

A man found dead in the parking lot of a tavern has a bitemark on his arm, with a head injury as the cause of death. Police have no witnesses. A suspect is ordered to give bitemark exemplars. The state's experts in odontology and digital evidence are prepared to testify as to the theory and technique supporting the analysis and methods of application used to reach the conclusion that the bitemark of the suspect is consistent with the bitemark on the body.

Classroom exercises based on these simulations include witness preparation by the prosecution, defense pretrial questioning, and preliminary hearing and trial testimony presentations. Seasoned lawyers from the Indianapolis area participate in the simulation exercises, providing co-counsel for each team. Students perform the simulations, with critique, feedback, and opportunity for reflection.

Results Obtained: Law students and forensic science students are capable of identifying and examining the differences, as well as the similarities, between legal roles and forensic science roles. Future prosecutors and defense attorneys can be taught the need to obtain and understand the raw data supporting the expert opinion and prepare to admit, prevent admission, and/or make the record for appeal. Future forensic scientists can be taught to recognize the importance of witness preparation between the expert and the lawyer offering the evidence. Stressing ethics in training results in application of ethics in a real world setting.

Conclusion: Interdisciplinary training for law and forensic science students advances the future professional competence of lawyers and forensic experts and, thus, the pursuit of justice.

F36 Analysis of Drug Paraphernalia in a Wrongful Death Case

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The goals of this presentation are to: (1) describe the case facts; (2) explain the use of Direct Analysis in Real Time Triple Quadrupole linear ion Trap/Mass Spectrometer (DART® QTRAP®/MS) platform for drug confirmation from drug paraphernalia; and, (3) understand how this technology may aid forensic analyses and investigations.

This presentation will impact the forensic science community by introducing novel methods to identify drugs associated with paraphernalia recovered at the scene of a death. The information from the analysis can be used and interpreted by forensic toxicologists, pathologists, and attorneys to aid in wrongful death litigation.

Hypothesis: It is suspected that in addition to injecting an oxycodone solution, the decedent also injected the residue obtained from her fentanyl patch.

Statement of Content/Methods: The patient was admitted to a community hospital for renal and gastrointestinal issues. According to the medical records, the patient was prescribed a 75 microgram/hour fentanyl patch, hydromorphone, and promethazine. Promethazine was administered intravenously through a Peripherally Inserted Central catheter (PIC line). The patient was found dead, locked in the bathroom of her hospital room. Drug paraphernalia (syringes and pills) were located near the body and the syringe contained a pink residue; the fentanyl patch was not located. At autopsy, changes in the lungs consistent with Intravascular (IV) drug use were noted and toxicology specimens were collected. Both drug paraphernalia and toxicology specimens were sent to the University of Alabama at Birmingham Forensic Toxicology Laboratory for analyses. The drug paraphernalia included: a prescription bottle of pink pills labeled 10mg oxycodone, one needle containing dried powder, three syringe barrels (two 10mL capacity and one 1mL capacity), and a medicine cup containing a white glue like substance. Postmortem toxicology specimens analyzed included peripheral blood and urine. Toxicology analyses were performed on the decedent's samples using Enzyme Multiplied Immunoassay Technique (EMIT), Gas Chromatography/Mass Spectrometry (GC/MS), and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The drug paraphernalia were rinsed with methanol and analyzed by EMIT and DART® QTRAP®/MS.

Summary of Results: Postmortem toxicology results in the peripheral blood included: ethanol-negative, promethazine (0.473mg/L), hydromorphone (0.009mg/L, fentanyl-0.011mg/L), and oxycodone (0.090mg/L). Drug paraphernalia EMIT results were positive for opiates (300ng/mL cutoff) for the needle and the two10mL capacity syringes and positive for oxycodone (300ng/mL) for the needle and all three of the syringe barrels. Confirmation analysis was performed on the five samples via DART® QTRAP®/MS in enhanced product ion mode (EPI mode). The acceptance criteria included peak area exceeding three times signal-to-noise, acceptable library match (minimum fit quality of 75%) obtained for two of the three samplings, acceptable negative control (blank sampling device) and acceptable positive control (certified reference material). Fentanyl was detected in the needle, one 10mL capacity syringe (#1) and the plastic medicine cup wash. Oxycodone was confirmed in both 10mL capacity syringes (#1 and #2). Hydromorphone was not detected in the drug paraphernalia.

Conclusion: The manner and cause of death were determined to be accident and fentanyl toxicity, respectively, with contributing factors of oxycodone and hydromorphone use. From the DART® QTRAP®/MS results for the drug paraphernalia, it is clear that the patient not only self-administered unauthorized medication but also circumvented the designed route of administration for the prescribed medication, fentanyl.

DART® QTRAP®/MS, EMIT, Wrongful Death

F37 The Total Team: The Investigators, Forensic Scientists, and the Attorneys in a Capital Case From Kansas

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After attending this presentation, attendees will learn that the total team concept is essential to establish truth and justice in a capital case.

This presentation will impact the forensic science community by illustrating how: (1) each individual must concentrate on their specific duties; (2) each must collaborate together; and, (3) the attorneys must assemble the individual pieces of the puzzle into a picture detailed enough for the jury to interpret.

In early May 2013, the Franklin County Sheriff's Office (FCSO) was contacted regarding a body found in an outbuilding at a rural residence in Ottawa, KS. Also, a 22-year-old female, who was known to frequent the residence, and her 18-month-old daughter were missing. The FCSO requested assistance from the Johnson County Sheriff's Criminalistics Laboratory (JCCL). Officials searched the house and two additional bodies were found, one of whom was the woman reported missing. The Federal Bureau of Investigation (FBI) and more than 30 other agencies assisted with this investigation. The 18-month-old baby girl's body was found days later in a suitcase in a creek in Osage County, KS. In total, there were five scenes where forensic evidence was recovered, numbering more than 1,000 pieces. The FC District Coroner requested the forensic pathologist from the Frontier Forensics laboratory to join him at the rural scene.

The Crime Scene Investigation (CSI) supervisor recognized that the male victim in the house was covered by thousands of maggots. The victim in the outbuilding, who was covered with a tarp, was also showing an infestation of larvae, mainly in the head area. Because the CSI supervisor had been trained by a Forensic Entomologist (FE), he knew the procedures necessary for gathering the proper entomological specimens and requested assistance from an FE. The FE called upon two additional FEs and a forensic climatologist. What was peculiar about the two victims in the house was that the male possessed tens of thousands of maggots, while the female had no insect evidence on her at all.

The primary indicator insect species for the victim from the outbuilding was one of the blue bottle flies, *Calliphora vicina,* a cool-weather species. The forensic climatologist was called in to calibrate the outbuilding scene to the weather station. The forensic climatologist adjusted the inside temperatures, which the FEs used for determining a range of when colonization most likely occurred (namely, as late as April 20 to as early as April 18, 2013).

The primary indicator insect species from the male body in the house was the black blow fly, *Phormia regina*. From photographs of the remains and scene, huge numbers of maggots were observed on the remains (tens of thousands), indicating hundreds of female flies must have been present over a period of a few days with warm temperatures and had access to the remains for extensive egg deposition. These warm temperatures only occurred from April 28 through May 1, 2013. Investigators determined that, during this time, unscreened windows were open. Therefore, reliance on weather patterns, as opposed to calculating the period of colonization, was used to estimate the time of death. A similar approach was used to determine when the 22-year-old female was murdered. By May 2, temperatures were well below the lower flight limit. With cool temperatures, there was a lack of flight activity; also, covering the remains with clothing and the enclosed structure provided the reasons why this victim had no insect colonization. The female was killed and immediately covered during the afternoon of May 1, then temperatures dropped to below egg-laying activity levels until discovered.

With the vast amount of evidence collected, the multiple crime scenes involved, and with every death occurring at a different time, it was challenging for the prosecutor and FS team to reconstruct events to make sense to a jury. This challenge is why it is critical that forensic scientists work as a team when prosecuting complex cases, and why attorneys, whether prosecution or defense, need a comprehensive working knowledge of the forensic sciences.

Blow Fly, Climatology, Capital Murder

F38 Are All Instruments Always Valid and Reliable in Ascertaining a Crime?

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After attending this presentation, attendees will better understand of the role of instruments and metrology in forensic sciences and whether instruments used in ascertaining crimes are valid and reliable, under the considered circumstances, in order to help the trier of facts in employing the results of scientific tests and in rendering a decision beyond any reasonable doubt.

This presentation will impact the forensic science community by providing a clearer insight into the basic concepts of metrology and illustrating that instruments often used to understand how a crime has been committed and who committed it are not always valid and the obtained result is not totally reliable. This presentation will broaden understanding among the law community of how the fundamental concepts of metrology can help clarify how valid and reliable the measurement results are and, consequently, quantify the doubt on how correct a decision is based on an experimental test.

Forensic measurements are an important source of evidence in criminal trials and investigations. As a result, metrology, the science of measurement, is critical to both the field of forensic measurement as well as the implementation of justice.[1,2] The ubiquity of measurement tools in daily life leads to their often being employed outside their primary scope in an attempt to ascertain facts or provide scientific support to other propositions and pieces of evidence. One of the most commonly used such instruments is the cell phone as its location in space and time can be estimated utilizing phone company records and triangulation measurements. This makes it possible, in principle, to locate a suspect by tracing his cell phone.

More recently, smart meters have been used in Italy to assess, through the analysis of the electric loads in use in the victim's house, when the murder has been committed and to verify the defendant's alibi. Other measuring instruments are similarly used in so-called technoprisons, to locate prisoners and identify their activities.[3]

Such unvalidated use of these instruments raises critical questions about the reliability and validity of the use to which they are put. It is generally accepted that a measurement is valid to the extent that the instruments relied upon are employed within the primary scope for which they have been designed and validated.[4] On the other hand, metrology clearly shows that measurement results are never totally reliable and quantifies the lack of total reliability with the metrological concept of measurement uncertainty.[5]

It is evident that a cell phone's primary function is to facilitate communication, and that the ability for it to assist in determining locations is more an accidental consequence of the way it is operated. Similarly, the primary function of a smart meter is measuring the electrical energy flowing in through a meter, not that of tracking the time of operation of the single loads. When such measurements are made utilizing instruments beyond the scope of their validation, the reliability of measured results must be carefully considered in order to avoid drawing incorrect conclusions. This will be discussed in addition to some practical examples illustrating the limits of the validity of measurement results obtained utilizing instruments outside their scope of validation.

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Forensic Metrology, Measurement Uncertainty, Improper Use of Instruments

F39 Method Validation and Admissibility of Forensic Alcohol and Drug Tests

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After attending this presentation, attendees will understand the legal issues that are presented in courts when analytical methods are challenged as not having been properly or fully validated prior to use.

This presentation will impact the forensic science community by advising crime laboratories of the need to perform full validation on all methods, old and new, so that courts will be able to properly determine the legal admissibility of testing results when such quandaries occur.

Recently, courts have been called upon to determine whether a crime laboratory has fully validated their testing methods for drugs and alcohol in accordance with acceptable guidelines or publications.

Non-standard or in-house analytical methods are being used to test for alcohol and drugs in biological samples in crime laboratories throughout the United States. The forensic community has promulgated standards for validation that would apply to all such methods, both new and old. But many of these laboratories have failed to take steps to validate their methods. Many attorneys now argue that results obtained from less-than-fully validated methods should be ruled inadmissible as a matter of law in court proceedings.

In the scientific community, it is widely recognized that analytical methods employed for the qualitative or quantitative determination of drugs or alcohol in biological samples must be fully validated to yield reliable results. It is also understood that each analytical technique must be so validated for each target analyte that it seeks to identify and/or quantitate (where quantitation is necessary). While there is general consensus that validation parameters should include accuracy, precision, selectivity, sensitivity, reproducibility, limit of quantification, and stability, many crime laboratories have failed to validate their methods utilizing all or many of these parameters.

There is not complete agreement in the forensic community as to how method validation should be performed in order to satisfy both the courts and the sciences. The International Organization for Standardization (ISO) 17025 includes a requirement that method validation be performed (unless the methods are found in "preferred methods published in international, regional or national standards"), but ISO 17025 fails to specify with particularity the requisite steps necessary to satisfy this standard.

The recently published Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines (2013) are the best attempt yet to govern the validation of new methods used in crime laboratories. The SWGTOX guidelines also apply to methods that were validated prior to the promulgation of the SWGTOX standards, allowing for the use of historical calibration and control data to fulfill some, but not all, of the validation parameters. Of note is that compliance with SWGTOX procedures is purely voluntary and ISO 17025 has not adopted or even acknowledged them as being satisfactory for ISO purposes.

Some crime laboratories have claimed that proficiency testing, coupled with periodic calibrations, along with the use of controls and standards, are suitable substitutes for the types of method validations found in SWGTOX and other scientific publications. Still others have simply claimed that their methods pre-date all guidelines and are thus exempted from any proof of validation in court – akin to a "take my word for I" approach.

Because of the lack of uniform application of published guidelines on method validation within the forensic and legal community, and because of the lack of published court decisions regarding challenges to the lack of full-method validation, courts have struggled with determining whether a method has been "validated" sufficiently to be accepted in court.

In one case that will be presented, non-standard methods have been employed for decades with no evidence of them ever having been subjected to true validation testing. This presentation will include a case study of the issue(s) and the legal process that was used to challenge method validation and admissibility of the result(s).

Method Validation, Guidelines, Admissibility

F40 From the CIA to CSI: How the Intelligence Community's Structured Analytical Techniques Can Offset Forensic Bias

Max M. Houck, PhD, 1825 2nd Street, S, St. Petersburg, FL 33705*

After attending this presentations, attendees will have knowledge of structured analytical techniques as a toolbox for offsetting cognitive biases in decisions, analysis, and problem solving.

This presentation will impact the forensic science community by informing attendees of pre-existing methods for alleviating cognitive biases that plague decisions and problem solving.

Like any other human endeavor, forensic science suffers from cognitive biases, thinking patterns that lead to irrational or illogical decisions. Numerous wrongful convictions have been shown to be the result of cognitive biases on the part of the police, attorneys, judges, and scientists. Although these biases have been pointed out in every aspect of forensic science, few agencies have taken the time to alter or adapt their policies and procedures to offset the biases under which their scientists labor.

The human brain makes mental models, or frames, to deal with the uncertainty in complex situations, such as criminal investigations. Mental models are necessary for people to process what otherwise would be a bewildering torrent of information. Sometimes called heuristics, they allow people to be mostly right about most things and have served an adaptive evolutionary purpose: rustling in long grass on the savanna usually means a predator, not a friend; however, in structure thinking mental models can cause investigators to ignore or reject important information or to not seek out missing information. With greater experience (which tends to reinforce the mental models) comes a greater chance of rigid thinking and less of a chance for transparent thought processes. Mental models lead people to perceive information consistent with their existing beliefs over contradictory, although more accurate, data. People are often unconscious of key assumptions that guide and support their decisions. Cognitive biases, such as only seeking out information that supports one hypothesis ("The husband is guilty."), are implicit in mental models and heuristics.

The intelligence community has addressed these problems with a set of methods that re-formulate information, challenge pre-existing assumptions, and explore alternative hypotheses and interpretations. Collectively known as structured analytic techniques, these methods are used to help prevent intelligence failures and provide supported outcomes through transparent processes.

This presentation will provide an overview of structured analytical techniques and how they can be applied to forensic processes.

Cognitive Bias, Heuristics, Wrongful Convictions

F41 Cooperation Between Law Enforcement and Entomologists Leads to Practical Research Questions

Kristi Bugajski, PhD, 1610 Campus Drive, E, Valparaiso, IN 46385*

After attending this presentation, attendees will better understand how entomologists and law enforcement can work together during a case, and how case work can inform research questions.

If chemicals are applied to a body and that has an effect on the blow fly activity, then the estimation of the Postmortem Interval (PMI) is therefore compromised. The data obtained from this research will impact the forensic science community by helping to overcome this obstacle when chemicals are involved and yield more accurate assessments by forensic entomologists.

Hypothesis: It was hypothesized that chemicals would deter insect activity and when they are used in a suspicious death, it is important for law enforcement and the entomologist to understand the effects on PMI estimations.

Synopsis of Methods: This study sought to: (1) observe the effects of chemicals on blow fly oviposition timing; and, (2) observe differences among treatments in terms of blow fly development and species composition and the effect they would have on PMI estimates. Seven chemicals were tested: (1) ammonia; (2) bleach; (3) gasoline; (4) lime; (5) muriatic acid; (6) OFF!® (active ingredient DEET); and, (7) Raid® (active ingredients permethrin, tetramethrin, d-cis/trans allethrin). Pigs with no treatment served as controls. The experiment took place in the summer and fall of 2008, spring, summer, and fall of 2009, and the spring of 2010 in West Lafayette, IN.

Summary of Results: Significant interactions were found between event and season, season and treatment, and event and treatment. Pairwise comparisons found significant differences in the accumulated degree hour estimations between the control and bleach, muriatic acid, OFF!®, and lime. Pigs treated with Raid® were attractive to adult flies, but no oviposition occurred in six trials.

Conclusions: This research was conceptualized from previous work with law enforcement. The results reveal that it is important for forensic entomologists and law enforcement agencies to take chemical effects into consideration when providing PMI estimations. This information was later helpful when collecting insects from a local murder investigation where muriatic acid was used on the corpse.

After attending this presentation, the audience will have a better understanding of the effects that household chemicals have on the insect's role in the decomposition of the human body. Forensic entomology uses data derived from insects to assist the criminal justice system.[1,2] There have been many cases to document the effects of drugs on blow fly growth and development but none on the effects of household chemicals. This research was inspired from a murder that occurred in Lafayette, IN, where the perpetrator sprayed Raid® on the body of the victim. This led researchers to question what effects Raid® and other household chemicals have on blow fly activity and, subsequently, estimations of the PMI.

Forty pigs (*Sus scrofa*) weighing an average of 1kg each were obtained from the Purdue University swine unit and frozen. Prior to the start of the experiment, the pigs were thawed for 15hr in a room without fly access. Pigs were placed in the field at 9:00 a.m. hours. Seven chemicals were tested: four liquids (muriatic acid, Clorox® bleach, Great value® ammonia, and Marathon® 87 octane unleaded gasoline); two aerosol sprays (Raid® (active ingredient 0.05% permethrin, 17.5% Tetramethrin, 0.05% d-cis/trans allethrin) and OFF!® Deep Woods Sportsman (active ingredient 15% DEET)); and one powder (hydrated lime). Pigs were treated with the chemicals on both sides of the body and open orifices in the following amounts: 500mL of the liquids, 382g of the powder, and until run-off occurred with the aerosol sprays (200g of Raid® and 80g of OFF!®). There were five replicates of each chemical per trial and five controls (no chemical treatment). Adults and larvae were collected following standard operating procedures outlined in Haskell and Williams each day to document any differences in species composition or development among treatments.[2] Larvae were collected in KAA (composed of 95% ethanol (77%), acetic acid (15%), and kerosene (8%)) and adults in 70% Ethyl Alcohol (EtOH).

Significant interactions were found between event and season, season and treatment, and event and treatment. Pairwise comparisons found significant differences in the accumulated degree hour estimations between the control and bleach, muriatic acid, OFF!®, and lime.

Forensic entomologists are often asked by law enforcement agencies to provide an estimation of the PMI using insects. If chemicals are applied to a body and that has an effect on the blow fly activity, the estimation of the PMI is therefore compromised.

Reference(s):

1. Byrd J., Castner J. 2010. *Forensic Entomology: The Utility of Arthropods in Legal Investigations, 2nd ed*. CRC Press, Inc., Boca Raton, Florida. 681 pages.
2. Haskell N., Williams R. 2008. *Entomology and Death: A Procedural Guide, 2nd ed*. Forensic Entomology Partners, Clemson, South Carolina. 182 pages.

Law Enforcement, Blow Flies, Forensic Entomology

F42 Organization of Scientific Area Committees (OSAC): Developing Forensic Science Standards, Identifying Research Needs, and Informing the Research Community and the National Institute of Justice (NIJ)

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After attending this presentation, attendees will understand how, during the development of standards, OSAC helps to discover a range of forensic science research needs and how they are communicated to researchers and to the forensic science community.

This presentation will impact the forensic science community by educating attendees on the processes employed by the OSAC to identify research needs and to inform the forensic science community. Attendees will also learn how the NIJ is capturing this information and implementing it into an effective forensic science research strategy that ultimately strengthens the underlying scientific foundations applied to the practice of forensic science.

OSAC is comprised of approximately 560 voting members and more than 250 affiliates appointed to 34 functional units consisting of 5 Scientific Area Committees and 25 Subcommittees addressing nearly two dozen forensic science disciplines. There are approximately 200 task groups assigned to the development of standards and guidelines. During their deliberations, these world-class subject-matter experts concurrently discover research gaps, which they now capture on the OSAC research needs template and forward to the Forensic Science Standards Board (OSAC's governing board). The OSAC Charter and Bylaws calls for the organization to "provide insight on each forensic science discipline's research and measurement standard needs." To date, more than 50 forensic science research needs have been identified and communicated to the forensic science community. The research needs are posted in the monthly OSAC Newsletter, an online service distributed to more than 10,000 subscribers. All of the research needs are chronicled on the OSAC website at http://www.nist.gov/forensics/osac/osac-research-development-needs.cfm.

In finding a more effective path toward the implementation of research avenues to inform and fund forensic science research, OSAC has teamed with the NIJ to provide them with its insights and to provide consultation, where required. The NIJ will use this information to inform the NIJ Technical Working Groups advising and prioritizing their list of research topics for NIJ. The NIJ ultimately uses this information to distribute solicitations to the research community reflecting those priorities. The NIJ awards the winning proposals and ultimately seeks to fill the gaps identified on the OSAC list of research needs.

Standards and Guidelines, OSAC, NIJ

F43 Changing Forensic Science Practice to Meet the Demands of Law and Science

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After attending this presentation, attendees will learn how forensic science fits into the broader area of applied science and how modest changes in practice could resolve long-standing problems with the use of science in the courts.

This presentation will impact the forensic science community by illustrating how science operates and how changes to forensic science in the legal system can meet the needs and expectations of science and the legal community.

Since its inception, forensic science has been caught between potentially conflicting demands of science and the practical needs of courts. For more than a century, establishing the validity of scientific information and methods has challenged courts. In the United States, the *Frye* and *Daubert* Rules are examples that attempt to address this issue, but continuing problems in the availability, admissibility, and accuracy of forensic science all illustrate the continuing dilemma of how to use scientific evidence in court.

Despite many claims to the contrary, there is no single scientific method nor a single arbiter of scientific validity. Fundamentally, science postulates that objective reality exists, and understanding this reality through observation is the scientific process. From these premises, science operates through: (1) direct observation; (2) manipulated observations (the experimental process); and, (3) predicted observation (with predictions arising from models of various types). Scientific truth is established through objective evaluations of data, the observations themselves, and through replication and review, evidence that observations are valid and repeatable. The applied sciences — agriculture, engineering, medicine, and forensic science — use science to address questions of practical importance. Most applied sciences are self-correcting, bad science or bad applications lead to obvious consequences; such as failing crops, dead patients, or collapsed bridges. In contrast, as currently practiced, forensic science has no self-correction mechanisms.

The problems identified by the National Academy of Sciences in their Report on forensic science speak to this lack of self-correction. Fortunately, such a solution exists in that most basic science also lacks mechanisms of immediate self-correction. Science has resolved this problem through its requirements of replication and peer-review of evidence. Open access to data, methods, and conclusions allow peers to evaluate and test (as appropriate) the legitimacy of those same data, methods, and conclusions. Thus, the solution for forensic science is to embrace this same mechanism.

The changes necessary to implement such a mechanism are straightforward. First, in science, the peer-reviewed scientific publication represents the definitive statement of a research finding. By analogy (although it has importance beyond the statement of an analytic conclusion), the case report of a forensic scientist offers the data, analysis, and conclusions from associated evidence. So, the first change needed is that written reports should be required for all scientific testimony, which is already required in some countries (e.g., the United Kingdom). Second, all case reports should be published and peer-reviewed after adjudication, with reviews published with reports. With the advent of electronic publishing, this requirement can be met through existing publication mechanisms, ideally through organizations, such as the American Academy of Forensic Sciences. Models for online publication and open review and commentary also exist through journals such as *PeerJ* and offer not only a method for validating findings, but also an opportunity for new insights and evaluations. Finally, indications of problems in a case report through publication and peer-review should be potential grounds for an appeal.

This approach leaves the questions of validity of techniques and conclusions to those most qualified to evaluate scientific information — other scientists. It provides a mechanism to ensure consistency in how experts apply their analyses and for self-correction currently lacking in forensic science. Ultimately, it allows courts access to not only experts in the case, but to the broader scientific community.

Science, Forensic Science, Reform

F44 Look! Up in the Sky! It's a Bird — It's a Plane — It's a BASE (Building, Antenna, Span, and Earth) Jumper?

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After attending this presentation, attendees will understand how law enforcement was able to use license plate readers, videotapes from surveillance cameras, social media pages, cell phone records, and cell site information to ascertain the identity of everyone involved.

This presentation will impact the forensic science community by detailing the forensic trail of evidence that three BASE jumpers and a lookout left behind, which foiled their "perfect jump" from the newly built and iconic One World Trade Center in Lower Manhattan. A case study will be presented of an intense investigation to learn the identity of the reckless BASE jumpers who penetrated the security of New York City's most sensitive and iconic landmark, which replaced the Twin Towers destroyed in the terror attacks of September 11, 2001.

One World Trade Center, also known as the Freedom Tower, overlooks the 9/11 Memorial Pool in Lower Manhattan. It is a stunning edifice, visible for miles, whose beauty will always be tinged with sadness for all Americans who witnessed the terrorist attacks on the Twin Towers in 2001. Imbued with this historic mantle, the Freedom Tower stands on sacred ground; however, to four young men on September 30, 2013, the building represented a challenge: to be the first people to jump from the top. Extreme sports aficionados and thrill seekers have developed cult followings for this very dangerous behavior called BASE jumping (with parachutes). Whether performed by semi-pros or amateur copycats, these acts are surreptitious and potentially dangerous, even lethal, in an urban setting. New York State has enacted a statute prohibiting this most extreme of extreme sports, but some jumpers feel it is worth the risk.

The evidence overwhelmingly established that the defendants made elaborate plans to enter the still unfinished Freedom Tower (enlisting a fellow jumper who worked there to help gain entry). The 1,784 foot Freedom Tower has five below-grade levels, 104 stories, and a spire on the roof that projects more than 400 feet above the roof level. The Tower narrows toward the top, and there are three communications rings at the base of the spire. They have no railings or walls, which would impede broadcasting and reception, but they are not meant to be traversed other than to install or monitor equipment. Those rings are the equivalent of levels 106, 108, and 110. They are concentric, supported by steel struts and guide wires to protect the spire from the ferocious 50mph-60mph winds that occur at the top, and share the same vertical plane as the narrowest part of the Tower. The jumpers leapt from the communications crown above the top of the 104-story building, wearing hand-packed parachutes and armed with helmet cameras to record the jump. They landed on the 8-lane highway below at 3:00 a.m. A lookout, stationed below, also tried to record the jump.

After reviewing security footage after the fact, Goldman Sachs notified police that two men dressed in black had landed in front of their building in the dead of night on West Street.

Still smarting from bad publicity generated by a teenage trespasser's entry into the Freedom Tower a week before, law enforcement executives from the New York Police Department (NYPD), Port Authority Police Department, and other agencies were livid at the ease with which the jumpers had penetrated security, and a manhunt began. Investigators sought and found witnesses to the jump, and surveillance tapes were pulled from every nook and cranny of the area. High-tech forensics and good old-fashioned detective work soon nabbed the lookout, who gave up his cohorts, one of whom was an iron worker on the site with an access pass. The defendants were tried and convicted by a jury in June of 2014 and sentenced to 200-300 hours of community service.

Lawyers may be surprised to learn that such a dangerous act constituted only the misdemeanor level of reckless endangerment. Although the prosecutors also charged the perpetrators with burglary, the judge determined that since the defendants' crime was committed from atop the Freedom Tower, and a necessary element of the crime of burglary was that they "enter a building with the intent to commit a crime therein," he would not instruct the jury that thereon was the same as therein. The defendants were convicted of BASE jumping and misdemeanor reckless endangerment.

F45 Vitreous Chemical Analysis in Postmortem Cases: Reliability of Reported Methamphetamine Concentrations

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After attending the presentation, attendees will appreciate the need for accurate and precise postmortem toxicology confirmation of methamphetamine levels utilizing vitreous chemical analysis and its reliability compared to that of central and peripheral blood.

This presentation will impact the forensic science community by illustrating the reliability and usefulness of vitreous chemical analysis by a real-life example of the sampling, analysis, and reporting in a second-degree driving under the influence murder case in California in which the decedent had significant methamphetamine in his central blood, peripheral blood, and vitreous.

This begs the questions: (1) Is there a correlation between postmortem methamphetamine levels in the chest cavity, peripheral blood, and vitreous eye fluid?; and, (2) What affect does postmortem distribution or diffusion have in obtaining reliable methamphetamine levels at the time of death when sampled from different areas of the body?

Methamphetamine use has become prevalent among both adolescents and adults in America due in part to the addictive nature and multiple routes of ingestion. Arrests and deaths related to driving with measurable and/or impairing amounts of this central nervous stimulant in the body have increased in recent years. The need for accurate and reliable toxicology results in these cases is critical to insure that valid science is presented in the courtroom.

Sampling the vitreous (the acellular, viscous, translucent fluid made up of water, glucose, acids, collagen, and inorganic salts found in the eye) can be accomplished by inserting a narrow-gauge needle with syringe into the globe of the eye at the lateral canthus with low-pressure draw on the syringe to extract a sample of the 2+mL of fluid. One benefit of sampling the vitreous is that some literature indicates that the sampling may be conducted three to five days postmortem without compromising the reliability of the results. Analysis of the sample can be accomplished presumptively with Enzyme-Linked Immunosorbent Assay (ELISA) immunoassay analysis and confirmed by Gas Chromatography/Mass Spectrometry (GC/MS).

The vitreous concentrations of several drugs have been studied and have been correlated with premortem levels sampled from other parts of the body; however, since postmortem diffusion of certain drugs from the brain into the vitreous may occur, the value of the results must be viewed with a critical eye. Studies reveal that hydrophilic drugs are more likely to have concentrations similar to that of whole blood or plasma compared to drugs that are highly protein-bound, like benzodiazepines. Interestingly, it has been reported that with cocaine, the vitreous concentration can increase with time, leaving courts and juries to wrestle with the value of the proffered evidence.

There is evidence that postmortem methamphetamine distribution in the vitreous compared to central and peripheral blood can be assigned a reliable ratio, provided the proper uncertainty ranges are included in responsible reporting.

Vitreous Chemical Analysis, Methamphetamine Postmortem, Methamphetamine Distribution

F46 Is Your Handwriting Expert's Testimony Admissible? Navigating *Daubert/Kumho* Challenges to Enhance the Likelihood That Gatekeepers Will Find Handwriting Comparison Evidence Sufficiently Reliable To Be Admissible Under Federal Rule of Evidence 702 (Fed. Rule 702)

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After attending this presentation, attendees will better understand the factors that have led some courts to conclude that a handwriting expert's proffered testimony, as applied to the facts of that particular case, fell short of the reliability threshold required by Fed. Rule 702. Forensic Document Examiners (FDEs) will learn what they can and should consider doing to enhance the likelihood that gatekeepers will find handwriting comparison evidence sufficiently reliable to be admissible under Fed. Rule 702.

This presentation will impact the forensic science community by providing FDEs and other attendees with more effective foundational principles and tools for persuading gatekeepers that handwriting comparison evidence and the methods and reasoning underlying an FDE's opinion in a given case satisfy the reliability threshold of Fed. Rule 702.

In *Daubert*, the United States Supreme Court charged trial judges with the task of screening proffered expert testimony by making "a preliminary assessment of whether the reasoning or methodology underlying the testimony is scientifically valid and of whether that reasoning or methodology properly can be applied to the facts in issue."[1] In *Kumho Tire*, the Court held that the trial court's gatekeeping responsibility applies to "all 'scientific,' 'technical,' or 'other specialized' matters within (Rule 702's) scope," emphasizing that a trial court's role is "to determine reliability in light of the particular facts and circumstances of the particular case."[2]

FDEs have traditionally premised the scientific validity and reliability of handwriting identification on three principles or tenets: (1) handwriting is unique, meaning that no two people write exactly alike; (2) no person can produce an exact duplicate of his or her signature or write exactly the same way twice; and, (3) no person can exceed his or her writing skill level at a given point in time. Recent district court decisions excluding or limiting the proffered testimony of handwriting experts have concluded that, regardless of whether handwriting analysis is characterized as science or art, there has been indequate testing and insufficent data to support the scientific validity of the first two fundamental principles that have been espoused by virtually all FDEs as the underlying basis for handwriting identification. Perhaps most noteworthy is United States District Court Southern District of New York(SDNY) Judge Jed S. Rakoff's May 6, 2016, decision in *Almeciga v Center for Investigative Reporting, Inc.*, where the court considered "how well handwriting analysis fares under *Daubert* and whether (the proffered handwriting expert's) testimony is admissible as "science" or otherwise."[3] In granting the defendant's motion to exclude Wendy Carlson's "expert" testimony, Judge Rakoff found that "handwriting analysis in general is unlikely to meet the admissibility requirements of Fed. Rule 702 and that, in any event, Ms. Carlson's testimony does not meet those standards."

The rationale underlying Judge Rakoff's analysis of the traditionally offered tenets of handwriting comparison and the validation studies offered in support of those tenets will be reviewed as will his Fed. Rule 702 analysis of the handwriting comparison process in general and as applied by the proffered "expert" to the facts of the particular case at hand. Some other recent court decisions limiting testimony on the part of handwriting experts will also be discussed.

Finally, a somewhat different, task-specific basis for establishing that handwriting comparison evidence in a given case is sufficiently reliable to be admissible under Fed. Rule 702 will be offered. Methods and strategies will be suggested for providing a court with the necessary baseline level of confidence to enable it to find that a proffered handwriting expert's testimony rests on a reliable foundation as related to the particular task at hand (without having to reject Judge Rakoff's decision in the 2016 *Almeciga* case).

Reference(s):

1. *Daubert v. Merrill Dow Pharmaceutcals, Inc.,* 509 U.S. 579 (1993).
2. *Kumho Tire Co. v. Carmichael,* 526 U.S. 137 (1999).
3. *Almeciga v. Center for Investigative Reporting, Inc.*, 2016 WL 2621131, F.Supp.3d (2016).

Handwriting Analysis, *Daubert/Kumho* Factors, Federal Rule 702

G1 The Third Molar Cut-Off Value in Assessing the Legal Age of 18 in the Saudi Population

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The goal of this presentation is to assist forensic odontologists with the age assessment process for cases in which there is a question as to whether or not legal age has been attained, with emphasis on the use of the method with a specific ethnic population.

This presentation will impact the forensic science community by providing population-specific evidence of the cut-off values in dental development for the age of 18 years.

Teeth can play a major role in forensic sciences, especially in age assessment of an individual, which can be an issue in various criminal or civil matters. Teeth can be of significance due to the fact that they survive in extreme conditions and are affected very little by exogenous and endogenous factors. The third molar is the only tooth that is still developing at the age of 14 years and also during the legal age of adulthood, which is generally 18 years. The consequences of a criminal conviction can strongly affect the individual's subsequent life; therefore, it is crucial to properly set different parameters to predict whether an individual is a minor or an adult in the absence of acceptable supporting documents. Depending on the different legal requirements, such parameters can be set above a 90% probability for criminal matters and at 51% for civil matters (but note that "90%" is not a defined standard for criminal matters).

Goal: The goal of this research was to find the appropriate cut-off value of third molar development for the legal age of 18 years among Saudi individuals by using the third molar maturity index method by Cameriere et al.[1]

Materials and Methods: This was a cross-sectional study using 300 archived Orthopantomograms (OPGs) of healthy Saudi patients between the ages 14 and 22 years attending the Dental Hospital at King Saud University, Riyadh, Saudi Arabia. All OPGs were taken with a PLANMECA ProMax machine and were evaluated using Romaxis® software. The inclusion criteria were good quality OPGs taken during the course of treatment. All patients were healthy with no systemic diseases or disorders with the presence of third molars and clear root apex.

The Lower Left mandibular third Molar (LL3rd M) was assessed using the third molar maturity index (I3M) in order to estimate whether the individual was younger or older than 18 years of age.

Results: The cut-off value of I3M for the Saudi population was (I3M <0.08). The sensitivity of this method was 51.7% and the specificity was 98.5%. Early mineralization was found in males, except in the I3M ranges (0.0-0.4) and (0.9-1.7). Cameriere et al.'s method results were reproducible with a good measure of realibility.[1]

Conclusion: This method is suitable for assessing the attainment of legal age of adulthood in the Saudi population, and the cut-off value of I3M is similar to other populations. Although dental age assessment by means of third molar development is useful, it still has some limitations because of variation in individual tooth position, morphology, and development.

Reference(s):

1. Cameriere R., Ferrante L., De Angelis D. The comparison between measurement of open apices of third molars and Demirjian stages to test chronological ages of over 18 years old in living subjects. *Int J Leg Med.* 2008: 122(6): 493-7.

Legal Age, Cut-Off Value, Age Estimation

G2 The Interpretation of Canine Scavenging Lesions on Human Corpses

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After attending this presentation, attendees will be better able to interpret forensic cases involving canine scavenging with certain specific postmortem lesions.

This presentation will impact the forensic science community by providing examples of two different suspicious death cases with specific lesions caused by dogs scavenging on human corpses, on different areas of the body, and with unusual mechanisms.

Canine scavenging typically results in skin and/or bone lesions with specific characteristics, but this aspect of the injuries can be difficult to interpret, especially when different mechanisms are combined. Two different cases are presented and illustrated. The first is the case of a 51-year-old man found dead in his garden by a neighbor, his left hand separated from his body, with his dog feeding on it. A bloodstained hatchet was found in the garage at the back of the garden, with blood spots all around the room. At autopsy, an association of different lesions was observed on the left upper limb: sharp, regular and deep cutaneous and bone wounds on the wrist, and irregular and wrinkled wounds, as well as a loss of soft tissue on the forearm. The histological analysis revealed that the lesions on the wrist were antemortem and the ones on the forearm were postmortem. Those findings reflect different injury mechanisms, which should be identified in order to reconstruct the facts: one part of the wound is typically associated with sharp objects, while the other one presents the characteristics of canine scavenging. Death was attributed to an external hemorrhage due to the amputation of the left upper limb by the hatchet found onsite. The manner of death was classified as suicide.

The second case concerns a 72-year-old woman found dead at home by her son, seated on a chair, with a deep loss of tissue around both eyes. The decedent was in a poor general condition and was the owner of a dog. External examination and autopsy revealed isolated loss of soft tissue in both of the periorbital areas, but sparing the eyeballs. No facial bone fracture and no other traumatic injuries were observed, only cutaneous lesions, with respect to her medical history. The aspect of the lesions on the periorbital areas is rarely described and was compatible with licks and bites from a dog. The histological analysis revealed that the facial skin wounds were postmortem. The cause of death was attributed to a cardiac decompensation and the manner of death was classified as natural.

This presentation highlights the unusual injury mechanisms that can be observed in cases of canine scavenging. The injuries are compared with the literature in terms of visual appearance, location on the body, motivation of the animal behavior, and time interval between death and the canine scavenging. An increased knowledge of those lesions, as well as a close collaboration between forensic pathologists and odontologists, can be particularly useful when confronted with similar cases.

Scavenging, Dog Bites, Forensic

G3 Dental Age Estimation: Combining Radiographic Information of Two Dental and Four Skeletal Predictors in Children

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After attending this presentation, attendees will be updated on the current scientific trends in research involving age estimation in children. Additionally, attendees will benefit from learning that the combination of radiographic dental and skeletal age predictors results in more accurate age estimates. Finally, attendees will be acquainted with the specific added value each skeletal age predictor provides when combined with the dental age predictor.

This presentation will impact the forensic science community by indicating the best practices in age estimation procedures involving children. In particular, it will reveal that multivariate analysis of combined dental and skeletal age predictors contributes to more accurate age estimates. Additionally, the optimal balance between the most accurate age estimate and minimal radiation dose will be proposed for application in forensic age estimation practices.

In children and adolescents, the age estimation process is based primarily on radiographically observed development of either the teeth or the skeleton, or both.[1-4] Combining several age estimation parameters for more accurate age prediction appears to be the general consensus[5]. In order to obtain more accurate age estimates in a safe manner, dental and skeletal developmental parameters should be combined with a minimal use of radiation (in some jurisdictions, there may be issues with exposing individuals to radiation for a non-diagnostic, non-therapeutic purpose such as age assessment). The goal of this study was to validate age prediction methods that combine dental development of the permanent teeth and third molars with the skeletal development of cervical vertebra, hand-wrist bones, and craniofacial bones.

Panoramic radiographs, lateral cephalometric radiographs, frontal cephalometric radiographs, and left hand-wrist radiographs were collected from 256 female (n=135) and male (n=121) Japanese subjects between 4 and 20 years of age. The set of four radiographs of the sampled subjects were all made the same day. On the panoramic radiographs, the development of the permanent left mandibular teeth were staged according to the technique of Demirjian et al., and the available third molars were staged according to the technique of Köhler et al.[2,6] The skeletal development of the hand-wrist bones from the left hand-wrist radiographs and the third cervical vertebra from the lateral cephalometric radiographs were staged according to the techniques of Greulich et al. and Hassel et al., respectively.[3,7] On the frontal cephalometric radiographs, the cranial width and the mandibular angle width were measured and recorded. The age estimation procedure was sex-specific and based on the application of Bayes' rule to a multivariate continuation ratio model for the distribution of the scores.[8] The correlation between variables was calculated. The age estimate performances were quantified for mean error, mean absolute error, and Root Mean Squared Error (RMSE). The Wilcoxon signed rank test was used to compare the error and absolute error between models containing different variables as age predictors.

Strong correlations were found between chronological age and hand-wrist development (females: correlation coefficient (Rho)=0.89, $p<0.0001$, males: Rho=0.84, $p<0.0001$), mandibular angle width (females: Rho=0.68, $p<0.0001$, males: Rho=0.62, $p<0.0001$), or third cervical vertebra (females: Rho=0.77, $p<0.0001$, males: Rho=0.82, $p<0.0001$). Combining all parameters was the most accurate technique for age estimation in children (RMSE: 1.14 years in females, 1.19 years in males; mean error: -0.04 years in females, -0.04 years in males; mean absolute error: 0.91 years in females, 0.95 years in males). The best performing single combination was the dental parameters combined with the hand-wrist parameter (RMSE: 1.19 years in females, 1.22 years in males; mean error: -0.06 years in females, -0.06 years in males; mean absolute error: 0.94 years in females, 0.98 years in males).

This study proved that combining dental and skeletal age predictors enhances accuracy of the age estimates. Combining the dental and hand-wrist development variables resulted in more accurate age estimates. The images could be obtained with minimal ionizing exposures.

Reference(s):

1. Willems G., Van Olmen A., Spiessens B., Carels C. Dental age estimation in Belgian children: Demirjian's technique revisited. *J Forensic Sci.* 2001;46(4):893–5.
2. Demirjian A., Goldstein H., Tanner J. A new system of dental age assessment. *Hum Biol.* 1973;45(2):211-27.
3. Greulich W., Pyle S. *Radiographic atlas of skeletal development of the hand and wrist.* Stanford: Stanford University Press, 1959.
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Forensic Odontology, Dental Age Estimation, Skeletal Age Estimation

G4 The Accuracy of Third Molar Development as an Indicator of Chronological Age in a Texas Asian Population Using Demirjian's Method

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After attending this presentation, attendees will better understand third molar development as a tool for age estimation in a Texas Asian population.

This presentation will impact the forensic science community by providing information about the reliability of third molar development as an indicator of chronological age in a Texas Asian population.

Age assessment and age estimation are integral components of forensic science. They aid in the identification process of unknown remains by narrowing search parameters for possible matches, aid in providing age estimation relating to illegal immigrants without proper documentation, as well as help the legal system determine whether or not a person in question is of legal age.

Asia is the largest continent, constituting nearly one-third of the landmass, lying entirely north of the equator, except for some Southeast Asian islands with more than half the world's population. The Asian population is a diverse ethnic group and encompasses individuals from Japan, Taiwan, Korea, China, Vietnam, India, the Philippines, and various other countries. In 2012, Austin, TX, had the highest percentage of Asians of all cities in Texas and the tenth highest of all cities in the country.

To date, there has been very little research concerning age estimation for Asian populations completed in the United States. "Asian" is a term that defines "a person of Asian origin, regardless of race."

The accuracy and precision of chronological age estimation based on the stages of third molar development is studied in a sample of panoramic radiographs of Asian patients, ages 13-20 years according to Demirjian's schematic definitions of crown and root formation. The data was collected from current and previous patients of The University of Texas Health Science Center in San Antonio, TX, as well as from pediatric and orthodontic private practices in the Austin, TX, area. Asian ancestry was established by one of the following methods: surname of unmarried individuals, self-reporting on health history forms, and/or heath provider assessment in certain circumstances. Males and females are equally represented and there is adequate representation of all ages included in this study.

The age estimated from scoring the radiographs will be compared with the chronologic age of the subjects at the time the panoramic radiograph was taken. This study explores whether Demirjian's method provides accurate results that can be used for age estimation in this Texas Asian population. The population studied may differ from other ethnicities in third molar development. This study focuses on the reliability and/or variance of third molar development as it correlates with chronological age in the Texas Asian population studied and provides needed population-specific data.

Age Estimation, Third Molar, Asian

G5 Utilizing Custom Spreadsheets for Age Assessment Cases

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After attending this presentation, attendees will have gained a basic knowledge of how to utilize Excel® spreadsheets to aid forensic odontologists in the calculation of estimated age for their cases.

This presentation will impact the forensic science community by teaching the forensic odontologist the basic principles related to a spreadsheet constructed to allow the forensic odontologist to precisely and accurately calculate the estimated age of an unknown individual utilizing established data and methods from prior published studies.

The forensic odontologist is often called upon by agencies to estimate the age of deceased or living individuals. Many methods used to estimate age have been based on the dentition and its developmental stages. To calculate the estimated dental age, the forensic odontologist commonly utilizes published studies that provide a method to analyze the dentition of the unknown individual and typically apply a mathematical formula and/or a table of reference data.

This study has developed custom Excel® spreadsheets called Draft Age Estimation Quicksheets™ for many of the studies that forensic odontologists might utilize for the particular cases they are investigating. This presentation will review the custom spreadsheets and educate attendees on how to properly input the measured variables based on the specific study being used. The spreadsheet will then utilize the data and formulas contained in the particular study and the entered variables to calculate the estimated age, associated rate of error, and age range automatically.

The main goal of utilizing the custom spreadsheet method is to increase the speed of the calculations for the forensic odontologist and reduce the need to access and reference the original study. The spreadsheet will also help reduce common mathematical errors and increase the accuracy of the calculations made for age estimation cases. The results of the calculations in the spreadsheets may also be utilized directly in the forensic odontologist's reports by importing the appropriate fields into the report.

The Draft Age Estimation Quicksheets™ were previously presented to the Odontology Section at the American Academy of Forensic Sciences (AAFS) meeting in Las Vegas, NV. The spreadsheets have been further developed and refined since the last presentation. This presentation will explain in greater detail the application of the spreadsheets and also preview what is being worked on next.

In conclusion, this presentation will demonstrate custom Excel® spreadsheets, which can be utilized for age assessment cases. Attendees will also be provided with copies of the custom spreadsheets via email for interested forensic odontologists to utilize.

Age Assessment, Spreadsheet, Odontology

G6 Forensic Age Estimation (FAE) By Third Molar Mineralization Using Digital Orthopantomography (OPG)

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After attending this presentation, attendees will better understand the principles of FAE, how assessment of third molar mineralization can increase the accuracy of FAE, and how the use of digital OPG can increase the precision of third molar mineralization estimates.

This presentation will impact the forensic science community by providing information about the role of third molar mineralization in FAE and will highlight the role of digital OPG in FAE.

Age estimation is of paramount importance in assisting law enforcement in some cases.[1] In many countries, the relevant age in regard to liability for criminal responsibility is 18 years. Maturation of sexual characteristics, as well as tooth and skeletal development, have been used to estimate age.[2] Dental techniques that use progressive morphologic changes have proven to be the most accurate methods for estimating the ages of infants, children, and adolescents when compared to other methods of age estimation.[1] Several studies have revealed a strong correlation between third molar development and chronological age.[3-5] The focus of these methods is on the stage of maturity of the wisdom teeth because these are the only teeth to continue root mineralization after the age of 16 years. The present study is designed to judge the reliability of third molar mineralization in age estimation of adolescents by using digital OPG.

A retrospective study analyzing 167 OPGs (85 males and 82 females) aged between 14 and 25 years was performed to assess the degree of maturity of third molars. Demirjian's staging system, which subdivides development of the third molar into eight stages, was used. The assessed mineralization stage for each third molar was recorded separately for each quadrant. The mineralization stage and chronological age were subjected to statistical analysis.

This study revealed there was no statistically significant difference between males and females attaining each stage of mineralization. There was no significant difference between the upper and lower third molars in their mineralization. There was no statistically significant difference between the two genders with respect to the distribution of developmental stage H over the age of 18 years. The predictive percentage is 96.59% for stage H over the age of 18 years for both males and females. Based on Demirjian et al.'s stage H, the likelihood of an individual being at least 18 years of age with at least one fully developed mandibular wisdom teeth of the four amounts to 100% for both males and females.

The present study concludes that, in the case of completed mineralization for at least one third molar (Demirjian et al.'s stage H) out of four, the probability of the individual being at least 18 years old is 100% certain for both males and females. The method may, therefore, be considered a valid aid in estimating legal adulthood. The advantages of digital OPG for dental analysis must be acknowledged.

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Forensic Age Estimation, Third Molar Mineralization, Orthopantomography

G7 Third Molar Magnetic Resonance Imaging (MRI) in Forensic Age Estimation: Protocol Development and Considerations for Use

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After attending this presentation, attendees will understand which factors influence MRI protocols for age estimation based on the development of third molars and which considerations should be made when third molar development staging techniques are applied to MR images.

This presentation will impact the forensic science community by: (1) providing an MRI protocol to study third molars for age estimation; (2) providing recommendations to apply staging techniques to these images; (3) acknowledging influential factors in the development of an MRI protocol to study third molars for age estimation; and, (4) comparing third molar radiographs with third molar MRIs in order to demonstrate the differences between these two techniques and their advantages and disadvantages.

Established dental age estimation methods in subadults mainly consider development of third molar root apices visible in radiographs; however, in living individuals, minimizing ionizing radiation is essential.[1,2] Studying dental development with MRI complies with this goal, adding the advantage of imaging in three dimensions. Several studies have investigated the use of MRI for dental age estimation; however, none of these studies discussed the differences between staging with radiographs versus staging with MRI.[3-5]

First, this study proposes to demonstrate development of a 3-tesla MRI protocol to visualize all third molar positions for forensic age estimation. Particular attention will be given to the development of the third molar root apices.[6] Second, this study intends to prospectively study root stage assessment of third molars using MRI. In order to provide a comparison of 2D staging to 3D staging, panoramic radiographs will be compared to MRIs.[7]

To develop a clinically feasible MRI protocol visualizing all third molars for age estimation, *ex vivo* scans of porcine jaws and *in vivo* scans of ten living human volunteers aged 17-25 years were performed. Studied parameters were T1 versus T2 weighting, Ultrashort Echo Time (UTE), fat suppression, in-plane resolution, slice thickness, 3D imaging, signal-to-noise ratio, and acquisition time. A bilateral four-channel flexible surface coil was used. Two observers evaluated the suitability of the images.

To compare third molar staging on MRI with staging on panoramic radiographs, all third molars of 16 participants between 14 and 26 years of age were evaluated. Three different staging techniques were used by two observers.[8-10]

T2-weighted images were found to be preferred to T1-weighted images. To clearly distinguish root apices in (almost) fully developed third molars, an in-plane resolution of 0.33 x 0.33mm² was deemed necessary. Taking acquisition time limits into account, only a T2 Fast Spin Echo (FSE) sequence with slice thickness of 2mm generated images with sufficient resolution and contrast. UTE, thinner slice T2 FSE, and T2 3D FSE sequences could not generate the desired resolution within the 6.5-minute acquisition time limit.

Lower third molars were equally assessable on both imaging techniques (93.8% MRI, 98.4% radiograph). Upper third molars were more difficult to evaluate on radiographs than on MRI (96.9% MRI, 43% radiograph). Inter- and intra-observer agreement for staging was higher for MRI than for radiographs. In both imaging techniques, lower third molar staging revealed higher inter- and intra-observer agreement compared to upper third molar staging. MR images in the sagittal plane proved to be essential for third molar staging. The most significant roots were the palatal in upper third molars and the distal in lower third molars.

The 3-tesla MRI of the third molars is a feasible technique for age estimation, in which a T2 FSE sequence can provide the desired in-plane resolution within a clinically acceptable acquisition time. Several considerations are necessary to transfer known radiographical 2D staging methods to this 3D MRI application.

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Forensic Odontology, Age Estimation, Magnetic Resonance Imaging

G8 Root Pulp Visibility (RPV): A Reliable Mandibular Maturity Marker (MMM) to Determine a Subject's Status at the 18-Year Threshold

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After attending this presentation, attendees will be aware of the use of RPV as an MMM to predict whether or not a subject of unknown date of birth is below or above the 18-year age threshold and will be able to apply assessment of RPV to cases that are presented for their consideration.

This presentation will impact the forensic science community by illustrating that the differentiation between RPV-A and RPV-B compared to RPV-C and RPV-D will enable assessors to predict with 100% reliability whether or not a subject is more than 18 years old.

Introduction: The potential for using the gradual disappearance of the root pulp of lower third molars as a marker to indicate an age above 18 years was first published in 2010 by Olze et al.[1] This approach was subsequently tested on a large group of subjects in England to provide a reference data set for United Kingdom Caucasians.[2] The high concordance of the results from these two studies led to the introduction of the RPV assessment for cases of forensic age estimation. As a consequence of this development, it was deemed appropriate to revisit the Dental Age Estimation (DAE) cases carried out by the Dental Age Research London Information Group (DARLInG) team.

Materials and Methods: All DAE cases from 2005 to 2015 were re-examined to determine the applicability of the RPV assessment when predicting whether subjects were above or below the 18-year threshold. A cascade of filters was applied to the data held on DAE cases including the presence of Stage H for LL8 (Fédération Dentaire Internationale (FDI) 38; if the LL8 was absent but the LR8 was present, LR8 was used as an alternate). When LL8 Stage H was present (or LR8H as an alternate), RPV was assessed using combined criteria[1,2] This enabled calculation of the probability of a subject being more than 18 years of age.

Females	**(years)**	**sd**	**p > 18 years**	**%-age > 18 years**	**n - tds for RPV Stages in females**
RPV-Af	21.44	2.03	0.966	96.6	4
RPV-Bf	22.10	2.03	0.990	99.0	22
RPV-Cf	23.64	1.58	1.000	**100.0**	9
RPV-Df	23.84	1.38	1.000	**100.0**	1
Males	**(years)**	**sd**	**p > 18 years**	**%-age > 18 years**	**n - tds for RPV Stages in males**
RPV-Am	21.27	2.05	0.977	97.7	40
RPV-Bm	22.61	2.15	0.993	99.3	87
RPV-Cm	23.34	1.98	1.000	**100.0**	26
RPV-Dm	23.46	1.67	1.000	**100.0**	2

Results: From the total of 410 forensic age assessments, there were 191 subjects with tooth 38 (FDI; the lower left third molar) at Stage H, which is completion of root development. Where appropriate, tooth 48 was substituted.

Using conventional probability estimates confined to the final stage of development of LL8H, the following probabilities that the subject is more than 18 years were derived for both sexes: (1) in females, tooth 38 (LL8Hf), the probability that the subject was more than 18 years was 0.867 (86.7%); (2) in males, tooth 38 (LL8Hm), the probability that the subject was more than 18 years was 0.750 (75.0%); and, (3) the probabilities of a subject being more than 18 years using RPV are shown in the table. For both female and male subjects with RPV-C and RPV-D, the probability of being more than 18 years is 1.000 (100%).[3]

Discussion: The presence of RPV-C and RPV-D is a clear indicator that the subject is more than 18 years old. For RPV-A and RPV-B, there is a small probability that the subject is less than 18 years old. The MMM are limited to subjects for whom the LL8 (or LR8) are present on the dental panoramic tomograph.

Conclusion: The presence of RPV-C for both females and males indicates a 100% probability that the subjects exhibiting this feature are more than 18 years old.

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Root, Pulp, Visibility

G9 The Integration of Vertebrae and Teeth Analysis for Age Estimation in a Population of Libyan Children

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After attending this presentation, attendees will possess novel information concerning age estimation in a population of Libyan children using an integrated model of vertebrae and tooth metric analyses to increase the accuracy of these estimations.

This presentation will impact the forensic science community by demonstrating how different approaches based on tooth development and skeletal development can increase the accuracy in age estimation in populations of northern African children. The northern coasts of Africa play an important role in emigration from Africa to Europe; thus, the availability of an accurate method to age children is fundamental from a legal point of view, especially in cases of non-accompanied children when adoption procedures are instituted.

Since 2000, age estimation of living individuals has become increasingly common in civil and criminal cases in order to address problems such as: the age of children in cases of adoption; criminals who refuse to provide their age; issues related to immigration and asylum requests; pedophilia prosecution; child pornography; and various other civil matters, such as pensionable age.

Estimating the age of a living person often requires an integrative approach that involves anthropology, forensic dentistry, and radiology. Human identification and aging using dentition have been well established in the forensic field and several methods based on sequential changes that occur to teeth during the aging have been developed. The goal of this paper is to validate, for the Libyan children population, three approaches: the first based on tooth development, the second on vertebrae development, and the third on the integration of these two approaches.

For this purpose, two samples of children from Tripoli and Misurata, two cities where different ethnic groups are represented in the population, were analyzed.

Panoramic radiographs (Orthopantomograms (OPTs)) and cephalograms from healthy living Libyan children aged between 4 and 15 years with no obvious development abnormalities were analyzed. The samples were obtained directly by digital radiological technology and were collected from February, 2015 to June, 2016. Radiographs were performed for clinical purposes, and consent to use them for research purposes was obtained from the patients' relatives. Panoramic images with lost or extracted single-rooted teeth, as well as those with fillings, crown restorations, severe caries, or other abnormal dental anatomy which might cause difficulty with measurement were excluded from this analysis.

Results obtained based on the tooth development method allowed researchers to generate a new formula for age estimation with a higher coefficient of determination (R^2) when compared with the previously published data from the European and Mediterranean area.

Vertebrae analysis reveals a positive correlation between the Vba index and the patients' age, but here a low correlation coefficient was obtained. This value is lower compared to the previously published data.

The integrated model (teeth+vertebrae) allows the generation of a new formula that increases the accuracy of the age estimation, with a reduction of the estimated error of approximately 5%.

In conclusion, the tooth-based method results in a more accurate age estimation in children when compared to the vertebrae method. Moreover, the integration of the two approaches results in the reduction of the estimated error associated with the age prediction.

This conclusion has important applications in the Libyan forensic context, where the necessity for reliable and accurate age estimation techniques has never been greater than in the last five years, mainly due to armed conflicts within the country. The lack of a validated method of age estimation for the Libyan population age is a fundamental issue both in world crime investigation and in the emigration/immigration control at national and international levels.

G10 A Novel, Semi-Universal X-Ray Sensor Holder

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After attending this presentation, attendees will have an appreciation of a novel X-ray sensor holder that has semi-universal applicability to a variety of non-traditional dental and potentially non-dental applications.

This presentation will impact the forensic science community by increasing awareness of a new design for intraoral X-ray sensor holders. Current commercially available sensor holders have limitations that include rigidity, multiple pieces, and the inability to be barrier protected.

The current commercially available X-ray sensor holders for intraoral radiography are generally proprietary and sensor specific. While universal solutions for holding X-ray sensors exist, they fail to address needs encountered in forensic odontology, veterinary medicine, or other non-traditional applications. As a result, low tech devices, such as hemostats, tape on a stick, or even finger placement, is sometimes the sensor holder of choice. Commonly encountered problems in forensic odontology, when imaging decedents during postmortem examination, include access, orientation, and cleanliness of the sensor and sensor holder. A novel solution, to address limitations of currently available sensors in a postmortem setting, is the radiographic paddle (RadPad®). The radiographic paddle is a semi-universal sensor holder approximately 12 inches in length, expandable to approximately 18 inches. The handle of the RadPad® houses an expandable, rubber-coated wire attached on the terminal end to a hexagon-shaped, neodymium-surrounding magnet. The terminal end of the handle attaches to the X-ray sensor by insertion of the hexagon-shaped male portion into the receiving hexagon-shaped female portion. The neodymium magnet provides retention of the handle to the sensor. Several methods of connecting the female portion to the sensor are currently being investigated, with two different methods being utilized to acquire images in actual postmortem examinations.

Potential advantages of the RadPad® include: (1) only one handle and one sensor holder required for a 20-film Full Mouth X ray (FMX), with minimal manipulation of the sensor; (2) with some planning, a single handle can be adapted to any sensor, allowing for universal application and quick change between sensor brands; (3) flexibility of the rubber-coated wire allows for flexibility not offered in most X-ray sensor holder systems; (4) expansion of the handle affords the operator to reach intraoral locations not easily accessed by straight and rigid X-ray systems. Additionally, the handle expansion allows for decreased radiation exposure by allowing the operator to have greater distance between the X-ray beam and operator hand; (5) potential for complete barrier protection; (6) possibility to utilize in other non-traditional X-ray applications; and, (7) while some technical skill is required, a RadPad® can be constructed with materials available at most hardware stores, minus the neodymium magnets, which are readily commercially available.

Potential disadvantages of the RadPad® include: (1) not commercially available; (2) handle to sensor retention in the event of tight spaces that require moderate or greater force; (3) flexibility of the rubberized wire in the event of tight spaces that require moderate or greater force; (4) lack of X-ray tube head positioning; (5) bulkiness, which might preclude utilization in awake/alive patients; (6) handle cannot be heat sterilized; and, (7) possible durability.

The RadPad®, while not without limitations, has distinct advantages when utilized in a non-traditional setting. The potential for decreased radiation exposure to the operator while simplifying the sensor holder armamentarium and increasing cleanliness through complete barrier protection are three primary advantages.

X-Ray, Sensor, Holder

G11 The Accuracy of the London Atlas of Human Tooth Development and Eruption in Dental Age Estimations of Saudi, Spanish, and Italian Children

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After attending this presentation, attendees will better understand similarities between various populations and that new evidence-based methods of age estimation can be used internationally without a need for a population-specific method.

This presentation will impact the forensic science community by increasing understanding as to why certain age estimation methods are applicable to variable populations with good measures of accuracy and why others are population-specific.

Schemata of dental development are used frequently to assess maturity and estimate dental age, yet there is still little evidence of their accuracy when used for various ethnic populations. The goal of this study was to test and compare the accuracy of The London Atlas of Tooth Development and Eruption when used for dental age estimation in Saudi, Spanish, and Italian populations.[1]

Materials and Methods: The sample consisted of 400 Saudi, 400 Spanish, and 300 Italian males and females between the ages 6-15 years. Inclusion criterion was: good quality, clear panoramic dental radiographs (Orthopantomograms (OPGs)) of healthy patients with no medical history of systemic diseases/disorders. Exclusion criteria were: unclear radiographs; hypodontia (one or more missing teeth); hyperdontia (one or more extra teeth); gross pathology (taurodontism, microdontia, amelogenisis imperfecta, dentinogensis imperfecta, tumors, abscesses, fractures, etc.); presence of gross caries; or previous orthodontic treatment. Age estimation was performed using The London Atlas of Tooth Development and Eruption on the left side of both upper and lower jaws by direct comparison with the diagrams.

Chronological age ("Real Age" (RA)) was blinded from the researchers until all radiographs were assessed and age estimation was completed. All data were managed and analyzed using the Statistical Package for the Social Sciences (SPSS) program (v24). Inter- and intra-examiner reliability tests were performed on a random 10% sample from the radiographs and the appropriate kappa statistic was calculated.

Results: Intra-examiner reliability test kappa was (0.9) and the inter-examiner reliability test kappa was (0.87), which indicates excellent agreement.

Mean difference between Estimated Age (EA) and RA in all populations combined was 0.21 years with Standard Deviation (SD) of 0.978 years, and absolute mean difference of 0.645 years.

The Saudi population revealed the mean difference between EA and RA to be 0.247 years (SD 0.769 years) with no bias (p 0.08), and the absolute mean difference was 0.620 years. The Spanish population exhibited the lowest mean difference (0.099 years), but with the largest SD (1.09 years) with no bias (p 0.071), and the absolute mean difference was 0.857 years. In the Italian population, the mean difference was 0.451 years with a standard deviation of 0.965 years, with positive no bias (p 0.006), and the absolute mean difference was 0.782. Thus, there were no statistically significant differences between populations.

Conclusion: The London Atlas of Tooth Development and Eruption is applicable to the Saudi, Spanish, and Italian populations with good measures of accuracy.

Reference(s):

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The London Atlas, Age Estimation, Accuracy

G12 Evaluating a Bitemark in Light of the Scientific Research Regarding Skin Distortion

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After attending this presentation, attendees will better understand the issues at hand with regard to traditional bitemark analysis.

This presentation will impact the forensic science community by highlighting the fact that traditional analysis of bitemark evidence cannot be viewed as axiomatic.

Bitemark analysis, as has been taught and accepted in this country (as well as in others) for the past 30^{+} years, has been based on the intuitive concept that teeth will leave marks on a bitten surface that are distinct and attributable. A basic tenet underlying this conceit, according to opinion, is that each human dentition is unique. Early methods of analysis involved the use of tracings of the biting edges of the anterior dentition on acetate and the superimposition of said tracing on a photograph of the mark in question (as opposed to comparison to the mark itself, due to the obvious logistical complications that would be associated with such an approach). The fabrication of these overlays has evolved from tracing on acetate to computer-assisted overlays, and the comparisons to the marks are also computer assisted. Additionally, metrics are used.

Research on the fidelity of skin as a recording medium, particularly with regard to bitemarks, led the researchers to caution that this approach was scientifically unsupported as early as the early 1970s, yet this research seems to have been marginalized or ignored.[1-3] More recent research has led current researchers to the same conclusions.[4-7]

The case to be discussed involves a bitemark on the right forearm of a deceased 5-year-old mentally disabled child. The child was received at the Kings County Office of Chief Medical Examiner of the City of New York in 2012. In addition to the bitemark, there were multiple bruises consistent with physical abuse. The cause of death was determined to be drowning.

As the bitemark was in a position where it might have been self-inflicted, impressions of the child's dentition were made and stone models created. Photographs of the bitemark were taken with an American Board of Forensic Odontology (ABFO) ruler in place. The subcutaneous tissue displayed a somewhat sharper image of the mark and it was photographed as well.

By convention, after first identifying the mark to be a bite, the next question which must be posed is, "Does the mark have sufficient clarity and definition to warrant further analysis?" Should one answer, "Yes," then a traditional forensic dental workup (measurements, overlays, etc.) is initiated, as in this case, to determine first whether the wound was self-inflicted; however, the far more important question is, whatever the determination of the analyst with regard to the source of the bite, can the analyst reference a scientific underpinning to support it?

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Bitemark, Distortion, Evidence

G13 The Use of Age Estimation Analysis for the Identification of Four Male Siblings in a House Fire in Kane, Manitoba, Canada

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After attending this presentation, attendees will be able to recognize how dental age assessments could be utilized to aid in predicting the ages of siblings. Attendees will also be able to appreciate the steps used in the analysis of the dental remains for estimation of age.

This presentation will impact the forensic science community by familiarizing attendees with the unique features of this case and discussing how forensic odontology provided a method to aid in estimation of the age of the four male siblings who perished in a tragic fire.

On February 25, 2015, close to midnight, a rural house in Kane, Manitoba, was completely destroyed by a fire. In the house were the mother and her seven children, while her husband and eldest son were at work. The mother and her three youngest children managed to escape, whereas the four older male children were trapped on the second floor of the house and perished. Unknown #1 (15 years old), Unknown #2 (12 years old), Unknown #3 (10 years old), and Unknown #4 (9 years old) died in the blaze.

The fire destroyed the house entirely and, initially, the remains of three of the victims were retrieved from the extinguished inferno by the Provincial Fire Commissioner's office. A cadaver dog was utilized to locate the remains of the fourth victim. The Office of the Chief Medical Examiner's Office of Manitoba received all remains and the forensic odontology team was consulted to provide expert assistance in identifying the victims.

Due to the financial constraints of the family, the decedents had never attended a dental appointment and, as a result, no clinical dental charting records or antemortem radiographs were available. As all four of the victims were siblings with the same parents, conventional parentage DNA analysis could only verify that the decedents were siblings, but could not confirm individual identities. Fingerprint evidence was not available due to the extensive damage from the fire to the remains.

The remains of each of the victims suffered various degrees of damage. Postmortem dental examination and radiographic images were collected for three of the four victims. No dental examination or radiographic images were completed for the fourth decedent (Unknown #2) because the skull and dentition were missing. A single tooth fragment that appeared to be fused with the spine was visualized on a chest radiograph.

Due to the lack of antemortem records, the Moorrees, Fanning, and Hunt dental age estimation method was utilized for three of the four unidentified remains.[1] Analysis of the postmortem radiographs revealed the following three age estimates: Unknown #1 (mean age of 14.5 years with an interval including 13-16 years), Unknown #3 (mean age of 10.25 years with an interval including 9-11 years), and Unknown #4 (mean age of 9 years with an interval including 8½-10¼ years). A range of +/-1 standard deviation was included for each of the analyses.

The forensic odontology analysis based on the Moorrees, Fanning, and Hunt charts and tables provided age estimates for three of the four decedents that were coincident with the known ages of the siblings. The forensic analysis using age estimation was a useful and expeditious method for identification of the siblings. The family was able to move forward with a funeral and burial with four separate caskets holding the remains for each of their children.

Reference(s):

1. Moorrees C.F.A., Fanning E.A., Hunt E.E. (1963). Age variation of formation stages for ten permanent teeth. *Journal of Dental Research.* 42, 1490-1502.

Age Estimation, Fire, Dental IDs

G14 The Kwok Bitemark Case Revisited

Duane E. Spencer, DDS, 1844 San Miguel Drive, Ste 112, Walnut Creek, CA 94596-5282*

After attending this presentation, attendees will better understand a criminal case that included bitemark evidence presented in court with a conclusion of "reasonable dental certainty."

This presentation will impact the forensic science community by illustrating a case in which the defendant and the victim bit each other. The bitemark on the victim was considered of good evidentiary value in 1996, with the defendant eventually admitting in court that he inflicted the bite.

Bitemark evidence has been admitted into the courts of the United States for a number of decades. In recent years, numbers of criminal cases that involved bitemark evidence have shown that evidence to be faulty, misanalyzed, or perhaps wrongly presented.

This 1995 case involved a dental hygienist who later became a registered nurse. Upon completing her nursing curriculum, she became reacquainted with her pharmacology instructor at her graduation party. For some months, they saw each other frequently for dinner. He gave her gifts and told her he was separated from his wife (he was not). He had a romantic goal and she did not. She told him he needed to be more outgoing. Eventually, he hatched a plan to "play a joke" on her. Without her knowledge, he had a key made for her home. Later, when he knew she would be working late at the hospital, he entered her home, dressed all in black, wearing a ski mask and black leather gloves, and carrying a bag filled with numerous items.

Arriving home late from work, she went to bed. He then proceeded with his "joke," which he said was patterned after the movie *True Lies*. On the way to her bedroom with his bag, he also picked up a knife from her kitchen. Asleep in her bed, she was awakened by someone on top of her, grabbing her throat, and heard, "Shut up, I'm going to kill you," which was from a recording of Eddie Murphy in *Beverly Hills Cop II*.

Thinking she may die, she fought back, not knowing her assailant. Falling off the bed, he slammed her head against the floor. When he put his hand over her mouth, she bit down on his thumb and held on. She struck him with her bedside radio. He lost consciousness and she thought he was dead. Running next door, she called 911. The police found the assailant unconscious by her bed. She now learned his identity. They were both transported to the local hospital, where he spent two days in the Intensive Care Unit (ICU).

Ten months after the incident, the forensic odontologist was contacted by the district attorney to evaluate "bitemarks" on a leather glove. After examining the tooth marks on the glove, the odontologist reviewed a number of photographs taken in the Emergency Room (ER). Noting a photo with marks on the victim's neck, the odontologist was told they were "finger marks." They were not finger marks, but a bitemark, which included two arches and drag marks! This new evidence caused the trial to be rescheduled. The odontologist took evidence from both the defendant and the victim and had three board-certified odontologists independently review the evidence and submit reports. Bitemark evidence was presented at trial by the prosecution. No defense odontologist testified. The defendant did testify and stated that it was when he was being bitten on the thumb and struck with the radio that he "must have bitten her." The distinct quality of the bite injury indicated that the bite was most likely inflicted when she was sleeping. She awoke to the bite, thinking she was being strangled.

A conviction was rendered in the case. The nurse went on with her life and the pharmacist went to prison. This case is being presented again after 20 years in the interest of the status of bitemark evidence today.

Bitemark, Drag Marks, Leather Gloves

G15 Domestic Violence and the Death of a Child

Barry E. Lipton, DDS, 2549 Eagles Crossing Drive, Clearwater, FL 33762*

After attending this presentation, attendees will understand: (1) the need to properly document a case and preserve the evidence, taking into consideration the time frame that may occur between the actual crime and potential litigation of the case; (2) how to prepare a case for trial even though testimony may be years in the future; and, (3) dental anomalies and their occurrence in the general population.

Testifying in a court of law is not the normal environment for a forensic dentist. As such, this presentation will impact the forensic science community by educating attendees on how to be: (1) prepared to recall the details of an investigation that may have occurred several years previously; (2) able to educate the judge and jury as to the findings; and, (3) able to render a professional opinion.

On the morning of May 5, 2009, the Hillsborough County Medical Examiner's office requested a forensic dental consultation to examine and evaluate a soft tissue injury located on the left chest of a 2-year-old child who was found in the median of Route 275, a high-speed roadway that passes through Tampa, FL. Based on the initial investigation, this child was believed to have been alive prior to being thrown from a moving vehicle that was traveling above 65 miles per hour. Based on the examination of the decedent, this specific injury was found not to be a human bitemark.

Following established protocol, on May 6, 2009, the Hillsborough County Sheriff's office produced several photographs of three soft tissue-pattern injuries present on the arms and right shoulder of the child's mother, an emancipated teen. These injuries were determined to be human bitemarks, and arrangements were made for a forensic dental examination and analysis of these bitemark injuries. On May 7, 2009, a forensic dental examination was conducted on the child's mother, who was herself the victim of domestic violence. During this examination, two additional human bitemarks were found on the victim. These additional injuries were located on her left cheek and left neck. The entire examination was conducted and the evidence documented in accordance with the then-current American Board of Forensic Odontology (ABFO) Bitemark Guidelines.

On May 10, 2009, the suspect in this case, the boyfriend of the mother, was examined per a court-ordered search warrant. The forensic dental evidence, findings, opinion, and court testimony will be presented.

This presentation will review the techniques used in documenting these multiple-patterned injuries, obtaining the dental records from the suspect, the results of the dental comparison of the injuries on the day they happened, and the physical and visual changes observed in these injuries over several days' healing. In addition, potential problems associated with multiple-patterned injuries (bitemarks), such as whether there was more than one assailant, the involvement of a forensic odontologist immediately after the injuries occur, the need for proper written documentation, photographs, collection of evidence, and preparation for trial, will also be considered.

Multiple Bitemarks, Record Keeping, Observation

G16 Analysis, Comparison, and Misinterpretation: Do You Really Want to Ban All Bitemark Evidence?

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After attending this presentation, attendees will have learned the importance of bitemark evidence as a tool for law enforcement, the prosecution, and the defense. The analysis of a patterned injury to determine whether or not it is a human bite will be shown and explained. The analysis — not comparison — has many applications, none of which involve matching bite to biter.

This presentation will impact the forensic science community by demonstrating the value of bitemark evidence with emphasis on the exculpatory use of this evidence. Determination that a patterned injury is a human bitemark is the first and most basic step in the analysis process. Some important historical cases will be presented to illustrate the value of this evidence for exclusion of a suspect, as well as for inclusion. The mistakes caused by incompetence, manipulation of the evidence, and other misuses will be explained and illustrated by actual case material.

Bitemark evidence in some cases has been misused, and occasionally abused, but that does not render it useless. Banning all bitemark evidence would be similar to banning fingerprint evidence because the Federal Bureau of Investigation (FBI) misidentified a print in the *Mayfield* case. Can bitemark evidence be misused, misinterpreted, and manipulated? Yes, but it can also be a valuable and valid forensic tool under the right circumstances, especially for the exclusion of a suspect. The statement by the late Dr. Joseph Davis (Chief Medical Examiner, Miami-Dade County), "It's not the science that's faulty, it's the people," is applicable with fingerprint and DNA analysis, as well as with bitemarks.

In the textbook *Dental Autopsy*, ten issues are listed with which bitemark analysis may be able to provide assistance in the pursuit of justice.[1] The analysis of a bitemark should not in any way be confused with comparison or "matching" to a suspect. When the term analysis is used, it should mean "the separation into its constituent parts for individual study." In the book *Bite Mark Evidence*, an entire section is devoted to the usefulness of bitemarks as exculpatory evidence.[5] It is pointed out that if the bitemark clearly shows marks from six maxillary teeth (i.e., analysis) and the suspect has only three maxillary teeth, he can be excluded, regardless of any such complications as skin distortion, occlusal wear, bite force, etc. — there are simply insufficient teeth to produce the mark. Why would anyone want to ban the use of bitemark evidence that could prevent an innocent person from being falsely accused?

Misuse of bitemark evidence is not a reason to ban it entirely. Rather, put rules in place, guidelines for use, restrictions guarding against misuse, and limitations on when and how this forensic tool can be used. There should be punishment for deliberate misuse. An excellent example of limiting the use of bitemark testimony was the ruling by the Michigan Supreme Court that banned the use of statistics when testifying as to a particular level of certainty. Michigan did not ban the use of bitemark testimony altogether, but rather put appropriate restrictions on the odontologist's testimony.

Most of the miscarriages of justice were brought about by faulty bitemark comparison (defined as "examination in order to note the similarities or differences"). In these cases, bitemarks were used without corroboration to identify a specific biter — and the analysts were wrong. These tragic mistakes have resulted in false convictions in several cases, of which the Kennedy Brewer case from Mississippi is probably the most egregious.[1,3-5] A classic example of bitemark manipulation is the case of Ray Krone and the matching (i.e., comparing) of a bitemark to his dental arrangement.[2,5] There was only one expert for the prosecution, while a plethora (4^{+}) of board-certified experts excluded Ray Krone as "the biter." Nevertheless, Krone was convicted and the jury recommended the death penalty. Ten years later, DNA evidence excluded Krone, and the DNA from the bitemark was "matched" to the real killer, whose teeth "matched" the bitemark.

To repeat, "It's not the science that's faulty, it's the people."

Reference(s):

1. *Dental Autopsy.* Silver and Souviron, CRC press, Taylor/Francis group, 2009. Chapter 13 PP-151-194.
2. *Forensic Dentistry Second Edition.* Senn and Stimson, CRC press, Taylor/Francis, 2010. Chapter 14 PP-305-367.
3. *Medicolegal Investigation of Death.* Spitz and Fisher, 4th Edition, Charles C. Thompson Publisher LTD. 2006. Chapter VI PP-264-275.
4. *Forensic Pathology.* Dolinak, Matshes, and Lew, Elsevier Academic Press, 2005. Chapter 27 PP-615-629.
5. *Bite Mark Evidence.* Edited by Robert B.J. Dorion, Second Edition, Marcel Dekker, 2005. Chapter 28 PT 555-556.

Comparison, Analysis, Bitemarks

G17 Human Bites: A Diagnosis

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After attending this presentation, attendees will recognize how the diagnosis of a human bite is different than assessing the evidentiary value of a bitemark, and how the diagnosis of an injury as a bite is useful in legal proceedings.

This presentation will impact the forensic science community by considering the value of the diagnosis of a bite injury without the inclusion or exclusion of a specific suspect.

Odontologists, Emergency Room (ER) physicians, pediatric child abuse specialists, medical examiners, and other mandated reporters may be asked to examine injuries suspected to be human bites. How does one diagnose an injury as a human bite? How is a diagnosis of human bite different than assessing the evidentiary value of a bitemark?

First, what is a diagnosis? Diagnosis may be defined as: (1) the act or process of identifying or determining the nature and cause of a disease or injury through evaluation of patient history, examination, and review of laboratory data; and, (2) the opinion derived from such an evaluation. Diagnosis is also based on the experience of the clinician as well as epidemiologic studies. Diagnoses are based on observed signs and symptoms, patient history, laboratory tests, and clinical experience — also known as evidence-based medicine.

Characteristics diagnostic for a bite injury are: a circular, oval, or curvilinear pattern or patterned injury consisting of two opposing arches, often, but not always, separated at their bases by space. Individual marks, abrasions, contusions, or lacerations may be found near the periphery of each arch. The marks present should reflect the size, shape, arrangement, and distribution of the contacting surfaces of human teeth. The size and shape of each arch visible is consistent with the size and shape of the human dentition.

The hypothesis that a human bite will cause an injury is reliable and valid. Reliability refers to the extent to which results are repeatable. If a bite is made with sufficient force, there will be an injury (mark) produced. "Validity" addresses the issue, "Is this statement true? A human bite with sufficient force will cause an injury." Again, data and experience support the validity and reliability that injuries can be caused by human teeth. The many studies on bitemark analysis have all started with an actual or experimental bite. There has been no disagreement that a visible injury was caused; however, assessing the evidentiary value of a bitemark is different, as the reliability and validity of bitemark analysis has been questioned. Studies are anticipated to better define parameters for evidentiary classifications and linkage to include or exclude suspect dentitions.

The 2014 Freeman-Pretty study was to test the first step of the American Board of Forensic Odontology (ABFO) Bitemark Algorithm. The question was essentially, "Is this mark evidentiary?" The disagreement between examiners cited by bitemark critics was not based on recognition of an injury as a bite, but rather whether the injury had sufficient evidentiary value to include or exclude a suspect.

The presentation will use case studies to review signs and symptoms of human bites, discuss differential diagnoses, discuss terminology, discuss the importance of a diagnosis without linkage to a suspect, and address differing opinions between clinicians.

Bitemark, Diagnosis, Odontology

G18 How the Lack of Human Dentition Uniqueness in Orthodontically Treated Patients and Twins Affects Bitemark Investigations

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After attending this presentation, attendees will be better informed regarding the research on the Uniqueness of the Human Dentition (UHD). Specifically, attendees will be aware of the most advanced pathways for investigations in the field, and will obtain scientific evidence to indicate whether or not the UHD is able to support current bitemark analyses. Finally, suggestions for undisputable bitemark analyses will be presented.

This presentation will impact the forensic science community by scientifically demonstrating the lack of UHD among orthodontically treated patients and twins. Additionally, this presentation will reveal the most advanced approach for investigations on the UHD on the level of bitemarks, encouraging the use of this methodology for further research in the field. This presentation may reinforce the fact that bitemark analyses should be performed with caution in the forensic practice, confirming that only trained professionals should become involved, and that specific case selection should be considered.

The UHD became one of the most polemic fields in forensic dentistry in the last decade. Criticism arose in the scientific community[1], especially in the interface of the UHD and bitemark analysis.[1-4] The scientific literature demonstrates a dissonant opinion concerning the existence or not of the UHD on the level of bitemarks.[5-7] The present study proposed to investigate the UHD at the level of bitemarks in human skin, systematically modifying the amount of dental material analyzed in the anterior dentition.

The sample consisted of 445 dental casts digitalized with an automated motion device (XCAD 3D®) and implemented in the Geomagic Studio® software package. The dental casts derived from randomly selected patients (n=22), orthodontically treated patients (n=59), twins (n=344), and orthodontically treated twins (*n*=20). Additionally, casts (*n*=20) were collected from subjects at two different times to establish threshold values indicating whether or not two dentitions were equal. Within each group, pair-wise comparisons were performed with the Geomagic Studio® software, using automated superimposition. The comparisons were performed on four levels, systematically reducing the amount of dental material analyzed (level 1: 3mm of the incisal edges; level 2: 2mm of the incisal edges; level 3: 1mm of the incisal edges; level 4: a slice of 1mm not including the incisal edges of the anterior dentition). The morphological differences between dentitions were quantified separately for the maxilla and the mandible, for each group, and for each level, and expressed in four values: the maximum positive and negative deviations, the average and the standard deviation. The four quantified values were log transformed in a single mean Euclidean distance and compared with the threshold of the respective level, using the Analysis of Variance (ANOVA) test with a significance interval of 5%. A Receiver Operating Characteristics (ROC) analysis was performed to test the predictive value of the threshold group to classify unique and not unique dentitions.

In all the study levels, the mean Euclidean distances of the maxilla and the mandible of orthodontically treated patients, twins, and orthodontically treated twins remained below the threshold ($p<0.05$). The opposite was observed with the randomly selected subjects. The resulting ROC curve was optimistic for the study levels 1, 2, and 3, reaching sensitivity and specificity rates between 70%-100%, while it decreased to 55%-80% in study level 4. Apart from that, the Area Under the Curve (AUC) remained >71% in all the study levels, suggesting a proper threshold for the classification of "unique" and "not unique" dentitions.

The lack of the UHD on bitemark levels observed in the present study was proven with advanced scientific standards; however, this finding must be carefully interpreted. Specifically, the bitemark practice must not be disregarded, but applied after careful case selection.

Reference(s):

1. Committee on Identifying the Needs of the Forensic Sciences Community NRC. *Strengthening Forensic Science in the United States: A Path Forward.* 2009.
2. Pretty I.A., Sweet D. The scientific basis for human bitemark analyses—a critical review. *Sci Justice.* 2001;41(2):85–92.
3. Pretty I.A. The barriers to achieving an evidence base for bitemark analysis. *Forensic Sci Int.* 2006;159(1):110–20.
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7. Kieser J.A., Bernal V., Neil Waddell J., Raju S. The uniqueness of the human anterior dentition: A geometric morphometric analysis. *J Forensic Sci.* 2007;52(3):671–7.

Forensic Odontology, Uniqueness of Human Dentition, Bitemark

G19 The Torgersen Tooth Mark Case From Norway — An Enigma

Tore T. Solheim, Institute of Oral Biol, Box 1052 Blindern, Oslo 0316, NORWAY*

After attending this presentation, attendees will be better informed regarding tooth mark examination and the current American system.

This presentation will impact the forensic science community by emphasizing that examinations and reporting should be as neutral and objective as possible.

Torgersen, then 24 years old, was in 1958 sentenced for the murder of a 16-year-old female in Oslo, Norway. Among several types of evidence were toothmarks around her left nipple, which were interpreted as a bitemark. All experts agreed that the marks were made close to the time of death as the indentations were rather clear and there was little, if any, bruising. At the court case, two Norwegian forensic odontologists testified independently of each other that the marks were nearly certainly made by Torgersen's teeth. Torgersen never admitted to having made the bites nor murdering the girl. In 1973, a petition for reopening the case was filed. The tooth marks were then examined by a third Norwegian forensic odontologist with the same result as before, and reopening the case was not granted. In 1998, another petition for reopening the case was presented. The court then went to Great Britain and found two of their most experienced forensic odontologists who concluded that the bite was very likely made by Torgersen's teeth, even though they made a mistake in orienting the upper jaw teeth. The defense now produced one American forensic odontologist, who said that he could exclude Torgersen as having made the bite. He also consulted three other board-certified American forensic odontologists who all agreed that Torgersen could be excluded. Reopening the case was not granted by the Norwegian High Court, even after hearing the evidence of the British and American experts. Next, a special committee was convened in Norway for assessing the possible reopening of criminal cases. This commission held a hearing with the British experts and the American expert in 2006. In addition, another expert was asked to assess the possibility that the marks were made by the teeth of Torgersen and also comment on the other experts' reports. It was concluded that the marks were in all likelihood made by the teeth of Torgersen. Reopening the case was again not granted.

This presentation will examine the findings that indicated that Torgersen made the marks and some of the arguments used by the American forensic odontologist to exclude Torgersen as having made the marks. A couple of those same facts were even found to be possible evidence that Torgersen did, in fact, make the bite. Some comments on the possible reasons for the discrepancies in interpretation and conclusions will be provided. Torgersen died last year and took his secret with him the grave.

Tooth Marks, Examination, American Forensic Odontology

G20 Hazards with Bitemark Identifications

Cheri Lewis, DDS, 8500 Wilshire Boulevard, Ste 805, Beverly Hills, CA 90211-3106; and Leonor A. Marroquin, MS, 10717 Woodruff Avenue, Downey, CA 90241*

After attending this presentation, attendees will understand how the position of bitemark photographs impacts the resultant reliability. Attendees will be able to identify how skin distortions impact the reliability of bitemark interpretation and will understand the limitations related to working with victims, given that dead men tell no lies and live victims are not reliable in describing exact body positions at the time of a bite.

This presentation will impact the forensic science community by explaining the need to limit the use of bitemark identifications in the judicial system.

In recent years, human bitemark interpretation has experienced a large degree of scrutiny due to the subjective aspects of the interpretation process. Analysis has never passed scientific examination.[1-3] Some critics contend the rate of error or of false identifications may be as high as 91%. High-profile cases in which people have been convicted largely on the basis of bitemark analysis and later proven innocent through DNA testing include Willie Jackson in Louisiana, Ray Krone in Arizona, Calvin Washington in Texas, James O'Donnell in New York, and Dan Young in Illinois.[4]

Attendees will be introduced to alternative measuring considerations when utilizing the standard American Board of Forensic Odontology (ABFO) #2 reference scale to evaluate bitemarks.

Devore and Harvey recognized that, while the human dentition may be unique, bitemarks made on malleable mediums, such as human skin, are not.[5,6] During a physical altercation, skin will stretch and distort as victims alter their body position while fighting for their release.[7] After an assault, bitemarks on living tissue can experience inflammation and hemorrhaging.[1,8]

Tattoos and external bite stamps have been utilized as substitutes for bitemarks by Lewis and Marroquin.[9] Each substituted mark has been photographed using an ABFO #2 reference scale with measurements taken at multiple locations. Changes in body position were photographed at each location. A representative maxillary arch width measurement of 40mm was used for comparison based on work by Rawson et. al. in which 397 dental arches were examined with the determination that maxillary arch widths range between 21.3mm and 41.0mm.[10] P. Magne, G.O. Gallucci, and U.C. Belser provided a related study examining maxillary anterior tooth widths and found central incisors had maximum widths measuring 9.10mm-9.24mm.[11]

In Devore's 1971 study and Lewis's and Marroquin's 2015 study, an inked stamp was placed on the surface of living human tissue as a representative bitemark to show positional distortions.[5,12]

A bruise by definition is a "contusion usually producing a hematoma without rupture of the skin".[13] Bitemarks produce hematomas that manifest with various degrees of organization and discoloration below the epidermis or outer skin layer. Tattoos similarly result in discolorations below the epidermis. Tattoos produce identifiable colored marks placed below the epithelial skin layer.

Distortions of 52.5% and 76.3% have been measured along the horizontal and vertical axes, respectively. Observed distortions were found to be non-uniform.[9]

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Bitemarks, Identification, Skin Distortions

G21 The Greening of Cremation: The Elimination of Cremercury

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After attending this presentation, attendees will understand the past and present impact that cremating mercury fillings has had on the environment and the future possibilities to see the elimination of this effluent. Attendees will be informed regarding the trends in the usage of mercury fillings and the practice of cremating teeth with amalgams. The goal of this presentation is to provide a review of the negative effects of dental amalgam fillings after remains are cremated, present options for reducing dentistry's contribution to the heavy metal bioburden, and introduce novel approaches for mitigation.

This presentation will impact the forensic science community by providing a review of crematory mercury emissions worldwide and the significant effect amalgam fillings have on the environment. This presentation will educate the community on responsible alternatives that can be used by scientists for global public health and the environment.

The practice of cremation is ancient, and there is presently a growing trend of enormous increase in the number of cremations throughout the world every year. This rising popularity is attributed to several factors, including consumer cost considerations, fewer religious prohibitions, changing consumer preferences, such as the desire for simpler, less ritualized funeral practices, decreasing burial space, and environmental concerns. Cremation has become socially acceptable. In the United States in 2015, the cremation rate was 48.5%. In 2016, the rate surpassed the burial rate. That rate is projected to grow to 78% by 2035, far exceeding the burial rate.

An area of concern is the environmental impact that human remains containing toxic substances, such as amalgam dental restorations, contribute to the release of the heavy metal mercury and its effects on the living and the environment. Dental amalgam is already releasing a significant amount of mercury into the environment. The Environmental Protection Agency (EPA) has proposed effluent limitations guidelines and standards for the dental category but does not address cremation.

There is a wide variation in the number of amalgam restorations placed in developed countries, and many dentists in North America no longer place amalgam restorations; however, amalgam is still being used at least some of the time by the majority of practitioners in North America. What should be noted is that, even though in some parts of the world amalgam use may be decreasing, it is rising in others. There is a need to look at the fact that dental caries is becoming an increasing problem in middle- and low-income countries as they adopt Western lifestyles, including high consumption of sugars, but have not yet begun widespread preventive programs, such as fluoridation. As a result, the need for filling materials is expected to grow in these countries.

The population that will likely be dying in the next 20 years comes from a time when amalgam placement was more prevalent that it is now. In underdeveloped countries, amalgams placed currently will remain in the population the next 30 to 50 years. Because of the increase in the number of cremations yearly, it is forecast that an increased amount of mercury will be released in the next few decades due to an increase in the number of cremated decedents that have retained their teeth with amalgam restorations. This increase is expected to be followed later by a decrease in mercury emissions in industrialized countries, as the next generation of people have both fewer cavities and fewer amalgam restorations.

A deputy associate administrator for congressional affairs in 2010 reported that, as of 2007, dental amalgam remained the second-largest category of mercury use in all products. It is estimated that in the United States in 2005 nearly 3,000 kilograms (6,613 lbs.) of mercury were released to the environment from crematoriums. At this time, no federal or state regulations restrict mercury emissions from crematoriums, and the EPA does not plan to regulate human crematoriums at this time.

Generally speaking, there are no legally recognized property rights in a dead body, and laws in the United States regarding the treatment of dead bodies derive from the government's police power to guard public health. Based on its police powers, the state probably could mandate the removal of amalgam fillings from dead bodies to prevent mercury releases to the environment.

Cremercury, Cremation, Amalgam

G22 Looking Into the Past: Challenges With Dental Identifications of Historically Unidentified Individuals

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After attending this presentation, attendees will be aware of the challenges associated with the identification of historically unidentified remains and the difficulty in matching these cases to long-standing missing persons. After identifying these specific challenges, solutions are proposed that may assist in refining current practices in antemortem and postmortem data collection and storage.

This presentation will impact the forensic science community by bringing to light globally reoccurring issues experienced by many dentists and related agencies as they try to gather antemortem and historically available postmortem information to facilitate the identification of human remains. Furthermore, this presentation will emphasize: (1) the need for increased funding and enhanced legislation to assist in compiling these antemortem and postmortem data sets; (2) the need for content experts to interpret and record this data; and, (3) a call for more effective communication between various holders of information.

In 2012, the Ontario Forensic Pathology Service and the Office of the Chief Coroner undertook a comprehensive review of all unidentified remains files dating back 50 years. Hundreds of cases were reviewed by content experts, including a forensic odontologist, anthropologist, pathologists, and pathologists' assistants, and data was entered into a relational database capable of performing case comparisons with missing persons based on specific variables. During this review, postmortem dental information was routinely found in these historical files. Although it was recognized that postmortem dental data had been collected relatively well over the years and largely by content experts, there remained numerous obstacles to the collection of a reliable postmortem data set. Dental information varied from robust to incomplete, coding methods were not uniform, and radiographs were limited or in the custody of now-deceased odontologists and could not be located.

There are also issues with regard to the collection of antemortem dental data for long-standing missing persons. In Ontario, these records are only mandated to be kept by a dentist for ten years from the last entry in record or ten years after a minor has turned 18 years old. The opportunity to retrieve these records diminishes with time if they are not seized immediately.

Additional challenges exist when there is a possibility that the missing person may have died out of province or out of country. Canada and the United States share land and water borders. Missing persons and unidentified remains data is not fluidly shared between provinces and states and their respective agencies. The dental nomenclature used by each country, namely the universal tooth-numbering system in the United States and the Fédération Dentaire Internationale (FDI) in Canada differs and would provide a challenge for anyone but a content expert.

Although there are many difficulties in the historical postmortem data collection of dental information, some of the former practices of forensic odontologists have actually proven useful in assisting current technologies. The dated practice of removing the jaws from unidentified remains and retaining them indefinitely, while no longer considered dignified, has provided a means to obtain DNA. The availability of these jaws has also enabled new sets of radiographs, photographs, and charts to be completed should the originals be unavailable.

Dental Identification, Historically Unidentified, Dental Challenges

G23 Third Molar Maturity Index (I3M) for Assessing the Age of Majority: The Experience in Several Different Countries

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After attending this presentation, attendees will be able to use this technique in their daily practices. In addition, this presentation will inspire researchers in other countries to contribute to a project that already involves several nations.

The goal of this presentation is to evaluate a unique method for estimating the 18-year age of majority.

Assessment of legal age, also known as age of majority, is a controversial issue as there are few body biomarkers or other evidence available during late adolescence that may be used to reliably differentiate a subject as being a minor or an adult. Third molar development has been recognized as a suitable subject for age examination in late adolescence.[1,2]

In the past few years, the study of third molar development, and specifically the evaluation of cut-off value, I3M=0.08, has been studied in several samples from different countries, namely Australia, Turkey, Colombia, Albania, Serbia, Libya, Brazil, Peru, Botswana, and Italy.[3-6]

All Orthopantomograms (OPTs) were recorded in JPG format and ImageJ software, version 1.48v, was used to examine the images. OPTs without the accompanying subject's full dental records, lack of birth date, and date when the OPTs were taken, as well as those OPTs of children with proven hereditary or systematic illnesses, malnutrition, severe destruction, extraction, or hypodontia of permanent teeth, and where the third molars were missing, were excluded from the study.

The left lower third molar was assessed using the I3M without knowledge of the subjects' date of birth in order to avoid bias during measuring of specific projections of third molars on OPT, as proposed by Cameriere et al.[7] Briefly, I3M is a ratio of the sum of projections of open apices in multi-rooted teeth or apex width in single-rooted teeth and a tooth length of the mandibular third molar during growth. If third molars were found with entirely closed roots, then I3M=0.00 was recorded. The cut-off value of I3M=0.08 and additional cut-off values close to I3M=0.08 were tested to discriminate adults (≥18 years) and minors (<18 years). For all research and samples, sensitivity, specificity, and correct classification were evaluated.

As regards the Turkey sample for females, the sensitivity was 85.9% (95% Confidence Interval (CI) 77.1-92.8%) and specificity was 100%. The proportion of correctly classified individuals was 92.7%. For males, the sensitivity was 94.6% (95% CI 88.1-99.8%) and specificity was 100%. The proportion of correctly classified individuals was 97.6%.[8]

In the Australian sample, the results demonstrate that the sensitivity is 0.90 in males and 0.90 in females; associated specificity values are 0.85 and 0.87, respectively.[4]

In the Botswana sample, values of sensitivity of the test or the proportion of participants being 18 years and older were 0.88 (95 % CI, 0.87 to 0.90) in males and 0.88 (95 % CI, 0.90 to 0.93) in females, while values of specificity or proportion of individuals younger than 18 who have I3M <0.08 were 0.94 (95 % CI, 0.91 to 0.96) in males and 0.96 (95 % CI, 0.94 to 0.98) in females.[5]

In the Colombian sample, age distribution gradually decreases as I3M increases in both females and males. For females, the sensitivity test was 95.1% (95% CI 87.1%-95%) and specificity was 93.8% (95% CI 87.1%-98.8%). The proportion of correctly classified individuals was 95.1%. For males, the sensitivity test was 91.7% (95% CI 85.1%-96.8%) and specificity was 90.6% (95% CI 82.1%-97.8%). The proportion of correctly classified individuals was 89.7%.[6]

In the Serbian sample, the results demonstrated high sensitivity (0.96, 0.86) and specificity (0.94, 0.98) in males and females, respectively. The proportion of correctly classified individuals was 0.95 in males and 0.91 in females.[9]

In conclusion, the suggested value of I3M=0.08 can be used in several countries with a high rate of accuracy.

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Age Estimation, Third Molar, I3M

G24 Are Texas Dentists Prepared to Recognize and Report Elderly Abuse?

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The goal of this presentation is to provide awareness and education to dentists in diagnosing and reporting suspected cases of elder abuse.

This presentation will impact the forensic science community by demonstrating how general dentists in Texas are underprepared to diagnose and report suspected cases of elder abuse.

Background: In the United States, the elderly are the nation's fastest growing population, with estimates that 20% of the population will be age 65 or older by the year 2030.[1-5] Abuse of individuals of all ages in the population has become epidemic; however, abuse of the elderly has been greatly underreported.

Purpose: A random, cross-sectional survey was taken to determine training, knowledge, and the ability to recognize signs of elderly abuse, as well as proper protocol in reporting suspected abuse to the proper authorities, by licensed Texas general dentists during patient examination and evaluation.

Methods: With prior consent, a 25-question survey was emailed to 500 randomly selected state-licensed Texas general dentists, and data received were analyzed. A protocol was developed for reporting cases of suspected elder abuse to proper authorities, and a library of elder abuse resource material will be formulated and recommended.

Results: The results of the survey will be presented.

Conclusion: The results of the survey will be discussed.

Introduction: In the United States, the elderly are the nation's fastest growing population, with estimates that the elderly will comprise an estimated 20% of the population by 2030.[3-5] The number of individuals who are victims of elder abuse has reached epidemic proportions.[3,6] Studies from around the United States estimate that between 200,000 and 2.5 million individuals are abused annually.[7,8] It has been generally recognized by the medicolegal community that there are currently six types of elderly abuse: physical abuse, psychological abuse, sexual abuse, neglect, financial exploitation, and passive abuse.

Elders, on average, make two visits per year to their dentists.[9] Due to the frequency of these visits, dentists are uniquely in a position to discover and report cases of elder abuse to the proper authorities and to provide victims access to helpful resources.[3,10] In cases of physical abuse alone, more than 50% of such abuse involve the head and neck region, again making dentists among those on the front line of identification and reporting.

In Texas, as in all states in the United States, dentists are mandated by law to report cases of suspected abuse. Several studies reveal that dentists, among other health care providers, are neither knowledgeable or comfortable in recognizing or reporting cases of suspected elder abuse.[3,11] Older persons are abused only slightly less than children, yet the reporting rate is half that of reporting child abuse.[12] This study will discuss current educational curriculum regarding elder abuse in Texas dental schools and will recommend an educational template based upon findings of the study.

Discussion: Discussion will follow collection and analysis of all data collected in the study.

Conclusion: Dentists have an ethical and lawful obligation to recognize and report elder abuse to provide for their patients' well-being and safety. Conclusions will be offered concerning the following: (1) current levels of education in Texas dental schools regarding recognition and reporting of elder abuse; (2) reasons for the apparent underreporting of elder abuse by state licensed Texas dentists; (3) recommendations to increase awareness and diagnosis of elder abuse in the Texas dental population by Texas dentists through development of an examination protocol which addresses this issue; and, (4) education in the reporting process in areas suspected of elder abuse and identification of current resources to help victims of elder abuse.

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Elder Abuse, Elder Abuse Diagnosis, Elder Abuse Reporting

G25 The Lake Ontario Floater: Identifying "John Doe"

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After attending this presentation, attendees will be aware of the assistance that organizations such as the National Missing and Unidentified Persons System (NamUs) can provide in identifying a cadaver that could literally have come from anywhere. As odontologists, we can profile and chart, but that information doesn't help much if we have nothing to compare it to. NamUs is one repository for information that can be provided on unidentified persons, which will: (1) cross match information that is input about an unidentified person with information posted by other agencies about missing people; and, (2) look for matches or similarities between cases. It is amazing that a detail of a case which may seem insignificant can lead to an avenue that, when followed, leads to closure of a case that wouldn't have been closed otherwise.

This presentation will impact the forensic science community by increasing awareness of a valuable search tool to help generate leads in cases of missing or unidentified remains.

The type of case that is being presented comes to every medical examiner's office at some time and presents the problem of a set of unidentified human remains. The body in this case, that of a decomposed white male wearing shorts and an overcoat, with no identification, drifted into Irondequoit Bay from Lake Ontario on June 23, 2015.

This person could have originated from nearly anywhere. The Great Lakes are a connected system of lakes and waterways that also are part of the border of the United States and Canada. A body found in Lake Ontario could have originated from any of the Great Lakes and from either the United States or Canada. Complicating issues is the fact that foreign ships also use all of the Great Lakes, entering and leaving the system via the Saint Lawrence Seaway.

In international cases, the jurisdiction falls to the country and county where the body is found. A dental workup was performed for this decedent at the Monroe County Office of the Medical Examiner in Rochester, NY. The dental structures were in excellent condition. In the first weeks, there were several missing persons reported from Canada and the United States. These missing persons were excluded as a "match" by comparisons of dental records.

The dental records and all other information from the Monroe County Office of the Medical Examiner were entered in NamUs. Two weeks later, there was a "hit" on the information provided to NamUs, which led to the positive identification of this "John Doe".

This case emphasizes the need for a database that can find similarities in two different sets of data (the missing person data and the data gleaned from the remains) and narrow the list of possible matches that exist. That need has been met in the NamUs system. NamUs represents an excellent tool that can help identify missing persons.

Search Engine, Missing, Unidentified

G26 "Three Dog Night" or Death by Dog

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The goal of this presentation is to highlight animal bitemark evidence in an event involving dogs. Special consideration is critical in the process of evidence collection and the requirements for the presentation of evidence within the judicial system in order to secure an equitable solution.

This presentation will impact the forensic science community by calling attention to a rare case of multiple dogs participating in the mutilation and death of an elderly woman, followed by a request from the owner for the return of the offending dogs. It is important to understand the process whereby a hearing is held and a judgement is rendered for the disposition of the offending animals.

It is unusual for multiple dogs to be involved in an incident in which an elderly adult is killed directly as a result of the canine activity. From the time of first notification to the forensic odontologist of a possible bitemark to the results of the hearing for the return of the dogs, strict adherence to new protocol was required due to the unusual nature of the victim and the suspects. The victim was examined and a general autopsy was performed by the medical examiner as well as a routine examination for bitemark analysis by the forensic odontologist. The three canine suspects were immediately placed in custody of Miami Dade County Animal Control. It was requested that an examination be made by the forensic odontologist to determine which animal, or animals, might be responsible for the death of the elderly woman. The situation required the expertise of an anesthetist skilled in the care of animals. Impressions of the dogs' dentitions were obtained with standard polyvinyl siloxane putty, and the impressions were poured in acrylic resin to prevent fracture of the relatively long canine teeth. These acrylic models were used to identify bitemarks directly on the skin of the victim in areas where the skin had not been torn away. Acetate film was used to record as many marks as possible for comparison with the models of the various dogs' teeth. There appeared to be a significant difference in intercanine distance between the three different breeds (American Bulldog, Rhodesian Ridgeback, and German Shepard).

Following the identification of all three dogs as perpetrators in the death of the victim, the Miami Dade Animal Control Service requested euthanization of all three dogs. This was met by resistance from the owner of the dogs, who was both the grandson of the deceased and the person who had left the dogs with his grandmother. According to Miami Dade County ordinances, the owner of the dogs had a right to appeal the ruling and to appear before a hearing officer for a decision after presentation of evidence by the county and the appellant. The county's case was presented by the county attorney, the Director of Animal Services, and the forensic odontologist. The appellant was represented by his attorney. A three-judge panel heard the case, and the entire proceedings were videotaped by a local television station for presentation on the air (the video will be shown during this presentation).

Since the decision of the hearing officer was to euthanize the three dogs, an appeal period of 30 days was granted but was never exercised.

Dog, Bitemark, Death

G27 Marking Dental Prostheses

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After attending this presentation, attendees will understand the importance of marking dental prostheses as an aid to help identify the victims of accidents or disasters, people with Alzheimer's disease, the senile, the elderly, and people who are undocumented or lost. Alternatively, marking helps provide recognition of a lost prosthesis in nursing homes. Attendees will also understand how to recognize such a marking.

This presentation will impact the forensic science community by illustrating that the marking of a prosthesis is reliable and reasonably priced. This study completes the research undertaken in the identification of victims at the Forensic Odontology Department of the Institute of Criminal Research of the French Gendamerie (IRCGN).

The marking of dental prosthesis has two major benefits: (1) to help locate or identify an accident or disaster victim; and, (2) to help prevent loss, disappearance, or exchange of dentures among nursing home residents. Requirements for applying markings to dental prostheses is dependent on the country. In the United States, 22 states require dental surgeons to mark the dentures. In Sweden, 35% to 50% of the dentures are marked. In France, a survey by the National College Council of the Dental Surgeons reported that, in retirement homes, only 7% of dentures are marked.

To be accessible to all patients, the marking must be easily applied, should not affect the strength of the denture, and have a reasonable cost. The marking should not be uncomfortable for the patient. Moreover, the marking must withstand saliva, fire, and stand the test of time. In forensic odontology, the marking must allow for the recognition of crowns, bridges, and partial or full dentures. Finally, marking must be precise to eliminate confusion.

The catalog of different marking techniques used for dentures will be presented with the methods divided into two groups: engraving or inclusion. References will be provided to inform attendees as to which techniques may be available for creating such marks.

Currently, miniaturized technologies should be considered as the most interesting. An embedded microchip can contain very important information and enables the rapid identification of the wearer of the denture. The transponder that supports such data in microchipped full and partial dentures has a very low space requirement. Research has been undertaken in the Forensic Odontology Department of the IRCGN to apply this marking technique in fixed dental prosthesis, to some extent. Such a possibility should encourage dental surgeons to chip dentures of personnel at risk (e.g., firemen, flight crews, soldiers, policemen, and hospital staffs), after obtaining their permission.

Marking, Dental Prosthesis, Identification

G28 The Yarnell 19: The Deadliest Wildfire in Arizona History and the Successful Implementation of Rapid Identification of the Granite Mountain Hotshots Firemen

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After attending this presentation, attendees will understand the importance of scientific identification protocols, mass fatality planning, logistics, and multidisciplinary coordination efforts to obtain antemortem identification data.

This presentation will impact the forensic science community by providing specific procedural guidelines for the identification process in a mass fatality and will also stress the significance of inter- and intra-agency coordination for management of a mass fatality event.

The Granite Mountain Hotshots are a specialized team within the fire department who are highly trained in fighting wildfires. On June 28, 2013, with the thermometer showing triple digits and ground conditions extremely dry, a wildfire was ignited by lighting near Yarnell, AZ. The Yarnell Hill Fire claimed the lives of 19 Granite Mountain Hotshots firemen and destroyed nearly 8,500 acres. The winds rapidly changed while the firemen where clearing away brush in the mountain area of a small town, trapping them as the fire came full circle around them, blocking their exit. The chief medical examiner for Maricopa County Medical Examiner's Office in Phoenix, AZ, was contacted by the Yavapai County chief medical examiner requesting assistance, due to Maricopa County having more resources — a full-time forensic odontologist, a forensic anthropologist, ample cooler space, larger staffing levels, and the ability to handle a surge incident while still maintaining daily operations.

Following guidance provided in the office draft mass fatality plan, along with the experience and expertise provided by staff with prior mass fatality knowledge, the office prepared for overseeing this event. The chief medical examiner and the chief medicolegal death investigator (mass fatality coordinator) established a work flow and oversight for the logistical coordination of this incident. Logistical considerations were made regarding staff, supply needs, response to a remote scene, transportation of remains to the office, coordination of release to the funeral home, grieving peers, safety issues, media, and the examination and identification process.

Establishing a single point of contact, the community liaison unidentified decedent coordinator, for the oversight of the identification process allowed for efficient organization and management of antemortem and postmortem identification data. This was essential during this incident because of the condition of the remains, which were classified as decomposed or burned. This was due to the fact that a number of the decedents were found in fire safety shelter tents while others were outside of the tents. Due to the condition of the remains, dental identification was the preferred method.

Communication between the community liaison unidentified decedent coordinator, police agencies, and families was essential. In this specific situation, it was advantageous to have all antemortem identifiers organized in a spreadsheet and ready to be compared to the postmortem findings. By working closing with families, law enforcement, and dental offices, the antemortem identifiers began to be collected immediately and were fully obtained in two days. Following a set standard, the autopsies for all 19 decedents were completed in one day, and the postmortem dental and anthropology exams were completed over the course of two days. This allowed the identification of all 19 firemen to be confirmed in two days.

The preparedness and oversight provided for this incident allowed the majority of identifications to be made using scientific methods. This included six decedents being identified by comparison of antemortem and postmortem dental radiographs, seven decedents being identified through written dental records that were consistent with the postmortem dental exam, and two decedents being scientifically identified by medical hardware radiographs. The remaining four decedents were identified presumptively though tattoos and demographic information. The rapid confirmation of identifications allowed the timely release of all 19 decedents for memorial services.

This presentation will illustrate the importance of mass fatality planning, identification protocols, and coordination between multiple agencies. This presentation will further include a review of the after-action report, which provides lessons learned, successes, and critical points for future planning and integration.

Dental Identification, Firemen, Multidisciplinary Effort

G29 "Oscar" the 1985 Unidentified Person (UP): Forensic Evolution — Odontology to Phenotyping

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After attending this presentation, attendees will appreciate the efforts and perseverance demonstrated in a 1985 UP case. Attendees will also appreciate the evolution of forensic sciences in attempting to identify this UP case.

This presentation will impact the forensic science community by visualizing the evolution of techniques for UP cases.

In Glen Burnie, MD, circa 1985, the skull of a murdered man was found in a trash can. As expected, this case became an Unidentified Case associated with the office of the Chief Medical Examiner of the State of Maryland. To this date, this decedent remains unidentified as UP Case #142852A or UNK 85-33.

As time and case investigators have moved on, this unidentified person has affectionately become known as "Oscar." Continued attempts to identify this unknown decedent via dental examination have been unsuccessful. Subsequently, the skull of "Oscar" has been submitted to forensic artists to provide sketches and a facial reconstruction with the hopes that the public would take notice and contribute information that would assist with an identification of "Oscar." The July 2016 issue of *National Geographic* depicted "Oscar's" skull during the artist's rendition of what "Oscar" is perceived to look like. Despite these efforts, "Oscar" still remains unnamed and unclaimed.

Many traditional disciplines have been employed over the past 30 years with respect to this unidentified case. The investigators have also been willing to use any and all modalities available for forensic identification. Traditionally, with skeletonized remains or a skull needing identification, we have always looked toward forensic odontology to contribute to the identification of the unknown individual. "Oscar" has restorations in nearly every tooth. The restorative dental materials include amalgam, composites, and also gold foil. All of the disciplines of forensic science have evolved over the years as technology has developed. "Old school" methods are still used on a daily basis; however, new technologies are emerging and are actively being investigated in forensic science.

"Oscar's" case was presented in the 2003 Bring Your Own Slides session at the American Academy of Forensic Sciences (AAFS) annual meeting. This 2017 presentation is intended to update the case and demonstrate how forensic science has evolved over the intervening years. We now have the ability to take a DNA sample and produce expanded identification capabilities. "Oscar's" genetic sample was sent to Parabon® Nanolabs in December, 2015, for analysis.

This presentation will show the current capabilities of Parabon® Nanolabs to take the decedent's DNA sample and, using their current technology, produce a phenotype report based on various predicted attributes of the person. These include skin color, eye color, hair color, freckling, genomic ancestry, and face morphology.

There is now a computer-generated image of how agency case #1985-055625-20150401, also known as "Oscar," has evolved. Who is "Oscar"?

Human Dental Identification, Facial Image Technology, Dental & Phenotype Comparisons

G30 "Oscar" the 1985 Unidentified Person: A Career Development Adventure

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After attending this presentation, attendees will recognize the evolution of advances in identification investigation through the examination of a 1985 unidentified male case study, who remains unidentified today. Attendees will integrate retrospective and evolving unidentified person investigation processes by law enforcement, laboratory methodology, and forensic odontology from the 1985 "state-of-the-art" forensic sciences to the present. Attendees will learn a career experiences to potentially model and create opportunities for developing their professional and interdisciplinary careers.

This presentation will impact the forensic science community by teaching attendees the value of perseverance in successful evidence collection, for personal career development and interaction with their local jurisdictions, even when the case study identification remains unknown.

This presentation will chronicle the evolution of an unidentified man's antemortem life, and the advancing investigative technology since his death in 1985. In 1985, law enforcement could only nickname the unidentified victim as "Oscar" because he was found in a galvanized trash can. This became his moniker.

"Oscar" was found four miles from the author's resident community of 50 years. It is reasonable to think that these two lives have had common experiences and acquaintances in the 20 years prior to "Oscar's" death. The need for "Oscar's" identification and the criminal investigation into his death began with active inquiries to multidiscipline investigators in order to assist law enforcement to coordinate antemortem and postmortem "Oscar" data. In retrospect, the unsolved case may serve as a model for newer colleagues in forensic and police sciences far beyond our own discipline. While law enforcement must thinly spread their resources over many missing and unidentified persons' cases, this research was able to focus on "Oscar" alone, which provided an introduction to a wide range of forensic technologies, as well as judicial and human resources.

As one comes to know professional friends longer, one learns more about them. Through evolving technologies, we have learned more about "Oscar" than about many others who have been successfully identified with less information. "Oscar" becomes an ever-closer friend because he continues as the model for implementing new technology and inspires new investigators to persevere in the investigation.

"Oscar's" cadre of friends have grown over time. With them have come radiographic films, Computed Tomography (CT) head and dental images, anthropologic and dental age estimation, dental charting and coding into the National Crime Information Center (NCIC) and the National Missing and Unidentified Persons System (Name's), forensic artists' sketchings and clay reconstruction, and shoe-sole patent symbols that may date fabrication. Sometimes friends leave the community due to job opportunities. This is true for "Oscar" who has had, at minimum, seven missing and unidentified persons' police investigators responsible for his case. With each change, "Oscar's" friends welcome a new investigator who learns about his life and the maturing postmortem data. This is a useful evolution for the newer forensic odontologist who gains experience in the process.

The "Oscar" circle of friends has continued over the decades, yielding technical satisfaction, but no resolution. In the past year, "Oscar's" friends have brought him international recognition in the pages of *National Geographic Magazine*.

The companion portion of this presentation will explain how new phenotyping analyses have helped us to better know and see "Oscar," as well as enriching colleagues who can share these findings with local law enforcement for other unidentified persons.

Human Dental Identification, Facial Image Technology, Odontology Career Development

G31 Fractalyse Software — The Analysis of the Trabecular Bone in Identification

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After attending this presentation, attendees will possess new information regarding the use of mandibular trabecular bone patterns to establish positive identification.

This presentation will impact the forensic science community by demonstrating a method of calculating the significance of mandibular trabecular bone patterns in arriving at a positive identification.

According to University of California, Berkeley's orthopedic biomechanics research, the trabecular bone can be classified as a porous cellular solid, consisting of an irregular 3D array of bony rods and plates, called trabeculae, which are composed of a calcified matrix. Bone marrow fills the spaces of the pores. In addition, because all free bone surfaces are covered with bone cells, bone is a living tissue that is self-healing and has the ability to adjust its morphology in response to changes in its mechanical environment, the so-called but poorly understood phenomenon of bone remodeling. As such, the mechanical complexity of this two-phase biological tissue surpasses any engineering material making it a fascinating subject of study, regardless of clinical applications.

The process of dental identification compares postmortem to antemortem data. It involves the analysis of different factors such as: the presence and absence of teeth; crown and root morphology and their interrelationships; the evaluation of the periodontal status; the type and extent of restorative, endodontic, fixed, removable, and implanted materials; tori and sinus configuration; anomalies and pathologies of teeth and bone; and trabecular pattern morphology.

Few studies have been conducted on the statistical reliability of trabecular bone patterns for identification purposes. Some deal with algorithms, a mathematical expression that produces the answer to a question or the solution to a problem in a finite number of steps. Others deal with fractal analysis, consisting of assigning a fractal dimension or other fractal characteristic to a dataset. The theoretical dataset, pattern, or signal extracted from a phenomenon can include natural geometric objects, sound, market fluctuations, heart rates, digital images, molecular motion, networks, etc.

Some of the studies apply fractal geometric techniques: (1) to the study of trabecular bone; (2) to fractal analysis of radiographs by the assessment of trabecular bone structure and prediction of elastic modulus and strength; (3) to methodological principles for fractal analysis of trabecular bone; (4) to digital image analysis of cadaver mandibular trabecular bone patterns; (5) to fractal dimension and lacunarity analysis of dental radiographs; (6) to technical factors in fractal analysis of periapical radiographs; (7) to the morphodigital study of the mandibular trabecular bone in panoramic radiographs; (8) to fractal analysis of mandibular trabecular bone using the tile counting method; (9) to the anatomical variations of trabecular bone structure in intraoral radiographs using fractal and particles count analyses; (10) to the analysis of trabecular bone using site-specific fractal values calculated from cone beam Computed Tomography (CT) images; and, finally, (11) to the fractal dimension of the mandibular trabecular bone measured on digital and digitized images.[1-11]

As a continuation of previous research projects, the current research focused on the multifunctional uses of Fractalyse software, a free open source software, as an aid and noteworthy tool for trabecular bone pattern morphometric analysis and comparison.[12-15] For that purpose, 20 digital panoramic radiographs from ten patients treated in implantology and orthodontics were analyzed and compared. For each panorex, 12 designated squared areas were selected on the mandible, of which 11 were adjoining and shifted by the equal distance. The resulting 240 radiographic areas were analyzed and compared with one another, taking into account various factors. This process was also conducted independently by a second researcher and the results compared. Outcomes suggest that the development of a new software with a revolutionary approach to handling human trabecular jaw bone patterns for identification purposes could be possible. This approach would be especially practical in mass disaster situations involving large numbers of edentulous victims and/or with fragmented remains.

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Fractal Analysis, Trabecular Bone, Identification

G32 Using More Than Radiographs in Identification: A Crown, a Scanner, and a Denture

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After attending this presentation, attendees will better understand how dental information other than radiographs can be used to assist in the identification of individuals, and why it is vital to have all dental antemortem information available.

This presentation will impact the forensic science community by illustrating the need to collect all available antemortem dental information and by discussing ways in which valuable aids to identification can be found in sources other than dental radiographs.

Case 1: On October 30, 2015, a home in Cayuga Ontario, Canada, was leveled to the foundations by a fire which was so intense that the entire house collapsed into the basement. The remains of two severely burned individuals, presumed to be the couple who owned the home, were retrieved.

The first victim was the wife who had been to the dentist just two months prior to the fire and had bitewing radiographs taken. She was quickly and positively identified by dental radiographs with no inconsistencies found.

The body of the second victim suffered more severe damage. The forensic anthropologist determined that the victim had suffered gunshot damage to the head as well as severe thermal damage. Examination revealed a large hole in the cranium, macerated brain tissue, and separate areas of internal and external beveling. These findings are indicative of gunshot damage and not thermal damage. The cephalogram also displayed multiple small radio-opacities known as "lead snow storm," also the result of a gunshot injury to the head. The mandible was found underneath the body of the victim and the maxilla was near the body. This damage is also consistent with the cranium nearly exploding from the gunshot injury at close range to the cranium.

The forensic anthropologist was able to retrieve bits of calcined jaw bones and seven roots. Also found were a gold crown and the shell of two porcelain-fused-to-metal crowns, which had fused together by the melting of the porcelain from the heat of the fire. The antemortem dental records included 2-month-old bitewing radiographs as well as models for crowns on the maxillary right premolar teeth and the opposing mandibular model.

Three methods were used to compare the antemortem and postmortem records: (1) radiographic comparison of the gold crown and the antemortem radiographs; (2) the gold crown and the mandibular model were digitally scanned and a comparison of the dimensions of the two scans was completed; and, (3) the occlusal profile of the gold crown was also captured in impression putty and sectioned at various intervals. These indices were then placed on the antemortem model and the fit was compared.

Case 2: On May 11, 2016, a body was retrieved from the Niagara Whirlpool in the Niagara River and brought to Hamilton, Ontario, for identification. The only identification found on the decedent was her Casino Niagara Players Advantage Card. The card was last used on March 29, 2016. Investigation by Canadian and American Border Agencies determined that the decedent may have been an Australian national who had been traveling for two years with infrequent contact with family in New South Wales, Australia, with a history of depression and suicide attempts.

After the initial dental examination in May, it was determined that antemortem dental records were not available, and identification would be attempted by DNA. The request for a DNA swab from the presumptive son in Australia brought to light the fact that a denture existed in Australia which belonged to the suspected decedent.

The denture and swab arrived in Canada on June 7, 2016, and the denture was swabbed for DNA analysis. At that time, it was also made known that the decedent was wearing a partial denture when retrieved from the Niagara River, which was being held by the police. Both dentures fit well in the decedent's mouth.

An impression of the tissue surface of the two dentures was made and positive models of the tissue surface were produced. A comparison of the tissue surfaces of the two dentures and of the decedent's palate was completed.

Summary: The basis of most dental identifications is a comparison of antemortem and postmortem radiographs. When antemortem or postmortem records are limited by availability or damage of the oral structures, peri-mortem or postmortem, other sources of comparison must be sought and used.

Prosthesis, 3D Scanner, Complete Dental Records

G33 The Princes in the Tower: Dental Age Estimation of the Archived Records of the Extant Remains of Edward V and Richard, Duke of York

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After attending this presentation, attendees will be aware that the skeletal remains of Edward V and Richard, Duke of York, are entombed in Westminster Abbey, London. Attendees will have the opportunity to consider the information on the dental development of the two princes and be able to assess the impact of dental age estimation on the identity of the two princes.

This presentation will impact the forensic science community by investigating the probability that the ages of the extant specimens of Edward V and Richard, Duke of York, call into question the reliability of the identification of the skeletal remains entombed in Westminster Abbey, London.

Introduction: King Edward IV ascended the throne in 1461 and brought stability to the kingdom. His younger brother was Richard, Duke of Gloucester — later Richard III. In April, 1483, Edward IV died unexpectedly. His successor, the 12¾-year-old King Edward V, journeyed from Ludlow Castle to London for the coronation and entered the Tower of London, a royal residence, on May 4, 1483. His younger brother, who was ten years old, was brought to the Tower of London in July, 1483, as a playmate. They never left the Tower and were not seen after the end of July, 1483. It is alleged that they were murdered by smothering on September 3, 1483.

In 1647, workmen clearing a staircase to the White Tower discovered a wooden trunk containing the skeletons of two children who were thought to be the missing princes. King Charles II ordered that the skeletal remains be interred in Westminster Abbey.

Materials and Methods: The data available for study are from the 1935 paper published in *Archeologica*.[1] The article contains skiagrams (the former name for radiographs) of the long bones, and the jaws of the remains of two young children.

In this presentation, only the dental findings will be considered. Edward V — the specimens comprise part of a jaw bone without teeth. There are sockets of teeth in the lower left premolar-molar region. The Duke of York — the jaw contains three teeth *in situ*.

The tooth development stages were assessed using the eight stage system.[2] The probability that the subjects were below the age claimed was calculated using the United Kingdom Caucasian Reference Data Set.[3] From this, the probability that the two specimens had ages that were concordant with the known ages was calculated using the method of probability estimates.[4]

Results: Edward V — the two sockets on the skiagrams present are consistent with the UL5Fm (#25) and the LL7Em (#37) and these give an age estimate of 10.49 years. Compared to the calendar age of 12.84 years, the probability that the subject is *below* that calendar age threshold is p=0.993 (99.3%). This is outside the limits of the interquartile width.

Edward V — likelihood of being less than 12.84 years is p=0.993 (99.3%), p >12.84=0.007 (0.7%).

Richard Duke of York — the three teeth on the skiagrams are consistent with LR5Dm (#45), LR4Em (#44), and LR3Fm (#43). These give a calculated age of 9.09 years compared to the calendar age of 10.05 years. This is within the interquartile width. Likelihood of being less than 10.05 years is p=0.737 (73.7%), p >10.05=0.263 (26.3%).

Discussion: The results illustrate that these alleged princes were younger than the stated calendar age, the young King by a difference of more than two years. Is this sufficient evidence to set aside the belief that these skeletons are from the royal bloodline?

Conclusion: These results support the ongoing request from modern scientists to gain access to the marble urn in Westminster Abbey to determine if the skeletal material is related to the princes' presumed uncle, Richard III.

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Princes, Tower, Dental Age

G34 Dental Identification of World War II Remains: The Battle of Saipan, June 1944

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After attending this presentation, attendees will understand the role of the forensic odontologist as it applies to the identification and repatriation of decedents from the Battle of Saipan during World War II.

This presentation will impact the forensic science community by demonstrating the importance of using dental comparison as a means of identification.

This presentation will supply a brief historical overview of the fierce fight to control a Japanese-held island in the Pacific Ocean, the disposition of American remains, and the attempt at identification and repatriation of several United States Marines. Several example cases of previously unidentified United States Marines will be cited, and the implementation of military records to positively identify previously unidentified American heroes will be shown.

The Battle of Saipan marked the beginning of the end of World War II. The second largest island in the Marianas chain is located 1,200 miles southeast of Japan. The significance of its capture by United States troops was due to the location of the base, which enabled United States bombers to fly non-stop to attack the Japanese mainland. This relatively small island, 72 square miles, was the first location that United States Marines placed foot on Japanese soil during the war. Seventy-five Landing Vehicles Tracked (LVTs), amphibious landing vehicles, attacked from the sea, carrying nearly 8,000 United States Marines on D-Day, June 15, 1944. Seven hours into the attack, having gained only 400 yards of beachfront, 2,000 Marines had been killed or wounded.

The final battle for control of the island featured the largest Banzai attack of the war, when 3,000 Japanese soldiers stormed the American positions under the cloak of darkness. By day's end, 3,500 Marines had been killed and 13,000 were wounded as the United States gained control of the island. Due to the vast number of casualties, there was little time left for proper burial and many of the deceased were placed in mass grave trenches on the beach. Several other brave men were lost at sea and their bodies never recovered.

Six years later, in 1950, the trenches were excavated, the remains disinterred, and either repatriated to the United States or reburied in the present-day Manila American Cemetery and Memorial in the Philippines.

This presentation will demonstrate the identification process utilizing antemortem and postmortem military dental records.

Forensic Odontology, Unidentified Remains, Battle of Saipan

G35 The Analyses of Dental Remains From 1607 Jamestown, Virginia, Using Micro-Computed Tomography (micro-CT) Imaging, Scanning Electron Microscopy With Energy-Dispersive X-Ray Spectroscopy (SEM-EDX)/Raman Spectroscopy (RS), and Paleobotany

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After attending this presentation, attendees will be familiar with advanced radiographic imaging and spectroscopic and paleobotanical techniques for analyzing skeletal and dental remains in order to reconstruct details of a deceased person's life and to determine events prior to and surrounding death and burial.

This presentation will impact the forensic science community by demonstrating how evidence-based science and technology can improve efficiency, quality, accuracy, reliability, and functional excellence in forensic sciences.

The first English settlement in North America was established in 1607, at Jamestown, VA. The first casualty in Jamestown was a 15-year-old boy (specimen 1225B), the apparent victim of an Indian attack.[1] His excavated remains reveal an arrowhead at the thigh, broken left collarbone, and anterior mandible with Ellis Type III fracture of mandibular left central incisor (#24), with significant periapical pathological bone resorption.

The objectives of this multidisciplinary study are to elucidate the nature and chronology of odontogenic cause(s) of mandibular pathosis evident in 1225B skeletal remains, and analyze contents of fractured teeth root canals to assist assembling a corporeal and physiological history.

In this study the following technologies were used: Cone Beam Computed Tomography (CBCT) for morphological and chronological assessment of 1225B skull; 3D intraoral visual imager to non-invasively examine and manipulate occlusion; 2D intraoral X-rays to document individual teeth; micro-CT (mCT) to image intact and fractured teeth and root canals; Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), and confocal Raman Spectroscopy (RS) for nanoscale imaging and elemental analysis of root canal particulate and soil surrounding skeletal remains; focus stacking light microscopy for spatial assessment of carious lesion; and polarized light microscopy for analyzing root canal contents of fractured tooth.[2-4]

MCT of fractured tooth #24 revealed arrested root canal maturation and established the boy's age as eight years old at the time of the trauma to anterior dentition; CBCT scans were consistent with the presence of mandibular, intra-alveolar pathosis for seven years of age; SEM/EDX and RS established the organic nature of #24 canal particulate, of possible fungal origin; intraoral imager supported the feasibility of reconstructing the fragmented cranial base; transmitted light microscopy of root canal contents indicated the presence of cerealia, tree pollen, starch, and fungal hyphae.[5] Polarized light microscopy clarified starch identifications.

In conclusion, this research demonstrated the effectiveness of using evidenced-based technology to reconstruct cause and chronology for disease processes and death from skeletal remains. Future research: refine and expand preliminary studies; plaque and canal particulate analyses using lipid studies and Next Generation Sequencing (NGS); comparison of 1607 and current plaque compositions; digital reconstruction of the cranial base for orthodontic analyses; evaluation of skeletal remains for evidence of scurvy, periodontal disease, and skeletal growth

plate deficiencies; multi-disciplinary analysis of grave-site soil adjacent to critical anatomy; excavation of adjacent Jamestown adolescent's grave for comparison studies.[6]

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Teeth, micro-CT, Spectroscopy

G36 When the Hammer Falls: Patterned Injury Comparison in a Blunt Force Homicide

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After attending this presentation, attendees will have a new tool to consider when assessing patterned injury in bone.

This presentation will impact the forensic science community by demonstrating a novel use for a well-established technique and increasing collaboration among forensic odontologists, forensic anthropologists, and forensic pathologists.

During the course of an autopsy on a female homicide victim, the pathologist observed deep incised injuries on a small triangular portion of frontal bone. She removed the segment and requested an analysis from the forensic anthropologist. The specimen was macerated in order to preserve the marks. After the maceration process, additional superficial etched patterns became visible on the bone. Law enforcement was contacted by the forensic anthropologist who requested that they include a jewelry hammer in their suspect searches, based on research reviewing different types of hammer heads. One possibility that might explain the distinctive pattern was the interchangeable heads used in making custom jewelry. During the course of a search, the detective located a worn and obviously used hammer/hatchet combination (Q1) with very similar markings on the hitting surface, and attempts were made to determine whether it should be included as a possible suspect weapon in the homicide. Ultimately, the weapon could not be ruled out as the cause of the marks on the bone.

The cranial vault of the decedent in this case was badly fractured, and the segment containing the marks was separate from the rest of the vault. The original bone markings observed by the pathologist were relatively deep semicircular score marks in the external table of the left frontal bone. The additional markings observed after the bone was cleaned appeared to be superficial, roughly parallel V-shapes. The V pattern appeared in small (~1 mm-~2mm) Vs and also as larger overlapping K parallel shapes (~4mm-~6mm) arrayed in a shallow arc. The superior portion of the arc contained inverted Vs ranging in size from ~0.1mm-~1.0mm. There were three larger incised defects: one curvilinear (~1.8cm) and two that were roughly parallel and measured ~0.8cm and ~1.2cm, respectively.

The suspect hammer in the case was badly worn, with a misshapen metal head and dilapidated handle. The hitting surface contained multiple overlapping marks that resembled both Vs and Ks. They covered the head of the hammer and were depressed in some instances and elevated in others. There was one portion of the head that contained an arced area of elevated metal with multiple overlapping Vs and Ks and two or three roughly ovoid depressed pits. This area became the focus of the investigation comparing the two etched surfaces.

Multiple techniques were used in attempts to transfer the pattern observed on the skull and the pattern observed on the hammer onto similar surfaces in order to make a comparison. The hammer was overlaid with thin tracing paper and a charcoal pencil was passed softly across the surface. This same technique was used on the bone segment containing the pattern. This process was very successful; however, it produced "negatives" of the pattern rather than the actual pattern. The hammer was punched into clay, a corkboard, and Styrofoam® in an attempt to determine which portion of the head "marked." These techniques were less successful than the tracing paper method.

The forensic odontologist determined that a cast could be made of the head of the hammer, and this would allow determination of which portion of the head might have created the marks on the bone. Dental polymer impression material was used to cover the head of the hammer, and a dental stone model was made from the resulting impression. Comparisons of the marks made by the suspect hammer and the marks on the bone were conducted using a dissecting microscope with magnifying lenses. The suspect hammer could not be ruled out as the cause of the marks on the cranial vault.

In order to determine whether other hammers could have similar markings on the hitting surfaces, four other used hammers (K3, K4, K5, and K6) were obtained and tested. In addition, a new ball-peen hammer (K1) and a new (unused) hammer/hatchet combination (K2) similar to the suspect hammer were tested. All of the tested known hammers could be ruled out, as they did not have similar characteristics to the marks on the bone.

This case is a good example of successful multidisciplinary cooperation and creative thinking. By involving several types of experts in the case, evidence could be examined in a novel manner. The defendant in this case ultimately pleaded guilty.

Pattern Comparison, Dental Casting, Evidence Preservation

G37 Testing a Novel 3D Printed Radiographic Imaging Device for Use in Forensic Odontology

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After attending this presentation, attendees will better understand specific forensic odontology challenges related to radiology, including lack of occlusion and difficulties in aligning X-ray equipment to teeth of interest, and will learn more about a new device.[1]

This presentation will impact the forensic science community by providing research on how 3D printing technology can be used to create a novel alignment device, the Combined Holding and Aiming Device (CHAD), as a way of addressing the positioning limitations of current aiming devices and those specific to forensic odontology.

Distinct dental features remain one of the most efficient Postmortem (PM) identifiers.[2] Dental identifications have been made on a single tooth alone.[1] An important part of the dental identification process is accomplished by comparing Antemortem (AM) radiographic images and dental records to PM images. Limitations of radiographic identifications based on AM and PM image comparisons are well described in the literature as "labor-intensive, subjective, of poor image quality, and containing insufficient dental anatomy for differentiation among teeth and other dental anatomy."[2,3] Specifically, common technical errors related to dental radiographic exposure include film packet and/or sensor placement and angulation discrepancies.[1] 3D printing technologies have been deemed "the next industrial revolution" and are predicted to change health care delivery models in both medicine and dentistry.[4] 3D printing has gained popularity in dentistry because parts, equipment, and products can be customized and produced at a low cost.

This presentation contributes to the forensic community by proposing a new device designed to help hold X-ray aiming equipment onto teeth of interest for the purpose of allowing dental professionals to more accurately obtain precise X-ray images on victim remains. The CHAD has a sliding lock mechanism that securely adheres to and adjusts to the tooth, including those that may be broken or chipped (Table 1). Overall, the CHAD has the following capabilities: (1) it allows portable X-ray equipment to align with the CHAD, and, (2) it keeps the X-ray sensor or film in place and in alignment with dental remains while PM X-rays are taken; and, (3) it facilitates infection control as it is made of disposable biodegradable plastics.

	Mean	Standard Deviation	p value
Total Error Scores			
Device A	14.833	5.18	0.0015
Device B	9.853	4.63	
Device C	13.333	5.05	
Packet Placement Errors			
Device A	4.250	2.967	0.1716
Device B	2.958	2.255	
Device C	4.208	2.750	
Angulation Errors			
Device A	7.166	2.098	<.0001
Device B	4.500	4.500	
Device C	6.750	6.750	
Miscellaneous Other Errors			
Device A	0.041	0.204	0.6965
Device B	0.125	0.612	
Device C	0.041	0.204	

Angulation Sub-Categories			
Device A	0.042	0.204	0.0206[a] 0.0498[b] 0.0206[c]
Angulation 4	0.666	1.167	
Angulation 5	0.250	0.608	
Angulation 6	1.041	1.267	
Angulation 7	1.292	1.398	<.0001
Device B			
Angulation 4	4.500	1.933	
Angulation 5	0.333	0.963	
Angulation 6	1.083	0.974	
Angulation 7	0.250	0.675	<.0001
Device C			
Angulation 4	6.750	2.251	
Angulation 5	0.125	0.448	
Angulation 6	1.958	1.681	
Angulation 7	0.042	0.204	<.0001

Table 1: Total and Angulation Error Sub-categories for Device A, B and C

Means (M), Standard Deviations (SD), p values for comparisons of each technique by total error scores and error scores by category.

[a]*ANOVA*

[b]*Levene's Test for Homogeneity*

[c]*Brown and Forsythe's Test for Homogeneity*

Fragmented real human skulls were used to test and compare the CHAD to existing holding devices, specifically wax and a Modified External Aiming Device (MEAD). Participants (N=24) exposed six X-rays per device for a total of 432 X-rays scored.

Analysis of Variance (ANOVA) was used to compare sum of the errors for each device A (wax), B (MEAD), and C (CHAD). A significant difference was found at the .05 level between the three devices (p=.0015) (Figure 3). The means showed that devices A and C performed about the same (p=.3152); however, devices A and C were significantly different from B. In other words, B had significantly lower errors than devices A and C. Devices B and C were now compared. As expected, device B performed better in terms of minimal error than device C (p=0.0102). Comparing devices, A and B, the p-value revealed that there was a significant difference in overall errors (p=.0006).

The ANOVA test exhibited no significant difference in total "packet placement" errors between the three devices (p=.1716). Results are summarized in Table 1. A t-test analysis found there was significant difference between all four of the subcategories of angulation errors (Angulation error 4-7) (p<.0001) with incisal edge/apices cut off as the highest number of errors (M=4.500) and horizontal overlap as the next highest error (M=1.083) within device B. Overall, total errors were higher in device A (soft dental wax only), and device B and C performed better than device A — these results support existing literature on the use of holding devices in PM radiographic imaging.

In conclusion, the CHAD combines the benefit of being an "all-in-one" device because it is able to be 3D printed with its own holding and aiming mechanisms — in this way, the CHAD can keep the X-ray sensor or film in place while PM X-rays are taken. Additionally, it needs no modifications or wax for use. Identification of ways to minimize retake errors is needed to ensure radiographers can take accurate dental X-rays with proper angulation and in an efficient way for AM and PM records comparison and victim identification efforts.

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Forensic Radiology, Forensic Odontology, Victim Identification

G38 A Distributed System for Human Identification

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The goals of this presentation are to: (1) distinguish between single computer, local network, and internet-scale computer applications; (2) describe infrastructure as a service; and, (3) discuss the success and challenges in the development of an internet-scale human identification database application.

This presentation will impact the forensic science community by presenting a novel method for collaboration on human identification projects. An internet-scale application allows geographically dispersed teams to collaborate efficiently, in a secure environment, with many tools tailored to the unique task of human identification.

In a disaster with multiple casualties, human identification is essentially a database task. Each set of remains must be compared to hundreds or even thousands of antemortem records looking for the best match. A computer database, WinID, has repeatedly shown its value in organizing and filtering records to find the most likely matches between antemortem and postmortem records

While the value of WinID is difficult to overstate, the program has a few limitations that were typical at the time it was written. Most specifically, WinID runs on a single computer or a local network of computers. Large-scale human identification projects depend on a team of skilled professionals to code and evaluate the records. Typically, the entire team must travel to the location of the disaster and work on a small network of computers running WinID.

This presentation introduces WinID for the Web, a distributed system for human identification. Due to WinID for the Web's permanent, web-based infrastructure, a new team or incident can stand up and begin working within minutes. With worldwide reach, geographically diverse teams can collaborate remotely without consuming scarce resources near the disaster site. Images, documents, and database information are seamlessly delivered to distant team members in an integrated workflow.

As WinID grows from a small network of computers to a worldwide identification system, security and data integrity are of paramount concern. WinID for the Web implements state-of-the-art industry-standard techniques for privacy and authentication to ensure that authorized users can constantly access the data they need and others cannot.

WinID for the Web supports teams that cross organizational boundaries. A free, downloadable lite version allows anyone to collaborate. User customizable forms permit teams to experiment with novel techniques or adapt data collection to the needs of a particular incident. Users who object to storing their data on an internet server can set up a local workgroup without dedicated information technology support.

WinID for the web builds on the strengths of WinID. The new, Web-enabled implementation adds additional distributed collaboration and security features to a tried and trusted tool.

Human Identification, Database, WinID

G39 Dental Identifications Without the Use of Dental Antemortem Radiographs

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The goal of this presentation is to demonstrate how positive dental identification may be accomplished by means other than a comparison of dental radiographs.

This presentation will impact the forensic science community by illustrating how positive dental identifications may be accomplished via comparison by means other than dental radiographs.

Identification based on dental record comparison is a common method of postmortem identification when the condition of the decedent does not allow for more traditional methods, such as fingerprints or visuals. The common technique applied is the comparison of dental radiographs that have been obtained from the decedent's dentist to postmortem radiographs taken by the forensic odontologist. Reasons that the dental comparison is a good tool for the coroner include the accuracy of dental radiographic comparison and the availability of dental radiographs, which are greater in the general population than fingerprints especially among persons under the age of 18 years old. Unfortunately, antemortem dental radiographs are not always available for various reasons. The following abstract details four cases in which dental identifications were accomplished by means other than the use of antemortem dental radiographs.

The first case involved a decedent who was a 60-year-old White male residing in an apartment in San Diego, CA. The decedent was seen in the parking lot of a department store in San Diego pacing around his vehicle. Minutes later, witnesses noted his vehicle engulfed in flames with him sitting in the passenger seat. The autopsy confirmed the cause of death as inhalation of products of combustion and the manner of death as suicide. The investigator summoned the forensic odontologist to complete a dental identification. Only medical radiographs of the head and neck were available. These radiographs were a frontal and lateral skull series highlighting a medical implant on the cervical vertebra. The dentition was visible on both radiographs but, because of the radiographic orientation, the lateral film showed the lower teeth more clearly and with more detail. Postmortem dental radiographs were taken and the positive identification was completed based on the comparison of these dental restorations.

The second case involved a decedent who was a 60-year-old transient. A resident near Buena Creek called 911 after finding the decedent floating in the creek. The autopsy confirmed the cause of death as atherosclerotic coronary artery disease with contributing factors of drowning and chronic alcohol abuse and the manner of death was accidental. Only medical radiographs of the head and neck were available. These radiographs were frontal and lateral Computed Tomography (CT) scans and, even though the dentition was visible in both views, the lateral view displayed greater detail of the teeth. The positive dental identification was based on the comparison of the medical lateral CT scan and postmortem dental radiographs.

The third case involved a motor vehicle collision. A Sports Utility Vehicle (SUV) was stopped in traffic when it was rear-ended by the intoxicated driver of a full-size pickup truck who was driving home from an impromptu Christmas party with co-workers. The driver of the truck was able to exit his vehicle unscathed, but both occupants of the SUV were killed upon impact. The bodies of both decedents were charred to a level four-degree postmortem state. One of the victims had been undergoing orthodontic treatment in Tijuana, Mexico. The death investigator discovered that the Mexican orthodontist had not taken pre-orthodontic treatment radiographs of any type and the only pre-treatment records the orthodontist possessed were study models, which were obtained for comparison. The postmortem condition of the oral cavity was unaffected by the fire and was sound. The jaws were resected, alginate impressions were taken, and stone models were poured. Once the models were trimmed and finished, they were compared to the antemortem models. There were numerous consistencies in the patterns of the rugae and this identification was clearly to the level of positive.

The fourth case involved a male individual who committed suicide by driving his rented vehicle off a 300-foot ravine. The body was discovered two weeks after he had been reported missing. There were no antemortem dental radiographs available, but there were recent family photographs, some of which depicted the decedents maxillary anterior dentition (6-11) clearly. The decedent's maxillary jaw was resected and photographic images were taken.

With the use of Photoshop®, both antemortem and postmortem images were superimposed to complete a positive identification.

These four cases are examples of the manner in which positive dental identifications can be completed by means other than radiograph comparison.

Dental Identification, Medical Radiographs, Photographic Superimposition

G40 Tarawa Revisited: A Summary of Results

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After attending this presentation, attendees interested in dental identification will be better informed regarding the identification of recently recovered United States Marine Corps and Navy personnel who died during the World War II Battle of Tarawa.

This presentation will impact the forensic science community by summarizing the identification outcomes from the two missions in Betio, Tarawa, in the nation of Kiribati, and by explaining the multidisciplinary approach to the identification process by odontologists, anthropologists, DNA experts, and historians.

The Battle of Tarawa was an early battle in the Pacific Theater of World War II that was fought from November 20 to November 23, 1943. The battle took place in and around the southwest corner of the Tarawa Atoll in the Gilbert Islands. Nearly 6,400 men died during the battle, including American and Japanese servicemen plus Koreans who were there as Japanese-forced laborers. The majority of those men died on the island of Betio.

The remains of at least 42 individuals were recovered from a previously undiscovered post-battle cemetery on Betio Island, Tarawa Atoll, between March 2015, and March 2016. Anthropological examination results reported that the individuals recovered were males of predominately European or mixed European ancestry. Additional findings included that the remains were associated with many uniform and equipment items issued to United States military personnel. The remains of Japanese individuals were also recovered.

Antemortem military dental records were examined for 532 unrecovered service personnel presumed to have died during the battle. The associated oral and dental remains were examined, photographed, and radiographed. Re-association was required for some fragmented remains. WinID computer software was employed to aid in postmortem and antemortem data comparison. Dental age assessment techniques were applied to estimate the age intervals for the remains. Comparisons to OdontoSearch databases were performed to assess the relative comparative incidence of dental patterns for these servicemen when compared to a large database.

The combined efforts of the professional disciplines involved with the identification process resulted in a much higher rate of positive identification than was initially thought to be possible with recovered human remains of this type, especially when considering the nearly total lack of antemortem radiographs. The process of each discipline working in isolation to minimize confirmation bias and a subsequent reconciliation of the various results is an important feature of the approach taken in Tarawa. This presentation will detail the interplay of the various disciplines and their contribution to that process.

Forensic Odontology, World War II, Tarawa

G41 Identification Challenges With Fragmented, Disassociated, and Commingled Remains

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After attending this presentation, attendees interested in dental identification will be aware of a specific case of recently recovered United States Marine Corps and Navy personnel who died during the World War II Battle of Tarawa.

This presentation will impact the forensic science community by offering insight into the methodology, sequencing, and challenges of the potential identification of fragmented skeletal remains from a different period of time.

The Battle of Tarawa was a battle in the Pacific Theater of World War II that was fought from November 20 to November 23, 1943. It took place at the Tarawa Atoll in the Gilbert Islands. Nearly 6,400 Japanese, Koreans, and Americans died in the fighting, the majority on the island of Betio.

The remains of at least 42 individuals were recovered from a previously undiscovered post-battle cemetery on Betio Island, Tarawa Atoll between March 2015 and March 2016. Anthropological examination results reported them to be males of predominately European or mixed European ancestry, and the remains were associated with many uniform and equipment items issued to United States military personnel. The remains of Japanese individuals were also recovered.

Antemortem military dental records were examined for 532 unrecovered service personnel presumed to have died during the battle. The dental remains were examined, photographed, and radiographed. WinID computer software was employed to aid in postmortem and antemortem data comparison. Dental age estimation techniques were applied to the remains. Comparisons to OdontoSearch databases were performed to establish population incidence of dental patterns for the deceased.

Dental identification is usually based on the comparison of the antemortem and postmortem records of a similar nature. The comparison of postmortem evidence to antemortem charting alone is not a common forensic odontology practice. The case presented will demonstrate the challenges encountered by the team when dealing with commingled and fragmented remains postmortem in which the only available antemortem dental information consists of dental records and charts without dental radiographs. This case also reinforces the collaborative effort of different forensic disciplines.

The case presentation will also explain some of the uncertainties and limitations of the comparison of dissociated orofacial remains solely with written records and the possibility of confirmation bias in a combined-discipline approach to identification.

Forensic Odontology, World War II, Commingled

G42 The Strengths and Weaknesses of Missing Persons Apps for Smartphones: A Forensic Expert's Perspective

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After attending this presentation, attendees will possess a deeper understanding of how social media and smartphone applications could become a valuable investigative tool in the search for missing and wanted persons. Crime investigations can benefit from the vast amount of information available online, such as videos, photos, and text posted by criminals, victims, or witnesses.

This presentation will impact the forensic science community by increasing awareness concerning how missing persons apps work, and by considering improvements that could be introduced to make their use possible in human identification scenarios.

There has been a rapid growth in the creation of applications for smartphones that can assist in the search for missing persons. The goal of this presentation is to evaluate features of some of the apps currently available, focusing on the similarities between them and the functions that are unique to a specific app.

Thirteen apps were downloaded and analyzed: Missing Persons (Astutech, United Kingdom), Missedperson (Vijayanand Infotech Pvt. Ltd.) Missing Person's App (Eventa, Saudi Arabia), Missing People Find (QuarkSystem, India), Missing Persons Ireland (Davis College Mallow, Ireland), Missing People Sweden (Red Dawn, Sweden), Safety Savvy (Texas Center for the Missing, United States), MissU (Ashwin Wadte, India), Lost or Found (TeckMapz, India), Alert Missing (Alert Missing), Missing People Canada (Website integration, Canada), ReUnite (National Library of Medicine at NIH, United States) and Scomparsi (SoftFobia, Italy). The apps were installed, tested, then uninstalled on an Android™ phone in order to analyze one app at a time.

The inclusion criteria were: free to download and no parental supervision required. All applications include a repository section where images and descriptions of the missing person can be uploaded, plus a view of all those reported missing: however, not all apps have search filters. "Reunite" has the most complete search and upload features including, when relevant, the ability to plot the location of disasters. The best quality in reporting features are found in those apps which require registration, such as (Reunite and Missing Persons), whereas others can update possible sightings to the app and provide a Global Positioning System (GPS) location. Some apps can only be used in a specific country, although this cannot really be considered a weakness. Nevertheless, the missing person phenomena can, of course, involve people traveling outside the country of origin. Finally, most apps have connections with social media such as Facebook®, Twitter®, and Google+®, but these merely link to the site through the app, they do not share details of the missing report.

The main focus of all the apps tested was on finding the missing person "alive," whereas the use of the app as an aid to identify a deceased person is not considered. The connection with social media networks could be enhanced in order to contribute to antemortem secondary identifiers data collection and possible human identification of missing and unidentified persons through visual recognition including forensic dental identification.

Missing Persons, Social Media, Dental Identification

G43 Case Study: Identifying Balloon Fatalities in Central Texas

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After attending this presentation, attendees will better understand how to plan and organize local efforts to identify the deceased in the event of a multiple fatality event at the local level.

This presentation will impact the forensic science community by explaining how a mass fatality response team can be created and mobilized in a small community with seemingly minimal resources.

On Saturday morning, July 30, 2016, shortly after 7:00 a.m., 16 people climbed into a hot air balloon at a rural private airfield between San Antonio and Austin, TX, for a "bucket list" pleasure ride over the prairies of Central Texas. The scheduled ride of one hour ended tragically approximately 30 minutes later when the balloon collided with a major power line. The power company reported that its lines were tripped at 7:42 a.m., and the first 911 call came in approximately one minute later. Exact events are unclear, but it is known that the gondola and its contents ignited, and all 16 souls aboard perished. There was an initial response of local fire fighters and law enforcement, followed by numerous federal agencies investigating in their areas of authority.

A private autopsy service within the county was selected to conduct forensic autopsies and identifications. Due to the condition of the remains, it was realized from the outset of the tragic event that odontology support would be needed to complete the identifications. A local odontologist was contacted and his support was requested. The odontologist in turn surveyed resources at a local university forensic anthropology program and a nearby military facility. Three additional experienced team members were willingly recruited (two anthropologists and one military dentist). The blended odontology and anthropology team came together at the morgue on the second day (Sunday) and began dental autopsies in conjunction with the forensic autopsies. On the third day (Monday), dental offices were contacted to begin the collection of antemortem dental records. Postmortem dental data from autopsy and antemortem dental data from private offices were continuously entered into WinID as they were collected to facilitate identifications. Every effort was made to work in a digital world. The first dental identifications were completed on Monday and work continued until completion of the dental data collection on Friday, the sixth day. At the end of work on Friday, 15 of the 16 decedents had been positively identified: 12 by dental means and 3 by other means. The final unidentified individual had a tentative identification by the process of elimination, but dental records were not located until the following week. Identification by familial DNA analysis was initiated. Dental records were finally located early the next week to complete the final identification.

Lessons learned: (1) well-trained individuals from diverse backgrounds can work together very well. The blended team of anthropologists and odontologists performed flawlessly together; (2) preparation and equipment availability are essential to perform well in a stressful environment. Computers, digital radiology, and copy stands or scanners are essential; (3) all phases have their challenges. Dental autopsies, collecting data from offices, putting it all together for comparison, collaborative reviews, and writing final reports are all equal parts in the process; and, (4) prepare for disasters with complementary daily practices. Each single human identification by dental means must be conducted following a meticulous protocol that is exactly the same as a disaster identification. Only the scale of the events differs.

This tragic event unfolded with an expected amount of chaos that was quickly organized into a successful identification process. The local institutions of state and county government benefited from the relatively rapid response of the odontology team. The ultimate benefactors are the families and loved ones who received a respectful final answer as to the identity of their decedent.

Dental Identification, Balloon Fatality, Local Disaster Response

G44 The Ability to Discern Dental Restorative Materials on Radiographs for Dental Identifications

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After attending this presentation, attendees will realize that many of the newer Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) restorations may appear "tooth colored" and natural in the mouth, but actually appear radiopaque on dental radiographs. Forensic odontologists routinely observe and compare postmortem and antemortem dental radiographs.

This presentation will impact the forensic science community by increasing awareness of the fact that it is no longer possible to distinguish a metallic vs. a non-metallic restoration simply by viewing dental radiographs alone, and forensic odontologists must examine the decedent or refer to antemortem treatment notes in order to determine the type of restorative material present. This presentation will strongly encourage medical examiners and coroners to request a consultation with a forensic dentist as often as possible to ensure correct dental charting.

This research project is the result of participation in the University of Texas (UT) Health School of Dentistry's 2016 Summer Research Program. This project developed a database of radiographic images of newer restorative dental materials designed to be used as a reference source in forensic dental identifications. There have been several studies investigating the characteristics and radiopacity of ceramics, composites, and other restorative materials, but none contain a significant grouping of images to serve as a reference source.

After receiving Institutional Review Board (IRB) approval for the study, a retrospective chart review was performed to gather post-treatment radiographs of various types of dental restorative materials. Constructing the database of radiographic images involved utilizing the school's electronic health records to view patient radiographs after a restoration. By using the Info Manager Search feature, a text search was performed for current dental materials or brand names in the Progress Notes. Once the specific dental material or brand name was confirmed, a search for a post-restoration radiograph ensued. The radiograph was collected and used in assembling the databank of images. The types of restorations collected were amalgam, direct composite resin (ex: Filtek™ Z250), glass ionomer (ex: Fuji LC®), gold, indirect restorative composite (ex: Belleglass or Premise™), IRM®, monolithic zirconia CAD/CAM crown (ex: BruxZir®), porcelain-fused-to-base-metal, porcelain with ceramic substrate (ex: Procera®), and stainless steel.

Next, an online survey was developed to assess the ability of forensic odontologists, dental faculty, and dental students to discern recently introduced dental restorative materials from dental radiographs. A letter of invitation for the online survey was sent to the following groups of individuals: School of Dentistry faculty, School of Dentistry students and residents, Diplomates of the American Board of Forensic Odontology, and members of the American Society of Forensic Odontology. The survey consisted of ten demographic items and ten query items and was estimated to take less than ten minutes to complete. The survey items displayed a dental radiograph and prompted the participant to identify the restoration's material from a multiple-choice list, stating that all responses would be grouped and no individual would be identifiable, thus all responses were completely confidential.

At the time of this abstract proposal, the online survey was still active, so results will be summarized during the presentation. It is presumed that the survey results will confirm the hypothesis that we will no longer be able to distinguish metallic vs. non-metallic restorations on radiographs in the future. The study results will encourage medical examiners to consider requesting forensic dentist consultation as often as possible to ensure correct dental charting.

Forensic Science, Forensic Dentistry, Forensic Dental Identification

G45 The Germanwings Air Crash in the French Alps (2015): Victim Identification

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After attending this presentation, attendees will understand the importance of appropriate training, planning, and collaboration of national and international teams in order to apply the International Criminal Police Organization (INTERPOL) Disaster Victim Identification (DVI) protocol. Procedure was of particular importance in this challenging DVI case as complicated site conditions, the state of the human remains, and the different nationalities represented on the flight all combined to render a successful outcome very difficult.

This presentation will impact the forensic science community by providing deeper insight into the response given by the French identification team to these DVI challenges. Special attention will be paid to techniques for collecting and identifying fragmented and commingled human fragments in an inaccessible and steep mountain range. This presentation will also help attendees understand the role the dental team played in the identification of the 150 victims of 18 different nationalities.

On March 24, 2015, an Airbus A320-211, Germanwings flight 9525 from Barcelona, Spain, to Düsseldorf, Germany, crashed 100 kilometers (62mi) northwest of Nice in the French Alps within the Massif des Trois-Évéchés.

All 144 passengers, 4 crew members, and 2 pilots were killed; there were no survivors and no external victims. The inaccessible and steep crash site covered two square kilometers (500 acres).

Immediately, the French national gendarmerie victim identification team was activated. The inaccessibility and dangerous conditions surrounding the crash area necessitated the use of specialized forces, such as the high-mountain gendarmerie helicopters, to recover remains.

An inquiry into the accident was conducted by the Transport Accident Investigators of Gendarmerie a few days after the crash. The voice recorder was recovered and examined, and the cause of the crash was determined very early, which is quite rare in an air crash investigation; the co-pilot deliberately crashed the aircraft. The aircraft was travelling at 700 kilometers per hour (430mph) when it crashed into the mountain.

Less than 24 hours after the crash, an ad hoc morgue was set up in a technical room in Seyne-les-Alpes near the crash site. All the forensic identification teams, pathologists, odontologists, fingerprint examiners, DNA analysts, and sealing procedures were organized into subsectors.

Crash investigators, police, gendarmerie, and INTERPOL worked together at the scene. At the same time, an Antemortem (AM) team was set up at the Institute de Recherche Criminelle de la Gendarmerie Nationale near Paris. Due to the 18 nationalities involved, the collection of AM data took place in different countries according to INTERPOL DVI protocol.

The forensic odontology teams were composed of well-trained forensic dentists from the Medical Reserve Corps. Six dentists alternated shifts on the Postmortem (PM) team in groups of two or three for ten days. They participated in the human fragments triage, performed photography and radiography, charted PM odontograms of the dental fragments, and performed tooth extraction for DNA analysis when necessary. The AM team consisted of only one dentist because there were no French victims. His role was to transcribe the dental data collected by INTERPOL. A third forensic odontology team composed of three pairs of dentists was set up three weeks and five weeks after the crash, and comprised a reconciliation team responsible for comparing data and preparing it for presentation to the Identification Board.

Human fragment recovery and forensic examinations onsite lasted for ten days. Thousands of human fragments and 1,200 fingers were collected, and more than 3,000 fragments were analyzed. Nine days after the crash, 150 different DNA samples were isolated.

All victims were identified; results will be presented and discussed.

This multiple fatality incident generated huge media coverage and political interest. French President Francois Hollande greeted members of the police, army, and rescue workers along with German Chancellor Angela Merkel and Spanish Prime Minister Mariano Rajoy as they visited close to the crash site.

Each disaster is different and each one must be managed appropriately and as efficiently as possible.

The INTERPOL victim identification protocol was followed and required the mobilization of multidisciplinary teams. Respecting the procedure allows us to standardize methods and work with the different countries involved. The goal is to return the bodies to their respective families as quickly as possible and with absolute certainty of identification.

Forensic Odontology, Air Crash, Identification

G46 Identification of Sex Using Melanoderm Lip Prints: A Clinical Study in Senegal

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The goal of this presentation is to predict the sex of an individual from the architecture of a melanoderm lip print.

This presentation will impact the forensic science community by providing results from a clinical study. This presentation will add to research currently being conducted in cheiloscopy analyzing various materials from the crime scene, such as clothing, cups, glasses, cigarettes, doors, etc. The patterns of lip prints demonstrated significant sexual dimorphism between males and females.

Materials and Methods: This study was conducted on 197 melanoderm subjects in Dakar, Senegal. All the subjects (females = 97, males = 100) were 30 to 60 years of age. Non-gloss lipstick, cellophane tape, white bond paper, scissors, calipers, and a magnifying hand lens were used for analysis. The length and thickness of the upper and lower lips were measured with sliding calipers. For all lip prints, the thickness of the lip was measured at the center of the lip.

Results: Lip prints for each individual were collected to obtain the clearest and most complete print as noted by examination with the magnifying hand lens. Overall accuracy by the examiner was found to be 100% (all 197 subjects were diagnosed correctly). No significant difference was found in lip thickness between males and females ($P \leq 0.71649$ for upper lip, $P \leq 0.30718$ for lower lip, but there was a significant difference in widths of the mouth between males and females ($P \leq 0.00018$). For distribution of types of labial groove patterns, type V is predominant in all areas, whereas it is only in areas 2, 3, and 4. Type I is very important in all areas of females, but is present only in area 1 of males. The average difference in the lip length found between males and females was 7.63mm.

Conclusion: In the present study, none of the lip prints of any subject "matched" any of the others. This study demonstrates that lip prints have the potential of predicting the gender of the person, but in the future, a database for a population could be very important in criminal investigations.

Cheiloscopy, Melanoderm Lip Prints, Sex

G47 Identification of Charred Victims

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After attending this presentation, attendees will understand the value of applying a strict protocol for different phases of charred victim identification. For road accidents and air disasters, data synthesis was conducted during dental examinations.

This presentation will impact the forensic science community by illustrating that, even within the same accident, the techniques used to preserve the maxillaries, to examine dental arches, and to photograph and radiograph the heads of charred victims are specific for each victim. Carbonization of the victim differs with the length of exposure to the flames.

The soft tissues are very poor thermal conductors. The adipose tissue of the hypodermis will burn, but is a fuel quickly exhausted. In fact, human tissues are combustible only if they are kept in contact with fire. Several types of carbonization are described. Of these types, accidental carbonization takes a special place because it is very often present after aircraft disasters and road accidents. Observations reveal that the state of the body depends on the temperature of the heat source, on the location in relation to the heat source, and on exposure to flames. In one and the same fire, the variable extent of sometimes extensive carbonizations leaves bodies with different stages of preservation.

Whatever the degree of carbonization, it is observed that the dental system is usually well-protected by surrounding soft tissues and hard tissues, and the teeth have particularly good resistance to fire due to their high degree of mineralization; however, the high temperatures and direct action of the flames have some destructive effect. It follows from these findings that the dental system is the structure of choice for the identification of charred victims, while in extreme carbonizations the use of DNA analysis may be impossible.

Common carbonization phenomena have resulted in the development of procedures for identifying charred victims. Several studies have led to the establishment of a classification of the degrees of carbonization of the head and teeth and the standardization of examination protocols for each degree of carbonization.[1-3]

After explaining the establishment of appropriate protocols for dental identification of charred bodies in disasters, case studies will be presented.

The results illustrate that the percentage of dental identifications of charred victims is high, and this type of identification should usually be attempted first.

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Identification, Char, Disaster

G48 The Development of an Ultraviolet Photography Protocol for Composite Restorations

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After attending this presentation, attendees will better understand the development of a protocol for utilizing ultraviolet fluorescent photography to document the presence of composite restorations during dental identification procedures.

This presentation will impact the forensic science community by demonstrating a protocol to be used in taking ultraviolet photographs of composite restorations.

On November 10, 2015, a Hawker 125-700A private jet crashed in Akron, OH, killing seven passengers and two pilots. Anthropologists recovered nine bodies from the wreckage in less than 24 hours following the crash. Antemortem dental records were located for all nine of the victims. Records for five victims were provided within five hours of the accident. The seven passengers were identified within 48 hours using dental records. The pilot and co-pilot were identified by DNA analysis within 72 hours of the crash. Antemortem dental records were first compared to full body X-ray scans. In addition, focused dental autopsies were performed. This procedure provided access and exposure to specific areas of the dentition to allow comparison of antemortem and postmortem dental data for the basis of identification. Digital dental radiography was accomplished using DEXIS® software and a portable NOMAD™ X-ray unit, allowing side-by-side comparisons of antemortem and postmortem radiographs. Dental identifications of the pilot and co-pilot were made difficult by translation and interpretation issues with the Spanish language antemortem dental records and the quality of the antemortem radiographs. Ultimately, the pilot and co-pilot were identified by DNA analysis.

Antemortem radiographs indicated that one victim had a composite restoration on tooth number five (Universal system). This restoration was not visible on the postmortem full body radiographs and not readily visible during the initial oral examinations. Examination with an Ultraviolet (UV) light source was used to fluoresce the restoration in tooth number five to see that it was, in fact, present. Although examination using an ultraviolet light source allowed visualization of the fluorescence from the composite restoration, attempts to photographically document the fluorescence proved difficult with the equipment available in the morgue. This led to the development of a protocol for taking photographs that demonstrates the fluorescence of composite restorations using an ultraviolet light source in a morgue setting. The equipment required was: (1) a high-resolution digital camera that allows photography without a flash; (2) a UV light source producing UV at a wavelength between 365nm and 395nm; (3) an orange composite-curing-light filter; and, (4) a tripod or unipod to hold equipment.

The protocol was: (1) turn the camera flash off; (2) place or mount the orange filter directly in front of the camera lens; (3) direct the UV light onto the restoration; (4) place equipment on a tripod or unipod if taking the photographs unassisted; and, (5) collect images with and without an American Board of Forensic Odontology (ABFO) #2 scale in place.

Details of the identification of the victims of the crash and further discussion and details of the procedures employed and images produced by the protocol developed will be presented.

Ultraviolet Photography, Dental Identification, Composite Restorations

H1 A Postmortem Microbiological Evaluation: Notable Infectious Diseases in Forensic Autopsies

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After attending this presentation, attendees will be familiar with: (1) the most frequently detected notifiable infectious diseases encountered during forensic autopsies; (2) frequencies of those notifiable infectious diseases found during forensic autopsies; (3) frequency of the cases with notifiable infectious diseases that were undiagnosed before forensic autopsies; (4) possible biosafety risks of performing autopsies in those previously undiagnosed cases; and, (5) the help of postmortem microbiological analysis for determining the cause of death.

This presentation will impact the forensic science community by increasing awareness of the necessity of postmortem microbiological analysis and the detection of the notifiable infectious diseases during forensic autopsies.

Frequencies of infectious diseases and the fight against them are accepted among the success criteria for evaluation of national health systems. The first step in the fight against infectious diseases is collecting and interpreting the epidemiological data. Notifiable infectious diseases are groups of diseases determined by then World Health Organization and those infectious agents pose important risks to public health. Many notifiable infectious diseases, which were not diagnosed and notified before, can be detected during forensic autopsies. The goal of this study, is to determine the frequency of notifiable infectious diseases encountered during forensic autopsies and to emphasize the need for proper notification of those cases which were undiagnosed before. Additionally, autopsy is a highly hazardous health care service with regard to the potential of encountering with infectious agents. The risk of encountering infected materials during autopsy is very high for health care professionals. Another goal of this study is to emphasize the biosecurity hazards of the notifiable infectious diseases, which can be detected during forensic autopsy.

In this study, 23,079 autopsy cases performed in the Council of Forensic Medicine in Turkey between 2011-2015, have been examined retrospectively. The total number of cases included in this study is 271. Postmortem microbiological cultures of cerebrospinal fluid, blood and internal organ samples, Polymerase Chain Reaction (PCR) analysis, histopathological findings, and medical documents were used to determine the cases to be included in this study.

Of the 271 cases included in this study, 120 were detected with tuberculosis, 108 with viral hepatitis (HBV, HCV), 25 with HIV, 23 with echinococcosis (hydatid cyst), 5 with malaria, 4 with H1N1, 1 with toxoplasmosis, and 1 with meningococcemia; 81.5% of the cases were male, and 24.4% were foreign nationals. Medical reports of only 20.2% of the cases include data concerning the infectious agents. In 79% of the cases, histopathological evidence supporting the reported infectious agents were detected. In 43.5% of the cases, death was caused by the detected infectious diseases.

There are many cases of infection diseases present in forensic autopsy that warrant mandatory notification; however, these cases can be detected by postmortem microbiologic examinations. The higher prevalence of HBV, HCV, and HIV in forensic autopsy cases compared to the general population has been subject to several research papers. These agents cause risk of serious infection during an autopsy, which can lead to life-threatening infections for autopsy personnel. Availability of appropriate postmortem microbiological analyzers make it possible to identify a significant number of communicable infectious diseases in forensic autopsy cases. Awareness of these

factors has highlighted the importance and the need to use biosecurity measures, which result in reduction of the risk of transmission during an autopsy. Identification of these factors during an autopsy via microbiological analyzer has been helpful in determining the cause of death in cases in which the cause of death was unidentifiable due to the lack of microbiological analysis. Also, the identification and proper notification of the national authorities of the presence of these infectious agents in cases in which there has not been a definite diagnosis of disease prior to autopsy can help to curb epidemiological data inaccuracies.

Postmortem Microbiology, Autopsy, Notifiable Infectious Diseases

H2 I Like Your Shoes: The Utility of Barnacles (Crustacea: Cirripedia) in Forensic Investigations in a Marine Environment

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After attending this presentation, attendees will understand that barnacles are potentially useful when human remains are found in the sea by allowing estimation of the overall duration of the time spent in the water and hence contributing to the determination of the minimum time since death.

This presentation will impact the forensic science community by reviewing the first study involving the identification, colonization, and the growth rate of barnacles associated with shoes placed in a marine environment. This data will be useful in cases in which human remains and their garments are found in the sea.

Estimating the minimum Postmortem Interval (minPMI) is a necessary part of a forensic investigation. MinPMI can be estimated using forensic entomology, the scientific discipline that considers insects and other arthropods in legal investigations. In an aquatic environment, insects as well as crustaceans have the potential to provide data regarding the time the remains spent in water, Floating Time (FT), and Post Mortem Submersion Interval (PMSI), and this can also assist in determining the minPMI.

Barnacles (Crustacea: Cirripedia) are common crustaceans that colonize hard substrates in marine environments and they can often be found in association with human and animal remains floating in the sea. Barnacles are typically found colonizing shoes. Barnacles can colonize both floating remains and submerged remains and their growth rate is dependent on the water temperature. Despite their potential to be indicative of the FT and/or PMSI, at present, research is depleting and only a few case studies have considered it for this purpose.

This study is focused on the barnacle colonization of different type of shoes (plastic/sporty vs leather/elegant) placed in the sea (Boston Harbor, Boston, MA). The objectives of this study are: (1) identification of barnacle species of barnacles that colonize shoes; (2) identification of the settlement preferences of the barnacles associated with the shoes; and, (3) identification of the growth rate of the barnacles associated with the shoes.

In the experiment setup, 64 plastic shoes and 64 leather shoes were placed in the harbor at the same depth (-8/-10 meters), in early March 2016 and remained untouched for one month to allow for colonization to occur. Data loggers were placed with the shoes to record temperature throughout the course of the study. Four of each shoe type were removed every two weeks from April 2016 to October 2016 inclusive. Once the shoes were removed from the water, they were photographed and the barnacle colonization was documented. Individual barnacles from each shoe were sampled and measured to determine species, age as well as the overall colonization density, and settlement preference. Growth rates were calculated based on barnacles sampled from each sampling period correlated with the temperature data. Preliminary results to date reveal that barnacles can colonize both plastic and leather shoes, but also that leather shoes show a higher colonization density.

The results of this study will help determine whether barnacles provide accurate estimations of the time spent in water by the different type of shoes, expanding the field of forensic science and giving examiners more tools in the ever-difficult task of estimating FT, PMSI, and minPMI.

Barnacles, minPMI, PMSI

H3 Ketamine and Blowflies: An Entomotoxicology Study

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After attending this presentation, attendees will understand the capability of entomotoxicology and how the presence of ketamine, (a medication mainly used as an anesthetic), commonly known as the drug used in facilitating sexual assault, can affect the survival, the size and the developmental rate of blowflies, which have a consequence on the estimation of the minimum Postmortem Interval (minPMI).

This presentation will impact the forensic science community by providing both chemical and entomological data that will be useful when determining the minimum time since death of human remains exposed to ketamine.

The branch of forensic entomology that studies insects used to detect drugs or other toxic substances in decomposing tissues is known as entomotoxicology. In the absence of tissues or biological fluids, insects developing on a human or animal cadaver present a reliable alternative for analysis of toxicological substances, especially because chemical methods validated for keratin substrates (e.g., hairs) can be generally applied to chitin, the exoskeleton of an insect.

The main focus of forensic entomology is the estimation of the minimum time since death of a deceased human or animal using the succession status of carrion insects, but in numerous studies it has been demonstrated that such time frames may be severely compromised by drugs and toxins. Therefore, entomotoxicology also investigates the effects of these substances on insect development, survival, and morphology. While the detection of drugs, metals, pesticides, and alcohol has been reported in entomotoxicological studies, only one incomplete study regarding the effects of ketamine on blow flies is available in the literature.

Ketamine is a medication mainly used for anesthesia in both humans and animals. Currently, ketamine has been used as a recreational drug, as well as a drug associated with sexual assault, and has been implicated in several deaths globally. To note, ketamine has also been implicated in the suspicious deaths of animals (e.g., fatal sedation due to a wrong dose of the drug).

The present study for the first time describes the development and the validation of two suitable analytical methods to detect ketamine in larvae, pupae, empty puparia, and adults of *Calliphora vomitoria* L. (Diptera: Calliphoridae). One method is based on Gas Chromatography/Mass Spectrometry (GC/MS), the second on High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS). Furthermore, this study also considers the effects of ketamine on the survival, the developmental rate, and the morphology (length and diameter of pupae and larvae) of *C. vomitoria*.

C. vomitoria were reared on substrates (beef liver) spiked with two different concentrations of ketamine, a recreational use concentration (300ng/mg) and a concentration that could cause death in either a human or an animal (600ng/mg). Another untreated liver was used as control.

The results demonstrated that: (1) both the GC/MS and the HPLC-MS/MS methods are applicable in detecting ketamine in *C. vomitoria* because all the required parameters (linearity of method, coefficient of determination, detection limit, quantification limit, extraction recovery %, precision, selectivity, and carry over) were satisfied; (2) only the GC/MS method demonstrated positive results on *C. vomitoria* adults; (3) the presence of the two ketamine concentrations in the food substrate significantly modified the developmental time of *C. vomitoria* by slowing down the time to reach the pupal instar and the adult instar; (4) the survival of *C. vomitoria* is negatively affected by the presence of ketamine in the substrate; and, (5) the resultant lengths and diameters of larvae and pupae exposed to both concentrations of ketamine were significantly larger than the controls.

Entomotoxicology, Ketamine, *Calliphora Vomitoria*

H4 The Things You Find When Fishing: The Forensic Investigation of a Human Skull From the Sea

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After attending this presentation, attendees will understand the capabilities and limits of forensic pathology, anthropology, and zoology in order to determine the cause of death, the circumstances of death, and time since death associated with human remains found in the sea.

This presentation will impact the forensic science community by highlighting other marine invertebrates (muricids, a marine gastropod mollusk) that may have the potential of providing data that will be useful in estimating the overall duration of time that human remains have spent in the sea. As with other marine invertebrates, this could well be a vital component when determining the minimum time since death.

During April 2015, a human skull was found lacking its lower jaw, with only nine teeth of the superior arch present, no visible injuries on the skull cap, and covered by seaweed. The skull was recovered from a fishing net approximately three to four nautical miles offshore from Porto Tolle, Emilia-Romagna, Italy, in the northwestern Adriatic Sea. In the absence of any associated evidence, the Italian public prosecutor decided to forensically investigate the skull with the goal of gaining as much information as possible about the skull (e.g., human vs non-human, identity, cause of death, time spent in water). As a consequence, numerous experts in forensic pathology, anthropology, and zoology were invited to investigate the skull.

An analysis of the skull's anatomy, supported by X-rays, revealed it to be that of a young Caucasian male (23-35 years of age), with no injuries evident either ante- or postmortem. Suprisingly, the decomposition of the remains was not a consequence of being in the sea, as it lacked any abrasive marks usually associated with marine flora or fauna, and it was determined that it had spent only a short period of time in the sea.

The numerous brownish-yellow structures, each approximately 3mm-4mm that covered much of the skull and its interior, and initially considered to be seaweed, were identified as egg capsules of the marine gastropod *Ocinebrina* cf. *aciculata*. *Ocinebrina aciculata* is a representative of the Muricidae, a group of active predators that access their prey (gastropods, bivalves, and barnacles) by drilling a hole with their radula. This activity pierces the shells of their prey using a combination of chemical etching and mechanical abrasion. Muricid eggs are deposited in protective, chitinous capsules from which either crawling juveniles or planktonic larvae hatch.

X-rays of the skull also revealed the presence of an adult *O. aciculata* in the skull's left maxillary sinus where it layed its many egg capsules. It seems this adult lodged itself in the sinus by using its radula to pierce the the thin bones within the nasal cavity surrounding the sinus. The presence of unhatched egg capsules advocates a short colinization interval. Like many other marine invertebrates, further studies on Muricid biology, ecology, and development will help provide more information concerning how long a corpse may have spent in the sea.

Skull, Muricids, Marine Environment

H5 An Analysis of Skin Potential Hydrogen (pH) and Oxidation-Reduction Potential (Eh) on Decomposing Swine Carcasses

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After attending this presentation, attendees will understand that the pH and Eh of swine (*Sus scrofa domesticus*) skin carcasses changes after death.

This presentation will impact the forensic science community by informing attendees of a significant negative correlation that was observed between Postmortem Interval (PMI) and skin pH. This relationship may be used to estimate PMI in medicolegal death investigations, following additional research.

It is well established that the decomposition of human remains is associated with significant chemical changes. For example, soils associated with decomposing remains tend to go through significant pH shifts during decomposition; however, one area that has yet to be explored is the chemical changes of skin during decomposition. Skin is advantageous as physical evidence because it is easily accessible, can be analyzed at the death scene, and can be analyzed non-invasively. The goal of the current study was to analyze the pH and Eh during decomposition to test the hypothesis that these chemical parameters are significantly correlated with PMI. If so, this relationship could provide an investigative tool of a regularly encountered occurrence during medicolegal death investigation.

Three swine carcasses (*Sus scrofa domesticus*), killed via stab wound through the neck, were decomposed on the soil of a tropical savanna ecosystem in Palolo Valley, Oahu, HI, from February 2016 to April 2016. The pH and Eh of carcass skin were collected using a portable meter (Extech® PH100 pH Meter, Extech® RE300 ORP Meter) twice per day (morning and evening) until larval mass migration (eight days postmortem), at which time measurements were collected once per day. Measurements were then collected once per week from 36 days postmortem through 71 days postmortem. Data were analyzed using Prism® 7.0a for Mac® OS X. The relationship between pH, Eh, and postmortem interval was analyzed using Pearson's correlation coefficient.

Skin pH was neutral (7.0 ± 0.2) at the time of carcass placement and gradually became more acidic until 71 days postmortem, at which time it equaled 4.5 ± 0.2. This decrease was relatively consistent with some variation observed on days of increased relative humidity and rainfall. Postmortem skin pH was significantly ($P < 0.001$; $R^2 = 0.427$) negatively correlated to PMI where $y = -0.0256x + 6.62$. In contrast, postmortem skin Eh was not significantly ($P = 0.949$; $^{R2} < 0.001$) correlated to PMI. Skin Eh indicated an oxygenated environment at the time of carcass placement (111 milliVolts ± 3 milliVolts), which persisted until 14 days postmortem. After this time, Eh was consistently negative until day 24, which indicates an environment low in oxygen where metabolic processes are primarily anaerobic. After this time, skin Eh was consistently positive until the end of the experiment.

This study is beneficial to the forensic community because it may lead to an innovative method to estimate PMI. Better understanding of the correlation of skin pH to PMI may lead to accurate estimates of PMI that are rapid, non-invasive, and inexpensive; however, this potential application requires much more research since, according to research, the current study is the first of its kind.

Taphonomy, Skin Chemistry, Postmortem Interval

H6 Small Asian Mongoose Scavenging Behavior on Oahu

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After attending this presentation, attendees will understand the role the small Asian mongoose (*Herpestes javanicus*) plays in carcass decomposition on Oahu, HI, and the potential impact this behavior may have on forensic investigations.

This presentation will impact the forensic science community by indicating that outdoor death investigations should consider the scavenging activity of mongooses, which can alter death scenes.

Carcass decomposition is site-dependent. In built environments such as a house or apartment, microbes and insects dominate the decomposition process; however, at outdoor scenes, vertebrate scavengers are often responsible for the majority of consumption, modification, and dispersal of remains. Familiarity with local scavenging behavior helps forensic investigators recover clandestine remains and distinguish damage caused by scavenging. According to research, this is the first formal study of carcass scavenging on Oahu, which has approximately 50 outdoor death scenes per year.

Many animals scavenge, and the members of a scavenging guild vary according to ecosystem and environment. On mainland North America, a guild typically consists of both birds and mammals, and prominent scavengers include black bear (*Ursus americanus*), coyote (*Canis latrans*), turkey vulture (*Cathartes aura*), and raven (*Corvus corax*). Due to its geographic isolation, Hawaii has a very limited number of species that may act as scavengers. Not only are there few species of mammals present, there are also no wild populations of carrion birds (e.g., crows, and vultures) and only occasional gulls (family Laridae). During this study, the only vertebrate scavenger observed was the small Asian mongoose (*H. javanicus*).

Three swine carcasses (*Sus scrofa domesticus*) were decomposed in a tropical savanna ecosystem located in Palalo Valley, Oahu, HI, from February to April 2016. The swine were killed via single stab wound to the neck, drained of blood, and immediately transported to the decomposition site. Scavenging activity on a single pig was monitored using a Reconyx® PC900 Hyperfire camera, which features dark Infrared (IR) illumination and motion detection. It recorded a rapid sequence of five photos when triggered by motion as well a still frame every 15 minutes, regardless of wildlife activity. Images were downloaded every one to two days. Animals were characterized to lowest possible taxon and timing of visits was noted along with displayed behaviors.

Two distinct periods of mongoose scavenging were observed. The first period of activity was on days four and five (89.5 - 122.5 hours postmortem), when the mongooses visited the carcass seven times and fed on areas where there was significant larval activity. Given that this species of mongoose is primarily insectivorous, it is likely they were feeding on larvae rather than pig tissue, but this is not clear from the images.[1] After the main larval migration event at 131.5 hours postmortem, there was almost no direct interaction with the carcass until day nine (213 hours postmortem), when the mongooses visited the carcass four times, fed on, dislodged, and carried off small pieces of skin, bone, and remaining tissue. There was considerable bone displacement in one of the unmonitored pigs during a comparable stage of decomposition, and although this may be attributable to environmental factors (wind, rain, etc.), the presence of mongoose scat amid the remains suggests herpestid involvement.

This study is significant for several reasons. Understanding the scavenging patterns of *H. javanicus* will better inform recovery and analysis of clandestine remains. In addition, mongoose interaction with the larval mass could affect PMI estimates based on larvae or any associated bacterial signature.

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Taphonomy, Decomposition, Mongoose Scavenging

H7 A Case of Fatal Sepsis by *Clostridium perfringens* After a Hepatic Liver Biopsy of a Rare Neuroendocrine Tumor

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After attending this presentation, attendees will understand that what was initially considered to be artifact, was in fact real pathologic findings due to antemortem sepsis and gas production by *Clostridium perfringens*, making this case an excellent example of how a systemic infection can mimic an advanced state of putrefaction.

This presentation will impact the forensic science community by the uniqueness of the case: (1) mode of dissemination of *Clostridium perfringens*; (2) presence of a rare Primary Hepatic Neuroendocrine Carcinoma; and, (3) body changes noted at the time of autopsy.

Introduction: *Clostridium perfingens* is a spore-forming and toxin-producing gram-positive bacteria that can be found in healthy humans, but in rare cases, cause sepsis and intravascular hemolysis in susceptible individuals. Presented here is a fatal case of *Clostridium perfringens* sepsis in a morbidly obese man who underwent a transcutaneous liver biopsy for the evaluation of multiple liver masses, later identified as hepatic neuroendocrine small cell carcinoma.

Case Report: Reported is a case of a 50-year-old morbidly obese man with hypertension and diabetes who was admitted to the hospital for right upper quadrant abdominal pain and unintentional weight loss of one month's duration. An ultrasound of the abdomen revealed a very enlarged liver with multiple nodules with central necrosis. To evaluate the hepatic lesions, a Computed Tomography (CT) guided liver biopsy was performed; the pathologic diagnosis was a hepatic neuroendocrine carcinoma, small-cell type. The following day, the patient complained of increasing abdominal discomfort with a significant drop in hemoglobin; he developed hypotension and multiple organ failure, followed by death. The postmortem external examination was remarkable for morbid obesity and multiple fluid- and gas-filled skin bullae. Internal examination revealed multiple tumor nodules in the liver without any other primary. There was evidence of gas formation in all organs, including the liver, heart, and brain; *Clostridium perfringens* was subsequently identified in blood cultures that had been drawn prior to death.

Discussion: Sepsis is a rare but deadly complication of *Clostridium perfringens* infection, characterized by rapid progression and death occurring in 24 to 48 hours; this case is unique in many aspects. First, the mode of dissemination of *Clostridium perfringens* was likely related to the CT-guided biopsy, since the patient's hemodynamic status deteriorated very rapidly following the procedure. Second, primary hepatic small-cell carcinoma represents the poorly differentiated end of the spectrum of neuroendocrine carcinomas and is very rare, with only a few reported cases in the literature. Due to its rarity, the clinical course, natural history, molecular profile, proper treatment, and overall prognosis have not yet been fully characterized. Third, this case is unique from a forensic perspective due to the external changes noted at the time of autopsy, namely the large fluid filled skin bullae that had been initially interpreted as accelerated decomposition due to obesity; however, in this case was initially considered to be artifact, were in fact real pathologic findings due to antemortem sepsis and gas production by *Clostridium perfringens*, making this case an excellent example of how a systemic infection can mimic an advanced state of putrefaction.

***Clostridium perfringens* Sepsis, Small-Cell Carcinoma, Therapeutic Complication**

H8 The Role of P2Y2 Receptors in the Pathogenesis of Hantavirus Cardiopulmonary Syndrome (HCPS)

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After attending this presentation, attendees will: (1) better understand the role of receptor expression and activation in productive hantavirus infection; (2) recognize the association of hyperinflammation and procoagulatory state with HCPS pathogenesis; and, (3) recognize the potential for translation of the HCPS disease mechanism to clinical intervention.

This presentation will impact the forensic science community by providing recent insight into the pathogenesis of HCPS, a poorly understood and rapidly fatal illness. This insight is possible due to the unique affiliation of the University of New Mexico School of Medicine with the New Mexico Office of the Medical Investigator and highlights the importance of collaborative efforts between forensic pathologists and research entities.

HCPS is characterized by the loss of pulmonary vascular endothelial integrity, resulting in massive, acute pulmonary edema.[1-3] There is no curative therapy, and treatment of severe disease is supportive, including the use of Extracorporeal Membrane Oxygenation (ECMO).[4] Hantaviruses are known to primarily infect capillary endothelial cells, most prominently in the lungs, spleen and kidneys; however, the mechanisms of infection and pathogenesis have remained poorly understood.[5,6] Although pathogenic hantaviruses have been shown to bind the inactive, bent $\alpha v\beta_3$ integrin structure on endothelial cells, the identity of additional proteins involved in integrin activation and hantavirus infectivity have been heretofore unknown.[7] Recent studies revealed that integrin activation is mediated by the $P2Y_2$ receptor ($P2Y_2R$), a purinergic receptor that responds to Adenosine Triphosphate (ATP) and Uridine Diphophate (UDP). P2Y receptors are ubiquitous G Protein-Coupled Receptors (GPCRs) known to participate in a variety of biological functions including immune response and platelet aggregation.[8] Considering that ATP and other nucleotides act as Damage-Associated Molecular-Pattern (DAMP) molecules released at high local levels following infection and tissue damage, it is not surprising that $P2Y_2R$ is upregulated in the setting of inflammation, including infection.[9-11] In addition to being associated with hyperinflammation and tissue damage during sepsis, $P2Y_2R$ has also been associated with procoagulatory states, including Tissue Factor (TF) and Plasminogen Activator Inhibitor-1 (PAI-1) upregulation.[10,12-16] Recently, a proteomic study of HCPS patient plasma found that activated PAI-1 levels increase up to 100-fold within the 48 hours prior to death, indicating a procoagulatory state.[17] Given the mutual association of $P2Y_2R$ expression and HCPS with a procoagulatory state, it was hypothesized that $P2Y_2R$ contributes to the pathogenesis and severity of HCPS. To test this hypothesis, a gene expression assay was used to analyze $P2Y_2R$ expression in formalin-fixed, paraffin-embedded tissue of HCPS subjects whose deaths were investigated by the New Mexico Office of the Medical Investigator (OMI). The mean $P2Y_2R$ mRNA expression in HCPS lung tissue was 22.2 ± 4.5-fold higher than in controls (gunshot fatalities). In addition, $P2Y_2R$ mRNA expression correlated positively with plasma levels of PAI-1 measured in HCPS decedents. Lastly, the preliminary data show that HCPS plasma stimulates endothelial cells to upregulated $P2Y_2R$ mRNA during short-term culture, and that this upregulation correlates with disease severity. Altogether, it is concluded that $P2Y_2R$ expression is upregulated in HCPS and that a proteomic milieu of circulating factors contributes to this upregulation. Thus, these indices of a procoagulatory state might be useful as prognostic biomarkers for HCPS severity and prognosis. Furthermore, studies indicate a need for further study of $P2Y_2R$ for consideration as a therapeutic target in the treatment of HCPS.

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Hantavirus Cardiopulmonary, P2Y2 Receptor, Procoagulatory State

H9 Teaching Morphological Species Identification to Forensic Science Students: Advantages, Problems, and Results

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After attending this presentation, attendees will have a better understanding of instructing students in morphological species identification through the use of dichotomous keys. A dichotomous key guides the user through species determination for a specimen by providing a series of dual-choice nodes that center around morphological differences. Each choice leads to either a new set of dichotomous choices or a species decision. Attendees will also observe the ability of students to successfully apply this method to unknown entomological specimens. Of central focus to training students in species identification is the idea that dichotomous key nodal decisions take the user down specific pathways to a final species designation by not focusing on the organism as a whole, but rather specific parts that the alpha taxonomist has designated as important diagnostically. Thus, if followed correctly, the user should arrive at the correct species designation as long as the species evaluated are included in the dichotomous key.

This presentation will impact the forensic science community by providing an understanding on how accurately students can identify adult blow flies (Diptera: Calliphoridae) using a dichotomous key. Insects present at crime scenes need to be successfully and accurately identified to aid in these investigations by providing information such as Time Of Colonization (TOC), which can be linked back to a time since death. Species identification using a morphological dichotomous key cognitively falls under pattern recognition, which is part of the perception and problem-solving aspect of cognitive science. The critical difference between other forms of pattern recognition and dichotomous keyed species identification is that the dichotomous key approach provides rigorous, step-by-step, pre-determined instructions to arrive at the pattern conclusion (a species). These patterns are grounded in an extensive scientific literature going back to the Systema Naturae by Carl Linnaeus in 1735 and currently outlined by the International Code of Zoological Nomenclature (ICZN code). If followed, this approach forces the user out of top-down processing mode and into a bottom-up processing mode, whereby the parts of the organism are first understood and, from those partial understandings, a full understanding of the species identity of the specimen is achieved. This bottom-up approach has a critical advantage — it eliminates the possibility of forming biases that result from top-down processing.

These data were evaluated from an introductory-level forensic analysis course to understand the student's ability to utilize a dichotomous key. There were several opportunities for the students to record their nodal decisions along with their confidence level with the use of a tabular format. For each decision the student made, they ranked their confidence level using a Likert scale (1-5). Along with individual decision recording, they also conducted a post-decision comparison with their partner, following a think-pair-share active learning model. If their answers were not the same, they re-evaluated their decision making, along with a re-analysis of the specimen until a mutual evidence-based decision was reached. How successful the students were in making the correct identification was analyzed, along with the examining the correlation between confidence and correctness. From these data this presentation seeks to improve student training in the use of dichotomous keys for species identification, which then can be used to provide standard operating procedures for how forensic entomologists should approach and document the pattern recognition task at hand in a way that limits the influence of bias.

Dichotomous Key, Species Identification, Student Instruction

H10 Predicting the Postmortem Submersion Interval (PMSI) Using the Microbiome of Waterlogged Bone

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After attending this presentation, attendees will possess a greater understanding of the use of longitudinal succession of the microbiomes of submerged bone and its use in PMSI estimation.

This presentation will impact the forensic science community by providing information concerning a novel area of research, the use of microbial succession for Postmortem Interval (PMI) and PMSI.

The ability to both identify an individual and estimate the PMSI may depend on DNA obtained from skeletal remains.[1] Currently, PMSI estimation is limited to when insects or other invertebrates have been found, or is based on when the victim was last seen alive, making it primarily applicable in cases of accidental water deaths rather than deposition of homicide victims in water.[2] Because microorganisms are present throughout decomposition, longitudinal succession (including aspects of richness and diversity) of the microbiome of submerged skeletal remains through 16S recombinateDNA (rDNA MiSeq® sequencing was used to provide a reliable method for PMSI estimation. The use of microbes in human decomposition studies began with Vass, when he proposed that the appearance or disappearance of microbes, like insects, could be used to estimate PMI.[3] Although originally abandoned by Vass (as there proved to be too many microorganisms to culture and analyze), recent studies by Benbow et al. and Dickson et al. have demonstrated that characterization of bacterial communities can be a useful tool for estimating PMI and PMSI due to advancements in metagenomic approaches.[2,4] None of these studies, though, have focused on skeletal remains.

In this study, samples from 12 pig (*Sus scrofa*) humeri and 12 pig ribs were divided into a total of 24 humerus and 24 rib samples. Between June and November 2015, cut bones and waterproof dataloggers were submersed in water and left outdoors; water temperature was recorded hourly. A total of six collections of submerged bone were taken at 500 Accumulated Degree Day (ADD) intervals. The collected bone samples were ground into a powder, and the DNA was extracted using ChargeSwitch® gDNA Plant Kit. 16S rDNA variable regions 3 and 4 were amplified with dual-index primer pairs according to Kozich et al.[5] Sequencing utilized Illumina's® MiSeq® 2X300 paired-end sequencing, and analysis was performed via Mothur version 1.36.1.[6] The quality scores were below acceptance; therefore, read one, which covered v3, was used for analysis.

When an Analysis of Molecular Variance (AMOVA) was performed, a significant difference was observed in bacterial structure between rib and humerus samples ($p < 0.0002$), leading to analysis by bone type. Each bone type suggested the following: changes in phylum-level abundance based on greengenes taxonomy classification over time; Principal Coordinate Analysis (PCoA) ordination and UniFrac weighted β-diversity clustering among different time periods; Operational-Taxonomic Unit (OTU) -based indicator taxa for time periods; and, a positive linear increase in Shannon diversity index across time periods for each bone type.

Overall, this study demonstrates that microbial succession may be able to provide a reliable method for PMSI estimation based on the identified indicator taxa and Shannon diversity index.

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Waterlogged, PMSI, 16S rDNA

H11 A Fatal Alligator Attack in South Carolina: A Case Report and Review of the Literature

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After attending this presentation, attendees will recognize patterns of injuries inflicted during an alligator attack and will have an appreciation of the difficulties in interpreting antemortem from postmortem wounds in aqueous environments.

This presentation will impact the forensic science community by providing characteristics of alligator injuries and by illustrating the criteria used by forensic pathologists in the determination of the cause of death in cases where bodies recovered from water have injuries consistent with animal predation.

Twenty-four reports of fatal alligator attacks on humans have been reported in Florida since 1973. Several other deaths have been linked to alligator attacks in Texas and Georgia.[1] When bodies are recovered from water, drowning is high on the differential as the cause of death; however, drowning is essentially a diagnosis of exclusion, a determination assigned by forensic pathologists when all other causes of death have been excluded. When bodies are recovered from water with injuries consistent with animal predation, the question often arises, "Did the decedent die from their injuries from the attack, or did they drown and then become prey for the animal?" In aqueous environments, evaluation of wounds for vital reaction in tissue, a phenomenon seen only in injuries occurring during life, is obscured by the leaching of blood out of the wound and into the surrounding water, leaving a pale wound that may be mistaken for a postmortem wound.[2]

In the following case, which has been confirmed as the first death by alligator attack ever recorded in South Carolina state history, this issue was of utmost consideration.[3] In July 2016, an elderly woman with early dementia living in an assisted living facility in Charleston, SC, left her room shortly after midnight. When she was determined to be missing the next morning, a search of the property identified a disarticulated left arm near the bank of a retention pond adjacent to the facility. Her body was recovered from where it was floating in the water approximately 20 feet away. The injuries seen on the body were consistent with those inflicted by an animal. Alligators from a nearby swamp were known to inhabit the shallow pond; a necropsy later recovered the decedent's partially amputated right arm from the suspected alligator. Those involved in the death investigation sought to determine if she had drowned and was subsequently attacked by the alligator, or if the attack itself was responsible for her death.

Comparison with other cases of alligator wounds known to have been inflicted postmortem is presented to illustrate the criteria used by forensic pathologists for determination of antemortem versus postmortem injuries.

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Water-Related Deaths, Alligator Attack, Postmortem Wounds

H12 Pneumococcal Meningitis Associated With Phlegmonous Gastritis and HIV/AIDS

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After attending this presentation, attendees will understand a rare complication of HIV/AIDS and will be encouraged to pursue further studies in autopsy cases of individuals with HIV/AIDS.

This presentation will impact the forensic science community by illustrating a rare but important manifestation of HIV/AIDS, which led to the development of phlegmonous gastritis and pneumococcal meningitis in an individual with a history of substance abuse. The goal is to inform forensic pathologists of this bizarre association and to highlight the importance of performing complete autopsies on individuals in which drug abuse is suspected.

An elderly man with a past medical history significant for probable essential hypertension was found dead in an abandoned building. The building was known to be inhabited by squatters for housing and drug abuse. Drug paraphernalia was found at the scene.

An autopsy was performed at the medical examiner's office. The decedent was in an early state of putrefactive decomposition. The gastrointestinal surfaces were coated by a thick, purulent exudate. The proximal pyloric region of the stomach's external surface had a one-half inch area of perforation. Associated with the perforation was a thick, yellow, purulent exudate and omental adhesions. The distal half of the body and the proximal pyloric region had a firm, yellow, homogenous, infiltrative, seven centimeter, circumferential mass. Histological sections of the distal half of the stomach revealed infiltration by neutrophils within the gastric wall between the intact mucosa and serosa. There was vasculitis and vascular thromboses. Within the submucosa and vessel walls were many Gram-positive cocci in chains and pairs.

Gross examination of the brain revealed a thick, yellow, purulent subarachnoid exudate that tram-tracked along the cerebral vasculature. The 1,570-gram brain had cerebral edema. The cerebral hemispheres had an indistinct gray-white matter border. Histological sections confirmed the diagnosis of acute meningitis.

Cultures of the peritoneal and subarachnoid exudates both grew out *Streptococcus pneumoniae*. Blood samples were reactive for HIV antibody. Toxicology was notable for cocaine in the urine and benzoylecgonine without parent cocaine in the blood. The cause of death was certified as peritonitis, meningitis, and sepsis associated with phlegmonous gastritis due to AIDS.

Phlegmonous gastritis is a rare, rapidly progressive, and potentially fatal bacterial infection of the gastric wall and was first described as erysipelatous tumor of the stomach by Greek physician Claudius Galen (AD 138-201). Two forms have been described: localized and diffuse. Though the pathogenesis is unclear, this type of gastric abscess has been linked to gastric surgery, pre-existing mucosal injury, hematogenous spread from other infected areas, ingestion of caustic substances, and gastric carcinoma. Phlegmonous gastritis has also been associated with systemic disease and immunosuppressive conditions including diabetes mellitus, chronic ethanolism, and HIV.

In this case, the diagnosis of phlegmonous gastritis was made by autopsy and was associated with pneumococcal meningitis. The gastric wall abscess was localized to the distal stomach. Such abscesses can become diffuse through local spread, gastric wall perforation, or via exudate expressed from the serosal surface of the stomach. In previous reports, phlegmonous gastritis has been described as resulting from hematogenous spread from distant sites and has not been described to spread from the stomach to distant sites; however, the development of phlegmonous gastritis can occur slowly over weeks, so hematogenous spread from the gastric abscess to the brain is a possibility. Diffuse involvement of the gastric microvasculature and the presence of organisms within the vessel walls may contribute to such hematogenous spread.

This case highlights the importance of performing complete autopsies, especially in the setting of suspected acute drug toxicities.

In the practice of forensic pathology, HIV-associated phlegmonous gastritis should be considered and recognized as having potential association with pneumococcal meningitis. While it is strongly suspected that the stomach is the source of the infection, more research studies are needed to determine whether the stomach or meninges are the

initial source of the infection. The rarity of this finding may be a peculiarity of the systemic effects of HIV, may be related to ongoing drug abuse, or may represent a reflection of current attitudes toward autopsy in this patient population.

HIV/AIDS, Phelgmonous Gastritis, Meningitis

H13 The Application of Morphometrics to a Candidate Gene Approach for Identifying the Genetic Basis of Facial Morphology

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After attending this presentation, attendees will understand how morphometrics may be used to quantify aspects of facial morphology from 2D images. Attendees will also understand how the data produced may be used in conjunction with genotype information to identify genes affecting facial morphology.

This presentation will impact the forensic science community by introducing a low-cost method for the identification of genes that may prove useful in developing a method for the prediction of facial morphology from DNA. Such a method would provide a novel way to gain information from DNA samples from crime scenes or unknown remains that do not obtain a hit from database searches. Additionally, several of the techniques described herein may be generalized to other genotype-to-phenotype studies.

It has been shown that many aspects of the American criminal justice system result in bias that leads to false convictions. This bias often surrounds the identification of a suspect and may have a continued or compounded effect during the remainder of the criminal proceedings. The discovery of new, objective methods that use DNA to identify suspects could eliminate bias stemming from eyewitness testimony and malpractice in suspect identification. This could lower the number of misidentified suspects arrested and taken to trial, leading to a subsequent reduction in false convictions. It could also aid in the identification of unknown remains by providing an idea of the appearance of the deceased individual. This would help to solve cases that may have gone cold and bring closure to families.

In this study, candidate genes for facial morphology were identified from those implicated in craniofacial disorders and selected Single Nucleotide Polymorphisms (SNPs) found within them were genotyped. Facial morphology was captured from a set of landmarks mapped onto 2D photographs. Variation in the distances between pairs of landmarks was analyzed as a function of genotype for each SNP. Results revealed that specific inter-landmark distances varied significantly (alpha = .05) between genotype groups for two of the SNPs included in the study. This indicated that the genes may influence those aspects of facial morphology. The first of these SNPs is located in the POLR1C gene, which has been implicated in a form of Treacher Collins syndrome. This craniofacial disorder includes hypoplasia of bones in the mid-and lower face in its description.[1,2] The second is located within the LMNA gene, which has been implicated in Restrictive Dermopathy. This disorder includes abnormalities of the midface as part of its phenotype.[3,4] The SNP in the POLR1C gene indicated an effect on several measurements of the mid-and lower face. These measurements were a mixture of overlapping and discrete measurements and covered all three dimensions of the face. The other SNP, located on the LMNA gene, indicated an effect on measurements of the midface around the nose. LMNA's effect was only seen for measurements reflecting height and width of facial features. It was concluded that the POLR1C gene may play a role in the development of the bones of the mid-and lower face and that the LMNA gene may have some effect on the morphology of the midface, specifically the nasal region. The study is currently being expanded to a larger population. This will also involve the consideration of more polymorphisms.

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Human Identification, Facial Morphology, Morphometric Polymorphism

H14 Alternative Food Sources for Entomological Evidence: A Practical Tool for Crime Laboratories

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After attending this presentation, attendees will have an appreciation of how entomological evidence can be used in forensic investigations. Attendees will also develop an understanding of suitable food sources for rearing forensically important larvae. This presentation will review factors affecting the employment of such methods, including approximate costs and protocols to implement these methods so as to successfully rear blow flies both in the field and in crime laboratories.

This presentation will impact the forensic science community by providing an overview of alternative food sources for rearing forensically important blow flies (Diptera: Calliphoridae), specifically in crime laboratories. In laboratory settings, blow flies are commonly reared on beef liver. Obtaining this substrate in a crime laboratory environment can pose several problems, including procuring the liver, thawing it (if previously frozen), and maintaining a fresh supply. Indeed, the difficulty in creating an ideal rearing situation for entomological evidence can be a deterrent in itself. Therefore, several food sources that are easily accessible, cost efficient, and have long shelf lives will be discussed in this presentation. This presentation seeks to provide a cost-efficient and simplistic approach for entomological rearing protocols and to increase the use of collecting entomological evidence in crime laboratories across the country.

Forensic entomology is a well-established tool for evaluating death, or abuse, of a person or companion animal.[1,2] Insect evidence provides valuable information as related to time of colonization and movement of remains from one location to another. Blow flies are commonly found on human remains throughout most stages of decomposition and, consequently, when entomological evidence is collected, these tend to be the most abundant taxa; however, very few crime laboratories across the country have established collection and rearing protocols for these forensically important insects. Some of the main challenges for collecting and rearing blow flies are likely that proper collection techniques are not always widely disseminated, and it can be difficult to have access to an appropriate food source. Further, the majority of crime laboratories are not equipped with a forensic entomologist on staff. Thus, when crime scene investigators or pathologists collect these insects, they are often mishandled (e.g., placed into containers with no air holes, no food, or a food source that is not sustainable for their development).

This study analyzed alternative food sources for blow flies that are easy accessible and cost efficient, including tuna, dry cat food, wet cat food, and beef liver as the control. Blow fly mortality, development, and dry mass were examined for each food source. This experiment will provide an overview of possible food alternatives that could be used as a sustainable food option in laboratories when immediate assistance from a forensic entomologist cannot be obtained.

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Forensic Entomology, Food Source, Laboratory Protocol

H15 Insects of Forensic Importance: Seasonality and Georeferencing in the Mexican Territory

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After attending this presentation, attendees will understand the importance of collection, identification, and preservation of insects from different regions and seasons for forensic purposes.

This presentation will impact the forensic science community by providing information that will be transformed into a web tool to assist forensic investigations involving insects.

Forensic entomology is a discipline of utmost importance in the legal medical field because it contributes information to establish the time a body has been accessible to insects, possible circumstances of death, determination of toxic agents in the body, and even possible postmortem relocating.

Research on these issues is scarce in Mexico and the information occasionally used in forensic cases is usually of foreign origin, which are not always applicable to this region. For this reason, is necessary to generate research to know and georeference insects of forensic importance from Mexico.

This study references are insects of forensic importance, their geographical location, and the time of year in which each species can be found in Mexico. The purpose of this study is that the information generated can be found by researchers or related personnel to this area on a website, which will contain a database with information on the species found, as well as its georeferencing and time of year it can be found. It will also contain 2D and 3D insect pictures, which will serve as support in legal investigations in which insects or other arthropods of forensic importance are involved.

The results of this study will show the habitat where insects can be found (i.e, the different species can be associated to a type of environment, such as coast, mountain, forest, desert, or even if they are a synanthropic species, in other words, if they are species commonly associated with urban environments or humans, which in forensic investigations is useful to determine if an insect found in a site corresponds to that area or comes from other site). In this study can also contribute to the investigation of determining a possible postmortem relocation. Another scope of this study is that it can determine the presence or absence of different types of insects depending on the time of year, which will help establish in which season the death may have occurred.

During this study, insect collections were conducted using two methods: (1) by use of animal carcasses; and, (2) with scavenger insect traps. Samples collected were identified, labeled, and preserved by mounting with entomological pins. All samples were photographed with their data, including species name, location, time of year in which they were collected, biological characteristics, and the name of the person who made the identification, and were stored in a database. The insect collection work began in 2015, but there is no completion date, as sampling work will take time to cover most of the country. On the other hand, the website and database were started in October 2015, and in June 2016, the web system was developed. This allows the specific registration and administration of insects. The system involves a "model-view-controller design," allowing visualization information, programs for the control system, and a database for storing information. It also allows you viewing of 3D images of insects registered in the system. It will allow georeferencing of each insect within Mexico.

Currently, collections have been gathered from three areas of the country: Coahuila, Mexico City, and Yucatan. Some of the insects that have been found in these samples are: the order Diptera, the Calliphoridae family containing the species, *Calliphora vomitoria, C. vicina, C. coloradensis, Lucilia sericata, L. mexicana, Pollenia rudis, Cochliomyia hominivorax, Compsomyiops callipes, Chrysomya rufifacies, C. megacephala,* and *Phormia regina*. The Muscidae family includes *Musca domestica* and the Sarcophagidae family has *Sarcophaga carnaria*.

The order Coleoptera recorded the Staphilinidae family, the Silphidae family contained the species *Nicrophorus interruptus*, and the Histeridae includes *Xerosaprinus* sp. Nitidulidae contains *Omosita* sp., Dermestidae includes *Dermestes frischii*, *D. maculatus,* and *D. haemorrhoidalis,* and the Cleridae family has *Necrobia rufipes*. Parasitoids and other insect groups that mainly feed on other insects were also found, including the parasitoid *Pachycrepoideus vindemmiaelas*: Pteromalidae and fire ant *Solenopsis invicta*: Formicidae.

These preliminary results are the beginning of a long study that seeks to cover most of the country, so collecting and updating the database will continue indefinitely.

Forensic Entomology, Georeferencing, Insect Seasonality

H16 A Deadly Blade of Grass: A Case Report of a Penetrating Head Injury and Its Sequelae

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After attending this presentation, attendees will have a greater insight into the pathophysiology of penetrating head injuries and their sequelae.

This presentation will impact the forensic science community by providing information regarding low-velocity, foreign body, penetrating head injuries as they relate to cause and manner of death.

Traumatic brain injuries, including blunt force and penetrating head injuries, are commonly encountered in the field of forensic pathology. Approximately 1.7 million cases of traumatic brain injury occur in the United States per year, leading to approximately 50,000 deaths.[1] Concussions, as a result of blunt force trauma, represent 75% of traumatic brain injuries, making penetrating head injuries far less common.[1] Of the penetrating head injuries, the majority involve high-velocity projectiles, such as firearm injuries. Low-velocity foreign bodies are the least common and are rarely reported in the literature. They are more common in young children, when the bones are soft; however, in adults, they tend to occur in a transorbital location due to the thin nature of the bone. Reported low velocity foreign bodies have included a pencil, chopstick, TV antenna, tree branch and pool cue.[2-4]

A case is presented of a 40-year-old man who suffered a fatal pulmonary thromboembolism following prolonged immobilization due to a penetrating wound of the head by a single blade of grass, a rarely reported penetrating foreign body. At the time of autopsy, he was also found to have an associated skull fracture and cerebral abscess, both of which contained retained fragments of plant material. Gross and microscopic autopsy findings will be presented.

Penetrating injuries of the head are known to cause a variety of short- and long-term complications. These sequelae include meningitis, cerebral abscess, sepsis, focal neurologic deficits, intracranial hemorrhage, cerebrospinal fluid leak, and even death.[5] Factors influencing the development of sequelae are the location of penetration, velocity of the impact, size of the foreign body, and the treatment/management of the patient.

In all cases of penetrating head injuries, forensic pathologists should conduct a thorough autopsy to attempt to retrieve any retained foreign body and determine the wound tract, if possible. Depending on the post-insult latent period, external injuries may be healed at the time of death, making this more difficult. Therefore, proper death investigation, including gathering appropriate remote and recent medical history, is essential in determining a correct cause, mechanism, and manner of death.

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2. Gupta A., Chacko A., Anil M.S., et al. Pencil in the Brain: A Case of Temporal Lobe Abscess following an Intracranial Penetrating Pencil Injury. *Pediatr Neurosurg.* 2011;47:307–308.
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Penetrating Head Injury, Cerebral Abscess, Pulmonary Thromboembolism

H17 A Postmortem Diagnosed Case of Malaria in a Non-Endemic Country

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After attending this presentation, attendees will understand the importance of anamnesis in forensic pathology. In the presented case, that knowledge led to the selection of ancillary exams not commonly used in a forensic pathologist's everyday practice.

This presentation will impact the forensic science community by raising awareness of the growing incidence of sudden death cases related to diseases that are non-endemic in developed countries, probably related to the increased migration of populations.[1]

A 48-year-old man was found dead at home sitting on the toilet with his body surface partially covered in feces. The man was previously healthy without known hospital admissions. No information on his vaccination status, including anti-malaria prophylaxis, was available. He had been in Angola for some time and arrived at Portugal two weeks before his death. His sister reported that in the last days, he had complained of fatigue, anorexia, and dry cough.

The main goal of the investigation was to discover the cause and manner of death. Since the victim had recently returned from a malaria-endemic country, this disease was taken into consideration as a differential diagnosis and in choosing appropriate ancillary exams.

The external examination remarks were the yellowing of the sclera and the presence of feces covering the lower limbs, genital and abdominal areas. The internal examination revealed marked visceral congestion as well as hepatomegaly and splenomegaly.

Blood was collected to perform toxicological analysis (quantification of alcohol, prescription drugs, and illegal drugs). Blood, urine, stool, and cerebrospinal fluid specimens were taken and sent to the microbiology laboratory to perform microscopic observation and aerobic and anaerobic cultures. Tissue samples from the brain, heart, coronary artery, liver, kidney, lung, and spleen were collected in order to perform histological examination.

The Giemsa-stained thin blood smears revealed hyperparasitemia and numerous remnants of intraerythrocytic malaria parasites. *Plasmodium falciparum* was identified by the presence of the characteristic crescent-shaped gametocytes. Other different stages of its reproductive cycle were observed, namely ring forms, trophozoites, and schizonts, some arranged in rosettes.

The histological study revealed malarial pigment (haemozoin pigment) in erythrocytes that filled the vascular lumen of congested vessels in the gray and white matter of the brain, without hemorrhagic lesions; extensive malarial pigment was also seen in the red blood cells of the other studied organs and in liver Kupffer cells. The spleen revealed hemorrhagic necrosis lesions; signs of multiorganic failure were present. Toxicological analysis results are not yet available.

Malaria, being a cause of great morbidity and mortality, represents a significant cost in health care services. It's a prevalent disease in underdeveloped countries and is re-emerging in developed ones. This is due to increased migration to and from endemic regions.[1] *Plasmodium falciparum* is the most common pathogen from genus *Plasmodium*, being the one associated with cerebral malaria, the most fatal presentation of this disease. Along with the migration routes, the non-use of chemoprophylaxis and the increasing antibiotic resistance also contribute to malaria re-emerging around the globe.[2]

This is a report of a disseminated malaria case diagnosed in postmortem studies. This case led to a forensic investigation with a contribution of forensic fields not commonly involved in the work of the study's pathology unit. Due to the awareness of a higher prevalence of this tropical disease in travelers from endemic areas, a microbiologic analysis was performed. Malaria is a rare disease in Portugal and this case provided new technical and scientific knowledge to the forensic pathology unit.

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Malaria, Microbiology, Postmortem

H18 Forensic Sciences' Contribution to a Murder Case With an Incineration Attempt

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After attending this presentation, attendees will understand the importance of a multidisciplinary approach in forensic pathology cases of greater complexity, such as when a murder is suspected. Frequently, only cooperation between forensic sciences can answer the investigative questions.

This presentation will impact the forensic science community by drawing attention to the resources forensic pathology has available and the importance of knowing when these contributions are crucial.

A corpse was accidentally found in an old warehouse by a group of youngsters. It was in an advanced decomposition stage with some body parts and clothes burnt. It was supine, covered by a partially burnt blanket. After preliminary examination, the police found a granite block next to the body and evidence of physical violence, such as blood on the scene suggesting beating and dragging of the body, as well as various types of burnt waste suggesting an incineration attempt. Later, the authorities found a copper bar near the crime scene. The corpse was unidentifiable since the face was unrecognizable due to decomposition and blunt force trauma; the body had no tattoos or other signs that could allow a positive identification. It was only possible to determine that it was a male.

The main goals of the investigation were to discover the victim's identity and investigate the cause and manner of death. Due to the external examination findings and lack of information about the circumstances of death, the contribution of a multidisciplinary forensic expert team was required.

Skull X-rays performed prior to autopsy revealed several skull and facial fractures. Chest and upper limb X-rays didn't reveal acute traumatic bone lesions.

The autopsy disclosed an advanced stage of bodily decomposition with fungal and larvae colonization. There were no identifiable skin wounds due to the stage of decomposition and incineration attempt. It was possible to validate the imaging findings. Macroscopically, the brain tissue consisted of a mass of frothy paste and all the thoracic and abdominal organs were in an advanced putrefactive stage with no major abnormalities.

Subungual swabs and fingernails, several teeth, and a spleen blood sample were collected for genetic analysis. After cautious observation it was decided to study the granite block since it presented some hairs on its surface and the copper bar because of the possible presence of dried bloodstains. Lung, trachea, liver, and burnt skin were sampled for histological analysis.

Expert examination of forensic anthropology was performed along with the autopsy and revealed that the victim was a Caucasian adult male, aged between 45 and 60 years at death, and between 1.68m and 1.82m (66.14in and 71.65in) tall. An antemortem fracture of the left elbow was also helpful for the identification, considered an individualizing feature. With regard to acute traumatic peri-mortem injuries, fractures of the facial skeleton including the left ramus of the mandible, the right temporal, right and left parietal, left sphenoid, and right orbital bones were observed.

The genetic analysis confirmed the corpse's identity by comparing the victim's and the alleged mother's DNA profile: it was a homeless man who was declared missing two months previousy. It wasn't possible to obtain any genetic profile from the potential weapons that could establish their link to the death.

At the microscopic examination, no trace of soot was found in the trachea or lungs, nor was any vital reaction shown on the skin. These findings excluded, the possibility of the victim being alive during the cremation.

The anthropological analysis also contributed to clarifying that the cause of death was due to traumatic facial and skull fractures associated with meningo-encephalic injuries produced by violent blunt force trauma.

In conclusion, the link between the results obtained allowed for the identification of the victim, the determination of the most probable cause and manner of death (homicide with blunt force trauma) as well as the type of weapon used.

H19 Rat Bite Fever With Streptobacillus moniliformis Meningitis and Myocarditis Resulting in the Death of a 7-Month Old Infant

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WITHDRAWN

H20 Methods for Improvement of Allele Recovery With the GlobalFiler™ Assay

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After attending this presentation, attendees will have a better understanding of how the Polymerase Chain Reaction (PCR) cycle number, a 3500 instrument, and analysis parameters impact allele recovery with the GlobalFiler™ assay.

This presentation will impact the forensic science community by educating attendees of methods to improve their Short Tandem Repeat (STR) results with a challenged sample type.

Forensic casework samples, including bones, often have low DNA yields, degraded DNA, and PCR inhibitors. Newly developed STR genotyping kits including the GlobalFiler™ assay, are highly sensitive, robust to inhibitors and discriminating. This, combined with highly sensitive capillary Electrophoresis (CE) instruments, including the 3500 Genetic Analyzer, has resulted in useful STR profiles from previously untypeable forensic DNA samples; however, often, bone samples are still problematic and require modifications to both laboratory protocols and analysis procedures.

The GlobalFiler™ assay is the first 6-dye, 24-locus STR kit. It was designed with superior discrimination power, helping to enable forensic DNA labs to maximize information recovery and improve overall efficiency. The inclusion of ten mini-STRs maximizes results from degraded samples, such as bones. To increase the amount of useable information obtained with the GlobalFiler™ assay, forensics laboratories have options at multiple steps in the forensics workflow. During setup, the lab may increase the PCR cycle number from 29 to 30. Use of the 3500 CE instruments results in greatly increased sensitivity and reduced signal-to-noise ratios when compared with previous-generation CE instruments. Because of this, laboratories frequently use lower instrument and (dye-specific) Peak Amplitude Thresholds (PAT) to improve allele recovery.

To investigate the effects of PCR cycle number and reduced PAT on resulting STR profiles, three test sites processed both fresh and aged bone samples with commonly used sample preparation methods, followed by amplification with the GlobalFiler™ assay with 29 and 30 PCR cycles. For the majority of bone samples, less than 1ng DNA was added to the PCR reaction. The data were analyzed with both a 175 Relative Fluorescence Unit (RFU) threshold and a reduced PAT. This reduced PAT is specific to both the instrument and the dye channel.

The result of this analysis has been a comprehensive study comparing the effect of the PCR cycle number, PAT, and the 3500 CE instrument on the results obtained with compromised bone samples. As expected, the peak heights were increased by increasing the PCR cycle number. The effects of PCR cycle number and PAT on the number of alleles recovered wer substantial. The impact of these changes on the overall DNA profile and the potential analysis difficulties in distinguishing true signal from noise are also discussed.

DNA, Bone, GlobalFiler™

H21 A Brain Teaser: Two Atypical Meningoencephalitis Cases in Human Immunodeficiency Virus (HIV) Patients

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After attending this presentation, attendees will better understand the possible presentation of Neurological Immune Reconstitution Inflammatory Syndrome (Neuro IRIS) and appreciate the different atypical presentations of meningoencephalitis in HIV patients.

This presentation will impact the forensic science community by informing attendees of the differential diagnosis of lymphocytic meningoencephalitis, including unusual Central Nervous System (CNS) complications of HIV infection, and by describing approaches to conducting a work up of these cases at autopsy.

Two examples of a rare HIV complication, IRIS, are reported, which were diagnosed following an extensive autopsy. This presentation provides a case study of a rare complication with few reports in the literature.

IRIS is a paradoxical worsening of the patient's condition as a newly reconstituted immune system reacts to infection following initiation of Highly Active Anti-Retroviral Therapy (HAART). An overwhelming response involving T cells can result in an encephalitis as activated T cells infiltrate the brain to fight an underlying infection of the central nervous system (Neuro IRIS). Both IRIS and Neuro IRIS are associated with a breakdown of the blood-brain barrier.

The first patient was a 34-year-old black male inmate with a history of HIV infection and a plasma CD4+ lymphocyte count of 459/cmm who was not on HAART at the time of death. His clinical presentation entailed newly diagnosed schizophrenia one to two months prior to death, increasing disorientation, and profound terminal hypothermia with hypotension. He was treated for possible seizure and sepsis but died before a definitive diagnosis was made. At autopsy, the brain was edematous with bilateral uncal and tonsillar herniation. Microscopic examination revealed a diffuse, marked lymphoplasmacytic meningoencephalitis with non-specific features. Serologic testing on postmortem blood was negative for several arboviruses (including West Nile Virus), as well as Rabies virus and lymphocytic choriomeningitis viruses. Immunohistochemical staining for p24 antigen was diffusely positive; clusters of CD3+ and CD68+ cells were often found near positive-staining areas. The cause of Neuro IRIS in this case most likely represents a reaction to the HIV antigen itself.

The second patient was a 30-year-old Caucasian male inmate with a past medical history of AIDS diagnosed in 2010; he was started on HAART in 2013 and again upon re-incarceration in September of 2014. Recent blood work on 6/1/15 indicated an absolute CD4 of 143 and HIV viral load of 538 copies/ml. Approximately three weeks prior to his death on July 20, 2015, he began complaining of severe headaches, vomiting, and difficulty walking, and was admitted to the hospital. The Cerebrospinal Fluid (CSF) contained elevated protein and pleocytosis. At autopsy, there was marked cerebral edema with uncal herniation and a pronounced, diffuse lymphocytic meningoencephalitis with no visible viral inclusions or specific features. Postmortem Polymerase Chain Reaction (PCR) testing on frozen brain tissue by biofilm was negative for Eastern equine, Western equine, Venezuelan equine, cytomegalovirus, enterovirus, Herpes simplex 1, *Varicella zoster*, and *Cryptococcus*. Of interest, PCR was positive for Human Herpes Virus 6 (HHV-6). Since the CNS is known to be a site of latent HHV6, the significance of this finding is unknown. By exclusion, the most likely cause of meningoencephalitis in this case was IRIS.

Clinical presentations of subacute meningitis can be subtle and pose a diagnostic challenge, particularly in HIV patients, where a range of opportunistic infections, HIV encephalitis, and neurologic IRIS are all diagnostic considerations.

Autopsy, IRIS, Meningoencephalitis

H22 It Came Back to Bite Him: A Case of Snakebite in Combination With Multiple Toxicities

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After attending this presentation, attendees will appreciate the contribution of the bite of a snake non-native to North America in a case with multiple toxicities and the importance of identifying the snake species in such events.

This presentation will impact the forensic science community by reporting an unusual death in which multiple toxicities, including the bite of a snake not native to North America, resulted in an accidental death.

Death from snake envenomation is relatively rare in the United States. In the United States, the majority result from encounters with native species in the wild. Deaths due to envenomation from non-native snakes typically occur in the setting of snakes kept as pets or in zoos.

The case of a 41-year-old man with a history of bipolar disorder and alcohol abuse who was discovered unresponsive is reported. The previous night, the decedent had threatened his wife with a reportedly venomous pet snake. Per report, the decedent was also acting in an intoxicated manner. The wife departed the home that night out of fear for her safety and returned the following morning to find her husband unresponsive. The snake was in its enclosure in another room. Autopsy revealed numerous paired and densely arranged puncture marks on the left hand and forearm with underlying soft tissue hemorrhage. The hand was edematous. Toxicologic analysis of heart blood showed the presence of amphetamine (0.6mg/L) and alcohol (0.09%). The decedent had no known amphetamine prescription.

Considerable difficulty was encountered when attempting to identify the snake. The investigating police agency left the snake in the possession of the decedent's family rather than turn it over to animal control. Initially, the snake had been reported to be a poisonous cobra; further discussion with the family questioned the speciation of the animal. The animal was requested to obtain speciation by an expert; however, the family had disposed of the snake in the interim. Secondary to further discussion with the family, the snake was finally identified as an adult *Aspidelaps lubricus*, also commonly known as a shied-nosed cobra or coral cobra, which is native to Sub-Saharan Africa; however, the bites and venom of this particular breed are poorly documented. Bites are reported to cause local swelling, pain, and may cause non-specific systemic symptoms such as nausea, vomiting, and dizziness. A rare neurotoxic effect has also been reported, with possible moderate to severe flaccid paralysis; however, the potency of a single bite is widely considered to be low, and this species is often bred and sold in the United States as pets.

Autopsies involving snakebites present a unique challenge, in part due to their relative rarity in the United States. Careful examination at autopsy is required to determine if the decedent was bitten and determination of envenomation may be difficult. There is additional complexity if the involved animal is not native to the United States, as in this case. Identifying the species is crucial to determine if the snake is venomous and if so, the toxicity of the venom. In this case, the decedent was bitten multiple times, which in combination with amphetamine and alcohol intoxication, resulted in sufficient toxicity to cause death. Each substance in and of itself likely would not have resulted in death. Investigation revealed no evidence of current or prior suicidal ideation or attempts. This, in addition to the autopsy findings, was consistent with an accidental death. In such a complex case, the forensic pathologist needs definite identification of the snake and adequate resources to aid in the investigation of the toxicity of envenomation. Such resources include pertinent literature and expert opinions regarding the validity of the snake speciation and the relative toxicity of the snake's venom. In this particular case, the literature briefly touched upon the toxicity of the snake's venom with references to known complications as well as references to colloquial accounts of deaths related to a bite; however, additional expert opinions regarding the relative toxicity of the snake, including an opinion of an individual who had previously been bitten by a similar snake, were instrumental in determining the role that the snakebite played.

Snakebite, Accidental Death, Multiple Toxicities

H23 A Rare Snake Bite-Related Fatality: A Case Report

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After attending this presentation, attendees will understand the incidence of snake-related fatalities, the types of venomous snakes in South Carolina, the best immediate management of a bite, and the diagnosis of a snake-related fatality at autopsy.

This presentation will impact the forensic science community by demonstrating the methods necessary for the diagnosis of a snake-related fatality at autopsy in a state and institution where such cases are extremely rare.

Fatal snake bites are exceptionally rare, with less than ten reported nationally in the United States per year.[1] The incidence of fatal snake bites in the state of South Carolina is nearly non-existent.

This study illustrates a case of a fatality related to a venomous snake bite occurring in the state of South Carolina. The decedent was a 71-year-old male with extensive knowledge of the outdoors and local wildlife. The incident occurred in a Wildlife Refuge known to be a habitat for one or more types of venomous snakes. The decedent waded into the water and soon experienced a bite; just prior to being bitten, the decedent and a companion reported hearing a rattling sound, but neither one saw the snake. The decedent rapidly succumbed to the injury within 15 minutes of the bite.

On autopsy, two sets of puncture wounds were noted to the left leg with associated swelling and diffuse hemorrhage into the underlying subcutaneous tissue and musculature of the medial aspect of the leg. Additionally, the decedent was noted to have preexisting hypertensive and atherosclerotic cardiovascular disease, including an enlarged heart with evidence of prior myocardial infarctions. It was concluded that the decedent died of the toxic effects of snakebite with the hypertensive and cardiovascular disease playing a contributory role.

The state of South Carolina has 38 species of snakes of which only six are venomous, including the coral snake, copperhead, cottonmouth, eastern diamond back rattlesnake, timber rattlesnake, and pigmy rattlesnake.[2] The best and most effective protection from snake bite is knowledge and awareness of one's environment, avoiding areas where venomous snakes may be common.[2] It is recommended that when having recreational activities or working outdoors in areas where snakes may be encountered, thick boots and leg protection be worn.[2,5] If a snake bite does happen, the likelihood of survival is increased by remaining calm and getting the victim to a hospital for immediate medical attention.[3,5] An examination of the bite wound by a medical professional may be able to differentiate venomous snake bites, which usually have a set of two puncture marks, from a non-venomous snake bite.

The majority of snake bites are accidental and occur on the lower extremities. The key physical findings on autopsy in a case of snake bite/envenomation is the identification of the puncture wounds, localized swelling, and examination of the underlying soft tissue.[4,5] These findings may be subtle and can be easily missed. Additional autopsy findings may include anaphylaxis, hypotension, cardiovascular compromise, and myonecrosis.[4,5] A team approach is valuable in investigations of snake-related deaths as documenting the presence of a venomous snake and history of envenomation when combined with autopsy findings make a more accurate and specific determination into the cause and manner of death.

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Snakebite, Venomous Snakes, Envenomation

H24 Field Near-Infrared (NIR) Spectroscopy in Taphonomy Research

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WITHDRAWN

H25 A Correlative Forensic Approach to the Dynamics of Decomposition in a Tropical Environment

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After attending this presentation, attendees will understand the importance of the integration and correlation of multiple dynamics in determining the Postmortem Interval (PMI).

This presentation will impact the forensic science community by discussing the correlation of the results; the accuracy and reproducibility of PMI determination can substantially be improved by the innovation in standardization and sampling, along with a multidisciplinary approach.

Decomposition dynamics are the predominant factors that influence PMI in any given environment. This provides important evidence of the spatiotemporal events involved in a death investigation; however, current techniques in PMI determination are limited in precise scope and provide inconsistent chronology. This study seeks to determine the dynamics of decomposition in a tropical environment to provide a more accurate PMI determination through the simultaneous integration of a series of techniques using a correlative approach. This is part of an ongoing investigation (2014/2015) into processes of decomposition in Puerto Rico, in order to establish the causal relationships in a tropical monsoonal climate (Köppen-Geiger classification: Am). A model organism (*Sus scrofa*) and location (protected wetland area) was used for monitoring and standardization. The on-site environment and climate was monitored, along with morphology, internal temperature, and entomology. Sampling and data collection were conducted three times daily and in triplicate. Specifically, climate monitoring was normalized utilizing onsite data, a nearby weather station, and the national weather service station. Measurements included wind speed, direction, surface pressure, temperature, humidity and precipitation.

Rate of decomposition and changes, including internal and external temperature probes, morphology, and entomological succession, were monitored by photography and sampling in all stages of decomposition. The total estimation of the entomological populations, species identification, and photography was taken on site. Representative samples were taken for offsite morphological and genetic characterization and dissection.

Multiple representative samples were taken of the diverse anatomical microenvironments of the corpse including the nasal, skin, oral, aural and anal cavities. These were used for the DNA extraction and metagenomic sequencing, primarily to determine microbial diversity (16S rRNA) of the tropical necrobiome. The microbiome has a high initial variability between sites due to predominate host-microbiome microenvironments of the living organism. The correlation between initial microbiota and necrobiota is poorly understood and as demonstrated may also carry a chronological correlation between temperature, humidity, and necrophage colonization.

Corpse decomposition lasted approximately 25 to 30 days; this process identified the five main stages of decomposition and associated arthropods. Entomological succession initiated with the arrival of crickets (Gryllidae) and carrion flies (Calliphora), followed by the Sarcophagidae and Muscidae. Cockroaches (Blatellidae) and ants (Formicidae) were also present at the site. The number of estimated insects and predominant species of corpse decomposition in this environment, including diurnal effects, is also discussed.

Decomposition processes occur at an elevated rate in the humid tropics, with temperatures ranging between 20°C-35°C, and a predominantly high humidity, ranging between 65%-100% Relative Humidity (RH). Rainfall was variable but reached up 200mm of rain. These factors are favorable for accelerated decomposition due to elevated microbial growth rates, diversity, and biochemical degradation. Diurnal cycles (night and day) have been shown to affect both the rate of necrobiome and necrophage corpse decomposition. Internal microclimates, monitored within the corpse, detected temperatures exceeding those of the ambient at early stages of decomposition. The degree of entomological colonization and decomposition was significantly affected by rainfall, although this study was unable to determine the effect of wind as the wetland reserve area is relatively protected.

This experiment was a large-scale investigation to elucidate dynamics of decomposition in a tropical environment. A number of analyses are currently underway and the continuation of these will provide a clearer understanding of the relationship between the environment, the corpse, and the rate of decomposition.

The significance and importance of this research is consistent with an improved understanding of the rate of decomposition and the environmental factors that influence this rate. Additionally, through correlation of these

results, innovation in standardization and in sampling, along with a multidisciplinary approach, the accuracy and reproducibility of PMI determination can be substantially improved.

Tropical Environment, Multidisciplinary, Correlative

H26 An Autopsy Fingerprint Technique Using Fingerprint Powder

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After attending this presentation, attendees will: (1) understand that, in addition to traditional manual inked prints and digitally scanned prints, postmortem fingerprint records can be produced by the use of a method that employs fingerprint powder and adhesive address labels; and, (2) understand the technical aspects involved with employing the powder/adhesive label technique.

This presentation will impact the forensic science community by introducing a manual postmortem fingerprint technique that is simple, inexpensive, easy-to-perform, and offers several advantages over the traditional manual inked card technique.

Fingerprints are an important component of routine forensic autopsies and represent one of several potential methods for identifying a decedent. As such, fingerprints are typically retained as a part of the permanent autopsy record. Historically, fingerprints collected at autopsy involved a manual method using ink and cards.[1] Many offices continue to employ this method of fingerprint collection, as it is inexpensive and relatively easy to perform. Typical equipment required for taking manual inked prints at autopsy include an inking plate, a cardholder, printer's ink, a roller, a "coroner's spoon," and a fingerprint card.[1] Both flat and "rolled" prints can be made of the fingers, while flat palm prints are also sometimes created. Manually produced inked fingerprint cards can be photographed and/or digitally scanned or stored in "hardcopy" form. Some offices use fingerprint scanning machines, with the direct creation of digital prints.

A variety of challenges exist when attempting to take fingerprints from certain decedents. Decomposition, skin slippage, mummification, finger pad wrinkling due to water immersion, and trauma may cause great difficulty in obtaining suitable fingerprints at autopsy. Although the forensic literature provides recommendations regarding how best to overcome many of these challenges, the current study presents a simple method for obtaining high-quality finger, palm, and footprints at autopsy using fingerprint powder, a camel hair fingerprint brush, white adhesive address labels, and clear transparency sheets.[2-4] To employ this method, fingers and hands should be cleaned and dried, as a practitioner would do for traditional inking techniques. The skin surface of interest is lightly dusted with fingerprint powder, using the camel hair fingerprint brush. Next, the adhesive side of an address label is firmly applied to the powdered finger, followed by gentle removal. The adhesive side of the label, with adherent powder print, is then applied to the transparency sheet, thus preserving the powdered print, which is now visible through the opposite side of the clear plastic sheet. Similar techniques, with larger adhesive labels, are effective for obtaining palm and footprints. The technique is quick, easy-to-perform, and has the added benefit of allowing the adhesives to be labeled with pertinent autopsy information on the non-adhesive side. Additional benefits include a reduction in smudging (as is common with traditional inked prints), more easily obtained palm prints (as the adhesive sheets can more easily conform to the curved contours of the palms), and ease of filing, either via hardcopy storage or digital scanning.

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Fingerprints, Autopsy, Identification

H27 The Collaboration of Forensic Sciences in a Particular Case of Murder

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After attending this presentation, attendees will understand the necessity of cooperation between different forensic science disciplines in order to: (1) estimate the time of death; (2) determine the cause, mechanism, and manner of death; and, (3) provide information to the investigation authorities to reconstruct the course of events defining the type of offense committed.

This presentation will impact the forensic science community by presenting medicolegal difficulties in a murder case, especially when circumstantial information is limited and the corpse is in an advanced stage of decay. A multidisciplinary approach requires the use of entomological, radiological, and toxicological analyses, combined with autopsy and histological findings because, in cases with inconsiderable and discordant circumstantial information and in the event of tissue destruction due to putrefactive processes and maggots, the autopsy and histological analysis could provide very little data.

A 70-year-old man disappeared from his own home in Ferrara, Italy; the forensic examination of the house revealed signs of a fight and bloodstains in the dining room, so as to exclude a voluntary disappearance. The putrefied corpse of the man was found 12 days later in an abandoned cottage in the countryside of northern Italy. On-site forensic examination revealed a body in an advanced state of decomposition, with the head and thorax skeletonized by feeding insects. The corpse was found in a prone position, with an adhesive tape wrapped on the head and inserted inside the mouth and a T-shirt tied to the occipital region that caused a mechanical occlusion of the mouth. Moreover, supination movement was impossible because the upper limbs and hands were tied behind his back as well as the lower limbs being tied together.

Before completing an external and internal examination, a computed tomography scan of the body was performed and samples of maggots were taken to perform an entomological analysis. Histological analysis with Hematoxylin-Eosin (HE) and immunohistochemistry of the heart, lungs, and muscles were performed.

The entomological analysis found the presence of larvae of *Chrysomia albiceps, Lucilia sericata*, *Calliphora vicina,* and *Protophormia terranovae.* The data obtained, considering environmental data such as air temperature and relative humidity, revealed that the corpse had been colonized by feeding insects for 11-12 days, the exact time of disappearance. Entomological findings supplemented with a description of the stage of decomposition facilitated the estimation of time of death, which would later be confirmed by circumstantial evidence.

The attackers stated they had hit the victim on the head with a blunt object and the head colonization of maggots served as physical evidence of a wound in that body region.

Before completing an external and internal examination, a computed tomography scan of the body was performed and fractures to the rib cage and left fibula were found; this excluded the presence of skull fragmentation. External examination revealed the occlusion of the external respiratory orifices and bruises on the lower limbs. Internal examination did not provide any information due to a macroscopic alteration of the tissues caused by putrefaction processes. The histological sections of the heart and lungs revealed intense putrefaction changes, such as cardiomyocite disintegration, alveolar overdistension accompanied by alveolar rupture, and absence of macrophages. Immunohistochemically negative reactions were observed with C5b9, NP57, and CD65. The histological examination of bruises revealed the presence of hemorrhage in soft tissue without any sign of granulocyte invasion. The toxicological analysis revealed a low concentration of ethanol, probably a consequence of a postmortem production.

The cooperation of several forensic scientists permitted the estimation of the time of death and they correlated these data with the day of his disappearance to confirm that the death occurred after the seizure.

Radiological findings (fractures to the rib cage and left fibula) and the presence of contusions to the soft tissues of the legs suggested that the death had been violent, providing information about the modality of the aggression.

External and internal examination resulted in smothering as cause of death (presence of adhesive tape and t-shirt occluded respiratory orifices), to the exclusion of haemorrhages, vascular disease, and positional asphyxia, without any macroscopic and microscopic signs of asphyxia.

In a complicated case of murder, these elements provided information to the investigating authorities to determine the course of events, the conduct of the attackers, and the felony committed.

Murder, Forensic Radiology, Forensic Entomology

H28 The Implementation of a Quality Improvement (QI) Program for Death Scene Photography in a Medical Examiner Office

Kevin Jenkins, MD, West Tennessee Regional Forensic Center, 637 Poplar Avenue, Memphis, TN 38105; and Paul V. Benson, MD, UTHSC/Shelby County Medical Examiner, 637 Poplar Avenue, Memphis, TN 38105*

After attending this presentation, attendees will better understand QI measures for performance of death scene photography and diagram documentation as performed at the West Tennessee Regional Forensic Center (WTRFC) in Memphis, TN.

This presentation will impact the forensic science community by: (1) improving the attendees' knowledge of a QI project for death scene photography and diagram documentation; (2) teaching how to implement a QI project for death scene photography and diagram documentation; and, (3) reviewing death scene photography principles to improve investigator competence and performance.

In 2011, the United States Department of Justice (DOJ) published an updated guide; *Death Investigation: A Guide for the Scene Investigator*.[1] The publication describes topics of death investigation, including death scene photography. The publication states; "Photography allows for the best permanent documentation of the death scene. It is essential that accurate scene photographs are available for other investigators, agencies, and authorities to recreate the scene. Photographs are a permanent record of the terminal event and retain evidentiary value and authenticity. It is essential that the investigator obtain accurate photographs before releasing the scene."

This project will provide a retrospective review of a number of cases from 2016 at the WTRFC to determine if death scene photographs and diagrams adhere to the DOJ guidelines. Additionally, the scene photographs will be evaluated for focus/sharpness to adequately document a visual reproduction of the death scene. Twelve DOJ guidelines for death scene photography will be evaluated for each case and documented in spreadsheet format. For each case all 12 guidelines will be assigned "YES" or "NO," indicating case adherence status with that guideline. Photograph focus/sharpness will be subjectively evaluated and assigned "YES" or "NO," as well as recording the number of photographs in focus and out of focus in each case. Investigators responsible for taking the photographs in each case will be randomly assigned a letter (e.g., Investigator A, B) and that information will be kept confidential.

The significance of adequate death scene photography and diagram documentation is well established and clearly described in the DOJ guidelines. Photographs and diagrams should provide a visual reproduction of the scene such that evidence is preserved and evaluation of the photographs and diagrams provides the viewer with an accurate interpretation of the death scene. This project highlights the importance of evaluating death scene photographs and diagrams for adherence to DOJ guidelines within a specific medical examiner's office. The tabulation of data will enhance the development of a global death scene photography training plan and provide an evaluation process necessary for implementation of individual investigator performance improvement plans. Re-evaluation for adherence to DOJ guidelines and overall quality improvement will be performed after training as indicated by the outcome of this study.

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Death Investigation, Death Scene, Photography

H29 Facial Dissection: Two Case Reports Showing the Need for This Special Autopsy Technique

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After attending this presentation, attendees will have learned about a better forensic approach in investigating fatal cases where deep examination of the face is required.

This presentation will impact the forensic science community by discussing two fatal cases involving face trauma in which the standard autopsy technique did not provide all the answers to the autopsy objectives. Therefore, facial dissection was essential in the avoidance of mistakes and making correct autopsy conclusions. Although this type of technique is seldom or only periodically performed, it should be a part of all forensic pathologists' backgrounds.

Postmortem technique is of foremost importance in forensic pathology and should be adapted to the disease or condition of the deceased. Failure to do so can compromise the autopsy results and, consequently, the report conclusions. Facial dissection is a very specific autopsy technique which is rarely performed, not only because the facial area is normally not involved in the cause or circumstances of death, but also because of the possibility of disfiguration. Despite this, all pathologists dealing with autopsy technique should learn this technique and be aware of the situations when it must be used.

This study presents two homicidal cases of facial damage caused by traumatic injury. In the first, the facial internal trauma was caused by punches during a fight, leading to a hospital admission, with the death occurring later due to a respiratory infection. In the second, the deceased suffered a shotgun wound to the face with instant death because of the serious facial trauma. In both cases, it was necessary to extend the superficial subcutaneous dissection superiorly (from the neck) and inferiorly (from the head), displaying the underlying facial soft tissue and bone. In the first case, it was possible to detect the mandibular condyle fracture (already with signs of minor consolidation) resulting from the blunt force trauma. In the second case, and because the shot reached mostly the mouth and nose (with no exit wound) and came through a metal window grating, the facial dissection allowed better documentation of the facial trauma.

In conclusion, cases like those presented illustrate that forensic pathologists need to have specific training regarding autopsy technique, including the special autopsy dissections. In facial dissection, particular care needs to be taken in pursuing the plane between dermal and subcutaneous tissue. Patient dissection should enable precision in order that the overlying skin is not punctured (the latter is impossible to satisfactorily reconstruct invisibly). Directing the scalpel blade away from the epidermal surface at all times helps to prevent such "buttonholes." The use of this technique may be required when dealing with forensic cases that involve facial damage caused by traumatic injury, violation of human rights, natural disease (for example parotid gland disease), and Black people with asuspicion of facial trauma.

Forensic Pathology, Autopsy Technique, Facial Dissection

H30 The Postmortem Diagnosis of Pulmonary Thromboembolism in Decomposed Corpses: Limitations and Errors of the Autoptic Techniques

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After attending this presentation, attendees will be able to describe the impact of the problematic diagnosis of thromboembolism in decomposed corpses with venous leg ulcers.

This presentation will impact the forensic science community by demonstrating the importance of a correct autoptic technique in decomposed corpses in order to identify the fatal pulmonary thromboembolism in cases of uncertain deaths.

When a corpse decomposes, it is difficult to determine a cause of death diagnosis, especially when the cause is not a trauma. In fact, the transformative phenomena alters the internal organs, their location, and the skin. These modifications make it difficult to identify diseases or abnormalities as opposed to fresh corpses. Moreover, the histopathological examination of the decomposed organs can not identify histologic alterations due to colliquative necrosis, especially in the pancreas, brain, and lungs. Toxicological investigations are altered by postmortem acidification and anaerobic metabolism.

Deep vein thrombosis and pulmonary embolism represent the clinical manifestations of the same disease spectrum, known as venous thromboembolism, considered the third most common acute cardiovascular disease after ischemic heart disease and stroke. The autopsy of fresh corpses is considered the diagnostic gold standard.

Venous ulcers resulting from venous thromboembolism are the major cause of morbidity. It is a chronic disorder that can be highly debilitating, with a negative impact on the patient's quality of life.

The purpose of this study is to emphasize the role of the autopsy, through the analysis of skin ulcers, in the diagnosis of thromboembolism. The autoptic standard technique is characterized by a cut from the chin to the pubis and a craniotomy. This study emphasizes the role of the incision of other areas of the body, such as the lower limbs.

The case of an 81-year-old man found dead in his home after approximately seven days in the summer. An external examination and an autopsy were performed in order to determine the cause of death. The incision of the lower limbs was associated with traditional autoptic technique. The histopathological surveys and genetic testing for mutations of coagulation factors were performed. The corpse was in the emphysematous stage of decomposition with an initial skeletal reduction of the head. The analysis of the visible skin showed the absence of external traumatic injuries and the presence of gauze on the left foot. Also, in the nasal and ear bud cavities it was the presence of Calliphoridae and Sarcophagidae larvae. At external examination, it was possible to detect black eschar of the left foot corresponding to the cutaneous ulcer. The autopsy revealed the presence of blood clots in the heart and pulmonary vessels. The analysis of the lower limbs disclosed the presence of a deep ulcer on the left tibial region. The cutting of the ulcer revealed the presence of venous vessels with internal clots under the skin, which were also found in the left iliac vein. Histological analysis with hematoxylin-eosin staining and immunohistochemistry of clots found in the lungs and the left leg vessels exhibited histological features of thromboemboli back to several weeks before death while clots found in the heart were attributable to a postmortem origin from blood stasis. The investigation did not reveal genetic mutations. The cause of death was due to a cardio-circulatory arrest secondary to a massive pulmonary embolism proved by the presence of a large thromboembolism inside the pulmonary vessels and another in the left iliac vein and in the small vessels under he cutaneous ulcer. Therefore, this case exhibits the role of a correct autoptic technique choice of and the analysis of venous leg ulcers in the postmortem diagnosis of fatal thromboembolic events.

In the postmortem analysis, it is difficult to distinguish clots from thrombi, especially in decomposed corpses. In this case, the careful external examination was conducted, despite the limitations of the decomposition and the presence of the larvae, by choosing the incision of the lower limbs, then correctly analyzing the clots found. The

choice of the autoptic standard technique would not have allowed the identification of the thromboembolic origin and the correct postmortem diagnosis.

Thromboembolism, Decomposition, Autopsy

H31 A Cold Case in Fatal Precipitation: Suicide or Murder? The Role of Virtopsy in Forensic Cases of Exhumation

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After attending this presentation, attendees will be able to describe the impact of virtopsy in cold cases in which there were doubts about manner of death.

This presentation will impact the forensic science community by demonstrating the importance of radiological investigations in order to clarify doubtful cases of exhumed corpses.

The event is precipitated when a body, possibly already in motion, knocks itself to the ground by hitting another obstacle under gravitational force. The seriousness of lesions by precipitation depends on the precipitation height, even if the body weight can affect the traumatic extent. In precipitation from a height of more than ten meters, external injuries on the body are generally absent or scarce. The precipitation may occur as a consequence of murder, suicide, or accident. Often, the forensic pathologist solves real puzzles regarding mode of death in these cases. In particular, if a long time has passed between the death and the reopening of a cold case, differentiating a murder from a suicide is difficult.

A large priest died due to precipitation. At the time of inspection, the coroner revealed the presence of a swelling (large hematoma) on the left side of the head, which was identified as the point of impact. This injury and the circumstantial findings prompted the coroner to conclude that it was a suicide. Since the family of the priest did not believe the hypothesis of suicide, the unresolved forensic case was reopened after a year. The newly appointed forensic pathologist and investigators had to transfer the body in order to perform an exhumation by following a protocol for decomposed bodies. The radiological investigation revealed the presence of multiple fractures of the lower limbs with asymmetry of the pelvis, multiple fractured ribs (bilateral more right side), fracture of the third medium distal clavicle and right humerus, skull fractures, fragmented bone of the parietal region and right zygomatic arch, linear fracture on the left and right mandibular fractures. In precipitations, injury of the skull due to the primary head impact may occur; ring fractures of the cranial base linear or fractures of the pelvis and femoral neck from the impact or standing on podex (as in the case reported), and fractures of the heel and rib located on lines paravertebral to impact on the heels. Therefore, the forensic pathologist noted a discrepancy in the reconstruction made by the first forensic pathologist regarding the injuries found on the body. Fractures of the left side of the head, according to the identification of virtopsy, were linear, so this suggested an indirect propagation of a shock occurred on the opposite side (right) and, for this reason, were attributable to a backlash. According to the first description on the crime scene, the presence of bruising and swelling on the left, which was interpreted as a precipitation with the victim's head to the ground, was reported. The analysis of the characteristics of bone fragments instead suggested that the precipitation took place with the feet. Therefore, the bruising to the left side of his head was certainly attributable to precipitation but also to another traumatic unknown cause. The mode of death in precipitation cases is frequently suicidal; however, the execution of a correct inspection is essential in order to determine: (1) the distance between the body and projection to the ground of the supposed initial point of precipitation; (2) the characteristics of the walls along which the free fall occurs and the characteristics of the impact surface; (3) possible presence of obstacles encountered during the precipitation; (4) accessibility of the point of precipitation; (5) indicative elements of a specific victim's movements to reach the point of precipitation or to predispose him/her to precipitation; (6) height of precipitation; (7) signs on the body or clothing indicating attempts to arrest the fall or restrain the precipitation; (8) any signs of a struggle; and, (9) the state of the clothing. These analyses may reduce the risk of unresolved cases in which the hypothesis of murder or suicide are unclear.

Forensic Sciences, Exhumation, Precipitation

H32 Sudden Infant Death Syndrome (SIDS) and Tongue-Ties: Is There a Connection? Upper-Lip Frenum as a Predictive Marker for Unexpected and Unexplained Asphyxia in Infants

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After attending this presentation, attendees will better understand the research, anatomy, mechanism, logic, and evidence supporting a novel hypothesis for the cause of Unexpected Unexplained infant death by Asphyxia (UUA), also known as SIDS or Sudden Unexplained Infant Death Syndrome (SUIDS). Attendees will learn about a study published in 2016 that found a high and significant correlation between restricted lingual frenums (tongue-ties) and UUA. A brief overview of SIDS research to date will illustrate how this current study fits into the overall pattern. Pathologists and medical examiners will learn what to look for to help decide how to pronounce on these perplexing cases as well as what they can do to aid in future research in this area.

This presentation will impact the forensic science community by providing information about a new theory of causation for (or contribution to) UUA, SIDS, or SUIDS. Identifying a restricted upper-lip frenum could provide an additional piece of useful information when analyzing these difficult cases to determine cause of death. In cases in which a restricted upper-lip frenum exists, there is now another possible explanation for asphyxia to consider in addition to mechanical overlay. If a tongue-tie is suspected, inquiries can be made into the medical history of the infant with respect to untreated tongue-tie, reflux, gas, and success or failure of breastfeeding. These results support the need for further research into this theory of causation. It may also provide pediatricians with a practical tool for identifying infants at possible risk for UUA/SIDS.

Hypothesis: There is a correlation between tongue-tie (ankyloglossia) and UUA that is predictive of risk and may be causal.

Synopsis: The demographics of tongue-ties and SIDS have several things in common: both occur more in boys than girls at the ratio of 3:1, they occur in approximately 5% of the population, and they can run in families. Because tongue-ties limit tongue movement, they often interfere with an infant's ability to breastfeed. Successful breastfeeding has been shown to correlate with a reduced risk for SIDS. Rather than successful breastfeeding being protective against SIDS, perhaps it is the case that infants with an inability to breastfeed because of a tongue-tie may be at higher risk for SIDS. To investigate this possibility, this study investigated whether wanted to see if SIDS/UUA infants do, indeed, have a higher frequency of tongue-ties than the general population.

A retrospective review of 327 cases of UUA under one year of age was conducted. Cases were included if their official cause of death was SIIDS, SUIDS, unknown, undetermined, co-sleeping, probable overlay, Asphyxia or Accidental Suffocation and Strangulation in Bed (ASSB). Cases of probable overlay were included because this is often only suspected rather than proven. Upper-lip frenums were used as a proxy for tongue-ties because: (1) when an upper-lip tie is present, 99% of the time there is also a restricted tongue as well; and, (2) upper-lip frenum restriction is more reliably diagnosed, even in death. Using autopsy photos, upper-lip frenums were classified as restricted or not restricted. The occurrence of tongue-ties in the general population is generally accepted to be approximately 5%. If there were no connection between tongue-ties and UUA, one would expect to find the same 5% of restricted upper-lip frenums in the UUA population. It was found that 84% were restricted. This finding is statistically significant at the 99% confidence level. The use of upper-lip frenum restriction as a proxy for tongue-ties would tend to bias this study toward a rejection of the hypothesis since not all tongue-ties have an associated upper-lip frenum restriction. These results proved significant nonetheless.

Results: While a correlation does not prove causality, it is significant that 84% of the UUA cases had restricted upper-lip ties as compared with 5% incidence for the general population.

Conclusion: There is a strong statistically significant correlation between restricted upper-lip frenums (and, by proxy, tongue-ties) and cases of unexpected and unexplained infant death. This supports further research into this causal mechanism that relates restricted lingual frenums to UUA. Also, clinical findings of restricted upper-lip frenums may be useful for pediatricians to identify infants at possible risk for UUA/SIDS.

SIDS, SUIDS, Tongue-Tie

H33 Too Much, Too Little, Too Late: Postmortem Discovery of a Postpartum Endocrinomaly

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After attending this presentation, attendees will be able to: (1) recognize some common and uncommon disease entities associated with maternal deaths; (2) identify gross and microscopic features of postpartum lymphocytic hypophysitis; and, (3) identify postmortem testing applicable to confirming this rare disease entity.

This presentation will impact the forensic science community by serving as an important reminder of a rare cause of maternal death and in turn expand the attendees' knowledge base regarding the subtlety of disease manifestation in order to ensure that the most accurate and etiologically specific cause of death is determined, which directly contributes to public health awareness through mortality data reporting.

The physiological changes of pregnancy are generally self-limiting, extending into the postpartum period. Pathophysiological changes are associated with varying degrees of morbidity and potential mortality if not recognized clinically.[1,2] Maternal mortality trends are derived directly from cause-of-death information, such as autopsy reports and death certificates, provided by hospitals and medicolegal offices. Publication of these trends, along with disease-specific etiologies, serves as a reminder for the clinical practitioners aiding in timely recognition of signs and symptoms of disease entities, particularly those with lethal potential. Pregnancy-induced hypertension, cardiomyopathy, amniotic fluid embolism, thrombotic embolism, and infection are some of the more common death-related entities that have been reported.[3] Endocrine complications associated with pregnancy are clinically recognized, one of which is panhypopituitarism stemming from pituitary necrosis caused by obstetric hemorrhage, also known as Sheehan's Syndrome.[1,4] Infrequently, pregnancy-associated hypopituitarism can cause or contribute to death.[5] A much less common cause of hypopituitarism in the postpartum period is lymphocytic hypophysitis, a disease entity with variable and insidious clinical manifestations and with lethal potential.[6] A more recent report of death caused by this unusual entity is presented, along with distinguishing serological and histological features.

A 25-year-old female with a history of sickle cell trait who was three weeks postpartum following an uncomplicated delivery by cesarean section, died suddenly after a recent onset of lethargy and anorexia. At autopsy, adrenal gland atrophy, a 1.0cm thyroid gland nodule, visceral congestion, and an enlarged pituitary gland were found. Extensive necrosis, acute and chronic inflammation, lymphoid follicle formation with germinal center transformation, and fibrosis were discovered on microscopic examination of the pituitary gland. Immunohistochemical staining highlighted distinct B- and T-cell populations within the follicles, distinguishing the immunological component of this entity, while the fibrosis revealed by trichrome staining confirmed its chronicity. Negative immunoreactivity for Adrenocorticotropic Hormone (ACTH) correlated with the atrophic adrenal glands, marked hypoglycemia, and marked hypocortisolemia detected upon perimortem and postmortem serological testing. Serological evidence of thyrotoxicosis was an additional confounding finding in light of the negative immunoreactivity for Thyroid Stimulating Hormone (TSH). Moreover, the absence of immunoreactivity for TSH-secreting cells was consistent with the destructive inflammatory process of the pituitary gland and the explanation for the markedly low TSH level found on postmortem serological testing and not the result of negative feedback caused by the thyrotoxicosis. Thus, the thyrotoxicosis was due to the functioning thyroid gland adenoma. Lymphocytic infiltrates were seen in adrenal gland sections constituting additional evidence of an autoimmune-mediated disease process, but no such infiltrates were seen in sections of the thyroid gland. Vitreous fluid analysis revealed mild dehydration. The above findings defined a clinicopathologic state of hypopituitarism with profound adrenal insufficiency caused by lymphocytic hypophysitis, complicated by thyrotoxicolsis, terminating in death. Additional microscopic and laboratory examinations revealed evidence of peri-mortem ischemia and hypoxia as part of the terminal mechanism of death in this case.

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Lymphocytic Hypophysitis, Maternal Death, Sudden Death

H34 Two Instances of Sudden and Unexpected Infant Death (SUID) in Siblings With a Shared Mutation in Plakophilin 2 (PKP2)

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After attending this presentation, attendees will learn the value and limitations of the molecular autopsy in investigation of multiple cases of SUID within the same family and learn of a potentially arrhythmogenic mutation.

This presentation will impact the forensic science community by showcasing the evolution of investigations into multiple SUID cases, and the value and limitations of the molecular autopsy.

SUID remains one of the most actively studied and yet least completely understood areas of forensic medicine. More challenging still are instances of multiple SUID cases within the same family. Historically, some of these cases have resulted in criminal convictions, of which some of have been deemed wrongful and have been overturned on appeal.

More recently, non-structural genetic heart disease has been recognized as a potential cause of SUID, and, with postmortem genetic testing, is being identified more frequently. Presented are the cases of two full siblings presenting with SUID, with a shared genetic mutation that may account for death.

A 10-week-old girl was discovered dead in her crib approximately one to two hours after she was last fed. Resuscitation attempts were not successful. Scene investigation was non-contributory. An autopsy revealed no significant external or internal trauma or natural disease. Toxicological, biochemical, radiological, bacterial, viral, and metabolic studies were negative or non-contributory. A police investigation revealed no criminal suspicion. The cause of death was undetermined.

Approximately two years later, a 1-month-old full sibling was discovered dead in his bassinet approximately two hours after he was fed and put to sleep. The family history of SUID was disclosed immediately to death investigators. An autopsy was performed, with the additional steps of cardiac pathology and neuropathological consultation; however, no anatomical cause of death was identified. Similarly, scene investigation, ancillary tests, and the police investigation were negative. The cause of death was undetermined. The initial autopsy, including histology, was reviewed, with no new findings identified.

Postmortem genetic testing revealed both infants to be heterozygous for a recognized mutation of unknown significance of the PKP2 gene (c.473 G>A; p.R158K). Loss of function mutations in this gene have been associated with Arrhythmogenic Cardiomyopathy (AC/ARVC). This mutation has been reported in individuals with signs and symptoms of AC/ARVC, as well as asymptomatic individuals.

Following these tests, the cause of death in both cases was still given as undetermined, with discussion of the mutations included in the opinion. Family members met with the death investigation team, who conducted assessment at an arrhythmia clinic for first-degree relatives.

These cases illustrate the difficulties posed by multiple cases of SUID in the same family, the breadth and depth of the required investigations, and the value and limitations of the molecular autopsy in identifying potential disease-causing mutations.

Sudden Unexpected Infant Death, Molecular Autopsy, Arrhythmogenic Cardiomyopathy

H35 Ectopic Pregnancy-Related Deaths at Autopsy in North Carolina: A 25-Year Retrospective Review

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After attending this presentation, attendees will be able to identify trends in clinical presentation as well as document and diagnose pregnancy-related ectopic deaths at autopsy.

This presentation will impact the forensic science community by raising awareness of common clinical and autopsy findings in the rare entity of medical examiner/coroner jurisdiction ectopic pregnancy-related deaths.

Ectopic pregnancy-related deaths are not commonly discussed in the forensic literature. Additional information could help identify trends and provide insights into how they can be more successfully diagnosed prior to and after death.

Ectopic pregnancy, the implantation of a fertilized ovum outside the endometrial cavity, accounts for 1.5% - 2% of all pregnancies and is an important cause of morbidity and mortality in women of reproductive age. The ectopic pregnancy mortality ratio in the United States declined by 56.6% from 1.15 to 0.50 deaths per 100,000 live births between 1980 - 1984 and 2003 - 2007. This decrease is predominantly attributed to early diagnosis and treatment prior to rupture. Nevertheless, due to the relatively young age of the patient population and some with death prior to diagnosis, ectopic pregnancy-related deaths will be encountered in medical examiner/coroner systems.

To better understand ectopic pregnancy related deaths in the setting of advancing medical imaging and diagnosis, a retrospective review of electronic and paper records from North Carolina's Office of the Chief Medical Examiner database between 1980 and 2015 was performed to define the autopsy findings and clinical presentation of fatal ectopic pregnancies in the North Carolina medical examiner autopsy population. Demographic information, circumstances, antecedent symptoms, toxicology findings, autopsy findings, and cause of death were collected from death certificates, autopsy reports, and investigative reports. Eleven cases of fatal ectopic pregnancies diagnosed at medical examiner autopsies were identified. Decedents were predominantly Black (55%), and ranged in age from 19 to 40 years, with a median age of 30 years. Only three (27%) were educated beyond high school, with one, two, or four years of college, respectively; 82% of decedents were employed (including one college student). Review of investigative information revealed 82% displayed classic antecedent symptoms of abdominal pain, nausea, and vomiting, 33% of which sought medical care for the symptoms. The remaining 67% were found deceased at home. Ruptured ectopic pregnancy was the proximal cause of death in 82% of cases. The remaining 18% of deaths resulted from post-surgical complications (pulmonary embolism and diffuse alveolar damage). In all cases where ruptured ectopic was the proximal cause of death, the autopsy documented intraperitoneal hemorrhage. The average volume was 1,800ml. In nine (82%) of the cases, a fallopian tube was the implantation site. The pelvic sidewall accounted for one case (9%) and the implantation site for 1 case (9%) was not reported.

In conclusion, ruptured ectopic pregnancy is a relatively rare cause of death but is likely to be encountered in medical examiner/corner systems at autopsy. Massive intraperitoneal hemorrhage leads to rapid death, thus preventing many women from ever making it to the hospital to seek treatment. In this study, 67% of deaths occurred unwitnessed at home. Women of reproductive age without significant clinical history or an identifiable cause of death will often fall under medical examiner/coroner jurisdiction and likely undergo autopsy. Thus, all autopsy pathologists in medical examiner/coroner jurisdictions need to be familiar with the clinical presentation and autopsy findings in these maternal deaths to ensure this rare diagnosis is not missed. Additional studies with larger numbers in the forensic literature are needed to determine clinical presentation trends, symptoms, and risk factors in the hopes of ultimately preventing these deaths.

Ectopic Pregnancy, Maternal Death, Autopsy

H36 Congenital Heart Defects (CHD) in Children: A Retrospective Review of Autopsies From the Cook County Medical Examiner's Office

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After attending this presentation, attendees will understand the types of CHD commonly encountered in autopsies of children.

This presentation will impact the forensic science community by providing information about the anatomical defects (simple or complex, single or multiple) and the medical history in CHD cases of a medical examiner's pediatric population.

CHDs represent the most common type of birth defect. According to the Centers for Disease Control and Prevention, they affect nearly 1% of births per year in the United States. Anatomically and functionally, CHDs can be broadly classified as simple (involving one anatomic structure) or complex (involving multiple structures) with repairs varying in complexity. While some simple defects, such as atrial or ventricular septal defects, may resolve spontaneously, most CHDs require surgical correction at some point in life, depending on symptoms.

The files of the Cook County Medical Examiner's Office in Chicago were searched for cases of subjects less than 18 years of age involving "congenital heart disease" and "congenital heart defects" as a cause of death, from January 2006 to July 2016. Cases were reviewed for age, sex, race, cause and manner of death, medical history, and autopsy findings.

In this study population, simple defects consisted of Atrial Septal Defects (ASD), Ventricular Septal Defects (VSD), Patent Ductus Arteriosus (PDA), anomalies of the valves, and coarctation of the aorta. Complex defects consisted of Tetralogy Of Fallot (TOF), Hypoplastic Left Heart (HLH), Double Outlet Right Ventricle (DORV), and Transposition of the Great Arteries (TGA).

A total of 64 cases were identified: 35 males and 29 females; 38 African American and 26 Caucasian. Regarding age, 23 were ≤1 month old, 29 ranged between one month - one year, seven ranged between one-ten years old, and five were older than ten years. The cause of death was listed as "congenital heart disease" in all cases but two, where it was undetermined. The manner of death was determined to be natural in all cases but two (undetermined manner).

There were 31 (48%) decedents with a history of CHD prior to autopsy and, of these, 24 underwent a surgical repair. Five cases had a CHD associated with genetic syndromes (mostly Down syndrome).

Regarding the simple defects, coarctation of the aorta was observed in six cases. ASDs were observed in 4 cases as the only defect and in 4 in association with other defects. VSDs were never observed alone; in all six cases they were in association with other defects. PDA was observed alone in 1 case and in association with other malformations in five cases. Valvular stenosis was observed in 1 case alone and was associated with other defects in four cases.

Regarding the complex defects, TOF was observed in four cases. HLH was seen in 11 cases, DORV in seven cases, TGA in five cases, and valvular atresia in ten cases. AVSDs were observed in five cases alone and in association with other defects in six cases. Ebstein's anomaly was seen in six cases: one alone and five associated with other conditions.

This study reveals that, in the cases surveyed, children below one-year-old represented the vast majority of subjects (81%) with CHD. The most common simple defect was ASD (eight cases). The most frequent complex defects weres HLH and ASD (both observed in 11 children). Frequently undiagnosed conditions at autopsy were coarctation of the aorta (four out of six), HLH (five out of 11) and ASD (five out of 11). One case of TOF was not diagnosed prior to autopsy. When diagnosed, HLH, ASD and coarctation of the aorta were commonly surgically treated (4 out of six cases, five out of six cases, one out of two cases, respectively), while TOF was always treated.

It is important to recognize CHD as a potential cause of sudden unexpected death in children. Despite advances

in prenatal care and imaging, a significant number of cases of CHD go undiagnosed prior to autopsy (52% in this series). In children, and especially in infants, it is important to carefully assess for the presence of undiagnosed CHD as a potential cause of death, consulting a cardiac pathologist, if possible.

Congenital Heart Defects, Children, Sudden Death

H37 A Sudden Unexpected Infant Death (SUID) Associated With Coronary Arterial Fibromuscular Dysplasia

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After attending this presentation, attendees will appreciate some causes of sudden cardiac death in infancy, have an understanding of the pathophysiology, incidence and epidemiology of fibromuscular dysplasia, and be able to identify coronary artery abnormalities (or coronary artery hyperplasia) grossly and microscopically.

This presentation will impact the forensic science community by illustrating the aspects of a thorough and complete postmortem cardiac examination, stressing the importance of including sections of the coronary arteries for histologic examination in cases of SUID.

SUID is a traumatic burden for families and a significant diagnostic challenge for forensic pathologists. There is increasing evidence that certain cardiac abnormalities presenting as SUID may be underemphasized and may have implications for surviving family members.

Reported here is the case of a 4-month-old male infant who was laid down by his mother on the date of death and was found unresponsive approximately ten minutes later. Resuscitative efforts were unsuccessful. The infant had a history of prematurity, born at 29 weeks, 4 days, and weighing 1, 600 grams. At that time, he was hospitalized in the neonatal intensive care unit for 52 days with respiratory distress syndrome, apnea of prematurity, a transient flow murmur which resolved, and retinopathy of prematurity. His prenatal history was significant for maternal smoking (one-half pack per day) and a positive maternal drug screen for amphetamines and marijuana. According to his mother, the infant had upper respiratory symptoms prior to death.

Autopsy revealed a well-nourished, 7,400-gram infant measuring 59.5cm from crown to heel. The major autopsy finding was limited to the heart, which exhibited a 1.2cm x 0.8cm area of epicardial fibrosis on the left ventricular apex with scattered petechiae on the anterior visceral epicardium. Grossly, the epicardial coronary arteries were each diffusely thickened and fibrotic, although patent. Histologically, all of the coronary arteries were diffusely, mildly stenosed by a concentric musculoelastic intimal thickening. The subendothelial connective tissue contained an infiltrate of spindled mesenchymal cells set in an abundant fibrocollagenous tissue matrix. The proliferating cells showed smooth muscle actin and vimentin positivity and desmin negativity, confirming myofibroblastic origin. By elastic/trichrome stain, the internal elastic membrane and elastic fibers of the media were fragmented, especially at sites of greater intimal proliferation. An Alcian blue/Periodic acid Schiff stain revealed increased intimal mucinous ground substance. There was no evidence of inflammation, foam cells, cholesterol clefts, or other evidence of atheroma. Other anatomic regions of the heart, including the cardiac valves and the atrioventricular nodal region, were free of abnormalities. Other vessels, including the aorta, internal carotid arteries, and renal arteries, showed no vascular stenosis, aneurysmal changes or dissections. Cultures of the lungs, cerebrospinal fluid, and blood showed postmortem overgrowth, and a nasopharyngeal swab viral panel was negative. Postmortem toxicological analysis was negative. No other natural disease processes or injuries were identified that caused or contributed to the infant's death. Based on the lack of clinically significant coronary artery stenosis or chronic or acute ischemic cardiac lesions, the cause of death was listed as SUID, and the coronary arterial fibromuscular dysplasia was listed as a contributing condition.

A non-inflammatory, non-atherosclerotic disorder, fibromuscular dysplasia is characterized by abnormal fibroblastic proliferation in the walls of small- to medium-sized arteries leading to stenosis, aneurysm, dissection, or occlusion. The etiology is unknown but is believed to be related to hormonal, environmental/mechanical, and genetic factors. It is familial in 7%-11% of cases and commonly affects the renal (60%-80% of cases), extra-cranial cerebrovascular (25%-30% of cases), and vertebral arteries. Isolated involvement of the coronary arteries is rare and can present with unstable angina, myocardial infarction, left ventricular dysfunction, or even sudden cardiac death.

Sudden cardiac death in infancy can be attributed to cardiomyopathies, channelopathies, congenital cardiac anomalies such as valvular abnormalities, endocardial fibrosis, and coronary artery disease. Anatomic findings of

these entities can be subtle or sometimes obscured by prolonged survival time leading to secondary myocardial ischemia; however, a thorough cardiac examination can elucidate certain structural pathologies. This case highlights the importance of a thorough cardiac examination including microscopic sections of the coronary arteries in cases of SUID.

Sudden Unexpected Infant Death, Coronary Artery, Fibromuscular Dysplasia

H38 The Effect of History and Context on Consensus in Diagnosis of Patterned Injuries of the Skin

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After attending this presentation, attendees will have a better appreciation of how history and context affect medical diagnosis in forensic pathology.

This presentation will impact the forensic science community by emphasizing the importance of history, context, and the errors that will be created by decreasing access to these parts of the diagnostic process through regulatory standards.

In a previous study, a survey-based analysis of pathologist diagnoses of patterned injury was performed. Subjects were provided with photographs of "classic" injuries and asked to diagnose the lesion in the absence of history or context. There was a relatively low diagnostic consensus among respondents. A second survey suggested that the disparate answers were not due to a strong belief in different diagnoses, but instead reflected how the respondents dealt with ambiguity.

A third survey was created that asked participants to evaluate patterned injuries of the skin, but provided history and contextual information. Email invitations were sent to all members of the National Association of Medical Examiners (NAME) with email addresses on the roster, a total of 1,098. Two hundred sixty-nine surveys were started and 192 surveys completed.

The mean consensus per question with history provided was 91.3%. The distribution was skewed to the left because of two outlier questions; the median consensus was 97.9% with a standard deviation of 0.13, and standard error of the mean of 0.23. This is in contrast to the results of the third tier of the first survey (without history), which provided a mean consensus of 76.2%, median of 80%, standard deviation 0.18, and standard error of the mean 0.03.

The average confidence per question was 90.3, median 92.7, standard deviation 6.48, standard error of the mean 1.16. This compares to the results of the first survey, which provided a mean confidence per question of 58.7 (out of 100), median 56.1, standard deviation 15.2, and standard error of the mean 2.4. The mean confidence was 90 out of 100, median 91.6, standard deviation 8.02, and standard error of the mean 0.57. This compares to the first survey, which provided a mean of 73.2, median 79.0, standard deviation of 14.8, and standard error of the mean of 14.8.

A possible training effect was tested among those who took the first survey. There was no significant difference in consensus answers between those who had participated in the first survey and those who had not.

These studies demonstrate the importance of history in the diagnosis of patterned injury of the skin. Denying history produces significant lack of consensus, primarily due to issues of ambiguity rather than actual differing diagnoses. Providing history increased the degree of consensus by 20 points to near-complete agreement. This study and those of medical decision-making suggest that data hiding will decrease diagnostic accuracy.

Medical Inference, History, Medical Imaging

H39 A Gunshot Wound Trajectory Analysis Using Forensic Animation to Establish the Relative Positions of the Shooter and Victim

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After attending this presentation, attendees will better understand how forensic animation can be used to assist in gunshot wound trajectory analysis.

This presentation will impact the forensic science community by illustrating the value of forensic animation in examining complex gunshot wound trajectories and its use in investigations.

Few peer-reviewed articles or case reports detail how trajectory analysis is performed in complex gunshot wound cases with conflicting testimony. Forensic pathologists who autopsy the victims of gun violence are often called upon to answer questions in both criminal and civil courts about the relative position of the shooter and the victim. In this case report of an officer-involved shooting incident, the statement of the police officer contradicted the statements of other eyewitnesses. Trajectory analysis of the decedent's two gunshot wound pathways was performed in light of the physical evidence at the scene, including the final resting position of the decedent's body and forensic animation was used to create a court exhibit.

The officer stated the decedent had tripped and was on the ground, turning toward the officer in a threatening manner when the officer decided to shoot. Forensic trajectory analysis revealed that the decedent sustained two gunshot wounds: a penetrating wound to the lower right leg and a perforating wound to the head. The first gunshot wound to the right calf had a back-to-front, right-to-left, and upward trajectory. The bullet fractured the right tibia and lodged in the knee at the patella. It did not sever any major arteries and would not have been a lethal or incapacitating wound. The second gunshot to the back of the head entered in the inferior right occipital scalp and exited at the left forehead. The brain stem was transected at the pontomedullary junction. The direction of fire was back-to-front, right-to-left and upward. This second gunshot would have been instantaneously lethal and would have caused the immediate loss of all muscle tone. The decedent's final resting position at the scene was face down with his left leg externally rotated and extended and his right leg flexed at a 90° angle at the knee, with the right foot resting up against an adjacent tree. The left hand was palm up, down by the hip. His right hand was at the waist under his body. The final position of the decedent was consistent with the decedent running away from the officer, with his arms down, which contradicted the officer's testimony that he was on the ground and twisting to face the officer, with his arms poised as if to shoot. The use of forensic animation in this case allowed for all the evidence to be visualized to demonstrate the relative positions of the shooter and victim and demonstrate the sequence of events that occurred during the shooting. The legal case was settled to the satisfaction of both parties.

Gunshot Wound, Trajectory, Forensic Animation

H40 Postmortem Imaging of Coronary Arteries for Natural and Violent Deaths

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After attending this presentation, attendees will be knowledgeable regarding modern postmortem imaging of coronary arteries and its usefulness in forensic practice for diagnostic and documentation purposes.

This presentation will impact the forensic science community by raising awareness concerning the advantages and limitations of radiological postmortem examination of coronary arteries.

Evaluation of coronary arteries is one of the essential steps during autopsy investigations as many sudden deaths are related to coronary artery lesions. Postmortem imaging is playing an increasingly important role in forensic investigations, not only in violent deaths but also in cases of natural deaths, and represents one of the most important topics of research in forensic pathology. In general, coronary arteries are investigated morphologically during classical autopsy, followed by histological examinations; however, depending on the chosen method and case, the radiological examination of coronary arteries often provides the first appreciation of coronary status and allows documentation before performing a destructive autopsy. The goal of this presentation is to explain the current approach for postmortem radiological investigation of coronary arteries for natural and violent deaths.

Since 2009, more than 700 postmortem cases were analyzed with Postmortem Computed Tomography (PMCT) and Multiphase PMCT-Angiography (MPMCTA) in this study's center. An unenhanced PMCT and subsequent MPMCTA were performed before the classical autopsies, following standard protocols. Postmortem liquid samples for toxicological screening and the biochemistry/microbiology were collected using PMCT guidance. A full classic autopsy, including subsequent histological examination of selected tissue, was performed for every case. Autopsies were performed according to international recommendations for forensic pathology and cardiovascular pathology for presumed sudden cardiac deaths.

Among natural deaths, the most frequent cause of death was ischemic heart disease related to atherosclerotic coronary artery disease. According to research, PMCT permits the detection of coronary artery calcifications while MPMCTA additionally allows for the evaluation of coronary artery lumina, the detection of myocardial lesions such as myocardial rupture, and, in some select cases, signs of myocardial infarction. Thanks to a detailed comparison between radiological images, autopsy, and histology, a specific key for postmortem radiological reading of coronary arteries has been developed which will be presented. Postmortem imaging of coronary arteries in violent deaths represents an additional tool for more detailed documentation and allows for reconstructions of gunshot or sharp injury trajectories, especially important in cases involving a third person. The autopsy technique could also be adapted according to the radiological evaluation. Postmortem imaging of coronary arteries with surgical interventions appears especially useful and is recommended according to this research. PMCT allows for the detection of coronary stents, which can be difficult to visualize at autopsy in the presence of severe calcifications. MPMCTA can demonstrate the permeability of coronary bypass and help guide the dissection technique during autopsy.

Advantages and disadvantages of PMCT/MPMCTA will be discussed. The systematic postmortem evaluation of coronary arteries and the knowledge obtained by comparative studies with PMCT/MPMCTA could improve the understanding of radiological presentations of coronary morphology and pathologies in living patients. The key for postmortem radiological reading of coronary arteries needs to be evaluated in other centers and adapted, according to the results of further studies. It is felt that, the combination of classical autopsy and postmortem imaging of coronary arteries leads to an improvement of quality of postmortem forensic diagnosis and documentation and should be considered as a new gold standard if lesions of coronary arteries are suspected.

Sudden Cardiac Death, Coronary Arteries, Postmortem Imaging

H41 Burns, a Home Fire, Strangulation, Intoxication, a Knife, and Three Cadavers: A Criminal Novel

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After attending this presentation, attendees will understand the deductive path of a forensic pathologist during the various stages of the investigation of a complex criminal case, from the scene to the autopsy, including multiple suspicions and evidence that should be analyzed, interpreted, and selected across the process of investigation, until the clarification of the final puzzle. The need for a good basic medicolegal preparation in all different areas of forensic pathology is emphasized, so the forensic pathologist is able to interpret properly the features observed, both at the scene and at the autopsy, integrating them all in order to arrive at a definite and correct diagnosis.

This presentation will impact the forensic science community by drawing attention to the importance of the scene examination and the presence of the forensic pathologist, who can offer extra skills concerning the examination of the victim's body on the scene, later confirmed or contradicted at the autopsy.

The fire department of a small city in Portugal was called to put out a fire at a young couple's apartment. At the scene, the firemen found two apparently charred bodies (female and male) and a dead cat; they called the police and the medical examiner, who later autopsied the bodies.

This study analyzes the steps followed in the investigation of this scene: (1) burns and/or charring of a victim (the wife) with a knife nearby; (2) hanging of the other victim (the husband), who was suspended from the stair handrail with an incised wound on the wrist; (3) the furniture in disarray, covered with a veil of black soot; and, (4) chemicals possibly used to start the fire found inside plastic containers in the living room and garage.

The autopsies were ruled necessary to clarify this complicated scene. The burns of the woman presented postmortem characteristic and she did not inhale the fire. In addition, she exhibited lesions compatible with manual strangulation and no cut marks. The knife was used by the husband to try to committee suicide, without success. Finally, he was found hanged, having ingested a blue toxic liquid that was recovered from his stomach (yet another unsuccessful suicide attempt).

It was concluded that both the presence of the forensic pathologist at the scene and the integration of all the information gathered, together with the autopsy findings, were indispensable to clearly understand this crime. The autopsies of the two victims provided explanations for nearly all the questions that arosearoused during the scene investigation. The toxin ingested by the husband couldn't be identified; the cause of the cat's death was not investigated, even though carbon monoxide intoxication is the most likely explanation.

Manual Strangulation, Hanging, Scene Investigation

H42 Synchrotron Studies of Spectrometric and Chemical Changes in Aging Bruises

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The goal of this presentation is to express to the audience the trials, pitfalls, and achievements of using high-intensity, high-resolution synchrotron light to determine the spectrometric and chemical changes that occur over time in bruises.

This presentation will impact the forensic science community by highlighting the advantages of using synchrotron light to probe the changes in spectra and chemical composition due to aging in bruises. This fundamental knowledge can be applied in normal conventional laboratory instruments.

The chemistry that occurs during the formation, maturing, and fading of a bruise during its lifecycle is complex and may provide information about the age of the contusion, which may have medicolegal significance. Spectroscopy can be used to distinguish similarly colored compounds, including those known to be present in bruises: oxyhaemoglobin, deoxyhaemoglobin, methaemoglobin, biliverdin (green), bilirubin (orange-yellow), and ferritin.

Visible and near infra-red reflectance spectra were obtained using an Ultraviolet/Visible/Near-Infrared (UV-Vis-NIR) spectrophotometer with a 150nm Integrating Sphere accessory at Flinders University, Australia. Far Infrared (FIR) transmission analysis was conducted at the Australian Synchrotron to extend the investigation into the 10 cm^{-1}-1,500cm^{-1} range.

Significant differences between the spectra of bilirubin and biliverdin were found and will be presented. These investigations were carried out in solid phase samples.

Further work involving the development of novel diamond liquid cells with spacers of 5mm to 60µm was also undertaken at the Australian Synchrotron as measuring the spectra in solution would be more realistic to the biological solutions found in a bruise.

This study will present spectra from hemoglobin, biliverdin, and bilirubin as solids, in matrixes, in slurries, and in solution from 10cm^{-1} to 25.000cm^{-1} from room temperature to 78K. As water causes interferences in the spectra, a number of different solvents were tried. A balance needs to be determined in finding solvents in which the materials are soluble but do not have a spectrum that overloads the detector or interferes with the spectral information from the desired material.

The process of obtaining spectra of bruise components using a liquid cell system produced novel challenges. The development of the analytical method and the results in the fluid phase will be presented and compared with solid phase analysis. It is not intended that all forensic analysis of bruises be conducted using a synchrotron light source, but the information and knowledge that is obtained from these specialist studies can assist in the analysis of bruises using conventional laboratory equipment that is available to all.

Bruises, Synchrotron, Aging

H43 A Fatal Accidental Gunshot Wound During a Pursuit by Law Enforcement

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After attending this presentation, attendees will better understand the importance of the examination of clothing and firearms in cases in which autopsy findings do not correlate with the stated circumstances.

This presentation will impact the forensic science community by highlighting the need to carefully examine the decedent's clothing and firearm(s) when the autopsy findings do not support the initial investigative scenario.

According to the Centers for Disease Control and Prevention in 2014 there were 33,599 firearm-related fatalities in the United States. Of these deaths, 586 were unintentional, 270 were undetermined, and 32,743 were violence related. Violence-related fatalities were further broken down by homicide/legal intervention (11,409 deaths) and suicide (21,334 deaths).

Unintentional firearm discharges can happen in several situations. The major method of accidental or negligent firearm discharge is by inadvertently pulling the trigger. Many people who are not properly trained may place their fingers on the trigger instead of along the side/frame of the gun. Accidental discharges can occur with some weapons if the loaded weapon is dropped. Accidental discharges when a gun was dropped have been reported in single-action revolvers, old or cheaply made double-action revolvers, derringers, striker-operated semi-automatics, and certain external hammer semi-automatics.

Case Report: A 28-year-old man was a passenger in a vehicle that wasstopped by law enforcement personnel. The man exited the vehicle and ran. While running up an embankment, both the man and the pursuing police officer fell. The police officer heard a gun discharge. He temporarily retreated and waited for back-up assistance. The man got back up and continued to run for about 75 yards, then collapsed. A .40 caliber Taurus PT-140 semi-automatic handgun was discovered in some weeds approximately ten yards away. The man had a gunshot wound to his left leg and was transported emergently to the hospital. He was taken to surgery where the surgical team attempted to repair his femoral artery, but he died in the operating room due to acute blood loss.

At autopsy, the detectives believed that the decedent had been running with a gun in his pants when it discharged because they described a gunshot defect in his pants below the left front pocket and a corresponding defect in his underwear. His clothing was not available for examination at the time of the autopsy, but the detectives furnished images of the clothing that revealed the two defects in his pants and underwear. A surgical incision was on the decedent's left medial thigh and a circular gunshot wound with circumferential marginal abrasion was on his left lateral thigh corresponding to the defects in the clothing. Re-approximation of the surgical incision revealed an irregular edge along the surgical incision's border. No soot or stippling was on the skin around either wound. Radiographs did not disclose a bullet or bullet fragments; no projectile was found at autopsy. Because the wounds on the body did not support the detectives' account of the incident, the clothing was retrieved at a later date and carefully examined. The subsequent examination of the clothing revealed additional holes in the underwear and pants indicating the gunshot wound entrance was on the lateral thigh and the corresponding exit was on the medial thigh — reversed from the initial interpretation. No gunpowder particles were visible on the pants or underwear.

It was unclear how the firearm discharged causing the fatal wound until further investigation revealed that the Taurus PT-140 is one of the Taurus models that have been recalled because of a design defect that permits the gun to discharge when dropped. This case emphasizes the challenges that can arise when initial accounts do not match findings at autopsy. Gunshot wound fatalities with law enforcement presence must be meticulously scrutinized to avoid mistakes in interpretation.

Gunshot Wound, Unintentional Dischage, Law Enforcement

H44 The Diagnostic Accuracy of the Triad in Shaken Baby Syndrome: A Systematic Literature Review

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After attending this presentation, attendees will better understand the reliability of the literature pertaining to the accuracy of the diagnostic triad of subdural hematoma, retinal hemorrhage, and encephalopathy as a proxy for shaken baby syndrome.

This presentation will impact the forensic science community by providing results from a systematic literature review and suggestions on how to perform more reliable studies in the future.

In 1971, it was proposed that abusive shaking of an infant was closely associated with subdural hematoma, eventually together with retinal hemorrhage and encephalopathy referred to as a "triad." Later, a corollary was derived; if the triad was identified and no "acceptable" alternative explanation provided by a suspect caretaker, it was concluded that the infant had been intentionally shaken. Over the past decade, the relationship between shaking trauma and the findings used to make the diagnosis of abuse has become the subject of increasing criticism. Since evidence-based knowledge of the effects of shaking has important medical and societal consequences, it is important that the criteria for identifying shaken infants are reliable. The literature review presented here was directed primarily at evaluating with what degree of certainty the presence of the "triad" is associated with shaking.

A literature search was performed in the databases PubMed®, EMBASE® and Cochrane Library through October 15, 2015. All types of studies with ten or more study cases were included. The criteria for eligibility included: ("population") children <12 months of age; ("index test") the triad; ("reference test") confessed or witnessed shaking or other trauma; ("outcome measure") diagnostic accuracy. All studies of potential relevance according to the inclusion criteria were read in full text by two reviewers independently. The relevant publications were assessed for risk of bias using the QUADAS tool, and classified as having low, moderate, or high risk of bias according to defined criteria.

The search generated 3,773 abstracts, of which 1,145 were read in full text; of 43 included studies 41 were assessed as having high risk of bias, two as having moderate risk, and no study as having a low risk. Seven systematic reviews were identified and evaluated using the AMSTAR tool; all seven were found to have a high risk of bias.

The main conclusion was that there is insufficient scientific support to conclude that the diagnostic triad is an accurate test for the identification of shaken baby cases. There is limited scientific support to conclude that shaking can cause the triad, and for the conclusion that other conditions and events are associated with the triad.

The reasons for the low ranking of the majority of studies, including the systematic reviews, were methodological issues and circular reasoning. Apart from the usual issues connected with the retrospective studies, the age of the controls was often significantly higher than among the shaken babies. Further, the radiological and ophthalmological investigations were often not blinded, but when blinded, the inter-rater agreement was poor or moderate. The classification criteria were sometimes only a reference to the judgment of a "child protection team," a sometimes speculative conclusion regarding the injury potential of a fall. Consequently, the group of allegedly shaken baby cases may have included accidental trauma, and the control group may have included shaken baby cases.

Another reason for low quality was circular reasoning employed in the conclusions of some of the studies, resulting from the assumption by the child protection team that if the triad was observed, then the infant had been shaken, unless another "acceptable" explanation was provided; however, the basis for rejecting alternative

explanations by caretakers as unacceptable was not linked to any methodologically valid scrutiny. The inherent but invalid assumption in these studies was that the triad has near-perfect diagnostic accuracy. There were other methodological problems in the studies as well.

Future studies with acceptable methodological quality need improved planning, higher quality, and larger study numbers. This means prospective observational studies of reliably documented cases or confessed cases in which the risk of false confessions has been minimized, sufficiently large study numbers examined with uniform methods, age matched controls, detailed descriptions of how the study cases were collected and examined and how differential diagnoses were excluded, information on blinding of the examiners, and presentation of detailed results, enabling the calculation of diagnostic accuracy.

Triad, Shaken Baby Syndrome, Bias

H45 Child Abuse and Head Trauma: A Retrospective Analysis for Diagnosis

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After attending this presentation, attendees will understand the importance of considering head trauma in child abuse, one of the most dangerous findings since obscure and life-threatening lesions may be overlooked, leading to accelerated morbidity and even death.

This presentation will impact the forensic science community by showing the importance of a careful diagnosis, not only to provide adequate care, but also to ensure complete and accurate reporting of the event to the prosecutor's office.

At this time in Italy, there is no specific nationally approved protocol that has been adopted for investigating head trauma associated with child abuse. This study was initiated to establish whether or not certain anamnestic, clinical, or instrumental factors can be strongly associated with the diagnosis of abusive head trauma in children. In the event of child abuse, the most important aspect is to ensure an early and correct diagnosis.[1] In fact, according to the literature, a diagnostic delay is responsible for an increase in the death rate. Nevertheless, the lesions observed during the medical examination are rarely a certain sign of abuse because of their lack of specificity.[2] Also, a precise diagnosis is more difficult in children because of their limited verbal skills and often a lack of cooperation during the examination.

A retrospective analysis was conducted on cases of pediatric head trauma observed in one year (2011) in the emergency room of the Pediatric Hospital (Ospedale Infantile Regina Margherita) in Turin, Italy. The study sample included 658 selected children less than 40 months of age. Among all the variables registered, the analysis was focused on gender (A), age (B), length of time elapsed between the trauma and the access to the emergency department (C), congruity of the parents' story about how the trauma happened (D), previous contacts with the unit (Bambi) of the same hospital, which is dedicated to the evaluation of suspected abused children (E), ≥2 visits to the Emergency Room (ER) for other trauma (F), vomit (G), loss of consciousness (H), and other lesions such as bruises, abrasions, cranial or facial fractures (I). The sample included 361 males (55%) and 297 females (45%). Most of these children (46%) were between 0 and 12 months, with a peak between 6 and 12 months of age. The male children were generally younger than females, although there was no statistical significance in this association (p = 0.679). Seven logistic regressions were conducted to estimate the probability of the F-variable as a function of the B, C, D, E, G, H, and I variables. Statistical significance emerged from the logistic regression between the F-variable and B-variable: children with ≥2 admissions to the ER for other trauma were significantly (p = 0.007) older than children with <2 accesses. The same relationship was observed between the F-variable and E-variable (p = 0.001) and between the F-variable and H-variable (p = 0.002). Most of the children with ≥2 admissions to the ER for other trauma had already visited the Bambi unit, dedicated to the evaluation of suspected abused or mistreated children (E) and presented on arrival at the ER with unconsciousness (H). A multiple logistic regression was conducted between the F-variable and B, E, and H variables. After this, the odds-ratio was estimated: F-variable (OR = 33.5) and I-variable (OR = 4.6). This study demonstrates a statistical correlation between multiple admissions (≥2) to ER's for trauma and age, previous admissions dedicated to the evaluation of suspected abused children, and unconsciousness. This presentation should serve as a stimulus to heighten the awareness of the importance of systematically collecting clinical data about head injuries in suspected or confirmed cases of child abuse.[2,3] The analyses of multiple data points and clinical findings associated with head trauma serves to accelerate and improve clinical interventions. Furthermore, this study illustrates the utility of a unit dedicated to the evaluation of suspected abused children for the diagnosis of child abusive head trauma.

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Child Abuse, Diagnostic Findings, Head Trauma

H46 Butyrylfentanyl and Acetylfentanyl Levels in Driving Under the Influence and Overdose Cases

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After attending this presentation, attendees will better understand about fentanyl analogs acetylfentanyl and butyrylfentanyl and blood levels that may be consistent with intoxication and death.

This presentation will impact the forensic science community by enhancing knowledge regarding fentanyl analogs acetylfentanyl and butyrylfentanyl and their levels in fatal and non-fatal intoxications. This information can be used to determine if a given level of acetylfentanyl or butyrylfentanyl in the blood is clinically significant.

Analogs of the drug fentanyl are becoming increasingly common in the United States. While the potency of fentanyl is well known and recommended thresholds for therapeutic levels and toxic levels have been published, less is known about fentanyl analogs. A large number of butyrylfentanyl and acetylfentanyl overdoses and a smaller number of driving under the influence cases with butyrylfentanyl present have been seen. The goal of this paper is to assist toxicologists, forensic pathologists, and coroners in determining if a given level of butyrylfentanyl or acetylfentanyl is consistent with toxicity.

During the period of April 2015 to April 2016, 32 overdose deaths with butyrylfentanyl present and 10 overdose deaths with acetylfentanyl present were identified. During the same time period, 23 driving under the influence cases with butyrylfentanyl were identified. The level of butyrylfentanyl in overdose deaths ranged from 0.6ug/L to 129.8ug/L in the blood. When butyrylfentanyl was the sole drug identified in a drug overdose death, the levels ranged from 3.4ug/L to 85.7ug/L in blood. The level of butyrylfentanyl in driving under the influence cases ranged from 2.3ug/L to 51.4ug/L in blood. The level of acetylfentanyl in overdose deaths ranged from 1.5ug/L to 137.2ug/L in blood. There were no cases in which acetylfentanyl was the sole drug present. Other drugs present in the blood with butyrylfentanyl and acetylfentanyl included fentanyl, heroin (morphine), cocaine, oxycodone, hydrocodone, methadone, buprenorphine, trazodone, butalbital, phenobarbital, alprazolam, clonazepam, diazepam, quetiapine, sertraline, citalopram, pregabalin, diphenhydramine, and ethanol.

Fentanyl analogs, including butyrylfentanyl and acetylfentanyl, are observed with increasing frequency in the United States; however, there is little in the literature regarding blood levels in overdose and non-overdose situations. In this research, acetylfentanyl was never seen alone as a sole cause of death without the contribution of other drugs or medications. When it was seen alone, the lethal range of butyrylfentanyl seemed similar to that of fentanyl, and, like fentanyl, the level of butyrylfentanyl in non-overdose situations exhibited significant overlap with overdose levels.

Acetylfentanyl, Butyrylfentanyl, Intoxication

H47 A Comparison of Postmortem Serum and Vitreous Cortisol Levels

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The goals of this presentation are to: (1) describe the limitations of laboratory testing using postmortem samples; (2) identify clinical scenarios in which cortisol levels are useful in the determination of the cause of death; and, (3) understand why vitreous humor is not always a reliable alternative to serum testing in the postmortem setting.

This presentation will impact the forensic science community by demonstrating the variability and inaccuracies in quantifying vitreous cortisol as a surrogate test for serum cortisol levels.

Background: Accurate postmortem cortisol levels are of great importance in specific case types encountered by death investigators. The postmortem diagnosis of acute adrenal insufficiency, for example, is heavily reliant on circulating cortisol concentrations. Additionally, sudden death secondary to pituitary infarction or hemorrhage is increasingly reported, and the diagnosis may be greatly supported by low postmortem cortisol levels. Research has demonstrated that postmortem serum levels of cortisol reflect antemortem concentrations at or near the time of death; however, testing serum in the autopsy setting is often difficult due to the quality and nature of the specimens. Vitreous humor is considered a more stable postmortem specimen than serum for a variety of substances.

Purpose: This study seeks to determine whether vitreous humor is a potential alternative to serum for testing cortisol levels in the forensic autopsy setting. Per research, this is the first study that specifically quantifies vitreous humor cortisol concentrations as compared to serum samples drawn at the same postmortem time interval.

Methods: Thirty decedents of various ages undergoing routine autopsy were enrolled. Cases were excluded for insufficient sample quantity or quality. Postmortem samples of vitreous humor and heart or femoral blood routinely obtained during standard procedures were tested for cortisol via chemiluminescent immunoassay. The quantitative values of both serum and vitreous cortisol from each decedent were then plotted against each other graphically and a Pearson correlation coefficient was generated. The time between death and collection and the nature of the deaths were also analyzed in a similar manner.

Results: Nineteen men and 11 women were enrolled, with an average age of 46 years. The mean time interval between presumed death to collection time was 25 hours, with a range of 10-45 hours. All usual manners of death were represented in the study, which included two violent homicides. Serum cortisol levels ranged from 0.5ug/dL-67.1ug/dL. Vitreous cortisol levels ranged from 0.5ug/dL-26.3ug/dL. Significant variability in levels between individuals as well as between sample types for the same individual was identified. The case with the lowest quantity of serum cortisol had the same level detected in the vitreous; however, although the case with the highest serum level of cortisol also had the highest level detected in the vitreous, the difference in concentration between the two samples was 41ug/dL.

Plotting serum versus vitreous cortisol for each decedent yielded an R^2 value of 0.643; however, as serum cortisol rose greater than 25ug/dL, greater variability between samples was identified. Plotting the residuals against hours between death and collection yielded no significant correlation. Violent deaths or those with stimulant toxicity did not represent the cohort of decedents with the highest serum levels. Cases with serum cortisol greater than 30ug/dL included deaths attributed to pneumonia and oxycodone toxicity.

Conclusions: Although vitreous cortisol concentrations are typically lower than serum concentrations, the levels detected in the same individual varied widely. The generally positive correlation in the sample population is poor for accurate analytical testing. Numerous factors may contribute to this variation. Daily fluctuations in serum cortisol have been well documented and may contribute to the variability in the study sample. Additionally, a distinction between free and bound cortisol was not made, which may influence the ability of cortisol to concentrate within the vitreous humor. It remains unknown if a significant equilibration between serum and vitreous does occur and whether there is a calculable time course. Regardless, the vitreous cortisol level does not accurately reflect the serum concentration when drawn at the same postmortem interval and should not be utilized in isolation to estimate the serum level of cortisol after death.

Cortisol, Serum, Vitreous Humor

H48 Trends in Opiate Deaths — Summit County, Ohio: January 2009-December 2015

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After attending this presentation, attendees will understand the prevalence of opiate deaths and their public health significance.

This presentation will impact the forensic science community by increasing awareness of newly emerging synthetic drugs, deadly combinations of synthetic drugs, and the role of the medical examiner in promoting public health awareness with education and treatment initiatives.

From January 2009 through December 2015, the Summit County Medical Examiner's Office (SCMEO) in Akron, OH reported a total of 801 drug overdose deaths. Heroin and fentanyl cases together accounted for 74.3% of all overdose deaths in that time period. Nationally, there have been reports of increasing deaths due to heroin and fentanyl, and these cases will be the main focus of this presentation. The rise of overdose cases due to these drugs raises concern because fentanyl is approximately 50 times more potent than heroin, and its lipophilic nature enables a rapid transit across the blood-brain barrier to exert its respiratory depressive effects on the central nervous system in as little as six minutes. Most users begin with the intent of using just heroin, but are overdosing due to the product being adulterated with fentanyl without their knowledge, resulting in deadly outcomes.

A retrospective analysis for all drug overdose cases was conducted through the SCMEO database from January 2009 through December 2015. The database collected the case number, date of death, age, sex, race, manner of death, location of death, place of residence, cause of death, and the corresponding cases' toxicology results. Summit County has seen a 181% increase in overdose cases from 2009 through 2015, with a greater than 12-fold increase in fentanyl and heroin cases. The cases were reviewed and sorted based on toxicology results. Queries were also run for fentanyl and heroin blood concentrations. Groupings were determined based on the presence or absence of other illicit drugs. This study identified an upward trend in overall overdose deaths and fentanyl use. There was also an increase in the number of deaths from substances mixed with fentanyl and heroin; however, there was not an apparent trend in the quantity of fentanyl or heroin in the mixtures. Postmortem blood fentanyl concentrations ranged from 0.6ng/ml to 97.2ng/ml. Free morphine concentrations ranged from 20ng/ml to 3410ng/ml.

This current deadly epidemic poses serious public health concerns. As a result of these trends, there have been many state and county initiatives instituted to combat this dangerous drug trend. One of the main concerns in trying to combat this issue is that standard hospital toxicology screens do not detect fentanyl. This issue is problematic in a forensic setting when apparent overdoses present and the admission drug screen result is negative. This presentation will summarize the case findings over the previous six years, review the local and regional initiatives developed to combat the crisis, discuss the anticipated future trends, and include a review of the literature.

Fentanyl, Heroin, Drug Trends

H49 Periosteal Tracheal Granuloma: A Fatal Complication of Tracheostomy in Infants

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After attending this presentation, attendees will have learned about a rare complication of tracheobronchomalacia, tracheal granuloma, which is potentially fatal and can cause unexpected death in infants.

This presentation will impact the forensic science community by describing a short, never-described timeline of the appearance of the granuloma and its possible implications in the forensic investigation of unexpected infant deaths.

Respiratory diseases are among the ten most frequent causes of death in infants. Tracheobronchomalacia is one of these diseases. It is defined as a flaccidity of the tracheal support cartilage, which leads to tracheal collapse. This pathology can affect the trachea, the bronchi, or both. Treatment depends on the severity of the flaccidity of the respiratory tract. Most of the time, non-specific treatment is sufficient. Non-invasive ventilation is only a temporary measure. Tracheostomy is one of the treatments that can limit this collapse in severe cases, but it can lead to an inflammatory response. Reported here is the case of a nine-month-old girl who was suffering from tracheomalacia and had a tracheostomy after the neonatal period. She was found dead in her bed. The tracheostomy tube was not in place. Fifteen days before her death, a bronchoscopy was performed, which revealed a normal respiratory mucosa without inflammation. The tracheostomy tube was inserted correctly. An autopsy of the body and histology exhibited a complete obstruction of the tracheostomy opening by a hyperplastic tissue growth — a periosteal inflammatory granuloma. The rest of the autopsy was unremarkable. The cause of death was determined as a mechanical asphyxia due to obstruction of the upper respiratory tract by the granuloma and the accidental expulsion of the tracheostomy tube. A review of the literature provides some insight into tracheal granuloma as a complication of tracheostomy. It is more frequent after a surgical tracheostomy than after a percutaneous procedure, usually happens after some delay, and is rarely fatal. In this case, the granuloma was not seen 15 days before, during a bronchoscopy. This short timeline had never been described in the literature; a minimal period of four weeks is usually observed between either the procedure or a normal fibroscopy and the appearance of a granuloma. This granulation tissue develops in the edge of the ostium and in the wall of the tracheostomic tract. In this case, with the proliferation, it protruded internally into the tracheal lumen. Therefore, the obstruction appeared when the tracheostomy tube was accidentally removed. Granuloma is a complication that can happen more quickly than described in literature. Forensic pathologists should be aware of this infrequent, but potentially fatal, complication of tracheostomy in infants with tracheobronchomalacia.

Forensic Pathology, Sudden Death, Tracheal Granuloma

H50 Sharp Force Homicides — Men Versus Women

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After attending this presentation, attendees will understand the general differences of a number of inflicted wounds and locations on the victims' bodies between male and female perpetrators of homicidal sharp force deaths.

This presentation will impact the forensic science community by stressing the importance of documenting and reporting sharp force injuries to law enforcement to help streamline the process of identifying the suspect and potentially solving the murder.

It is suspected that typically, men aggressively inflict sharp force injuries multiple times all over the body and women more effectively inflict sharp force injuries fewer times, most often in the chest. Consequently, when assaulting their victims, men and women often differ in aggression by the number of sharp force injuries and the distribution of wounds on the body. If able to characterize homicidal sharp force injuries on a victim, an advantage for law enforcement officials may help identify the likely sex that inflicted the lethal wound or wounds and may help solve the crime.

Homicidal sharp force injuries in Cuyahoga County between 2003 and 2016 were reviewed in an attempt to identify patterns of wound characteristics on victims correlated with the known sex of the assailants. Original case files of the homicidal sharp force victims, including autopsy reports, and police reports as well as the Cuyahoga County Prosecutor's database, were reviewed. Data was collected and entered into an Excel® chart where the sex, based on convicted or suspected persons, of the assailant was noted as well as the classification of the victim's sharp force wounds, the number of wounds, and the number of regions on the body the wounds were inflicted. The regions of the body with wounds were divided into three categories: head/neck, trunk, and extremities.

Of 171 homicidal sharp force injury deaths between 2003 and 2016, 117 of which were male victims and 54 were female victims, 105 known male perpetrators and 56 known female perpetrators were identified. Data revealed that male perpetrators inflicted multiple sharp force wounds on male victims 38 out of 67 times versus a single sharp force wound, which occurred 29 out of 67 times. Of the 38 multiple wound victims, 8 were located in one region of the body, 17 were located in two regions of the body, and 13 were located in three regions of the body. Female perpetrators inflicted multiple sharp force wounds on female victims four out of ten times versus a single sharp force wound, which occurred six out of ten times. Of the four multiple wound victims, two were located in one region of the body, and two were located in three regions of the body. Female perpetrators inflicted multiple sharp force wounds on male victims 12 out of 46 times versus a single sharp force wound, which occurred 34 out of 46 times. Of the 12 multiple wound victims, five were located in one region of the body, six were located in two regions of the body, and one was located in three regions of the body. Male perpetrators inflicted multiple sharp force wounds on female victims 35 out of 38 times versus a single sharp force wound, which occurred three out of 38 times. Of the 38 multiple wound victims, 6 were located in one region of the body, 8 were located in two regions of the body, and 21 were located in three regions of the body. Ten cases remained unsolved without a clear indication of the sex of the suspect.

Determining the sex of the assailant is an important initial step in profiling the perpetrator of sharp force homicides. Therefore, classifying the typical characteristics and differences in the way men and women inflict sharp force wounds will aid largely in recognizing patterns within homicidal sharp force injuries, which could potentially be used as a resource for anyone involved in working sharp force homicide cases. When presented with a new homicidal stabbing victim, knowledge of the likelihood of the assailant's sex will be a crucial step in streamlining the process of identifying the suspect and potentially solving the case.

Sharp Force Injury, Homicide, Perpetrator

H51 U-47700: A Synthetic Opioid of Unknown Significance

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After attending this presentation, attendees will havelearned about the synthetic opioid U-47700 and blood levels that may be consistent with intoxication and death.

This presentation will impact the forensic science community by enhancing knowledge regarding synthetic opioid U-47700 and its levels in fatal and non-fatal intoxications. This information can be used to determine if a given level of U-47700 in the blood is clinically significant.

Synthetic opioids are becoming increasingly prevalent as drugs of abuse around the world. One of the newer drugs available is U-47700. U-47700 was manufactured by Upjohn in the 1970s for use as a short-acting opioid analgesic; however, it was never put on the market. As it was never introduced for medical use, there is no clinical data about the effects of the drug or its pharmacodynamics or pharmacokinetics. Over the past year, there have been increasing reports of the presence of U-47700 in toxicology specimens from fatal drug intoxications. Multiple intoxication deaths and driving under the influence cases with the presence of U-47700 in the blood have been seen. The goal of this presentation is to assist toxicologists, forensic pathologists, and coroners in determining if a given level of U-47700 is consistent with toxicity.

During the period of February to May 2016, there were 27 fatalities associated with a mixed drug intoxication that included U-47700. During the same period, there were nine driving under the influence cases that included U-47700. The blood level of U-47700 in overdose deaths ranged from 1.5ug/L to 45.2ug/L, and the blood level of U-47700 in driving under the influence cases ranged from 1.3ug/L to 27.3ug/L. There were no cases in which U-47700 was the sole drug present. In all of the deaths due to intoxication, the other drugs present (such as fentanyl) were at levels high enough to be expected to cause death on their own without the additive effect of the U-47700.

Despite the increasing prevalence of synthetic opioids such as U-47700 in the United States and around the world, little is known about their clinical effects, including what blood levels would be expected to be associated with an adverse event. Despite having numerous cases of multi-drug intoxications with U-47700 present, this study has not investigated any cases in which U-47700 was the sole drug causing intoxication. Further, in every case of death due to intoxication, there were other drugs present in the blood at levels expected to cause death on their own without the additive effects of the U-47700. Nine cases of non-fatal U-47700 intoxication were also investigated, giving an indication of blood levels that at least in certain individuals may not be considered high enough to cause a fatal outcome. Until a case of a pure U-47700 intoxication is discovered, it is unclear if this synthetic opioid is able to cause fatal intoxication by itself and, if so, what blood levels could be required.

U-47700, Synthetic Opioids, Intoxication

H52 Cardiopulmonary Resuscitation (CPR) -Related Fractures and Their Differences From Fractures Due to Blunt Force Trauma

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After attending this presentation, attendees will have gained an appreciation of the difficulties in discerning the differences between fractures resulting from CPR maneuvers and fractures due to other blunt force impacts to the chest.

This presentation will impact the forensic science community by comparing CPR-related fractures with other blunt force-related thoracic fractures in medical examiner cases as well as discussing the problems of interpreting these injuries.

Chest compressions from CPR are part of an emergency procedure combined with artificial ventilation in an effort to restore spontaneous circulation and breathing in a person who is in cardiac arrest. Fractures of the thoracic skeletal structures can be seen as a result of these maneuvers, both manual and mechanical.[1,2] Although these fractures are seldom consequential, their presence can complicate the interpretation of antemortem blunt force injuries. This study evaluates and compares patterns of rib and sternal fractures in blunt force trauma cases without CPR administration and in non-traumatic cases in which CPR was administered.

In this study, the fracture and visceral injury patterns of two groups of medical examiner cases were compared. One group consisted of 50 cases of natural deaths that had CPR (manual and/or mechanical) attempted. The other group consisted of 50 cases of blunt force trauma deaths (accidents, homicides, and suicides) that had thoracic injuries and did not have CPR attempted. Cases selected for review included decedents of 18 years of age and older autopsied at the West Tennessee Regional Forensic Center from 2013-2015. The autopsy reports were reviewed and locations of fractures and visceral injuries were charted. The differences in fracture locations between the two groups of cases were compared.

In the blunt force trauma cases, fractures were observed in ribs 1-12 in the costovertebral, posterior, posterolateral, lateral, anterolateral, anterior, and costosternal portions of the rib. Additionally, there were fractures of vertebrae (T3-L5), sacrum, pelvis, clavicles, and sternum. In a subset of 17 drivers of motor vehicles where frontal impact occurred (and assumed to be similar to the location of impact sustained from CPR attempts), fractures were seen in all ribs (ribs 1-7 were the most commonly fractured) and across all portions of the rib. In the cases of natural deaths in which CPR was attempted, only ribs 1-10 were fractured, with ribs 3-6 being the most frequently fractured. These ribs were only fractured in the costosternal, anterior, and anterolateral aspects of the rib. Costosternal fractures were only seen in cases of mechanical CPR by the LUCAS™ device. The incidence of sternal fractures was much higher in CPR when compared to blunt force trauma. No fractures other than rib and sternal fractures were observed in association with CPR. Visceral injuries were also compared. In the blunt force trauma cases, injuries to the lungs, heart and associated vessels, liver, spleen, Gastrointestinal (GI) organs, kidneys, and pelvic viscera were noted, with the lungs and liver being the most affected. In the cases of natural deaths where CPR was attempted, there was a rare instance of heart, liver, and splenic injury. No other organs were noted to have sustained injury. Hemorrhage associated with CPR-related fractures was also noted, with the highest incidence occurring with the LUCAS™ device.

In conclusion, this study demonstrates that there are fractures of the ribs and sternum in both cases of blunt force trauma and cases of natural deaths with CPR, but CPR-related thoracic skeletal injury does not extend past the anterolateral aspect of the chest wall; however, it may be difficult to differentiate between antemortem injury from trauma and peri-mortem injury from CPR within this area. Also, visceral injuries can occur with CPR, but are rare.

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CPR, Blunt Force Trauma, Fractures

H53 An Unusual Mechanism of Fatal Pediatric Abusive Head Trauma (AHT): A Case Report

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After attending this presentation, attendees will understand that an isolated linear parietal fracture in an infant may not be accidental, and that head compression is a possible mechanism for AHT.

This presentation will impact the forensic science community by describing a case of AHT due solely to head compression. This unusual mechanism should be considered during autopsy of infants with craniocerebral injuries or in the clinical assessment of infants with closed head injuries.

AHT is the leading cause of fatal injury in infants and is usually incurred by shaking or impact with a hand or blunt object. Impact injuries can produce skull fractures that may cross suture lines and involve multiple bones. In contrast, accidental head trauma more commonly produces isolated linear fractures of parietal bones and results from a fall or an impact from a falling object. This study investigated a case of fatal AHT, which presented with an isolated linear parietal fracture. Additionally, the mechanism of compression force has not been previously described in association with AHT.

A 3-month-old male presented to the Emergency Room (ER) after he was found unresponsive, cyanotic, and apneic. Upon arrival, there was no visible evidence of trauma despite a history suspicious for abuse. The infant was resuscitated after one hour of pulseless electrical activity. A Head ultrasound revealed extensive left frontal intraparenchymal hemorrhage with surrounding edema and midline shift. Head computed tomography confirmed the ultrasound findings and showed a unilateral right parietal fracture, left hemispheric Subarachnoid Hemorrhage (SAH), Subdural Hemorrhage (SDH) along the falx cerebri and tentorium, and diffuse loss of gray-white matter differentiation. Skeletal survey revealed no abnormalities. There were no retinal hemorrhages on ophthalmic exam. Video electroencephalogram displayed evidence of diffuse severe encephalopathy. Support was withdrawn on admission day five and the infant expired.

At autopsy, there was no external evidence of trauma to the body, head, or face. Reflection of the scalp revealed a complete absence of soft tissue hemorrhage. There was a full thickness 5cm-long linear right parietal fracture that extended laterally from the mid-sagittal suture. Within the calvarium, a left SDH, diffuse flattening of the left hemispheric gyri, and multifocal SAH were observed. Serial coronal sections of the fixed brain revealed a large cavity that involved the anterior half of the left cerebral hemisphere. The cavity contained a small amount of residual blood clot and was surrounded by marked necrosis. The cavity connected to the brain surface through a tear in the anterior left frontal lobe. There was no evidence of direct parenchymal injury to the right hemisphere. Following autopsy, investigators elicited a confession from the caretaker. He had become angry with the infant and admitted to pushing the infant's head into a supporting surface/structure. This correlates well with findings at autopsy. The stress from anteroposterior pressure would put maximum stress at the apex of the parietal bone; when the fracture occurred, integrity of the cranial vault was lost, allowing crush injury of the brain, manifested by SAH, SDH, and intracerebral hemorrhage.

This unusual mechanism of compression force does not readily fit into any known category of abusive head trauma. It is inherently different from typical impact abuse and shaking-type trauma. It also differs from accidental crush injury because the application time is longer and the amount of force is smaller. Practitioners of pediatrics, emergency medicine, and forensic pathology should be mindful of this unusual mechanism for AHT.

Parietal Fracture, Compression, Abusive Head Trauma

H54 Postmortem Micro-Computed Tomography (micro-CT) Scanning of a Fetal Heart: A New Protocol for Determining Cause and Manner of Fetal Death

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After attending this presentation, attendees will better understand the importance of a micro-CT study of fetal heart specimens, which provides highly accurate 3D renderings of complex congenital fetal heart disease.

This presentation will impact the forensic science community by offering an alternative to postmortem dissection that permits a diagnosis in small hearts when a conventional invasive autopsy would have severe limitations.

Abnormalities of the heart and great arteries are the most common Congenital Defects (CHDs). They account for approximately 20% of all stillbirths and 30% of neonatal deaths due to congenital defects. Furthermore, cardiac defects are seen at autopsy in approximately 10% of sudden deaths in infants and could be the cause of death in up to 84% of these cases. Fetal and pediatric cardiac autopsies play a crucial role in the counseling of parents in regard to both the cause of death of their fetus or child and the implications of such findings for future pregnancies. They also serve as a tool for assessing quality assurance of antenatal screening programs and antemortem diagnostic procedures. In the past ten years, there has been a shift in the diagnosis of CHDs from the second to the first trimester of pregnancy; however, the feasibility and value of postmortem confirmation that can result in the modification or refinement of the prenatal diagnosis is only apparent in about 30% of cases, and findings are severely limited in small fetuses. When a determination is attempted in such small specimens, erroneous findings are more likely to result, and these can prejudice the diagnosis. Missed cardiac defects and discordance from prenatal diagnosis may also raise the potential for litigation. Several groups have proposed alternatives to postmortem dissection, including Magnetic Resonance Imaging (MRI). The resolution offered by scanners used for conventional MRI in the clinical setting is too low for the successful examination of fetuses in the first half of the pregnancy. More promising results have been obtained with high field micro-MRI scanners designed for imaging small animals; however, like all MRI-based approaches, their use in a clinical setting is limited by cost, time, and space constraints. For these reasons, the method based on micro-CT was explored. Micro-CT is X-ray imaging in 3D by the same method used in hospital CT scans, but on a smaller scale with massively increased resolution. The prefix micro-CT is used to indicate that the pixel sizes of the cross-sections are in the micrometer range

Methods: Iodine contrast micro-CT scannings were used to examine 22 hearts. Images were obtained from routinely fixed whole human fetuses (n = 7, weights 0.1g-90g, gestational ages: 7-17 weeks), isolated fetal hearts (n = 14, weights 0.1g-20 g, gestational ages: 11-27 weeks) and one isolated heart in a neonate. Samples were scanned using an isotropic resolution of 18µm. Findings were interpreted jointly by four fetal pathologists, a fetal cardiologist, and a radiologist. Samples with gestational ages ≥13 weeks also underwent conventional autopsy or dissection. Postmortem assessments were compared to prenatal findings.

Results: Micro-CT identified all anatomical structures and abnormalities documented by the echographic prenatal examination. In all seven cases involving first trimester pregnancies, micro-CT excluded the presence of structural anomalies. In 14 of the 22 cases, it provided all the information obtained with invasive autopsy or dissection, and in seven of these 22, it furnished additional diagnostic details. In one neonatal case, which was the object of litigation for a missed diagnosis of a complex cardiac anomaly, the micro-CT 3D rendering proved useful as a basis for discussion among courtroom experts about the features of heart disease and associated clinical implications.

Conclusions: Micro-CT study is useful for refining or modifying prenatal diagnosis later in fetal gestation on in neonates. Micro-CT 3D renderings of heart disease have been found to be very useful when a second opinion about controversial specimens is required.

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Fetal Death, Fetal Heart, micro-CT

H55 A Case of Sudden Death in Twin Infants Given Melatonin Supplementation: A Challenging Interpretation of Postmortem Toxicology

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The goal of this presentation is to raise awareness for the need to perform postmortem toxicological analysis for melatonin in cases of unexplained infant death in which the investigation suggests over-the-counter supplementation.

This presentation will impact the forensic science community by increasing awareness to the fact that, with heightened recognition for testing and reporting data for postmortem melatonin in infants, reference ranges could be established to aid with the interpretation of postmortem levels. This information may ultimately aid with the certification of cause and manner of death.

Melatonin is a compound normally produced by the pineal gland of humans (and other animals) to regulate the sleep-wake cycle. It is also available as an over-the-counter product, with numerous preparations available. It is used to treat jet lag, shift worker syndrome, sleep disorders in the blind, insomnia, and various sleep cycle disturbances. Melatonin is also administered intravenously in the Neonatal Intensive Care Unit to premature infants to regulate respiratory efforts. When given exogenously to adults, it is believed to cause few adverse effects. Headaches, drowsiness, and nausea have all been reported in the adult population. There are few reports of significant toxicity in the literature, and the pathogenic mechanism is not yet fully understood. To date, there are no reported fatalities from this compound. The following illustrates a case of unexplained infant death with increased levels of melatonin discovered on analysis of postmortem samples.

A 3-month-old female was found unresponsive in her crib with her surviving twin. Autopsy demonstrated no anatomic, traumatic, or infectious cause of death after a complete gross and histopathologic examination aided by full-body radiology and microbiology. Interviews with the parents revealed that the twins were both given eight to ten dissolvable tablets of melatonin, 5mg dosages, per day. Investigators discovered more than 20 bottles of over-the-counter melatonin at the scene, in various preparations. Postmortem toxicological analysis of the blood using Liquid Chromatography/Mass Spectrometry (LC/MS) revealed a melatonin level of 1,400ng/mL. Two tablets recovered at the scene were analyzed by LC/MS and Gas Chromatography/Mass Spectrometry (GS/MS) and revealed melatonin 3.7mg (+/- 0.15mg per tablet reported as free base) and 5.1mg (+/- 0.21mg per tablet reported as free base). The remainder of the complete toxicological analysis was negative.

The level of melatonin identified in the blood of the decedent was greater than 5,000 times the endogenous level of this hormone found in an adult (typically 0.02ng/mL – 0.2ng/mL based on the time of day and age). Oral use of 6mg of melatonin in 60 adult females produced an average peak concentration of 12ng/mL. While endogenous melatonin levels are likely higher in the pediatric population, no reference ranges currently exist for normal blood concentrations. Thus, in cases such as this of sudden unexplained infant death after a complete postmortem examination only positive for presumably elevated melatonin, the cause and manner of death are best certified as undetermined due to a lack of suitable pediatric reference ranges.

With the ubiquitous nature of over-the-counter melatonin, numerous formulations, unclear dosage recommendations, and poor manufacturing regulations, effort must be placed in testing for this compound in unexplained infant deaths and reporting the results to the forensic community. The establishment of a blood reference range for melatonin in the pediatric population would be invaluable in the evaluation of potential toxicity.

Melatonin, Natural Supplements, Interpretation

H56 It's Still the Wild West: A Case Report and Review of Arrow-Related Deaths in Oklahoma

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The goals of this presentation are to: (1) highlight the types of injuries sustained by crossbows as well as the different characteristics involved in crossbow deaths; (2) reiterate the need for adequate radiologic surveys when dealing with all projectile-related deaths for both accuracy and the safety of personnel working on the case; and, (3) delineate the differences between a bow and a crossbow and describe the different types of arrowheads commonly used.

This presentation will impact the forensic science community by introducing a unique method of suicide as well as details regarding the extensive injuries that can be sustained in a crossbow-related death. It is critical that all projectile-related deaths have proper radiologic surveys before beginning the autopsy.

Introduction: When one thinks of projectile injuries and death in a forensic setting, one's first thought is inevitably about bullets, pellets, and a gun that fires them; however, projectile injuries are not always induced by said ammunition. Different types of bows and crossbows that fire arrows and bolts are still often used for archery and hunting. They can also inflict injuries seen in decedents. This report describes a case of a 51-year-old White male who died after a self-inflicted crossbow injury and a review of the Oklahoma Office of the Chief Medical Examiner's (OCME's) electronic database for similar cases.

Case History: A 51-year-old White male was found unresponsive in his residence, seated in a chair with a crossbow between his legs and an arrow protruding from his neck. Shortly before, his wife had left the residence after a domestic dispute and went to a neighbor's house. This neighbor later found the man in the aforementioned position and summoned emergency personnel, who transported him to a hospital where he was pronounced deceased.

Results: The postmortem examination demonstrated a 21.75-inch-long arrow that perforated the skin and soft tissue of the left anterior neck and submentum before entering into the oral cavity. The arrow bypassed the tongue and perforated the soft palate and bones of the left cranial fossae before entering the cranial cavity. Grazing the brainstem, the arrow perforated the left cerebellum, left occipital lobe, left parietal lobe, and left parietal bone before penetrating the scalp. In addition to hemorrhage and maceration of the brain along the wound path, subdural hemorrhage, subarachnoid hemorrhage, and cerebral contusions were also identified. The arrow, a fragmented arrow tip, three retractable arrow tip blades, and metallic fragments were recovered. The cause of death was determined to be "Crossbow wound of the head and neck" and manner of death was "Suicide."

Discussion: Projectile injuries are common in a forensic setting; however, as a database query revealed, projectile injuries from arrows are quite rare. Multiple variously worded searches of the Oklahoma OCME electronic database revealed only three additional cases (two suicides and one homicide) with similar causes of death (sharp/arrow injuries of the head or chest) from its implementation in January 2000 to March 2016. Although approached much like any other type of projectile injury, an arrow is relatively unique given the potential for multiple detached sharp metallic parts and fragments, as seen in this case. Performing an adequate radiologic survey of the wound path before the postmortem examination is paramount to prevent injuries from occurring during evidence recovery.

Bow, Arrow, Projectile

H57 The Long and Short of It: A Skeletal Trauma Analysis of a Gunshot Wound Suicide From a .22 Caliber Long Rifle Round Shot Through a Handgun

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The goals of this presentation are to provide attendees with: (1) information concerning the diagnostic characteristics related to gunshot wound trauma to the head; (2)the order of operations implemented when law enforcement, forensic pathologists, and forensic anthropologists work on a case; and, (3) the logical and objective reasoning used to determine the manner of death.

This presentation will impact the forensic science community by filling a gap in the literature related to rifle ammunition that is shot from a handgun in cases of suicide.

This presentation discusses the analysis of trauma resulting from a suicidal gunshot wound to the skull. This study draws attention to the process of differentiating low-velocity projectile trauma from blunt force injuries while reinforcing how cooperation between the medical examiner, skeletal analysts, and law enforcement investigators leads to case resolution.

In 2015, the severely decomposed remains of a European (White) male were discovered within a wooded area in Venice, FL. A handgun was found at the scene. The forensic autopsy was conducted and the preliminary finding was a gunshot wound of the head; however, because of the extensive fracturing of the skull, the co-occurrence of blunt force trauma to the decedent's head could not be excluded. In addition, the positive identification needed to be established using an antemortem radiographic comparison. With these analysis needs in mind, forensic anthropological analysis was conducted at the request of the medical examiner.

Following best practices outlined by the Scientific Working Group for Forensic Anthropology (SWGANTH), the remains were macroscopically examined, photographed, and radiographed prior to rendering. After the maceration process, the fracture margins were evaluated using a 300X dissecting microscope; the skull was reconstructed using a quick-drying glue; the skull was radiographed and photographed post-reconstruction; the fracture lines were mapped onto anatomical figures. The examination revealed one entrance wound to the right temporal squama as evidenced by internal beveling while the exit wound with external beveling perforated the left temporal squama. Based upon the location of these entrance and exit defects, the projectile moved from right to left with a slight anterior to posterior trajectory. Multiple radiating fractures marked both defects and ran across the orbital plates, maxilla, and basilar portion of the cranium. Radiographic analysis revealed evidence of bullet wipe within the entrance and exit wounds as well as within the anterior cranial fossa.

Further information from law enforcement regarding the caliber of the gun and ammunition revealed that a Ruger® 22/45 (Model P512) was recovered near the body. Low-velocity projectiles are associated with a variety of handguns as well as 0.22 caliber rimfire rifles with muzzle velocities ranging from 650 to 1,400 feet per second. The 0.22 caliber Long Rifle (LR) round is the most powerful of the 0.22 short and long caliber ammunition. The injuries associated with these types of low velocity projectiles occur within the tissues and organs directly in the path of the projectile; although, 0.22 LR cartridges usually produce secondary fractures within the hard tissues — especially when the projectile impacts the head from a contact position. The pattern of low-velocity projectile injury contrasts markedly to the injury patterns that typify military ammunition or high-velocity (≥2,000 feet per second) center fire rounds (≥0.17 caliber) where tissue and organ damage can occur outside the projectile's path.

Although both gunshot and blunt force injuries create radiating and concentric fractures, they differ fundamentally in their direction, magnitude, and rate at which force is applied. For example, the rate of blunt force trauma is slow loading, allowing the bone to fail (or bend) over time, thereby creating plastic deformation, delamination, and sometimes tool marks. In this case, the radiating fractures, lack of delamination, fracture propagation, and the presence of beveling allowed us to rule out blunt-force traumato be ruled out. Therefore, the extensive fractures to the cranial vault, maxilla, and basilar portion of the skull were consistent with 0.22 caliber LR ammunition. The 0.22 caliber LR ammunition has a much longer cartridge and, as such, produces more gas at the time of discharge.

The discharge of the firearm in combination with the expansion of the gases confined within the cranial vault produces extensive fractures in a contact wound suicide.

Upon further communication with law enforcement and due to the evidence provided by the District 12 Office of the Medical Examiner, the decedent's skeletal remains were identified and it was confirmed that the skeletal trauma associated with a peri-mortem gunshot wound was consistent with a 0.22 caliber LR projectile.

Gunshot Wound, Suicide, .22 Caliber Long Rifle

H58 Fatal Tree Surgeries

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The goals of this presentation are to review workplace deaths related to tree surgery and to evaluate how these compare at the time of investigation, autopsy, and toxicological analysis to the literature on workplace deaths due to falls from heights.

This presentation will impact the forensic science community by comparing investigative and autopsy findings involving workplace deaths related to tree surgery to the literature on falls from heights in order to guide scene investigation and autopsy performance.

This presentation will inform attendees of patterns of injury in two workplace deaths related to tree surgery and the extent to which internal injuries can be correlated with external injuries in such deaths.

Fall from height is the second-leading cause of fatal workplace injury in the United States and head injury is the most common cause of fall-related death.[1-3] Reported here are two cases of fatalities due to falls related to tree surgery.

Case 1: A 49-year-old male tree surgeon, after trimming branches at 10-15 feet above the ground, was found on the lawn in full cardiac arrest. There was a history of alcoholism with seizures, but no heart disease. One coworker stated the victim fell from a ladder while reaching for a branch; another, that he fell out of the tree while using the chain saw. The orientation of the body was poorly documented in relation to the ladder, chainsaw, and tree. Reports conflicted on whether he had been drinking alcohol.

At autopsy, superficial injuries of the head and extremities were noted, with stasis dermatitis and pitting edema. Frontal, occipital, and left parietal subscalpular hemorrhage was not associated with skull fracture. Subarachnoid hemorrhage was limited to the posterior cerebellum. Neck dissection revealed fractured third and fourth cervical vertebrae, with partial spinal cord transection. Natural disease included hypertensive cardiovascular disease, coronary atherosclerosis, macronodular cirrhosis, and a previously undiagnosed right lobe hepatocellular carcinoma. Cause of death was ruled blunt force trauma to the head and neck and the manner of death was accident.

The inciting event was not identified; whether a seizure, acute cardiac event, judgement error due to intoxication, or other event such as slipping while reaching out with the chain saw. The site of primary impact to the head (frontal or occipital) was not clear from scene investigation and was not fully clarified by autopsy.

Case 2: A 38-year-old expert tree surgeon was working in a harness 25-30 feet above ground when the tree broke at the base, carrying him to the ground with it. Limited investigation was unclear whether he landed on the tree or the tree on him; his exact position was not recorded. The victim's girlfriend said he drank a "few beers" just prior to the tree surgery.

At autopsy, external injury was limited to the face; subgaleal and subarachnoid hemorrhage were not associated with skull fracture. There were lacerations through the left ventricle, the aorta, the lung, and the liver with multiple rib fractures and transection of the left renal vessels. A fracture of the anterior bodies of C2 and C3 suggested terminal spinal cord injury without transection. Cause of death was ruled multiple blunt force trauma and the manner of death was accident.

Discussion: According to published studies, in falls from 0-20 feet, most victims undergo primary head impact, and cranio-cerebral damage and cervical spine fracture are common.[2] External trauma in fall victims is concentrated in the region of primary impact; fatal head injuries are twice as likely to occur in conjunction with cervical fractures of C3 and below if the victim was inverted at the time of primary head impact.[4,5] Abdominal, thoracic, and limb injuries are more common when the fall is over 21 feet, regardless of site of primary impact.[2,3] In falls from greater heights, spinal fractures, particularly cervical, remain common when the primary impact site is the victim's head; primary head impact should be suspected when the victim presents with severe head injury and comparatively minor injuries to the rest of the body.[2,3,6] The liver is the most common solid organ to be injured in a fall; the

likelihood of liver injury increases with increased height of the fall.[7] Alcohol consumption is a major predisposing factor to any accidental fall from height.[8]

These cases concord with this literature review and confirm that the injury in fatal tree surgeries is primarily due to fall from height; external examination is likely to result in an underestimation of internal injury.

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Tree Surgery, Falls From Heights, Workplace-Related Death

H59 Suicides Using Atypical Methodology: A Case Series

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After attending this presentation, attendees will appreciate a wider range of suicidal methods, as well as the continued increase in the suicide rate in the United States.

This presentation will impact the forensic science community by increasing awareness of atypical devices — a sports utility vehicle, a hospital call-button cord, a homemade floor-mounted stabilizer, a nail gun, a meat thermometer, and a combination of a rotary saw and a neck tie — that may be used in committing suicide.

Suicidal acts are the tenth leading cause of death in the United States. Since 2009, there has been an increase in suicides in nearly all age groups nationwide. Men most commonly commit suicide by firearms, while women most commonly use poison/intoxication. Asphyxiation, which includes hanging, strangulation, and suffocation, accounts for approximately one in four suicides for both males and females. Suicides under the "other" category include cutting/piercing, drowning, falls, and fire; these comprise approximately 7% and 9% of suicides for males and females, respectively. For both sexes, falls from a height were the most common "other" methodology. Nonetheless, various measures may be employed by either sex. Since 2009, the suicide rate in Maryland has remained relatively stable, ranging from 5.4%-5.8%. The most common method of suicide in Maryland from 2009 to present was by firearms. Asphyxiation accounted for approximately 35% of suicides in Maryland, higher than the national rate.

To date, there have been case reports of suicide achieved by devices ranging from manual and power drills to complex cases involving multiple methods at once with cutting of the wrists, electrocution, and drowning. Maryland, has had cases of suicide with devices ranging from a meat thermometer and nail gun to the combined use of a rotary saw and hanging.

This presentation presents uncommon means of committing suicide and the injuries associated with each, expanding the compilation of reported atypical suicides. Case 1 is that of a 40-year-old White male who used a rope, fire hydrant, and his jeep to achieve decapitation. Case 2 is that of a 21-year-old White male who used a rope attached to the front tow hook of his sports utility vehicle and a tree to hang himself. Case 3 is that of a 76-year-old White male who hung himself using a hospital call button cord and a hospital lift. Case 4 is that of a 52-year-old White male who constructed a wooden mount screwed to the floor to stabilize and aid in discharge of a shotgun. Case 5 is that of a 53-year-old White male who discharged a commercial-grade nail gun at his left temporal region. Case 6 is that of a 44-year-old White male who stabbed himself through the right temple with a meat thermometer. Case 7 is that of a 51-year-old White female who used a combination of a rotary saw and a neck tie to end her life.

Presented is a case series of multiple suicides implementing unusual and atypical devices. Suicides can be very controversial cases, often times with family members contesting or questioning the mindset of their loved one. This issue is compounded in cases implementing an atypical method. Thorough and adequate investigation of the scene and case is a vital component in the decision-making process of the forensic pathologist. Additionally, the autopsy results must corroborate the results of the investigation in order for the forensic pathologist to arrive at a determination of suicide. It is therefore essential that law enforcement and forensic pathologists be aware of atypical and rare techniques for committing suicide and consider suicide as a potential manner in such cases.

Atypical Methods, Suicide, Device

H60 Fatal Propeller Injuries in a Non-Marine Context: A Work-Related Accident by a Concrete Mixer

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After attending this presentation, attendees will understand the importance of the interpretation of trauma patterns in determining cause and manner of death.

This presentation will impact the forensic science community by demonstrating that rapid identification of patterned lesions by the forensic pathologist can aid in the investigation and resolution of injury and death cases and that sometimes peculiar patterns are related to unusual investigative scenes.

Propeller injuries are rare reported events in forensic literature.[1] They usually result from nautical accidents, during recreational water facilities such as water skiing, boat racing, and skin and scuba diving.[2] Contact between a rapidly rotating propeller and a human body results in multiple impacts of great force on the human body within a split second.[3] The propeller has what is referred to as "blades" and makes linear lacerations in soft tissue that lead many to assume that the trauma is sharp. Propeller injuries have been described as hack or chop injuries.[4,5] The sharp propeller blades rotating at high speeds cause multiple and serious injuries such as deep lacerations, chop wounds, bone fractures, and mutilation of extremities. The available literature reveals an overall fatality rate of 15%-23%, and a similar rate of major amputation. Half of the deaths resulting from propeller injuries occur at the scene of the accident.[3] Work-related injuries are a societal burden worldwide. Approximately 337 million people have lost their lives to occupational accidents and more than 2.3 million die of work-related accidents or diseases each year.[6] A concrete mixer is a device that homogeneously combines cement, aggregate, such as sand or gravel, and water to formconcrete. A typical concrete mixer uses a revolving drum with a big propeller to mix the components.

Presented here is a case of a 32-year-old worker in the concrete industry. He was intent on washing the concrete mixer after the concrete production when he fell into the drum. The scene revealed that the body of the worker got struck by the blades. His trunk was in hyperflexion with the legs close to the head. The internal walls of the concrete mixer and its blades had diffuse bloodstains.

The external examination of the body exhibited the presence of cement dust on the worker's clothing (overalls) and on the skin. At the right frontal-parietal side and at the right zygomatic region were two large red bruise lesions with an irregular shape. The abdominal wall presented a laceration wound approximately 38cm long, directed from top to bottom and from left to right. This laceration permitted a nearly complete abdominal evisceration. This wound ended in the bilateral gluteal region and determined a comminuted fracture of the sacral-coccigea bones. The borders of these wounds were regular, with hemorrhage infiltration, and showing a wide diastase. The upper and lower limbs had numerous and irregular abrasions and lacerated lesions.

In conclusion, this case demonstrates that a pattern of lesions commonly related to mechanical injury needs an accurate scene investigation and external examination to arrive at a correct forensic interpretation.

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Patterned Injuries, Propeller Injuries, Concrete Mixer

H61 Severe Anogenital Injuries in Prepubescent Girls: Accidental Trauma Versus Sexual Abuse

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After attending this presentation, attendees will better understand the importance of recognizing the unique characteristics that distinguish accidental trauma from trauma associated with sexual abuse.

This presentation will impact the forensic science community by increasing understanding of the variables that are important for clinicians who evaluate anogenital injuries and who must provide input for confirming or refuting acts of sexual abuse.

The diagnosis of child abuse is based on the child's narrative associated with a general and anogenital inspection by a specialized medical provider. The genital examination, using a colposcopy and a camera, is performed with a separation and traction technique, while the young patient is in a supine and knee-chest position. The examiner tries to identify the most suggestive patterns of abuse, such as those described in the 2016 Adams Classification: (1) the complete hymen absence or the loss of posterior hymenal tissue; (2) hymenal lacerations; (3) external genitalia bruising; and, (4) scars of the posterior fourchette.[1] This presentation includes a brief review of the literature and describes two cases of severe genital wounds in prepubescent girls, each with a different etiology.

A review of the literature on anogenital traumatic injuries was conducted. The research was performed using the PubMed® electronic database with the following algorithm: accidental genital trauma and hymenal injury. Only five articles were found, underlining that traumatic hymenal injuries, such as lacerations or lacerated and contused wounds, are considered nearly pathognomonic of child sexual abuse. This seems to be the apparent conclusion, despite the unintentional genital traumas, such as straddle injuries, which can involve anogenital and perineal areas or external genitalia; however, injury of the hymen is felt to be a very rare event since this structure is well protected. Moreover, since it is shrouded by the labia and is essentially internal, the hymen is extremely unlikely to be injured during a straddling fall.

In this presentation, two suggestive cases of children's genital traumatic wounds were observed in the dedicated child abuse unit called "Bambi," which is located within Ospedale Infantile Regina Margherita, a pediatric hospital in Turin, Italy. Because of the severe wounds, both of these female children required surgery. The first case involved a 7-year-old girl who was brought to the emergency department with perineal bleeding. Her mother explained that her child slipped and fell astride the shampoo bottle while she was showering. During the examination, normal prepubertal genitalia were observed, with no evidence of vaginal trauma. The annular hymen was intact. The anogenital area was void of apparent injury, but was contaminated by fecal material. The wound was at the left-side margin of the perineal raphe, at about 1cm from the posterior labial commissure, and displayed a continuous oval appearance; it was approximately 4cm in length. The lesion was posteriorly extended up to the anal canal; wound margins appeared linear and close to this wound. There were also irregular ecchymosed injuries. The second case concerns a prepubescent girl who was presented to the emergency department with vaginal bleeding, needing surgery. In this case, the genital inspection revealed the near-total absence of hymenal tissue, a posterior vaginal wall tear that extended from the vaginal vestibule, also affecting the fork toward the inside of the vaginal canal, and continuing externally towards the median raphe for a length of approximately 10mm-13mm, without affecting the anal margin. Confirming the literature and the Adams Criteria, in the first case the perineal injury exhibits characteristics consistent with an accidental cutting wound (net margins, front tail) with perineal and rectum implications, but without the hymenal involvement.[1,2] In the second, instead, the genital wound refers to penetrating vaginal trauma after sexual abuse, with apparent hymenal and fourchette lesions.

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Genital Trauma, Child Abuse, Hymenal Injuries

H62 Victim Characteristics and Injury Patterns Associated With Intimate Partner Sharp Force Homicides

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After attending this presentation, attendees will be able to summarize the patterns of injuries associated with intimate partner sharp force homicides.

This presentation will impact the forensic science community by assisting law enforcement in narrowing the pool of potential suspects in sharp force homicide cases by identifying patterns of injury commonly seen in specific victim-perpetrator relationships.

Background: While it is known that females are more likely to be victims of intimate partner homicides and males are more likely than females to be killed by a stranger, the variability in injury pattern by victim sex in the context of intimate partner sharp force homicides has not been extensively studied.

Methods: A retrospective analysis was performed of the available forensic autopsy records for sharp force homicides perpetrated by adults in the populations served by Sparrow Forensics and the Department of Pathology in the Western Michigan University School of Medicine, which were either adjudicated or abated by suicide for the period from 2006 through June 2016. Law enforcement reports, court records, media coverage, and investigative reports from the medical examiner's office were reviewed. The victim-offender relationship, location of injuries, and number of injuries were analyzed.

Results: Among the 54 sharp force homicide cases analyzed, 14 cases were excluded, including 2 cases with multiple assailants, 4 cases perpetrated by juvenile offenders, and 8 cases in which a suspect was not identified. Of the remaining 40 cases, there were 24 male victims and 16 female victims.

Ten of the male victims were killed by female perpetrators. Nine of the ten cases perpetrated by females against males occurred between intimate partners, and the tenth case occurred between neighbors. None of the male victims killed by a female perpetrator suffered more than three wounds, no injuries occurred above the clavicles, and 90% of the victims suffered a single stab wound of the chest. Fourteen of the male victims were killed by male perpetrators, and the relationship between victim and perpetrator included relatives (n = 4), neighbors (n = 4), acquaintances (n = 3), and one case each of murder for hire, love triangle, and roommates. Six of the male victims killed by male perpetrators suffered a single sharp force injury, with five of the injuries occurring on the chest only. Victims who suffered more than five sharp force injuries were more likely to be related to their attackers (n = 4/7) and to have injuries occurring on the head, neck, or face (n = 4/6).

All sixteen female victims were killed by male perpetrators and included intimate partners (n = 9), strangers (n = 3), acquaintances (n = 3), and neighbors (n = 1). Two of these victims suffered a single stab wound of the neck. Two victims suffered two sharp force injuries, and in each case at least one of the injuries was of the head, face, or neck. The remaining victims (n = 12) suffered between 4 and 85 sharp force injuries, with the highest number of injuries occurring in victims who were strangers or acquaintances to their attackers. Eleven of sixteen female victims suffered at least one sharp force injury to the head, face, or neck. Seven of nine females killed by male intimate partners suffered more than one sharp force injury with at least one injury occurring on the head, face, or neck.

Conclusions: Patterns of injuries in sharp force homicides vary by the sex of the assailant and the relationship to their victim. Patterns of injuries inflicted by female perpetrators are different than those inflicted by male assailants. In the context of intimate partner violence, male perpetrators are far more likely to inflict injuries above the clavicles, and female perpetrators are far more likely to inflict a single sharp force injury of the chest.

Sharp Force, Victim Characteristics, Injury Patterns

H63 An Unexpected Toxicological Finding in an Infant: A Case of Chronic Intrauterine Fluoxetine Exposure

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After attending this presentation, attendees will understand the importance of a full forensic investigation in apparent sudden infant deaths. Attendees will also be informed regarding fluoxetine and its metabolite, norfluoxetine, from toxicological results that implicate possible drug toxicity in an infant.

This presentation will impact the forensic science community by discussing a specific case that emphasizes the need for additional investigation in a case of infant death.

Fluoxetine is a commonly prescribed yet atypical antidepressant that has been available for depression management since 1987[1]. Fluoxetine increases the availability of serotonin within the Central Nervous System (CNS) by inhibiting its neuronal uptake. Fluoxetine is absorbed in the gastrointestinal tract and metabolized in the liver to its metabolite norfluoxetine.[2] Less than 10% of the parent drug, fluoxetine, is excreted unchanged in the urine.[1] Fluoxetine and norfluoxetine have long half-lives, with detectable concentrations found in plasma long after drug discontinuation.[3] Single-dose studies have reported plasma elimination half-lives of 1-3 days for fluoxetine and 7-15 days for norfluoxetine; however, following the discontinuation of long-term therapy, plasma elimination half-lives of fluoxetine and norfluoxetine averaged 8 days and 19 days, respectively.[1]

This presentation reports a case of a one-month-old female found unresponsive in a portable crib/playpen. Following further inquiry of the mother, it was discovered that the infant was sleeping with the mother on an adjacent couch prior to being placed into the portable crib/playpen the previous evening. It is believed that the infant was alive at the time of this placement. She was delivered via cesarean section one month before the mother's due date and had no known medical history.

A subsequent complete postmortem examination yielded no immediate cause of death. Bacterial culture of Cerebrospinal Fluid (CSF) was negative and genetic testing was also negative. Microscopic examination displayed hyperexpanded alveoli in the lungs, but nothing to explain the sudden death of the infant. Toxicology was negative for nearly all substances, but positive for norfluoxetine in postmortem heart blood, with a quantitative result of 55.2ng/mL.

Subsequent investigation revealed that the mother had been prescribed fluoxetine throughout pregnancy and postpartum. The mother denied ever having breastfed the infant and additional investigation generated no indication that the mother had been administering the drug to the infant. Following further review of the literature, it was discovered that concentrations of fluoxetine could be detectable for more than four weeks after the discontinuation of the drug in patients with prescribed long-term therapy.[3] Concentrations of its metabolite, norfluoxetine, could be detectable for more than eight weeks after the discontinuation of the parent drug.[3] Although this information aids in the understanding of residual drug concentrations after long-term administration followed by discontinuation, intrauterine exposure has not yet been fully studied or documented.

This unusual toxicological finding in an infant was deemed incidental, due to chronic intrauterine exposure to fluoxetine. The immediate cause of death and manner of death could not be determined.

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Fluoxetine, Norfluoxetine, Intrauterine

H64 Bolt Gun Deaths — The Prevalence and Etiology in a Northern European Setting

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After attending this presentation, attendees will better understand the etiology and prevalence of bolt gun-related deaths in Sweden as well as autopsy findings associated with bolt gun injuries.

This presentation will impact the forensic science community by providing information on the prevalence, etiology, and associated autopsy findings of bolt gun-related deaths.

Bolt guns are devices used for slaughter in the meat industry. They consist of a cylindrical metal tube in which a bolt (metal rod) is placed. The cylinder is placed on the forehead of the animal and the rod is fired by the discharge of a blank round. After discharge, the bolt is retracted back into the bolt gun by spring tension.

Bolt guns produce different autopsy findings than regular firearms, namely a lack of explosive damage and a lack of exit wound.[1,2] Little is known about the demographics and variations in autopsy findings in human bolt gun deaths. The goals of this study were: (1) to expand the knowledge of autopsy findings in bolt gun deaths; and, (2) to examine the etiology and prevalence of such deaths in a north European medicolegal autopsy series. This study presents a series of all Swedish bolt gun deaths from 2007 through 2013, as well as a case report of a death from a bullet-loaded bolt gun.

The Swedish medicolegal autopsy database was searched for all deaths in which the Swedish equivalent ("slaktmask") of the word "bolt gun" was mentioned from January 1, 2007, through December 31, 2013. A complete autopsy was performed in each case. Police and hospital records were reviewed as well as the autopsy reports, including toxicological analyses.

There was a total of 17 cases during the study period, equivalent to 2.8 deaths annually, equivalent to 0.18 deaths per 100,000 citizens per year. These were 15 suicides, 1 homicide, and 1 undetermined manner of death. A majority (n = 15) of the decedents were male; the only two female decedents were one suicide and one homicide victim. The mean age of all decedents was 59 years (range 24-86, Standard Diviation (SD) 15) and, among the suicides, 62 years (range 41-86, SD 11). In four cases, the decedent survived initially but died later after admission to the hospital, with a survival time of, on average, one day (range 0-3 days). In all cases, the entrance wound was a circular defect in the skin and skull with an underlying cylindrical defect in the brain tissue. Most commonly, the entrance wound was located on the forehead (n = 9), followed by the crown (n = 4), the temple (n = 2), and the back of the head (n = 1). In the homicide case, the entrance wound was located on the neck. In seven cases, there were descriptions of secondary, radiating fractures of the base of the skull.

In one case, the decedent was found at a workshop next to his home with a bolt gun lying next to him. There was an empty 9mm shell casing lodged in the gun. At autopsy, a ragged circular defect on the forehead as well as a smaller L-shaped defect on the back of the head was noted. On internal investigation, there was a bullet trajectory in the brain, passing the left frontal lobe, slightly beneath the corpus callosum, and exiting the back of the left occipital lobe. There was a circular defect in the frontal bone with radiating fractures of the skull base as well as a smaller, uneven fracture of the back of the skull, also with radiating fractures to the skull base. This is, according to research, the first published death from a bolt gun loaded with a bullet, producing an exit wound while the core findings remained the same.

These findings are in line with the sparse previous literature regarding bolt gun deaths.[1,2] Common findings include a circular defect in the skin and skull with an approximately 15cm-deep cylindrical defect in the brain tissue. There may also be radiating fractures of the base of the skull. This presentation also describes a death from a bolt gun loaded with a bullet. In conclusion, bolt gun deaths are rare; however, they produce specific autopsy findings compared to regular firearm deaths.

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Forensic Science, Bolt Gun, Etiology

H65 Children's Hangings: A Case of an Accidental Event

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After attending this presentation, attendees will better understand the relevant roll of the forensic team in identifying (and notifying the pertinent authoritys of) potential risk and injury situations, especially those involving children and their environment.

This presentation will impact the forensic science community by drawing attention to the importance of scene investigations and interviews in cases involving accidental hanging, which may provide vital clues about the circumstances and etiology of the death, as in the presented case, in which doubts about the analysis of the death were raised by the public attorney.

Hanging is a type of violent death. Although most deaths by hanging have a suicidal medicolegal etiology, it's possible to have an accidental etiology; however, both suicidal and accidental hangings are considered uncommon in the pediatric age.

This study describes a case of an 11-year-old boy, whose autopsy was performed in the North Branch of the National Institute of Legal Medicine and Forensic Sciences, located in Porto, Portugal. Police records, social information, and autopsy data were analyzed.

The boy was found dead by relatives in his room from an incomplete hanging on a swing. No relevant personal or psychopathological background was found, nor any social concerns regarding his family. Attached to the police report, there was a product recall from the company manufacturer of the swing concerning to safety issues and injury risk.

The autopsy found the following: (1) a hanging mark on the left anterolateral aspect of the neck, incomplete, with a triple parallel groove, reddish, rising front to back and right to left and an incomplete single purplish mark, with the same orientation, on the right posterior aspect of the neck; (2) an ecchymotic mask ending in the hanging mark; (3) blood infiltration of the left sternocleidomastoid, sternothyroid bilaterally, and right omohyoid muscles; (4) a linear reddish area on the right, compatible with a swing strap abrasion; (5) epicardial blood suffusions; and, (6) diffuse cerebral edema. Toxicological screening was negative for alcohol, drugs, or medication. Histological lung findings (vascular congestion, multifocal alveolar distension, signs of recent intra-alveolar hemorrhage – petechial type) were consistent with a mechanical asphyxia. Thus, it was concluded that the cause of death was asphyxiation by hanging, with an accidental medicolegal etiology.

Although such deaths are uncommon, it is important to be aware of their existence in order to prevent their occurrence. Children, because of their curiosity and boldness, can very often put themselves in potential risk and injury situations. Hence, not leaving children unattended and paying special attention to toys and their materials, which could be potentially hazardous, are important to prevent most of these accidental deaths.

Hanging, Children, Accident

H66 Quantifying Physiologic Macrophages and Hemosiderin in the Dura Mater of Infants

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After attending this presentation, attendees will have an appreciation for the presence and distribution of macrophages and hemosiderin in infant dura mater under normal physiological circumstances. This will prevent misclassifying these macrophages as evidence of a three- to-four-day-old subdural hemorrhage in infants who demonstrate subdural hemorrhage at autopsy.

This presentation will impact the forensic science community by being one of the largest studies to microscopically examine non-traumatized infant dura mater and to directly focus on quantifying macrophages and hemosiderin and their distribution. Many other studies have described the features of evolving subdural hemorrhage; however, without well-defined characteristics of normal infant dura mater, it can be difficult to interpret the pathological significance of macrophages and hemosiderin when attempting to date a subdural hemorrhage. By defining the amount and distribution of macrophages and hemosiderin present under physiologic conditions, their significance in the context of subdural hemorrhage can be better understood.

Subdural hemorrhage is an important cause of mortality related to both accidental and homicidal injuries in infants and neonates. For medicolegal purposes, the ability to accurately estimate the age of a subdural hematoma at autopsy is of great interest. The first appearance of pigment-laden macrophages histologically is often referenced as evidence that the subdural hematoma is three to four days old; however, while the histologic features of evolving subdural hemorrhages are well described in the literature, the histologic features of normal, non-traumatized infant dura mater are not well defined. Further complicating the problem is that macroscopically normal infant dura mater, is not commonly examined histologically, and in contrast to adult dura mater, is more cellular and may contain macrophages. In order to accurately interpret the pathologic significance of macrophages, it is necessary to be aware of the number and distribution of macrophages present under physiologic circumstances.

This study examined the amount and distribution of pigment-laden macrophages present in 18 samples of dura mater taken at autopsy from infants without gross evidence of subdural hemorrhage or subdural neomembrane. Immunohistochemical staining with CD68 and staining with Prussian blue iron was performed to highlight macrophages and hemosiderin. The amount and distribution of macrophages and hemosiderin present in the macroscopically normal dura mater was then compared to 11 samples of dura mater taken at autopsy from infants with subdural hemorrhage or neomembrane.

Subdural Hemorrhage, Infant, Dura Mater

H67 Emerging Trends: Deaths Associated With the Novel Synthetic Opioid U-47700 at the Tarrant County Medical Examiner's Office in Fort Worth, Texas

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After attending this presentation, attendees will better understand the increasing trend in opioid deaths in the United States and specifically the presence of synthetic opioid analogs in forensic practices.

This presentation will impact the forensic science community by aiding public health agencies and law enforcement in addressing outbreaks of drug overdoses.

The Centers for Disease Control and Prevention reported in the January 1, 2016, Morbidity and Mortality Weekly Report that there was a 200% increase in the rate of opioid overdose deaths since 2000.[1] In addition, this Report notes that the age-adjusted rate of death from synthetic opioids other than methadone increased 80% from 2013-2014. There are many different synthetic opioids available on the internet, sold for research purposes, not for human consumption. These drugs may not readily be identified in forensic laboratories, as they are not on many general drug screens. This was the case in the laboratory at the Tarrant County Medical Examiner's Office (TCME) in Fort Worth, TX until 2016, when the standard for the synthetic opioid U-47700 was obtained from Cayman Chemicals and incorporated into the drug-testing library.

In 2016 at the TCME, there have been three cases in which the synthetic opioid U-47700 was identified. In two of those cases, the cause of death was associated with use of the drug. In the third case, the circumstances of death are still under investigation and further information is not available at this time. In all cases, initial enzyme-linked immunosorbent assay screening was negative for opioids.

The first case was a 38-year-old found unresponsive in the living room. He had a history of seizures, anxiety, and testicular cancer with hypofunction. Autopsy revealed pulmonary congestion and edema, mild cerebral edema, cardiomegaly, and moderate coronary atherosclerosis. A plastic bag containing white powder was found at the scene, reportedly field test positive for cocaine. This substance was analyzed in the forensic chemistry laboratory and was identified as U-47700. Toxicology testing on the decedent was positive for U-47700 in the femoral blood and urine by Gas Chromatography/Mass Spectrometry (GC/MS), as well as Tetrahydrocannabinol (THC) and ibuprofen in urine and alprazolam, 7 aminoclonazepam, and gabapentin in femoral blood. The cause of death was ruled as sudden death associated with synthetic opioid use with cardiomegaly as a significant contributory condition.

The second case was a 30-year-old male with a history of intravenous drug use and hepatitis C, found unresponsive at a friend's home. He was transported to the hospital and expired the next day. Hospital toxicology screen was reported positive for U-47700, THC, and amphetamine. Autopsy revealed pulmonary congestion and edema, cerebral edema with herniation, and moderate coronary atherosclerosis. At the scene, police found two syringes, a spoon with residue, and a plastic bag containing white powder reportedly labeled U-47700 that were brought to the forensic laboratory at the TCME for testing. The spoon tested positive for heroin while one syringe and the powder tested positive for U-47700. Hospital blood samples were tested by GC/MS and were positive for norfentanyl and U-47700. The cause of death was ruled sudden adult death associated with synthetic opioid use (U-47700) with coronary artery disease a significant contributory condition.

U-47700 (3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide), is a non-prescription synthetic opioid derived from AH-7921. AH-7921 was synthesized in the 1970s by Allen and Hanburys Ltd. as a potential analgesic medicine; however, development was abandoned due to addictive properties. In animals, it has m opioid receptor agonistic activities, though studies have not been performed in humans with activities similar to those of morphine, including analgesia, hypothermia, respiratory depression, and addictive behavior. U-47700 was synthesized from AH-7921 in the 1970s by Upjohn Laboratories and is currently marketed as a research chemical, not for human consumption. Due to structural similarity, the properties are assumed to be similar. Animal studies show a potency of approximately 7.5 times that of morphine. Recently, there have been a few cases of acute

intoxication associated with death reported in the literature, one due to the combined use of fentanyl and U-47700. There are no established reference ranges for this drug.

In summary, there is a growing problem of synthetic opioid use in the United States that can be associated with death. It is important for forensic pathologists to consider this in the setting of an apparent opioid death, even with a negative general opiate screen.

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U-47700, Synthetic Opioid, Opioid Overdose

H68 The Dark Side of Sudden Infant Death Syndrome (SIDS): When Isovaleric Acidemia Leads to Unexpected Death

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After attending this presentation, attendees will understand the causes of Sudden Infant Death Syndrome (SIDS), which are not yet clear to date.

This presentation will impact the forensic science community by demonstrating the importance of neonatal screening.

In neonates, fatal blood-chemistry changes could occur, such as Isovaleric Acidemia (IVA). This presentation will impact the forensic science community by illustrating that sometimes the causes of sudden deaths in the newborn are not always unknown, but may also result from acid-base balance alterations. Therefore, newborn screening is essential in order to prevent adverse outcomes such as sudden death, in particular in cases of consanguineous children with genetic mutations.

SIDS is a multifactorial disorder. The environmental risk factors are: prone sleeping, smoking during pregnancy, overheating, and co-sleeping. The biological risk factors may include mutations and polymorphisms in genes involved in metabolism (also in the immune system) and neurochemical alterations in the medullary serotonergic system. The genetic component of sudden infant death can be divided into two categories: mutations that are the cause of death or that might predispose infants to death in critical situations.

Newborn screening is a form of preventative health care that seeks to examine children in their first days of life to detect the presence of diseases whose main symptoms may not be obvious. The screening is performed on genetic, endocrine, metabolic, or hematologic diseases. The investigation of neonatal screening begins with the collection of blood samples from the baby's heel between the second and fifth day of life. If children are positive, surveys are necessary to start treatment within a few weeks after birth. The first case of a patient with isovaleric acidemia was described in 1966 and, several years later, was identified as a deficiency of isovaleryl-CoA dehydrogenase activity. Biological tests show a metabolic acidosis accompanied by increased blood ammonia concentration (hyperammonemia) and the reduction of calcium, platelets, and leukocytes. An important characteristic in isovaleric acidemia is an odor of sweaty feet due to the accumulation of isovaleric volatile acid. The treatment of patients with isovaleric acidemia is based on the restriction of a protein diet and on the administration, orally, of glycine and carnitine. Unfortunately, the isovaleric acidemia in neonates does not always cause important symptoms and signs; therefore, it cannot be recognized and will lead to death as in the case presented.

This study reports a case of sudden pediatric death in babies born to consanguineous parents of Indian nationality and residents in Calabria (Southern Italy). A 2-month-old baby was found dead in his home. This triggered the protocol for SIDS. At external examination, the infant exhibited abundant hypostases and lack of traumatic injury. At postmortem examination, the newborn had a smell of sweaty feet. Histological investigations were performed. At autopsy, the existence of an enlarged and edematous pancreas, with the peripancreatic exudates, confirmed the presence of acute pancreatitis. Analysis of the parents revealed consanguinity. For this reason, the forensic pathologist performed genetic testing, which confirmed the diagnosis of sudden death by isovaleric acidemia. This study has demonstrated that neonatal screening is characterized by simple and reliable investigations and, if the parents are consanguineous, the family must undergo genetic counseling before pregnancy to avoid fatal pediatric death and to improve neurologic and cognitive outcomes. Additionally, inserting the isovaleric acidemia among the cases of SIDS (poorly understood) is proposed as very often it is not preceded by recognizable signs and symptoms so this pathological condition can lead to unexpected and sudden death of the newborn. It was emphasized that investigations of sudden unexpected death are inconsistent, varying by jurisdiction and by the experience of the forensic pathologist. The addition of genetic testing to autopsy investigation substantially increases the identification of a possible cause of sudden death among neonates and infants.

Forensic Science, Isovaleric Acidemia, SIDS

H69 The Dark Side of Mothering: A Case of Infanticide

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After attending this presentation, attendees will be able to describe the impact of forensic investigations in cases of infanticide.

This presentation will impact the forensic science community by demonstrating the importance of a multidisciplinary approach with forensic pathologists, gynecologists, and anatomopathologists in order to clarify cases of doubtful abortions.

Filicide is the murder of a child by a parent, while neonaticide specifies the killing of a child on the day of birth. Although infanticide is often used to refer to as child homicide, the term has forensic implications and applies mainly to the killing of a child under the age of 12 months by a mother who has not fully recovered from the effects of pregnancy and lactation and suffers some degree of mental disturbance. Although maternal infanticide is a rare event, a high proportion of cases occur in the context of postpartum mental illness. This case report demonstrates that maternal mental illness is not always the cause of infanticide. Often the causes of infanticide can be financially motivated.

Reported is a case of a girl who went to the hospital with her lifeless fetus and placenta still in the uterus. The complete and spontaneous birth occurred on the same day at the hospital. The analysis of health records determined that in previous months the woman went several times to the hospital for abdominal secondary pain due to traffic accidents. Both the forensic pathologist and the gynecologist performed a forensic examination. During the consultation, the girl explained she had been involved in a car accident and, as a consequence of the impact, she gave birth. External examination of the woman didn't reveal traumatic injuries such as bruising, hematoma, bruises, abrasions, or lacerations. Speculum examination of the cervix revealed two lesions at the level of the anterior lip of uterine cervix, which were bleeding despite their cleaning with gauze. These injuries were attributable to fetal expulsion through the pinching of the cervix with surgical forceps. An ultrasound pointed out the absence of uterine disease or intracavitary pouring. Furthermore, an autopsy of the fetus was performed. External examination revealed that the fetus of 28 weeks had no malformations. The umbilical cord was inserted regularly and there were no abnormalities concerning it or the placenta. The examination of internal organs showed the presence of edematous lungs with subpleural and subepicardial petechiae. The histological examination revealed the occasional presence of squamous cells and amniotic fluid as gasping breathing, in the lungs. Therefore, pathologists confirmed that the fetus attempted to breath for few minutes. The maturity of the lungs in premature fetuses does not allow, in the absence of assisted ventilation, independent, physiological respiratory acts. Therefore, at the moment of fetal expulsion, it attempted respiratory efforts, which were demonstrated by the presence of subconjunctival petechiae and hemorrhage from the sclera, which resulted in acute respiratory failure and subsequent cardiorespiratory arrest. Fetal death was caused by a surgical abortion; the histological demonstration of respiratory attempts accomplished by the fetus after its expulsion asserts that this was a case of infanticide. The analysis of the clinical history and the psychiatric interview of the woman showed the absence of mental illness. The scientific investigations and the statements revealed the truth: the pregnancy had been used to stage an accident to collect the insurance on the death of the small fetus. Multidisciplinary and timely analysis in cases of doubtful abortions can determine the truth about the manner in which the event happened. The strict collaboration between the forensic pathologist, gynecologist, and pathologist played a key role. The postmortem diagnosis about the absence of fetal malformations and uterine pathologies, with a histological demonstration of gasping during ejection, confirm the vitality of the fetus and corroborate the suspects' statements.

Forensic Science, Pregnancy, Infanticide

H70 Pedestrian Road Trauma: The Role of the Judicial Inspection

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After attending this presentation, attendees will be able to describe the impact of forensic investigations in the reconstruction of pedestrian road trauma.

This presentation will impact the forensic science community by demonstrating the importance of judicial inspection and toxicological surveys to ascertain the driver's responsibility.

The number of traffic accidents has increased in the last decade and pedestrians are the most affected group. Collision with a pedestrian represents the most frequent type of road accident. It is often caused by a car and frequently involves the elderly. At autopsy, it is evident that the most common cause of pedestrian death is central nervous system injury, followed by skull base fractures, internal bleeding, lower limb hemorrhage, skull vault fractures, cervical spinal cord injury, and airway compromise. The attribution of accident responsibility can be realized through reconstruction of road accident dynamics, investigation of the scene, survey of the vehicle involved, and examination of the victim. Pedestrian collisions are very frequent, but often it is difficult to determine the dynamics of the car crash. The forensic pathologist plays a central role in determining the reconstruction of the accident. The correct results are obtained only by performing a thorough inspection of the vehicle, persons, or animals involved. Therefore, the forensic pathologist, in addition to determining the cause of death, must recognize the responsibility of the driver and the persons impacted.

This study reports two cases. The first was a case of an elderly man who was walking along a road when a Sports Utility Vehicle (SUV) knocked him down. The driver stated that the man was walking in the middle of the road. The body showed various contused and lacerated wounds at the level of the head (left parietal region) and face, with the presence of soil near the lips and on the entire face. The posterior right region of the singlet presented a large dark gray spot under which were bruises and abrasions. The inspection of the vehicle revealed the presence of a red cord (similar to the fabric of the man's shoes) at the lower portion of the right bumper. The right portion of the bonnet's bodywork was broken and presented hair fragments. The autopsy of the man revealed subarachnoid hemorrhage corresponding of the left (direct hit) and right parietal regions (rebound lesion). A chest examination revealed various rib fractures in the anterior right side and the presence of hemothorax, laceration of the descending aorta, and fracture of T6 and T7 vertebrae. Finally, a left tibia fracture was also revealed. In addition, a complete inspection was conducted on the implicated SUV. In this case, the evidence collected of the judicial inspection determined that the driver was not telling the truth because he was inattentive to driving. In the second, case, a young boy was involved in a pedestrian accident. The driver stated that the boy was walking at night in the middle of the road. In this case, the judicial inspection was not conducted since the victim was immediately transferred to the morgue. An autopsy was performed which showed multiple fractures and a severe spinal cord injury with brain hemorrhage. Toxicology revealed heroin and cocaine metabolites in his biological fluids. Therefore, the forensic pathologist concluded it was a pedestrian accident in which responsibility also fell on the pedestrian, who was under the influence of drugs although, in the absence of inspection, it has not been possible to determine the dynamics of the road accident and the real responsibility of the driver. In a pedestrian accident, it is fundamental to make an accurate survey of the scene. The autopsy alone cannot determine with certainty the dynamics of the event. The vehicle findings are important in order to locate the point of impact. Furthermore, it is important to perform toxicological investigations on the driver and on the victim, especially when a judicial inspection is not performed,, in order to reconstruct the dynamics of the collision and the responsibility of the driver.

Forensic Science, Pedestrian Trauma, Traumatic Death

H71 Suicide or Suspension of a Corpse? The Correlation Between a Hanging Mark and the Suspension Tool Used

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After attending this presentation, attendees will be able to describe the impact of the hanging mark and the differential diagnosis between suicide and suspension of the corpse.

This presentation will impact the forensic science community by demonstrating the importance of the correlation between the characteristics of hanging marks and tools of suspension found at the crime scene.

Introduction: Hanging is a type of asphyxial death occurring when the body is suspended in the air by means of a ligature. In most cases, hanging is due to suicide and only occasionally it is an accidental or homicidal event. Internal injuries of hanging are characterized by fracture of the hyoid bone and/or laryngeal cartilages, cervical fractures, muscle hemorrhages, and vascular lesions. At external examination, there are specific hallmarks of hanging. The wound examination is of prime importance in forensic pathology, and forensic pathologists are often asked to provide an opinion on how a wound was made and by what means. The most common types of traumatic skin injuries on the hanging mark are contusion (bruise), abrasion (scrape), laceration (tear), and blisters (vesicles) on the hanging mark. In particular, sulcus skin is the most common external sign of hanging. The sign of ligature is typically yellowish, such as leather, and the consistency is parchment-like; however, its characteristics depend on the ligature used. In order to demonstrate that the characteristics of the hanging mark vary in relation to the type of suspension and ligatures used, 20 cases of hanging marks are reported in which the association between the characteristics of hanging mark and the tool of suspension were analyzed. The purpose of this study is to illustrate that the characteristics of the hanging mark vary in relation to the type of suspensions and ligatures used. Also, this is important in order to distinguish a suicide from accidents and homicides.

Methods: This presentation reports on 20 cases in which the bodies were moved at the crime scene and occurred between 2013-2015 in an area of southern Italy. In these cases, the association between the features of hanging marks and the tools of suspensions and ligatures employed was analyzed. The analysis of the suspension tool was conducted through the evaluation of the nature of ligatures: soft, smooth, hard, and rough. Furthermore, a differential diagnosis between vital lesions of hanging marks and any other postmortem lesions was accomplished.

Results: Each suspension medium used was compared with the hanging mark. In nine cases, the correlation between soft and smooth ligatures with a hanging mark without abrasions is shown; the hanging mark presented a deep compression of the neck. In eleven cases macroscopic characteristics of the brownish hanging mark with hard and rough ligatures was correlated. In seven of these cases (death by full hanging), in which rough rope with a rough surface was used, serous vesicles were found. In four of these cases (death by partial hanging) the hanging mark showed negative impressions of fabric weft without vesicles.

Conclusions: Therefore, in cases in which rough tools are used, the lesions are certainly different than when smooth tools are utilized. Therefore, the crime scene in which a smooth tool is found and the groove on the neck is soft, the differential diagnosis between suicide and suspension of a corpse must be done very judiciously through the analysis of circumstantial data and any other signs of active or passive defense wounds. The analysis of hanging mark allows one to assess the method and tool of suspension used and to clarify the manner of death and the type of hanging. Although this is a descriptive matter, the purpose of this study is to emphasize the importance of judicial inspection and the crucial role of the forensic pathologist and the traditional forensic pathology in the evaluation of cases of suicide by hanging, in particular when the crime scene is contaminated and the body has been moved.

Forensic Science, Hanging, Suspension of Corpse

H72 Histopathology of Drug Abuse: A Retrospective Study on 312 Cases

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After attending this presentation, attendees will better understand about the postmortem histopathologic pattern in a large group of drug abusers.

This presentation will impact the forensic science community by providing the results of a histopathologic postmortem study, conducted on a large series of drug abusers in order to clarify the physiopathology of organ damage due to drug abuse.

The goal of this study is to evaluate the postmortem histopathologic pattern in drug abusers retrospectively analyzing the records of two institutes of legal medicine and two adjoining countries. The use of exogenous substances, both illicit and lawful, for recreational purposes is widespread and the effects of drugs can be observed in both surgical pathology and in forensic practice, as it is often the cause of death by itself or associated with other diseases. Multiple drug intoxication or abuse is also a great issue for the forensic pathologist because, in these cases, it is difficult to understand the effects of each substance. This retrospective research focuses, primarily, on cardiac and encephalic alterations due to both illegal drugs (most of which were heroin and cocaine) and lawful substances (benzodiazepines, barbiturates, etc.) and on the relationship between the type of substance, the cause of death, and the histopathologic findings.

This study included 312 cases of intoxication that were autopsied between 1999 and 2015. The police and medical records, the autopsy report, the results of toxicological exams, and the histology (on hematoxylin-eosin slides) were re-examined for each case.

Thirty-two cases were excluded because of putrefaction that hampered the histologic examination and the toxicological analysis. The entire series was classified into two groups: deaths due to drug intoxication (200) and deaths of drug abusers for other causes (80). The latter group included traumatic deaths, such as traffic accidents, homicides (mostly blunt force injuries and asphyxia), and suicides (fall from a height, hanging). The detected substances were arranged into illicit drugs (cocaine, synthetic stimulants, heroin, marijuana) and lawful substances (benzodiazepines, barbiturates, etc.), often associated with alcohol assumption. Overall, the most interesting microscopic alterations regarded the brain and heart. Concerning the heart, fibrosis, both interstitial and perivascular, small vessels disease, with thickening and vasculitis, fragmentation of cardiomyocytes, and atherosclerosis at a young age were detected. A broad spectrum of neuropathological changes were encountered among both groups. The most frequent findings were vessel thickening, micro-hemorrhages, edema, and metabolic disorders. Differences between the two groups were assessed, resulting in a significant contrast. Then, histopathological findings were correlated with the type of substance detected at toxicology (and/or known from circumstantial data and anamnesis), again with remarkable differences.

The most frequent cardiac disorders reported in drug abusers included vasoconstriction of coronary arteries (inhibition of NO synthesis and stimulation of endothelin-1 release) and decreased blood flow to the myocardium; alteration of coagulation (platelet function); endothelial dysfunction (induction of von Willebrand factor in endothelial cells), and prothrombotic state, which were consistent with the present findings. Regarding neuropathologic pattern, edema, vascular congestion, ischemic nerve cell damage, and neuronal loss were always found but were not specific. In light of this study, further research is needed in the field of neuropathology.

Drug Abuse, Histopathology, Intoxication

H73 A Singular Case of Suicide Committed With a Homemade Firearm

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The goal of this presentation is to emphasize how a detailed analysis of the crime scene as well as a very careful external examination of the corpse can shed light on the manner of death in a suicide case involving an unusual homemade firearm.

This presentation will impact the forensic science community by explaining how a person can create an effective firearm that is capable of killing, using simple materials that are easily bought in any hardware store and requiring only a minimal manual ability.

Shotguns are used in a large number of homicides and suicides, and gunshot wounds are widely reported in the literature. This particular case showcases circumstances and pathological findings that concluded in suicide with an unusual homemade firearm.

Reported is a case of a 50-year-old man found at the wheel of his car in a field. A forensic pathologist at the crime scene noticed an odd tool on the front passenger seat. This object was made of two different caliber scaffolding pipes; the thinner one was inserted into the larger, so one could glide inside the other. The latter was closed by a screw partially pivoted on a bolt. The thinner tube had open ends and the external tube was stained with blood. Each scaffolding pipe had a grip consisting of another shorter tube. Upon disassembling the weapon, a 12-gauge exploded cartridge was found in the large tube. Inside the car were bloodstains on the headliner, corresponding to the head and on the steering wheel were impact bloodstains. In addition, during the crime scene investigation, a 12-gauge unexploded cartridge was found in one of the jacket pockets of the dead man. After removing the body from the car with the approval of the prosecuting officer, abundant dried blood was found on driver's seat. At the external examination, on the left hemithorax between the first and the third intercostal space along the mid-clavicular line was a large blackish 10cm x 7.5cm stain from a round wound that was 3cm in diameter. This wound was inscribed in a grazed concentric blackish-brown area and was 4.5cm in diameter. The body exhibited no other wounds.

After having examined all the weapon components, as well as the analysis of the death scene, the functioning mechanism was understood, allowing the recreation of events: the man charged the larger pipe with the 12-gauge cartridge and glided the thinner pipe into the larger one; the screw acted as the firing pin and the bullets hit his right hemithorax.

All these scientific results demonstrated that the suicide was certainly premeditated and was, in the end, lucidly executed. This odd firearm had probably been tested and previously perfected, bearing in mind the close attention to details. Information gathered from the death scene was crucial in providing the precise sequence of events.

Suicide, Homemade Firearm, Shotgun

H74 Peculiar Suicidal Behavior: A Case of Multiple Fatal, Sharp, and Penetrating Wounds in the Neck

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After attending this presentation, attendees will be aware of a peculiar manner of suicide involving sharp penetrating wounds in the neck.

This presentation will impact the forensic science community by discussing an unusual type of suicide that often raises doubts about the differential diagnosis between suicide and homicide.

In this case, a 57-year-old male was found dead by his wife, lying in a large pool of blood in their apartment bathroom. Secondary droplets and blood spatter were found in the entire room, indicating the rupture of arterial high-pressure vessels. The body showed two patterns of injuries on both sides of the neck for a total of six sharp and penetrating instrument wounds, four on the right side and two on the left side. The prosecutor conducted a full autopsy to determine the cause of death and to gain information in order to establish suicide or to start an investigation for murder. External examination of the body was negative for any type of injury, apart from the two injury patterns on the neck — no bruising or signs of struggle and no self-defense wounds on the hands and arms to indicate that the subject tried to stop an aggressor holding a weapon. A plastic surgical clamp was found around the neck, compressing the soft tissues (not the airways), probably posed in order to highlight the blood vessels. Internal examination of the organs was negative for signs of pathology or internal injuries due to blunt trauma. The wounds on the neck were closely examined, both externally and internally. The wounds had the typical features of a weapon with a penetrating and sharp capability, with wide margins and clear signs of vitality. The weapon produced stab wounds that penetrated the neck skin and the soft tissues below, damaging the carotid and jugular vessels on the right side, while on the left side, only the carotid vessel was damaged. A full toxicological exam of the bodily fluids was conducted and was negative for all substances researched (both legal and illegal). The cause of death was determined to be exsanguination due to self-inflicted stabbing injuries; no signs of violence or struggle were detected except for the fatal injuries at the neck.

In this case, the interpretation of such a large number of wounds in vital areas is explained by the phenomenon of "test" or "incitation" wounds, meaning that the subject attempting to commit suicide makes some first attempts to harm himself to find the courage to deal the fatal blow(s). Cases like this are quite uncommon and can be considered borderline between suicide and homicide, since normally a suicide is committed by a single suicidal gesture (as falling from a height or hanging). Injury patterns and interpretation of injuries in suicidal deaths are important components of forensic examinations. The goal of this report is to analyze the different wound patterns present in this case, explaining how the investigation process has led the experts to the final diagnosis of natural death.

Reporting this type of case is of great value for the scientific community, proving how different patterns of suicidal behavior may challenge the expertise of the forensic investigator.

Suicide, Sharp Penetrating Wounds, Incitation Wounds

H75 Correcting the Count: Improving Vital Statistics Data Regarding Deaths Related to Obesity

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After attending this presentation, attendees will be aware of the importance of including the term "obesity" on death certificates of natural deaths for accurate vital statistics. Inconsistencies in death certification of natural deaths with the comorbidity of obesity encountered at the Jefferson County Coroner/Medical Examiner Office (JCCMEO) will be used to illustrate the importance of considering Body Mass Index (BMI) when completing death certificates.

This presentation will impact the forensic science community by increasing awareness of including "obesity" in Part II of death certificates when appropriate, enabling coroners and medical examiners to help track the obesity epidemic in the United States. The attribution of death due to obesity can be used to track the effectiveness of obesity prevention measures and guide future health care initiatives.

A recent issue of the Journal of the American Medical Association highlighted the prevalence and impact of obesity in adults in the United States. No significant change in trends was shownsince previous data published in 2005.[1] Obesity research has led to investments in preventative measures, advancements in clinical care, and development of programs to counteract obesity.[2] Although being obese is a well-documented risk factor in the development of several chronic diseases, obesity is rarely mentioned as a cause of death or as a contributing factor in death certification (Part I or Part II of the death certificate).[3] BMI, defined as weight in kilograms divided by the square of the height in meters (kg/m^2), is considered a valid metric to track obesity among the population. The classification of "obese" according to the World Health Organization, is a BMI $\geq 30 kg/m^2$.[4] Though classified as "obese class III," a BMI of $\geq 40 kg/m^2$ is commonly referred to as "morbid obesity." BMI could be utilized as an objective finding to support characterizing natural deaths due to obesity (in Part I of the death certificate). If an obesity-related illness was listed as the cause of death, then obesity could be a contributory factor in Part II. Either of these changes in practice among coroners and/or medical examiners across the country would more accurately depict the impact obesity has on death.

The JCCMEO Case Management Database was searched for cases with "obesity" listed in the cause of death or as a contributory factor to death. Thirty cases were identified among the 10,966 assumed cases from 1991 to 2015 (0.3%). Fourteen of the cases designated either obesity, morbid obesity, or cardiac complications of obesity in Part I of the death certificate, while 16 cases listed obesity or morbid obesity in Part II. A majority ($n = 25$) were natural deaths, but five cases were accidental. Of the accidental deaths, three were drug related, one case was due to complications from trauma sustained in motorcycle accident, and 1 case due to cardiac stress from a house fire. Overall the BMI ranged from $35 kg/m^2$ - $86 kg/m^2$ among 12 female and 18 male decedents aged 20 to 61 years old.

This prompted a search of the database to identify how often a postmortem exam was performed at JCCMEO on an obese or morbidly obese decedent. This review was limited to natural deaths due to conditions often encountered in the obese population from 2010 to 2015. Other exclusion criteria included decedents less than 18 years of age, deaths associated with drugs or alcohol, and decomposed or skeletonized decedents. The BMI was calculated for 507 total cases. Of these, 33% ($n = 166$) were obese, and 9.9% ($n = 50$) would have been classified as morbidly obese.

These data suggest that obesity may cause or contribute to death more often than reflected solely by the death certificate. The data also suggest that deaths with similar BMI vary with regard to listing obesity on the death certificate. A greater awareness of the role that obesity may play in death will lead to a more accurate attribution of obesity as a cause or contribution to death. This will add to the utility of death certificates for public health and help to accurately depict the impact of obesity in a population.

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Obesity, Death Certification, Autopsy

H76 An Unusual Sudden Death Due to an Invasive Hydatidiform Mole Responsible for a Uterine Rupture and a Massive Hemoperitoneum

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After attending this presentation, attendees will better understand an atypical cause of sudden death due to a gestational trophoblastic neoplasia, which is one of the gynecological malignancies with a usually favorable prognosis.

This presentation will impact the forensic science community by increasing awareness of the possibility of a sudden death presentation, leading to an autopsy of gynecological malignancies, such as gestational trophoblastic neoplasias.

Gestational trophoblastic neoplasias are rare tumors that constitute less than 1% of all gynecological malignancies. They are classified histologically into four distinct subgroups: invasive mole, choriocarcinoma, and the very rare placental site trophoblastic tumor and epithelioid trophoblastic tumor. Invasive mole as well as the other forms of gestational trophoblastic neoplasias could follow either a normal or abnormal pregnancy, but in most cases the antecedent pregnancy is a complete or partial hydatidiform mole. These tumors are generally highly responsive to chemotherapy with more favorable outcomes than other comparable malignancies; however if not diagnosed and treated early, invasive mole can result in serious complications, such as uterine perforation and hemoperitoneum. The most common clinical presentation of an invasive mole includes vaginal bleeding, an enlarged uterus, and high urinary or serum human Chorionic Gonadotropin (hCG) levels. Metastases are rare in cases of invasive mole and occur by hematogenous spread mainly to the lung but also to the vagina, brain, and liver. Color Doppler ultrasound is the imaging of choice. The pathological diagnosis of an invasive mole is rarely made because, most of the time, diagnosis is based on abnormal decrease of hCG levels according to the International Federation of Gynecology and Obstetrics criteria and patients are treated conservatively with chemotherapy, without any need for hysterectomy. Management includes treatment with chemotherapy as well as continued monitoring of hCG. Currently, cure rates are greater than 90% even in the case of widespread metastatic disease.

Presented is the case of a 15-year-old Comorian woman who was spending vacation at her uncle's house in France; she suddenly began complaining of abdominal pain and then collapsed. Despite resuscitation efforts by paramedics, she never regained a cardiac rhythm and expired. The only information concerning her past medical history was a metastatic "molar neoplasia" with clinical follow-up in Comoros. A forensic autopsy was performed to determine the manner and the cause of death at the Medical Examiner's Office of Lyon. External examination of the body was completely negative. The internal examination revealed the existence of a hemoperitoneum of 2,710cm^2. The gross examination of the uterus showed a large necrotic and hemorrhagic tumor that extended to the entirety of the wall. There were many vesicles within the endometrium and myometrium. The liver, kidneys and pancreas were bloodless. Histological analysis identified degenerated and edematous chorionic villi with trophoblastic proliferation and karyorrhectic debris that invaded deeply into the myometrium of the uterus. There were extensive necrotic and hemorrhagic areas. Some blood vessels were also invaded and, as a consequence of this vascular spread, metastasis of hyperplastic trophoblast were seen in lthe ungs. Additionally, the postmortem toxicological screening was negative. In conclusion, the cause of death was attributed to a uterine rupture secondary to an invasive hydatidiform mole resulting in a massive hemoperitoneum.

Invasive Mole, Uterine Rupture, Sudden Death

H77 Is Fluid in the Sphenoid Sinus a Valid Indicator of Drowning?

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After attending this presentation, attendees will better understand the potential problems associated with using fluid in the sphenoid sinus as an indicator of drowning.

This presentation will impact the forensic science community by challenging one frequently used indicator of drowning.

Drowning deaths are a diagnosis of exclusion. There is no definitive test for drowning and diagnosis is based on scene investigation and findings at autopsy such as signs of water immersion, frothy fluid or frank fluid in the airways, emphysema aquosum, water/sediment in the stomach and/or in the sinuses, and hemorrhage in the temporal bones.[1] The Office of Chief Medical Examiner in Balimore, MD, examines for fluid in the sphenoid sinus at autopsy in all cases of suspected drowning; however, several prior studies have shown that fluid is found in the sinuses of non-drowning cases. Bohnert et al. aspirated fluid from the sphenoid sinus in 52% of non-drowning cases.[2] Kawasumi et al. reviewed 111 cases with fluid in the sinuses on Computed Tomography (CT) scan (38 drowning and 73 non-drowning cases), although they report that the median volume of fluid was greater in the drowning cases.[3] Lundemose, et al. also found fluid in the sinuses on CT scan in all non-drowning cases studied, although they also report that the volume of fluid was significantly lower in the non-drowning cases.[4]

In this study, CT scans from January 1, 2015, to December 31, 2015, were examined for the presence or absence of fluid in the sphenoid sinus, comparing drowning cases to age-matched non-drowning controls. In addition, fluid was aspirated from the sinuses using a needle and syringe on random non-drowning cases and suspected drowning cases from July 2016 to November 2016, with documentation on the volume, color, and consistency of any fluid aspirated. Exclusion criteria in both portions of the study include age less than 16, presence of basilar skull fractures, and signs of decomposition. As of the time of this abstract, 12 cases from 2016 have been examined, and fluid was aspirated from the sphenoid sinus in three cases (25%) while no fluid was aspirated in nine cases.

The goal of this study is to corroborate prior studies that have shown that the mere presence of fluid in the sphenoid sinus cannot be used to determine death by drowning and to determine if it adds any additional evidence that drowning did indeed occur. By aspirating the sinuses, this research seeks to determine if the volume, color, and consistency of fluid can be used to help differentiate drowning versus non-drowning deaths.

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Drowning, Sphenoid Sinus Fluid, CT Scan

H78 Staining in Firearm Barrels: A Fundamental Study

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The goal of this presentation is to encourage medical examiners and police investigators to use firearms as a valuable source of victims' DNA.

This presentation will impact the forensic science community by revealing how staining in gun barrels develops and its importance for shot range estimation.

Background: In firearm suicides, backspatter is found on the hands of the deceased, which can be valuable evidence of a self-inflicted injury. Biological staining inside the barrel is increasingly revealed by endoscopy of firearms involved in contact gunshots to the head.[1] The Swiss National Foundation funded a three-year research project to investigate the conditions determining these traces inside barrels. The systematic experimental approach to the phenomenon is introduced.

Material and Methods: According to the "triple contrast method," 2ml acrylic paint, 1ml barium sulphate and 2ml human blood were mixed and sealed in thin, flat foil bags measuring 5cm x 5cm.[2] These bags were glued on a synthetic absorbent kitchen wipe on which 10% gelatin solution was molded to create cubes of 12cm in length. The kitchen wipe covering the paint pad on the inside formed the front of the cube. Following Fackler's recommendations, gelatin was stored at 4°C for at least 60h. Close contact shots were performed on the target models and recorded by high-speed videography (SA-X2, Photron). Common firearms covering the forensically relevant calibers 0.22, 0.32, 0.38 special, 9mm Luger® and 0.45 Automatic Colt® Pistol (ACP) were fired using non-deforming bullets. Immediately after each shot the barrel was examined using a rigid borescope and staining was documented by video. Samples were taken from the anterior and the posterior part of the barrel using the double-swab technique. The DNA yield was determined by quantitative Polymerase Chain Reaction (PCR).

Results: Endoscopy revealed a great variability of pattern and amount of colored material inside the barrels. In many shots, visible traces could be observed up to the chamber. For those without visible staining in the posterior part of the barrel, DNA analysis was positive. The comparison of the results demonstrated that the intensity of staining did not depend on the caliber, but on the system of the weapon. The greatest yield of traces was obtained with self-loading pure blowback pistols, followed by bolt-action rifles and high-power semi-automatic pistols. Revolvers yielded much less staining.

Close contact shots by the same firearms using blank cartridges caused analogous staining. Admittedly, the amount of staining was not comparable, because blank cartridges did not reach the same muzzle gas pressure as live ammunition.

The gelatin block was shortened up to an ultra-slim target containing only the kitchen wipe and the paint pad embedded in a small layer of gelatin. Close contact shots continued to demonstrate the typical pattern of staining in the barrel. As a consequence, the temporary cavity cannot be the principal force causing the staining inside barrels; however, the influence of the temporary cavity on the amount of traces has to be investigated.

Conclusion: The experimental study of close contact shots provided a reliable staining, in part along the entire barrel length. Different factors influencing the staining, such as the weapons' system, muzzle gas pressure, and the temporary cavity, have been investigated. In real cases, other factors, such as the positioning of the firearm or a temporary gap between muzzle and skin, have to be taken into account.

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Firearms, Contact Shot, Backspatter

H79 Identification of Bodies Via Unique Serial Numbers on Implanted Medical Devices

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After attending this presentation, attendees will: (1) understand a simple and definitive, but likely underutilized, method for identification of decomposed or otherwise visually unidentifiable bodies; (2) understand what types of implanted medical devices are likely to contain imprinted unique serial numbers; and, (3) understand how to perform dissections to reveal serial numbers on prosthetic joints and other implanted devices.

This presentation will impact the forensic science community by describing an underutilized method for rapidly and definitively identifying bodies and by providing data supporting the utility of this method.

Identifying bodies which are decomposed, charred or have extensive facial trauma is a common challenge faced by medical examiners and coroners. When visual identification is not possible, alternative methods of identification include dental or chest X-ray comparison, fingerprint comparison, or DNA comparison. A relatively underutilized method of identification is by comparison of unique serial numbers on implanted medical devices with the serial number recorded in the medical record at the time of implantation. This method of identification is particularly valuable because it uses a serial number that is specifically designed to be unique, as opposed to other methods of identification that are predicated on empirical observations of uniqueness. The utility of this method is likely to increase as implanted medical devices become more prevalent and medical device manufacturers develop more robust methods for tracking devices at the individual patient level. In order to assess the utility of this method of identification, a retrospective review of a regional medical examiner office's case files was performed for cases that required an alternative (non-visual) method of identification.

Between January 1, 2015, and March 31, 2016, 464 forensic autopsies were performed at the Southern Minnesota Regional Medical Examiner Office at Mayo Clinic in Rochester, MN, which provides medical examiner services to multiple counties in southeastern Minnesota. Of these 464 autopsies, 45 bodies were determined to be not visually identifiable due to extensive decomposition (n = 27), contact gunshot wound of the face (n = 8), extensive blunt trauma involving the face (n = 5), and charring of the entire body (n = 5). Of these 45 bodies, at least six (13%) had an implanted medical device with possible identifying information. Of these 6 cases, five (83%) could be positively identified at the time of autopsy by comparing the unique serial number on an implanted medical device to the serial number recorded in the medical record. The types of devices that were used for identification were prosthetic knee joints (n = 2), prosthetic shoulder joint (n = 1), implanted femoral rod (n = 1), and intrathecal medication pump (n = 1). The one case in which identification could not be positively made via a medical device was an ankle fixation plate, which contained an imprinted company logo, type number, and an apparent serial or lot number, none of which were recorded in the medical record.

In conclusion, comparison of unique serial numbers on implanted medical devices is a practical method for the identification of bodies and should be considered a first-line method for identification if there is a known implanted medical device likely to have a unique serial number. In situations in which there is a presumptive identification, the serial number can be compared with the serial number documented in the medical record. In situations in which there is no presumptive identification, the medical device manufacturer can be contacted to determine whether they possess patient-specific identifying information for that implanted device. Postmortem X-rays of unidentified bodies are advisable to determine whether implanted medical devices are present, to provide postmortem films for potential comparison with antemortem films, and to rule out the presence of ballistics. Devices in this study that contained unique serial numbers were prosthetic joints, femoral rods, and intrathecal pumps. Cardiac pacemakers, defibrillators, and Left Ventricular Assist Devices (LVADs) also contain unique serial numbers. Orthopedic fixation plates may contain imprinted numbers and logos, but the lack of consistent documentation in the medical records may preclude their utility as a definitive method of identification.

Identification, Medical Device, Serial Number

H80 Ischemic Heart Disease (IHD) as a Prime Cause of Sudden Cardiac Death (SCD) in Egypt

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After attending this presentation, attendees will have obtained current information regarding IHD, which is a cause of SCD. The causes, pathological and histopathological features, and epidemiological characteristics of SCD in Egypt will also be discussed.

This presentation will impact the forensic science community by reviewing a five-year study about IHD as a cause of SCD in Egypt. According to research, this is the first study investigating this issue in Egypt. This may have obvious implications in recognizing high-risk people attempting to plan different strategies for prevention of SCD.

One of the most frustrating challenges facing the forensic pathologist is the inability to determine the cause of death in a person previously thought to be healthy.[1] Cardiovascular diseases are the number one cause of sudden death worldwide.[2] Previous studies investigated epidemiological characteristics of SCD in Western countries.[3] Many studies are needed to detect the degree of agreement between characteristics of this disease in autopsied cases of the Egyptian population and those of other countries.

All cases with SDC during the period from the beginning of January 2010 to the end of December 2014 (five years) were thoroughly investigated with emphasis on death circumstances and postmortem histopathological findings. Epidemiological data were statistically analyzed.

Out of 535 cases of SDC diagnosed during the period of the study, IHD was the principle cause of SCD (87.66%). Sudden death was the first presentation in 67.29% of the cases. Coronary atherosclerosis was the largest contributor (89.55% of cases), hypertensive heart disease contributed in 19.61% of cases and chronic valvular disease in the form of aortic and/or mitral stenosis of rheumatic origin contributed in 4.69%. In cases with coronary atherosclerosis, there was a significant increase in males over females, people in their 50's and 60's, cases with severe stenosis and type IV atherosclerosis. Acute infarction was present in the myocardium of atherosclerotic coronaries in 55.47% of cases, while old infarction was present in 44.52% of cases. Acute coronary event proved to be a strong risk factor, as in all cases where it was present, acute infarction was detected.

IHD is a serious public health problem in Egypt as well as worldwide. This study revealed that demographic characteristics in autopsied cases with IHD in Egypt is in agreement with those of other countries. Moreover, chronic valvular disease contributes as a cause of SCD-related IHD to an extent that wasn't documented in previous studies. The challenge is to identify individuals at risk to decrease the liability for SCD among survivors.

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Ischemic Heart Disease, Sudden Cardiac Death, Coronary Atherosclerosis

H81 From Oxidative Stress to Proteins: Postmortem Interval Estimation Based on Biochemical Parameters

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After attending this presentation, attendees will be able to consider the possibility of using biochemical parameters related to cell death to study the early postmortem interval.

This presentation will impact the forensic science community by introducing novel quantitative indicators of postmortem interval in the first hours of death as a more accurate determination of this parameter.

Estimation of postmortem interval is one of the challenges in forensic sciences. Currently, it is analyzed using different approaches, including both physical and thanatochemistry methods; however, there are few reports studying this parameter from the point of view of cellular biology.

Decomposition is triggered by a process called autolysis, which induces destructive changes in tissues and cells involving cell death; however, the cell nucleus remains intact until four days after death, thus allowing/making feasible the application of cellular biology methods for time-since-death estimation. Nevertheless, current analyses based on this perspective have so far been focused on measurements of messengerRNA (mRNA) stability using human housekeeping genes.

The goal of this research was to study early postmortem interval (between two and eight hours) by applying various molecular and cellular biology approaches: (1) oxidative stress production; (2) mRNA expression analysis of cell death proteins; and, (3) expression of key proteins in the mitochondrial electron transport chain, such as cytochrome c.

Four adult male Wistar rats were euthanized at the same time with intra-peritoneal injection of 0.15ml/kg xylazine. Immediately after death, the rat bodies were placed in the laboratory under controlled conditions of temperature and humidity; 20mg of gastrocnemius muscle were biopsied from each rat at different time points (zero, two, four, six, and eight hours) after death. Each sample was divided in two halves that were placed into two independent sterile tubes containing standard lysis buffer for oxidative stress and protein expression analysis; the second half was preserved in a specific lysis buffer for posterior RNA extraction. Thus, a total of 20 muscle samples were obtained during the eight-hour period for each determination.

After tissue sample homogenization in lysis buffer, supernatants were subsequently divided in two to be used for both oxidative stress and protein expression analyses. The recovered supernatants for protein expression were quantified using a fluorometric approach. Protein quantification demonstrated variability between subjects and times, ranging concentrations between 0.41mg/ml-23.18mg/ml. Standard Western blot protocol was used to study cytochrome c expression in these samples, and its quantification was developed through imaging software.

The analysis of oxidative stress was conducted by specific fluorometric assay, analyzing all types of free radicals in the sample. The results obtained were expressed as Relative Fluorescent Units (RFUs).

RNA samples were extracted using a silica-based methodology. Quantification of RNA was performed using a fluorometric assay. Variability on RNA concentrations among the 20 muscle samples ranged between 1ng/µl-25ng/µl, reaching the minimal RNA concentration required for mRNA expression analysis by Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Hence, reverse transcription was the next step, followed by RT-qPCR to analyze mRNA levels of FasL (gene implicated in death cell signaling and inflammation) and PTEN (inhibitor of PI3K/Akt pathway, which regulates cell proliferation). The analysis of relative gene expression data was calculated using the $2^{-\Delta\Delta CT}$ method.

The results of protein and mRNA expression were similar. There was a time-dependent increase in the mRNA levels of FasL and PTEN as well as cytochrome c protein expression until 6 hours after death. Though, at 8 hours, the levels decreased, probably due to the degradation of mRNA and proteins as a consequence of the progress in the autolysis process. Using a regression analysis in the first 6 hours after death, a strong positive linear correlation was found between the mRNA and protein expression of these genes and the time-since-death. These results are in agreement with the initial hypothesis, since these proteins are implicated in cell death and inflammatory signaling pathways. In contrast, oxidative stress showed more variability, with an initial increase from 0-4 hours, a decrease at six hours, and a higher increase at eight hours, which can be correlated with the summit of autolysis process.

The findings from this research provide a novel quantitative tool for estimating early postmortem interval based on biochemical parameters. Future research may be able to expand on these results, searching for other cell death markers and extending time-since-death estimates.

Postmortem Interval, Cell Death Proteins, Oxidative Stress

H82 Development of a Statistical Model to Determine the Postmortem Interval (PMI) Using the Human Skin Necro-Microbiome

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The goal of this presentation is to educate attendees regarding the new predictive algorithm that was developed for determining the PMI using skin swabs of decomposing human cadavers.

This presentation will impact the forensic science community by reporting the development of a regression model which accurately predicts the PMI (to within ~2 days) of a decomposing human cadaver using bacterial skin swabs.

Efforts to establish new and more accurate methods to estimate the PMI of a human cadaver discovered in an uncontrolled environment have thus far met with limited success; however, breakthroughs in the ability to quickly, cheaply, and quantitatively analyze bacterial populations in the tiniest of samples have opened new avenues for these efforts. Thus far, much necrobiome research has focused on the gut microbiota; however, the bacteria of the skin might be a superior choice in this endeavor for a variety of reasons.

In this pilot study, bacterial swabs were collected from the ear and nasal canals of decomposing human cadavers at the Anthropological Research Facility at the University of Tennessee at Knoxville. Total DNA was extracted from the swabs and 16S rRNA metagenomic sequencing was performed, followed by complete phylogenetic analysis to identify all bacteria in the samples and their relative abundance. The data were then transformed to allow consideration of all taxonomic levels (species, genus, etc.): ear-only, nose-only, or jointly considered data; and curated versus non-curated lists of bacterial taxa. These variables were considered simultaneously using a variety of computational models, specifically regressors, each with their own advantages and disadvantages, which plotted the behavior of the various bacterial taxa as a function of time, that is, the progression of decomposition in an uncontrolled environment.

The goal of this computational analysis was to establish a predictive model such that the unknown PMI could be determined using skin bacterial swabs. To that end, these efforts have been successful. It was discovered that the k-nearest-neighbor regressor (at $k = 4$) can be used to predict the PMI of untested samples with an average accuracy of within 55 Accumulated Degree Days (ADD), which represents about two days in the summer months in most of the United States. It was also found that, quite surprisingly, higher taxonomic levels were more informative than lower taxonomic levels, increasing the likelihood that results of this pilot study will be generalizable. In addition, considering data from both ear and nose swabs is far more powerful than either alone, as is considering the entire dataset, rather than a manually curated list, another surprising result. In sum, this pilot study yielded a robust statistical model that performs well when challenged to predict the PMI using only bacterial swabs from the skin of a decomposing human cadaver.

Microbiome, Postmortem Interval, Computational Biology

H83 Interpretation of Postmortem Vitreous Concentrations of Sodium and Chloride

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After attending this presentation, attendees will be more confident in how to interpret postmortem vitreous samples regarding sodium and chloride in cases of dehydration, salt intoxication, water intoxication, or drowning. Analysis should be routinely considered at autopsy.

This presentation will impact the forensic science community by convincing attendees that vitreous sodium and chloride concentrations correlate well with antemortem serum concentrations when interpreted together with postmortem vitreous potassium levels or the Postmortem Interval (PMI), and that they can be used to diagnose certain causes of death that otherwise would not have been detected.

Vitreous fluid can be used to analyze sodium and chloride levels in deceased persons, but it remains unclear to what extent such results can be used to diagnose antemortem sodium or chloride imbalances. Further, there are no reference ranges for postmortem vitreous sodium or chloride concentrations. In this study vitreous sodium and chloride levels from more than 3,000 cases are presented. It is shown that vitreous sodium and chloride levels both decrease with approximately 2.2mmol/L per day after death. Since potassium is a well-established marker for the PMI and can easily be analyzed along with sodium and chloride, sodium and chloride levels have been correlated with the potassium levels and postmortem reference ranges relative to the potassium levels that have been presented. Virtually all cases outside the reference range exhibited signs of antemortem hypo- or hypernatremia. Vitreous sodium or chloride levels can be the only means to diagnose cases of water or salt intoxication, beer potomania, or dehydration. Forensic cases are presented in which the analysis of vitreous sodium concentration was the only means to establish the cause and manner of death.

The results of this study also illustrate that postmortem vitreous sodium and chloride levels correlate strongly and can in practice be used interchangeably if analysis of one of the ions fails.

It has been previously suggested that vitreous sodium and chloride levels can be used to diagnose drowning or to distinguish saltwater from freshwater drowning. In this study, vitreous sodium concentrations have been compared in cases of drowning in fresh water, drowning in brackish water, and non-drowning. The results reveal that in cases of fresh water drowning, vitreous sodium levels are in fact decreased, but that this mainly is an effect of postmortem diffusion between the eye and surrounding water rather than due to the drowning process itself, since the decrease in sodium levels correlates with immersion time.

Postmortem Chemistry, Vitreous, Sodium

H84 Bacteria as a Postmortem Interval (PMI) Clock? Successive Bacterial Colonization of Pork and Its Implications for Forensic Investigations

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After attending this presentation, attendees will understand the nature of bacterial colonization of a cadaver with time and appreciate how, in the absence of expensive high-throughput next generation sequencing, it is possible to use Terminal Restriction Fragment Length Polymorphism (T-RFLP) and proteomics techniques to identify bacterial taxa from decomposing animal and, by extension, human tissue.

This presentation will impact the forensic science community by providing data from one of the first attempts to find a cheaper and more easily accessible, culture-independent alternative to high-throughput techniques to establish a "microbial clock" for PMI estimations. This is the first study to identify Vibrionaceae as abundant on decomposing pork.

The accurate estimation of PMI is one of the most important and difficult tasks in forensic science, particularly of decomposed cadavers.[1] The decomposition process is driven by microorganisms, enzymes, and bacteria enteric and external to the body, but to date, little is known about the potential of bacterial identification to be used as a "postmortem clock," for estimating the PMI of unknown human remains.

To investigate the succession of bacterial populations in the decomposition process, three replicate pork loins were left to decompose for a total of 60 days in the University of Huddersfield's outdoor decomposition facility. Pork was chosen in order to establish a baseline of bacterial succession on which to build with further porcine and eventually human studies. Uniform porcine muscle was chosen in order to eliminate potential variations caused by factors intrinsic to the deceased individual, and external factors, such as insect colonization, microbial composition of soil, and local and seasonal variations were minimised. Bacterial communities from the surface of the pork were swabbed at regular intervals and sequenced using T-RFLP of the 16S rDNA to identify bacterial taxa. Previous research has highlighted a dynamic shift from aerobic bacterial colonization at low PMIs to anaerobic activity at higher PMIs, but these have relied on high-throughput techniques such as 454 Pyrosequencing or Illumina®.[2,3] T-RFLP is proposed as a cheaper and more accessible alternative to next generation sequencing. Matrix-Assisted Laser Disorption/Ionization-Time-Of-Flight (MALDI-TOF) peptide mass fingerprinting offers another low-cost method of bacterial identification, which was investigated here, supported by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) for sequencing. These techniques have been used to identify bacterial taxa in soil for forensic purposes, according to research, this is the first time they have been used in combination to isolate bacterial communities from animal tissue for the estimation of PMI.[4,5]

In general, a decreasing trend in bacterial diversity was evident during the first sampling time points. Eleven distinct bacterial families were identifiable at Day 0, but this figure dropped to three within 24 hours. The most abundant bacterial taxa found throughout the entire sampling period were Proteobacteria and, in particular, Gammaproteobacteria. Betaproteobacteria were mainly present in samples taken at low PMIs, and Alphaproteobacteria were mainly abundant after 15 days, which corroborates previous research.[2] Similarly, Cyanobacteria appeared at higher PMIs, again in accordance with previous studies.[6] Firmicutes were present within the first two days and reappeared in the swabs taken at 40, 50, and 60 days. Vibrionaceae (Gammaproteobacteria) was present in large amounts at almost all time points, but abundance reached a peak at five days, then tailed off gradually. Comamonadaceae (Betaproteobacteria) and Clostridiaceae (Firmicutes) were only present in samples taken up to 24 hours, which is partially contradictory to previous findings; however, it should be noted that up to

50% of the abundant bacterial phyla could not be readily identified by means of T-RFLP.[3] Proteomics results also showed similar changes throughout the time course, and appear to give better taxonomic resolution, however, this needs further study.

This study sought to establish the proof of concept that bacterial population succession can be determined using relatively low-cost methods that are readily available. It succeeded in identifying two out of three previously defined key bacterial contributors involved in the decomposition process and represents the first study to reveal Vibrionaceae as present on rotting pork at various PMIs. This study should serve as a springboard for further research targeted at the development of alternative methods for PMI estimations.

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Bacterial Succession, Decomposition, Postmortem Interval

H85 Using Increased Lead Impedance Information Obtained During Interrogation of Cardiac Devices in Decomposed Bodies to Narrow Time of Death

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After attending this presentation, attendees will understand how cardiac devices in decomposed bodies can show a more specific time of death and be able to look at a lead impedance graph to see when the lead impedance increases.

It is very difficult to determine a narrow window for time of death in a decomposed body, which can limit investigation and cause distress for family members. This presentation will impact the forensic science community by using increased lead impedance found during cardiac device investigation to more specifically determine a time of death in a decomposed body.

Decomposed bodies are a common part of the forensic practice. A specific time of death in these cases is very difficult to determine and can be a source of distress to families. When a decomposed body has a cardiac device, this can be interrogated to more closely define the time when the decedent passed. Interrogation of a cardiac device allows detection of a large increase in impedance in an device leads, which occurs within a day of death.

Thirty-Seven cardiac devices were interrogated as part of the POstmortem Systematic InvesTigation of Sudden Cardiac Death (POST-SCD) as part of a working collaboration with the cardioelectrophysiology department at University of California San Francisco, and these data have been previously published.[1] Every cardiac device found at autopsy (80 devices to date) continues to be interrogated, regardless of whether they fit the POST-SCD protocol or not in order to monitor device function or dysfunction and to determine the cardiac rhythm at the time of death. A large number of these decedents have an independently verified time of death, either dying after a hospitalization or in the presence of witnesses. On interrogation, 73 devices from decedents who died in a hospital or had a witnessed arrest had a large increase in lead impedance starting a day after death.

In the clinical literature, increased lead impedance after death is a known phenomenon, but has not yet been recognized in forensic pathology. Stroobandt et al. noted that lead impedance increases in all leads the day after death, and distinguished this rise in lead impedance from lead fracture.[2] Lead impedance is a parameter that is easily found at interrogation and can be easily noticed on the lead impedance graph which is available on all three cardiac device manufacturers that have been interrogated (Medtronic, St. Jude's, Boston Scientific/Guidant).

Lead impedance increases with increased electrical resistance, whether the increased resistance originates from a fracture of a device lead (which results in an increase in impedance in a single lead), or from the breakdown of cells, leakage of intracellular contents, and the resulting increase of electrical resistance after death (which results in an increase in impedance in all leads). This increase in impedance takes approximately 24 hours; approximately the amount of time for a body to begin significant autolysis.

To date, five cardiac devices from decomposed bodies (time from last being known alive "more than two days" to twelve days) have been interrogated. In each interrogation, a large increase in lead impedance was noted. In one case, where the decedent had not been contacted for twelve days, the increase in lead impedance happened three

or four days after the decedent had last been seen. This information was relayed to the decedent's son, who had requested device interrogation for the very reason of trying to determine a more specific date of death.

Implanted cardiac devices are not uncommonly found at autopsy. Previously presented data shows that interrogation of these devices can provide valuable information about the cardiac rhythm at the time of death, possible errors in programming of the device, and device failures, which can be helpful feedback to clinicians and manufacturers, as well as forensic pathologists. These devices can also provide information about the time of death, based on the detection of increased lead impedance at the time of interrogation.

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Lead Impedance, Decomposed Body, Time of Death

H86 The Design and Validation of a Finite-Element Body Cooling Model for Calculating the Postmortem Interval

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After attending this presentation, attendees will be informed regarding: (1) the latest developments in this research area; and, (2) the theory behind the mathematical cooling model and the performance of that model.

This presentation will impact the forensic science community by presenting a new model that has been validated using a body donation program and that describes the cooling of bodies much more accurately than current models.

Estimation of the time of death is an important issue in forensic medicine and may be one of the decisive factors in the investigations of homicidal deaths. For determining the time since death in the early postmortem period, body temperature is a commonly used parameter, a technique introduced in the 19th century. Since that time, many models have been developed to determine the time since death by measuring the body temperature, ranging from very simple one-parameter to complex models. The model that is routinely used in forensic practice is the empirical model by Henssche. The model is based on the assumption that every body follows a "typical," empirically derived cooling curve. This "typical" cooling curve is modeled by an exponential cooling rate, with the power of the exponent determined by the environmental temperature and the weight of the victim. Because the currently used empirical models are based on "typical" cooling curves, they will apply, in principle, only to those cases in which the victim has "typical" body dimensions, has a "typical" posture, is still intact, and is lying on a "typical" surface. To correct deviation from this standard correction, factors are applied. Resulting errors in the time-since-death estimation vary from 3 hours up to almost 12 hours on 20-hour timescales, making the results very unreliable.

This study developed a numerical model to determine the time since death based on body cooling kinetics. This model is based on the heat exchange in a final element grid. The advantage of the finite volume approach is that the posture and body dimensions are inputs to the model as well as the surroundings of the body, such as contact surface, (partial) submersion in water, partial coverage by clothes, etc. Furthermore, not only the core temperature will be sampled, but also the temperature on other different locations, such as the extremities, chest, and head. All these positions on the body have specific cooling rates, which will give, for each moment in time, a unique set of temperatures, which allow for an accurate optimization of the model. A literature search shows that exploratory work is performed using this approach but, when reviewing this literature, it becomes clear that a balance had to be found between technical accuracy and usability in forensic practice.

The model that will be presented requires input parameters, such as the dimensions of the victim's body. A better estimation of these parameters leads to a more accurate calculation of the time since death. The feasibility to use medical imaging techniques (Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) scans) to assess the body composition was investigated in collaboration with the department of radiology.

The thermodynamic theory behind the model will be presented as well as the performance of the model in controlled conditions. For this internal validation, this study measured the cooling dynamics of bodies at the morgue of the hospital using the body donation program. The results of this internal validation will be used to develop a statistical model, which will report the time of death and the confidence intervals. The results will be compared with the current gold standard, the Henssche model.

Body Cooling, Mathematical Model, Time of Death

H87 Jay Dix Day Memorial Lecture Series

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After attending this presentation, attendees will better understand how and why deaths related to the topics listed in the lecture series occur. Attendees will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

This presentation will impact the forensic science community by presenting a comprehensive review of what causes and contributes to deaths related to the specific topics covered in this lecture series. Attendees will be able to systematically evaluate deaths in which the specified topics may have played a role that they encounter in their daily practices.

A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual series of lectures is intended to provide the non-forensic pathologist and forensic scientist a comprehensive basic review of selected topics in forensic pathology in order to increase familiarity and understanding and enhance interdiscipline communication.

This year's lecturers will discuss the investigation of deaths due to the use of sedating agents, stimulants, electrocution, and firearm injuries and deaths consequent to asphyxia, sex crimes, and autoeroticism.

Electricity is a ubiquitous entity in our daily 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 negative 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 a 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 injuries constitute a major category of physical injury. Firearm fatalities are a major cause of non-accidental morbidity and mortality in the United States. The appearance of these injuries is affected by the firearm, ammunition, range of fire, victim anatomy, and, in some cases, intervening targets. Multiple factors and mechanisms are involved in injuries and deaths involving firearm injuries. Understanding and evaluating injuries and deaths in which firearm injuries may have played a role requires basic knowledge of injuries caused by firearms, 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.

The last 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 such as ketamine and GHB, and, more recently, by the proliferation of powerful derivatives of the analgesic agent fentanyl and other psychoactive agents such as "bath salts." A second pattern of expanded drug use involves the 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 these agents helps in assessing the role, if any, of these agents in particular deaths. These two lectures will provide a comprehensive review of both sedative and stimulant agents in the context of investigating deaths.

Human life requires the uptake and utilization of oxygen along with the release of metabolic waste. Failure of these processes leads to asphyxia. There are numerous entities — mechanical and chemical — that can cause asphyxia through a variety of mechanisms, present in a wide range of scenarios, and that can be associated with a broad range of physical findings. Proper evaluation of these deaths requires knowledge of the various entities that can cause asphyxia, mechanisms through which these agents affect physiological function, scenarios under which these deaths occur, and factors that contribute to these deaths. Some violent deaths have a sexual component that may be readily evident in some cases and subtle in others. The recognition and proper investigation of deaths related to felonious activity in which there is a sexual component may help properly identify the true nature of the incident, identify the perpetrator, elucidate the psychopathology involved, and link a particular death to other similar incidents. Inadvertent death is the occasional outcome of solo sexual activity (autoeroticism). Recognition of this entity is important in correctly classifying the death as accidental and excluding the death as being suicidal or homicidal. This lecture will discuss the medicolegal investigation of deaths consequent to asphyxia, sexual crimes. and autoeroticism.

Medicolegal, Death Investigation, Forensic Pathology

H88 Congenital Transmesenteric Herniation: A Case Report Illustrating a Rare Form of Internal Herniation Resulting in Small Bowel Strangulation

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After attending this presentation, attendees will be better equipped to recognize, describe, and document congenital transmesenteric hernias, a rare cause of sudden, unexpected death in the pediatric population.

This presentation will impact the forensic science community by reporting a highly unusual case of small bowel obstruction due to this uncommon type of internal herniation, thereby increasing awareness of this entity and contributing to the existing body of knowledge, as well as highlighting its potential medicolegal repercussions.

A previously healthy 7-year-old girl experienced abdominal pain, loose stools, and vomiting for approximately two days, which abruptly escalated to severe pain and shock, culminating in cardiac arrest and death. Since the cause of death was not known, the case was referred for a forensic autopsy. At autopsy, it was discovered that she had a strangulated internal hernia of the small bowel. A distended, dark red section of the ileum was seen protruding through a circular defect in an avascular region of the small bowel mesentery. The cause of death was subsequently determined to be infarction of the small bowel, and the consequences thereof.

Internal hernias, with an estimated incidence of less than 1% in the general population, are a group of unusual causes of small bowel obstruction, accounting for only 0.6%-5.8% of reported cases.[1-3] Transmesenteric hernias are a subtype, representing 5%-10% of internal hernias, and usually involve herniation of the small bowel through a defect in the mesentery.[4,5] Such a defect may be congenital or acquired (most often after abdominal surgery); congenital transmesenteric hernias can present at any age, but are more commonly seen in children[2,4,6]. The pathogenesis of these congenital defects is not fully understood, but it is thought to involve prenatal intestinal ischemia, since they are usually located in an avascular region of the mesentery of the terminal ileum, known as Treves' field.[1,3,7]

Due to its rarity, non-specific initial clinical features, and propensity for rapid progression to strangulation and necrosis, the diagnosis of a transmesenteric hernia is clinically challenging – and with a mortality rate approaching 50%, death often ensues before it can be identified.[3,5,7] Considering that the typical scenario is that of a previously well infant or young child who dies suddenly and unexpectedly, such cases should be referred for a forensic autopsy. This is particularly important when questions regarding the adequacy of medical care and parental attentiveness may arise. For the pathologist, a high index of suspicion, careful and methodical dissection technique, as well as a basic understanding of the relevant morbid anatomy, embryology, and pathogenesis, is helpful in distinguishing congenital transmesenteric hernias from other forms of small bowel obstruction at autopsy.

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Bowel Obstruction, Internal Hernias, Congenital Defect

H89 The Epidemiology of Suicides in Allegheny County, Pennsylvania: A 10-Year Retrospective Review (2000-2009) and Comparison to a Previous Review (1990-1999)

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The goal of this presentation is to summarize the observed epidemiological trends of suicide deaths in Allegheny County, PA, from 2000-2009, with a subsequent comparison to a similar review from 1990-1999.[1]

This presentation will impact the forensic science community by identifying trends in suicide methodology, as well as populations at risk, in order to benefit future investigative and preventative efforts.

Introduction: Suicides comprise approximately 12% of the caseload at the Allegheny County Medical Examiner's Office in Pittsburgh, PA. A better understanding of the methodologies utilized and the demographics of those involved, in addition to a comparison with previously published data from the preceding decade, may provide valuable information and guide recommendations regarding suicide prevention.

Materials and Methods: All cases identified on death certificates as suicide, defined as death due to self-inflicted harm or injury, from January 1, 2000, through December 31, 2009, at the Allegheny County Medical Examiner's Office in Pittsburgh, PA were reviewed, including the associated incident reports, autopsy narratives, anatomical diagnoses, death certificates, toxicology reports, death investigation reports, and other supplemental documents. Epidemiological characteristics, including age, sex, race, and marital status, as well as the mechanism of suicide, the month of the incident, the time of death, and the presence or absence of a suicide note, were analyzed.

Results: A total of 1,475 suicides were identified, comprised of 1,182 males (80.1%) and 291 females (19.7%). Two individuals were identified as transgender. By racial composition, 89.5% were White, 8.5% were Black, and 2% were from other races. The ages ranged from 8 to 93 years old, with the greatest percentage of suicides (24.2%) occurring between the ages of 41 and 50. Overall, 40.6% were single. The number of suicides was highest in August (9.4%) and lowest in February (7.1%), with the highest numbers occurring between 3:01 p.m. and 9:00 p.m. (35.7%). Firearm injuries (46.8%), hanging (25.1%), and drug overdose (10.5%) were the leading methods of suicide. Combinations of drugs were most commonly used in overdoses (65%), with opiates/opioids, antidepressants, and benzodiazepines the most frequently utilized.

Compared to the previous review (1990-1999), the rate of suicides averaged across ten years increased from 11.1 to 11.9 per 100,000, with the highest numbers in the 41-50 year age range, as opposed to the 31-40 year age range in the preceding decade. Among females, suicide by firearms injury (28.5%) surpassed both drug overdose (27.1%) and hanging (18.2%) as the leading method of suicide. There were fewer instances of lethal drug overdose involving the tricyclic antidepressants amitriptyline and doxepin than previously, while drugs such as alprazolam and propoxyphene (now discontinued) remained toward the top of the list.

Conclusions: In comparison to the previous study (1990-1999) in Allegheny County, there is an apparent shift in the most common age range for suicides, from ages 31-40 years to ages 41-50 years. Additionally, firearms surpassed drug overdoses and hangings to become the most prevalent methodology of suicide in the female population.

Based on these trends, recommendations include: (1) careful assessment of patients prescribed opiates/opioids and benzodiazepines; (2) enhanced suicide screening, including middle-aged individuals, by health care professionals; and, (3) the prevention of firearm access to those with suicidal intent.

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Suicide, Overdose, Firearms

H90 Diurnal Oviposition Timing of Blow Flies and DNA Identification of Early Arrivers

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After attending this presentation, attendees will better understand how DNA technology is used to identify blow fly oviposition timing.

This presentation will impact the forensic science community by providing research information in an area where very little research has been conducted. Blow flies are usually the first insects to arrive at a crime scene, so information about their oviposition is crucial for accurate Postmortem Interval (PMI) estimations.

Hypothesis: Based on previous research, it was hypothesized that blow flies would oviposit starting at three hours after sunrise.

Synopsis of Methods: Three pigs were placed in a field one hour after sunrise and observed hourly for the presence of blow flies and oviposition. DNA from egg masses was isolated and the mitochondrial Cytochrome Oxidase I (CO I) gene was amplified by Polymerase Chain Reaction (PCR). CO I sequences specific to individual blow fly species were identified using Basic Local Alignment Search Tool (BLAST).

Summary of Results: The earliest oviposition on pigs occurred four and one-half hours after sunrise, but adult flies were observed starting two hours after sunrise. The two blow fly species identified were *Lucilia coeruleiviridis* (Macquart) and *Lucilia illustris* (Meigen).

Conclusions: These results confirm previous findings that *Lucilia* species are early arrivers on carrion. Blow flies did not start ovipositing immediately after sunrise, and forensic entomologists should take this into consideration when making PMI estimations.

Abstract: Forensic entomology is the use of insects in the criminal justice system. Blow flies (Diptera: Calliphoridae) are usually the first insects to arrive and oviposit (lay eggs) on carrion.[1] Their early arrival makes the timing of blow fly oviposition critical for PMI calculations.[2] The PMI is the time that has passed between death and corpse discovery, and forensic entomologists can help provide an estimation of this interval.[2] There is little known about the diurnal timing of oviposition in forensic entomology. Previous studies found oviposition occurs as early as three hours after sunrise, but diurnal oviposition was not the primary focus of that study, nocturnal oviposition was.[3] Nocturnal oviposition has been well studied in forensic entomology, but the earliest diurnal oviposition has not.[3] This study documented the earliest oviposition time in relation to hours after sunrise.

Three pigs were placed in a field one hour after sunrise and observed hourly for the presence of blow flies and oviposition. Three bait cups filled with aged chicken liver were also placed in the field to note differences in oviposition timing and magnitude between pigs and liver. Pigs and liver were observed every hour to document the presence of eggs. The experiment was replicated three times in September 2015 and will be replicated in the late summer and fall of 2016.

No oviposition was observed on any of the chicken liver bait cups. The earliest oviposition on pigs occurred four and one-half hours after sunrise, but adult flies were observed starting two hours after sunrise. Temperature and light data were also recorded to try to determine if either have an influence on oviposition timing. Those results will be analyzed and presented at the meeting. Egg masses were collected from pigs immediately after oviposition to ensure the first blow fly species ovipositing was recorded. Egg masses were frozen in a -20°C freezer prior to DNA isolation. DNA from egg masses was isolated and the mitochondrial CO I gene was amplified by PCR. CO I sequences specific to individual blow fly species were identified using BLAST.

The two blow fly species identified were *Lucilia coeruleiviridis* (Macquart) and *Lucilia illustris* (Meigen). These results confirm previous findings that *Lucilia* species are usually the first to arrive on carrion.[1] It is important to note that blow flies did not start ovipositing immediately after sunrise, and forensic entomologists should take this into consideration when making PMI estimations.

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Calliphoridae, Oviposition, Diurnal

H91 Validation of Punitive Markers of Age in Immature *Lucilia sericata* (Meigen) (Diptera: Calliphoridae)

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After attending this presentation, attendees will have a greater appreciation and understanding of the role that genetic markers can play in predicting insect age, which can determine the Postmortem Interval (PMI) when certain assumptions are satisfied.[1]

This presentation will impact the forensic science community by identifying genes that are uniquely expressed in *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) at the third instar and pupal stages that can be used to improve the precision of entomological PMI estimates.

L. sericata is a cosmopolitan blow fly of forensic, medical, and veterinary importance.[2] In regard to their forensic application, *L. sericata* collected from remains can be informative as to estimating the PMI. Historically, characteristics such as body size or immature morphological feature have been used to estimate fly age, but are not useful for determining how long an individual has been in that stage.[3] This can be problematic in the stages that persist for relatively long periods of time, such as the third instar and pupal stages. Research has shown that there are temporal patterns of gene expression throughout the immature development of insects.[4,5] Dipteran gene expression, in particular, has been of recent interest in forensic entomology.[3,6-8] Such biological information can be used to break lengthier stages into smaller temporal pieces and improve the precision of entomology-based PMI estimates. While this is promising information, there is a need for validation of these markers. This study identified 45 promising loci for validation.

Immature, colony-reared *L. sericata* of a known age were sampled and flash frozen every 12 hours throughout their development. Sex of individuals sampled was determined by *tra* Polymerase Chain Reaction (PCR) assay. RNA was extracted following a standard trizol prep that is similar to a phenol-chloroform method followed by the addition of SUPERase•In™ to prevent RNA degradation. Extracts were obtained from a total of 96 larval and pupal specimens of eight different ages (12 samples per age, from four distinct biological replicates). The RNA was then purified using an on-column DNase treatment, then converted to complimentary DNA (cDNA) for quantitative PCR (qPCR). A total of 45 markers were chosen based on a previous *L. sericata* genomic screen.[9] Genes from different clusters of co-expressed genes were selected to maximize the power obtained from adding genes to the analysis. These genes were known to be expressed at relatively high levels in one or more of the time points studied and had evidence of biological support from other species such as *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) based on comparisons to expression reported in FlyBase.[10] Three housekeeping genes, *rp49*[6], a tubulin gene[6], and *Ef2b*[9] were used to measure and compare levels of expression. Primers were designed prior to qPCR so they could be tested to determine the appropriate concentration needed for the validated markers.

Uncertainty surrounding PMI estimates based on insect age has been a concern for the forensic entomology community.[11,12] Gene expression is one way to improve imprecision in age estimates. This project is focused on a subset of genes identified as promising candidates and evaluated differences in the sexes. Ultimately, the best markers of fly age will be validated across numerous genotypes, environments, and research groups. Outside of their forensic application, evaluating *L. sericata* gene expression could lead to better understandings of their evolutionary and developmental histories. Additionally, future research comparing lab-reared to wild-type gene expression profiles can also aid studies in development and behavior.

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Forensic Entomology, Postmortem Interval, Gene Expression

H92 Morphological Comparison of Insect Artifacts From Six Species of Necrophagous Flies

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After attending this presentation, attendees will understand what insect artifacts are, their importance to bloodstain pattern analyses, and limitations to current methods used for detection of insect artifacts.

This presentation will impact the forensic science community by expanding the knowledge base of insect artifacts associated with crime scenes and challenging current practices for detection of artifacts produced by flies.

Several species of insects are attracted to human remains and exuded body fluids as sources of nutrients. The best-studied examples are adult flies in the family Calliphoridae (blow flies), which walk along body surfaces or through pools of bodily fluids. Flies leave behind traces of their activity in the form of artifacts (e.g., stains, spots, and transference) that are deposited in numerous locations on and around the site of body decomposition.[1,2] Such activity has the potential to distort the shape of existing bloodstains as well as mechanically transfer small drops of wet blood to other locations. Compromising the physical evidence even further is that as a fly feeds, it will regurgitate and defecate some of the ingested food onto surfaces near the crime scene or other locations, resulting in intermixing of fly artifacts with human body stains.[2,3] Despite claims that fly artifacts can be detected based on morphological features, alternate lighting, and presumptive chemical tests, less than 0.2% of all forensic fly species (~1,400) known in the United States have been examined by the reported methods for discernment.[3,4] In this study, the morphological characteristics of artifacts (regurgitate and feces) from six species (*Calliphora vicina*, *Chrysomya megacephala*, *Ch. rufifacies*, *Sarcophaga bullata*, *Phormia regina*, and *Cynomya cadaverina*) of necrophagous flies were examined as the first step toward developing a method to distinguish insect artifacts from human body stains. Artifact shape, size, and color were compared between species, based on adult diet (liquid blood, fresh tissue, powdered milk, and mouse carcass) and on the length of exposure to a given food source. Regurgitate volumes were also estimated for each species and correlated with adult body mass and artifacts deposited on smooth surfaces. The results indicate that artifact shapes and sizes are highly variable and species specific. Larger-sized adults (*C. vicina*, *Cy. cadaverine*, and *S. bullata*) consistently deposited the largest artifacts, especially when feeding on blood. By contrast, *P. regina*, *Ch. Rufifacies,* and *Ch. megacephala* were more likely to yield transference stains from pulvilli after walking through liquid blood or moist tissue surfaces than the other species examined. The color of artifacts was dependent on diet of the adults, regardless of species. Longer exposure periods to food sources yielded more total artifacts deposited, especially defecatory stains, than when feeding was restricted to <24h. Stains resulting from regurgitation and defecation from all fly species fluoresced at 385nm – 400nm, but the number of artifacts that autofluoresced was highly variable between species and was influenced by diet. None of the artifacts could be distinguished by species, type of artifact, or from bloodstains based on morphological characteristics alone. This is consistent with the view that current methods of visual, contextual, and chemical analysis do not permit reliable or quantifiable discrimination between insect artifacts and human body fluids.[5] Thus, there is an absolute need to develop methods of identification for insect-based evidence such as fly artifacts that are precise, reliable, and are open to use by a broad cadre of well-trained forensic experts.

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Insect Artifacts, Blow Flies, Forensic Entomology

H93 Forensic Implications of Multi-Species Blow Fly Larval Masses and Their Associated Microbiomes

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After attending this presentation, attendees will be more familiar with previously unexplored aspects of postmortem larval insect communities, which include complex microbial communities, and how both insect and microbial evidence can offer insight into time-since-death estimates. Analysis of blow fly (Diptera: Calliphoridae) larvae and how they form masses throughout decomposition, as well as their associated microbiomes, will be presented to document interactions between necrophagous insects and microbial communities, thus providing new data that can potentially be used together for more accurate Postmortem Interval (PMI) estimates. Microbes that colonize cadavers are still largely unstudied and it is unknown how they interact with insects that forensic entomologists commonly use to estimate a minimum PMI (minPMI). A more thorough collection of entomological evidence and a greater understanding of how these communities interact could help investigators calculate a narrower PMI range.

This presentation will impact the forensic science community by offering new information on the community composition of postmortem blow fly colonizers and related microbes, which could be used by investigators to devise better methods for collecting evidence and improve accuracy of estimating the minPMI. Current common forensic entomology practice is to concentrate collections on the largest specimens: most often, only a few larvae are collected from just a single location on the body or within a larval mass. While this method seeks to include the most developed (presumed oldest) larvae in order to estimate the minPMI, it could be excluding other valuable information. By only collecting the largest larvae from a single location, investigators could be missing other species that may be in a different area of the body or smaller in size but of the same developmental stage, if not older, than the largest and most developed specimens. Knowledge of the variation in blow fly larval communities is important for forensic investigations, but it is also necessary to understand the reasons behind this variation. Microbial communities could be responsible for the fluctuation in presence, abundance, and size of blow fly larvae on cadavers.

To better understand insect larval community dynamics throughout decomposition, a survey study was conducted using six swine carcasses (*Sus scrofa* L.) in an open field in Indiana. Samples from the following areas were collected from each carcass: individual larvae from larval masses and their associated internal microbiota; the microbiome of the larval mass; and epinecrotic microbial communities of the skin. Samples were aseptically collected using sterile cotton-tipped swabs every 12 hours for eight days, which was the amount of time needed for the carcasses to fully decompose and larvae to migrate from the resource for pupation. Third instar blow fly larvae from multiple masses and within the same masses were identified as *Phormia regina*, *Lucilia coeruleiviridis*, and *Cochliomyia macellaria* with *P. regina* being the most abundant taxon collected and used for internal microbiome analysis. Using 16S rRNA V4 gene region amplicon sequencing, this study characterized the relative abundance and taxon richness of each microbial community over decomposition time. Relative abundance of blow fly species within the larval mass shifted as decomposition progressed and the relative abundance of microbial taxa followed trends over time as well. These findings suggest significant interactions between the environment, microbes, and blow fly larvae. Further, in support of recent studies showing that microbes associated with cadaver decomposition affect blow fly behavior, the temporally changing microbial communities may be driving the presence of certain blow fly species during the decomposition process.

There have been relatively few studies that have addressed the microbial ecology of postmortem blow fly larval masses, but understanding these interactions is becoming recognized as potentially important for forensic death investigation. Forensic entomology relies on incorporating numerous complex variables in order to draw a

conclusion about time since death. The more that is understood about the ecology of postmortem consumers, the more precisely investigators can estimate the minPMI.

Decomposition, Microbiome, Postmortem Interval

H94 Determination of Cause of Death in Criminal Cases Using Thanatomicrobiome

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After attending this presentation, attendees will understand how to use 16S rRNA gene amplicon-based sequencing to characterize the thanatomicrobiome of a postmortem liver and spleen from actual criminal casework. Specifically, attendees will learn methods to assess postmortem microbial diversity using cadavers for the determination of cause of death.

This presentation will impact the forensic science community by revealing the thanatomicrobiome succession during putrefaction using high-throughput next-generation sequencing of postmortem microbial communities found in cadaver internal organs to develop a framework for determining cause of death and to establish the Human Postmortem Microbiome Project (HPMP).

Is death the end of life? In some ways, it is; but in relation to the microbial activity on and in cadavers, there are abundant microbes in human death that may potentially assist in criminal investigations. Despite the knowledge of the abundance and activity of microbial decomposers in cadavers, there is a paucity of details on the specific microorganisms involved in the decay of human internal organs. Determinations of the precise cause of death and postmortem interval are critical data for the forensic science community. Modern methods to estimate postmortem interval have the potential to replace traditional methods, such as stages of decomposition, body temperature, and mortis (algor, livor, and rigor). Human microbiome studies have revealed that more than 90% of cells in the body prior to death are microbial; however, information on the human thanatomicrobiome (*thanato*, Greek for death) of the internal organs is in its infancy. It was hypothesized that the thanatomicrobiome exhibits a characteristic microbial diversity that is possibly a function of cause of death. The hypothesis was tested by surveying the thanatomicrobiome of two selected internal organs (liver and spleen) from 50 cadavers with postmortem intervals between 3h-70.5h and known causes of death. To characterize the composition and diversity of thanatomicrobiomic communities, DNA was extracted and Polymerase Chain Reaction (PCR) was performed PCR targeting the V4 region of the 16S rRNA gene using bacterial primers 515F-806R. Standard bioinformatics pipelines (QIIME™), visualization tools (Phyloseq, R), and custom software was used to identify coordinated changes in the thanatomicrobiome that correspond with cause of death. Firmicutes (which includes *Clostridium*) were detected in all liver and spleen samples. In cases of deaths by coronary heart disease and overdose, the order Clostridiales and the genus *Pseudomonas* were the most predominant bacterial decomposers. These results suggest that comprehensive knowledge of the number and abundance of each internal organ's signature microorganisms could be useful to forensic scientists as an innovative source of a biomarker for determining cause of death. Also, this thanatomicrobiome data can aid in the establishment of the HPMP.

Thanatomicrobiome, Cause of Death, Cadaver

H95 Embalmed Human Cadavers Are Not Sterile: The Impact of Two Embalming Methods on the Microbiome of Human Cadavers

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After attending this presentation, attendees will better understand the impact of embalming on bacteria associated with human cadavers and how this information can be used for medical and forensic purposes.

This presentation will impact the forensic science community by providing detailed information on key bacterial species associated with human cadavers after embalming, and on whether a bacterial succession-based Postmortem Interval (PMI) estimation method can be applied on embalmed human remains or not.

The act of embalming has been performed for at least 5,000 years, dating back to the Egyptians, who preserved their dead to ensure their entry into the afterlife. Today, embalming is a common practice in the United States that is used for the preparation of the deceased for funerals and wakes, but also for use in the medical field to prepare cadavers for medical teaching and research. The purpose of embalming is not to permanently preserve the deceased but to delay decomposition for a period of time. This study investigated which embalming technique, between the traditional formalin and soft cure, is most effective in stopping the growth of the microbiome associated with the anal/rectal region of human cadavers.[1] In addition, the structure of the postmortem microbiome was investigated in embalmed cadavers over a period of one month and whether the potential to infer the PMI by using indicator microbes could be achieved. To achieve this, DNA was extracted from the anal/rectal region of ten pre- and post-embalmed cadavers of each embalming technique using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction method.[2] Extracted DNA was amplified and sequenced for the variable regions of V3 and V4 of the 16S rDNA using the Illumina® MiSeq® sequencing platform.[3] Sequences were analyzed using the Mothur pipeline for hierarchical classification and diversity calculation[4]. Statistical analysis of the data was performed in R.[5]

No significant difference in bacterial taxa was observed between the control (non-embalmed) and embalmed remains by both embalming methods, which suggests that both embalming methods are effective in suppressing bacterial growth, at least for up to one month after embalming; however, embalming didn't suppress all bacterial growth. Relative abundance of Verrucomicrobia increased after embalming in both methods. In soft-embalmed cadavers, Proteobacteria increased 30 days post-embalming and Actinobacteria declined significantly after seven days post-embalming. There are potentially pathogenic phyla and genera present in both embalming methods at 30 days post-embalming, which should be made aware to the medical and teaching communities who use these cadavers. Bacterial succession associated with embalmed remains didn't provide enough information for the development of a model for PMI estimation. Studies on microbes associated with other body parts (e.g., skin, hair, buccal) may help in determining if those communities may offer more information on PMI estimation.

In conclusion, this is the first study that provides evidence that embalming, although mostly effective, doesn't suppresses all bacterial growth of human cadavers, and the bacterial community associated with the anal/rectal region of embalmed human cadavers are not very informative in developing a bacterial succession-based model for the estimation of PMI.

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Embalming, Postmortem Interval, Human Decomposition

H96 Internal Microbial Community Translocation Throughout Decomposition in a Controlled Vertebrate Model

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After attending this presentation, attendees will understand the fundamentals of microorganisms associated with decomposition of a vertebrate carcass, the benefit of studying decomposition in a sterile environment, and the microbial community changes in organs during the first seven days after death.

This presentation will impact the forensic community by providing some of the first data to characterize and document the pathways (translocation) of microbially mediated decomposition by commensal microbes without the influence of external environmental microorganisms in a model organism. This baseline is essential to being able to build on the current microbial forensics knowledge for potential estimation of the Postmortem Interval (PMI) range.

Decomposition begins immediately after death and is driven by two forces: autolysis and putrefaction. Putrefaction is the breakdown of the host tissue by microorganisms present in the interior and on the exterior surfaces of the host. These microorganisms may be part of the host's natural microbiota or may be introduced by the environment or manner of death. Although recent studies have detected changes in host-associated microbial communities and show distinct successional patterns during the decomposition process, these studies have included the influence of the environmental microbes present. This presentation describes how the microbiota of a living host changed and translocated within a body after death in a murine model during decomposition in a sterile environment to gain a better understanding of microbial activity solely dependent on the host microbiota after death. This baseline activity can then be built upon with the addition of the variables involved with individuals and multiple manners of death to provide insight on the impacts of these variables to the microbial communities and their relation to a PMI estimate.

This study investigated the postmortem microbial community structure in a murine model that was handled aseptically and allowed to decompose in individual sterile containers allowing only 0.2µm of filtered air to come in contact with the carcass. Immediately after sacrifice, a subset of mice was surface sterilized using a bleach solution to determine the impact of the external microbial communities throughout decomposition process. The mice were dissected at five discrete timepoints (1h, 3h, 5h, 24h, and 7d postmortem) to extract DNA from lung, intestine, heart, and bone marrow organ tissues in preparation for creating high-throughput sequencing libraries for whole genome shotgun sequencing. The 16S rRNA data was analyzed for community structure during the PMI to determine the baseline host microbiota communities associated with decomposition. Results indicate an increase in the relative abundance of *Lactobacillus*, *Clostridium*, and *Staphylococcus* as decomposition progresses through the first seven days. These methods provide original data to uncover how commensal bacterial populations translocate, colonize, and proliferate following death of a host organism, and how successional decomposition of the associated host has the potential be used to estimate the PMI range.

Microbial Translocation, Decomposition, Postmortem Interval

H97 Postmortem Microbiome Changes During Thaw for Autopsy: Two Pediatric Case Studies

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After attending this presentation, attendees will better understand how the postmortem microbiome changes as a frozen body thaws before autopsy. While several studies have documented shifts in microbial community structure (composition and abundance) from various locations on a body during the decomposition process, much less is understood regarding how its previous condition (e.g., frozen, buried, burned) affects the microbiome at the time of discovery. This presentation will include microbiome data collected from two pediatric deaths discovered frozen and concealed in a home in urban Detroit, MI.

This presentation will impact the forensic science community by providing the first case studies in which the bodies had been substantially altered and modified after death as part of the process of concealment. As the bodies were completely frozen, data for this presentation also represents new microbiome profiles for two frozen bodies with estimated postmortem intervals that ranged from 22 months to 32 months. Given the dearth of postmortem microbiome studies that have used samples taken during death investigation, this presentation will also impact the forensic science community by detailing the complexities associated with using microbiome information in one of a wide variety of death circumstances. This presentation also provides unique microbiome data that may be useful for establishing long-term postmortem interval estimations that result from concealing a body in ways that slow or limit decomposition and subsequent microbial activity.

Microbial samples were collected from two cases seen in the Wayne County Medical Examiner's Office in Detroit, MI. They consisted of male and female siblings that were hidden in a chest freezer in a residential location. The 9-year-old male was wrapped in a polychromatic bed comforter; his estimated frozen interval was 32 months. The 13-year-old female was loosely wrapped in a black plastic bag and yellow plastic band with a black cloth wrapped around the neck; her estimated frozen interval was 22 months. DNA-free (sterile) cotton-tipped swabs were used to aseptically collect microbial communities at three time points during the thawing process: when completely frozen, partially thawed (ca. 24h post-discovery), and when fully thawed (ca. 48h post-discovery) for autopsy. The microbial communities were sampled from six external anatomic locations at each time period: the external auditory canal, eyes, nares, mouth, umbilicus, and rectum. DNA was extracted using a modified protocol of a commercially available kit; all DNA was quantified to ensure quality samples. The 16S rRNA V4 gene amplicon region was sequenced for each sample using a 2 x 250 base pair, paired-end approach using an amplicon-based high-throughput sequencing platform.

There was a shift in microbial community composition from the external anatomical sampling areas of these bodies throughout the thawing process for autopsy. The mean (± standard deviation) Simpson diversity index increased as thaw occurred: 0.519 ± 0.294 (frozen) to 0.564 ± 0.330 (partially thawed) to 0.768 ± 0.173 (thawed). While the most prevalent increase in microbial diversity during the thawing process was documented in the nares, eyes, and rectum, buccal samples had the highest mean observed taxa, Simpson diversity, and Faith's phylogenetic diversity detected among sampling areas. Patterns of microbial taxon turnover during the thaw process were also documented. For example, the relative abundance of *Corynebacterium*, *Haemophilus*, *Fusobacterium*, and *Streptococcus* increased by 79.7%, 75.0%, 46.8%, and 31.0%, respectively, from frozen to thawed, while the relative abundance of *Staphylococcus* decreased 33.3% from frozen to thawed, and *Lactobacillus* became nearly undetectable during partially thawed and thawed sampling times (98.3% decrease). Overall, these data demonstrate that the postmortem human microbiome changes during the thawing process of frozen individuals.

In conclusion, this study contributes a unique data set to the studies of the postmortem microbiome; specifically, partnering with a medical examiner's office allowed the opportunity to characterize microbial communities associated with two unusual deaths that involved long-term freezing, with substantial altering of the microbiome profiles and changes in their communities during the thawing process. These data highlight the inherent variability

in circumstances of death in which the collected microbiome evidence may be complex in terms of analyses and interpretation, because microbial communities likely change when the cadaver has been moved or preserved for concealment.

Postmortem Microbiome, Forensic Pathology, Frozen

H98 Trace Evidence in Microbial Forensic Study Through Cluster Analysis

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After attending this presentation, attendees will understand: (1) the principles of trace evidence using microbial samples, including the strengths and limitations; (2) the mechanism of cluster analysis used in trace evidence; (3) two proposed methods based on cluster analysis in trace evidence; (4) performance comparison between the proposed methods and existing approaches; and, (5) how to practically utilize the proposed methods in trace evidence through microbial samples.

This presentation will impact the forensic science community by facilitating criminal investigation with more accuracy in estimating the probability of a suspect's presence at the scene of the crime based on microbial materials collected at the scene.

The human genome has become known as the blueprint of human biology, and the human microbiome has recently been recognized as human's the second genome. With more than 100 trillion microorganisms living in and on human bodies, microbes (and, in particular, bacteria) can be very useful in forensic investigations. Many human microbiome studies have determined that microbial compositions vary across different body sites, but there is also evidence that the microbiome has consistent differences between individuals, which indicate that the microbiome can be used as a tool for human identification with potential application in criminal investigations. In this way, the materials collected at crime scenes could potentially be connected to suspects through the information revealed by the microbial community. Quite a few research projects have investigated and evaluated the potential of using the microbiome as an indicator for forensic sciences. For example, metagenomic profiles of the skin, hair, bodily fluids (e.g., saliva), soil, and objects in the built environment have been demonstrated as potentials to become established methodologies for use in courts; however, there is a lack of statistical and computational methods for analyzing the microbiome data for this purpose.

In this research, two methods, Bootstrapping RElative Aitcheson Distance (BREAD) and Bootstrapping RElative Aitcheson and Source Track (BREAST), are proposed to accurately estimate the probability of suspects contributing to microbial evidence. The methods are based on a hierarchical cluster process with adjustment for baseline dendrogram structure. Aitcheson distance is used for cluster analysis, as the metagenomic sequencing data shall be treated as compositional data. Comprehensive simulation studies show that the new methods greatly surpass all currently available approaches in all situations, even when the data contains very much noise. In the simulation studies, real metagenomic data are used to generate/mimic the suspects and evidence. The methods and the software package developed in this research will allow other groups to analyze their own data in a more statistical manner and contribute to the field of microbial forensic analysis.

Microbial Forensics, Trace Evidence, Cluster Analysis

H99 Storage Conditions and Time Alter the Association of Known and Questioned Soil Evidence Derived Via Next Generation Bacterial DNA Profiles

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After attending this presentation, attendees will understand how storage conditions and time change bacterial profiles generated from soil evidence, which, if not considered, has the potential to negatively influence the association of a suspect, victim, or evidentiary item with a crime scene.

This presentation will impact the forensic science community by revealing how the transient nature of the bacterial makeup in soil and its traceability to a location of origin is affected by aging and storage conditions, which must be understood if bacterial profiling is to be used to individualize soil evidence. Further, the objective association of soil evidence with its habitat of origin, using supervised classification, is examined, as the ability to produce strong associations will increase the value of soil as trace evidence.

Soil is a common form of trace evidence, recoverable from clothing, tires, shovels, etc., although its forensic analysis is usually based on class characteristics. To overcome this, past researchers have tested microbiological methods to assess if the microbial makeup, primarily bacterial, of soil might be used for forensic identification; however, almost all of these studies involved assaying the microbial DNA shortly after soil collection or freezing the soil until processing. In contrast, soil evidence will not be submitted to a laboratory until sometime after a crime is committed, and during that interlude, the bacterial composition of the soil may change, perhaps substantially. Likewise, known soil samples will not be collected at the same time the crime occurred, and may be stored for extended periods prior to analysis. If soil microbial profiling is to be a viable forensic technique, understanding how soil profiles from different evidence types change temporally is requisite, as is identifying how known soil samples should be stored prior to analysis, such that the evidentiary soil best associates with them.

In the research presented, T-shirts, shovels, sneakers, and jeans were exposed to soil from one of three habitats (yard, agricultural field, and dirt road) and stored at ambient temperature. Three and ten months later, known soils were collected from the same three habitats at a center point and 5', 10', 15', and 20' in each cardinal direction. The knowns were stored in plastic bags at room temperature, 4°C, -20°C, and -80°C for one day, one week, one month, and two months prior to processing.

DNA was isolated from soil samples using a MO BIO PowerSoil® kit. A 16S rRNA gene fragment containing variable regions 3 and 4 was amplified using universal barcoded primers. Bacterial sequences were generated on an Illumina® MiSeq®. Bacterial DNA profiles from the evidence were associated with the known soils via non-metric multidimensional scaling. A random forests algorithm was used to objectively assign the aged soil evidence to a habitat, and scores were generated to measure the likelihood the given classification was correct.

The bacterial composition of the soil on evidence displayed specific and consistent bacterial changes over time in all habitats, most notably increases in the taxonomic classes Actinobacteria and Bacilli, and decreases in Acidobacteria and Sphingobacteria. In contrast, known soil samples maintained the overall bacterial makeup from the time of collection regardless of storage temperature. On the other hand, when the known soils stored at room temperature were exposed to air, bacterial composition changes mirrored those of the aged soil evidence.

As time passed, the aged soil evidence became less similar to their habitat of origin, with the evidence soils drifting away from the known soils in multidimensional space; however, the groupings between evidence and known soils tightened when knowns were stored at room temperature and exposed to air. This was further reflected in supervised classification, in which the highest median classification accuracy (98.7%) was obtained when known soils were stored under the same conditions as the evidence, which was approximately ten percentage points higher than known soils stored at -80°C. Therefore, unlike most biological evidence, cold storage may not be optimal for known soils.

Overall, next-generation bacterial DNA profiling proves to be a viable technique for forensic soil analysis and is more individualizing than traditional methods. Further, our understanding of bacterial composition changes in known and questioned soils, including how to best handle soils when submitted to a laboratory, will allow for highly informative comparisons.

H100 Conduction Disruption: A Rare Case of Sudden Death as the Initial Presentation of Metastatic Renal Cell Carcinoma

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After attending this presentation, attendees will be aware of a rare case in which renal cell carcinoma metastasized to the interventricular septum after an extended interval, without atrial or vena caval involvement, disrupting the electrical conduction system and resulting in sudden death.

This presentation will impact the forensic science community by demonstrating an unusual case in which the initial presentation of metastatic renal cell carcinoma from a remote prior malignancy manifested as sudden death.

Introduction: Renal cell carcinoma is known to metastasize to the heart, most often occurring as transvenous extension via the inferior vena cava and typically involving the right atrium.[1] It is exceptionally rare for metastases to occur in the left ventricle or interventricular septum without right heart involvement, as this suggests hematogenous spread rather than direct extension. Only a few case reports exist of interventricular metastases.[2-4] Here, an unusual case is reported in which the initial presentation of metastasis from a remote history of renal cell carcinoma was discovered upon autopsy.

Materials and Methods: The decedent was a 66-year-old Caucasian gentleman with a history of renal cell carcinoma, status post-nephrectomy 17 years prior, who presented to the emergency department for evaluation of two recent episodes of substernal chest pain. Electrocardiogram examination demonstrated normal sinus rhythm with few premature atrial contractions, and an echocardiogram revealed a dilated left ventricle with possible wall motion abnormalities, but no evidence of intracavitary masses. Cardiac catheterization demonstrated only mild to moderate atherosclerotic disease. The patient was treated for coronary vasospasm and was discharged after two days with nitroglycerin for use as needed. Hours later, he experienced an unwitnessed cardiac event, and despite extensive cardiopulmonary resuscitation with multiple rounds of defibrillator shocks and intravenous epinephrine, he was pronounced dead.

Results: An unlimited autopsy revealed widespread involvement by firm, well-circumscribed white nodules within the lungs, liver, right kidney, and lesser omentum. There were also two similar nodules within the interventricular septum of the heart, measuring 1.0cm and 0.4cm in greatest dimension. Histologically, all nodules were consistent with metastatic clear cell renal cell carcinoma. Sectioning the coronary arteries revealed only mild to moderate atherosclerosis, and there was no evidence of acute or subacute myocardial infarction. The cause of death was thus attributed to disruption of the His-Purkinje conduction system by metastatic renal cell carcinoma, resulting in sudden cardiac arrest.

Conclusions: Few reports exist of renal cell carcinoma metastatic to the heart without involvement of the right heart. Reported here is an unusual case in which the initial presentation of widespread metastatic disease from a remote malignancy masqueraded as acute coronary syndrome, eventually resulting in sudden death.

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H101 Fatal Complications of Aesthetic Techniques: Gluteal Liposculpture

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After attending this presentation, attendees will be able to: (1) list the complications found in six fatalities associated with aesthetic techniques, particularly gluteal injections; and, (2) list the demographics, concurrent procedures, and injection materials associated with six aesthetic procedure fatalities.

This presentation will impact the forensic science community by delineating the pathologic processes found in six deaths following gluteal aesthetic techniques.

Background: Cosmetic procedures are common and utilize many techniques to obtain aesthetically good outcomes for patient satisfaction with acceptable safety standards. Cosmetic procedures that involve the gluteal region are becoming increasingly popular as various procedures can target the gluteal region such as liposuction, tumescent liposuction, cosmetic filler injections, autologous fat transfer, depot drug delivery, and implants. Complication of cosmetic gluteal procedures can be localized or systemic with systemic complications being responsible for most deaths. These reported systemic complications include sepsis, thromboembolism, fat embolism with or without fat embolism syndrome, macroscopic fat embolism, anesthesia-related, and volume abnormalities. Herein are reported six deaths due to elective gluteal cosmetic procedures. Autologous fat transfer following liposuction resulted in three out of six fatal outcomes of gluteal aesthetic procedures.

Materials and Methods: The Miami-Dade medical examiner computer database was queried for all deaths relating to cosmetic procedures involving the gluteal region from the period 2003-2016. All manners of death were considered and included in the query.

Results: Six cases were found that resulted from gluteal procedures. All six decedents were female with an age range from 28-51 years and five of the women had prior pregnancies with live births. Three cases had fat emboli due to autologous gluteal fat transfer injections associated with liposuction. All three of the decedents were symptomatic within hours of the procedure, with one decedent dying after a ten-day interval. One of the three decedents had macroscopic fat emboli with paradoxical spread secondary to a patent ductus arteriosus. Two decedents died of septic shock following intramuscular gluteal injections. One decedent had transection of the deep sciatic vessels with systemic embolization of foreign material. The average combined lung and heart weights were 1,490 grams and 355 grams, respectively. All cases had pulmonary edema. Toxicology studies on four of the six cases showed no acute intoxications.

Discussion: Gluteal cosmetic procedures carry a risk of death, which may be related to the complex anatomy and rich vascular supply of the region. Fat embolism, microscopic and macroscopic, secondary to autologous gluteal fat transfer associated with liposuction accounted for three of the six deaths in this series. The forensic community will achieve competence in listing the complications that can lead to fatalities in cosmetic gluteal procedures.

Gluteal Injection, Plastic Surgery, Forensic Pathology

H102 Systemic Air Embolism Associated With Upper Gastrointestinal Endoscopy in a Patient With Whipple Procedure: An Unexpected Finding Diagnosed on a Postmortem Computed Tomography (CT) Scan

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After attending this presentation, attendees will better understand the association between a very rare, but fatal, air embolism after an Upper Iastrointestinal (UGI) endoscopy procedure. This possibility, which could be easily missed, should be added to the list of differential diagnoses. Attendees will also gain a better understanding of postmortem CT findings.

This presentation will impact the forensic science community by raising awareness of this rare complication and by ruling out this possibility in cases of upper gastrointestinal endoscopy, in addition to more common complications.

UGI endoscopy is commonly performed and carries a low risk of adverse events. Infection, bleeding, and perforation are among commonly reported complications. Air embolism, on the other hand, is a very rare complication of upper and lower gastrointestinal endoscopy but possesses the potential to be fatal. Reported risk factors for an air embolism include previous interventions, transhepatic portosystemic shunt, blunt or penetrating hepatic injuries, inflammation of the digestive system, post-surgical gastrointestinal fistula, and rarely after a Whipple procedure. A number of potential mechanisms have been described, which include intramural dissection of insufflated air into the portal vein, transection of duodenal vein radicles, and biliary-venous fistulas and shunts.

This study reports a 77-year-old African American female with a history of pancreatic cancer, status post-Whipple procedure nine months earlier. She had multiple readmissions for hematemesis and melena and had several UGI endoscopies and colonoscopies. During her last admission, she presented with septicemia and was diagnosed with mycotic thrombus of the retrohepatic inferior vena cava and portal vein, for which she underwent thrombectomy. A Magnetic Resonance Imaging (MRI) was performed and revealed changes suggestive of cholangitis and an endoscopic retrograde cholangiopancreatography was planned for the following day. A regular endoscope was used first, but could not reach the hepaticojejunostomy site due to tortuous limb. The endoscope was removed and a pediatric colonoscope was used instead. Before insertion of the endoscope, she became unresponsive.

An autopsy was performed at the Office of the Chief Medical Examiner in Baltimore, MD on the following day. As part of the routine protocol, Computed Tomography (CT) scan and a full-body X-ray with Lodox® StatScan™ were performed. Although air was suspected in the right ventricle of the heart on X-ray, the full extent of systemic air embolism, including air in both ventricles of the heart, main pulmonary artery, aorta, and cerebral circulation was identified on CT scan.

Air Embolism, Gastrointestinal Endoscopy, Postmortem CT

H103 Subtle Microscopically Abnormal Cardiomyopathies (SMAC): An Analysis of Ten Cases

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After attending this presentation, attendees will have an increased awareness of subtle cardiomyopathic changes, and how to differentiate potential primary genetic cardiomyopathies from acquired ones.

This presentation will impact the forensic science community by presenting ten cases in which either a cardiomyopathy was not suspected, or a novel cardiomyopathy was detected by genetic analysis. These cardiomyopathic changes can be subtle and may be missed if there is another cause of death. Nevertheless, it is important that these cardiomyopathic changes be recognized, and in some cases, investigated, as these diseases may have implications for living family members.

Reported here are ten cases of cardiomyopathies incidentally detected by microscopic examination. Five males and five females were investigated, ranging in age from 22 years to 68 years. Five were found to have genetic mutations in one or more genes screened using the GeneDx Comprehensive Cardiomyopathy Panel. Cardiac autopsy findings were generally unremarkable, with heart weights ranging from normal to slightly enlarged, based on biometric parameters. There were no obvious signs of hypertrophic, dilated or restrictive cardiomyopathies. With the exception of a lack of epicardial fat for two cases of extreme malnutrition, there were no other overt abnormalities of the heart on gross examination. Microscopic findings revealed cardiomyopathic changes, including hypertrophy of the cardiomyocytes with bizarre nuclei, increased lipofuscin accumulation, focal myofiber disarray, and interstitial fibrous tissue deposition. These subtle microscopic changes were reviewed with a cardiovascular pathologist. In five cases, the microscopic changes were significant enough to send blood for DNA analysis. The results of genetic analysis revealed mutations commonly associated with cardiomyopathies, such as the TNNI3, PKP2, or TNN genes.

The cardiomyopathic changes for the remaining cases were attributed to secondary factors, such as a nutrient deficiency or chronic alcohol use. Six individuals had a definite cause of death that was not related to the cardiomyopathy identified. Of the five individuals who had genetic mutations identified on the cardiomyopathy panel, all results were reported to the investigating coroner. These results were communicated with first-degree relatives of the deceased to ensure proper clinical follow-up for surviving family members. If cardiomyopathic changes are suspected in an otherwise normal heart, even if there is a separate, definitive cause of death, these changes should be investigated further to ensure follow-up for family members and for further data collection and analysis of emerging cardiomyopathies.

Cardiomyopathy, Genetic Mutation, Microscopic Changes

H104 Two Fatal Cases of Posterior Reversible Encephalopathy Syndrome (PRES)

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After attending this presentation, attendees will understand that PRES is a potentially fatal clinicoradiologic entity associated with a variety of risk factors. Few reports describing the neuropathology have been published. The information presented serves to increase awareness of this uncommon entity, its associated risk factors, and neuropathologic findings.

This presentation will impact the forensic science community by demonstrating the importance of a broad differential diagnosis in the combined setting of sudden natural death, peri-mortem neurologic symptoms, and relevant risk factors.

Introduction: PRES is defined by a constellation of clinical symptoms and characteristic radiologic findings of posterior cerebral white matter edema. PRES is associated with a growing list of risk factors including hypertension, immunosuppression, chemotherapy, and eclampsia. As the name reflects, the condition is usually transient (non-fatal); however, PRES may lead to progressive cerebral edema, infarcts, hemorrhage, and death in 15% of cases. Due to the low mortality rate, there are few reports describing the neuropathologic findings. Clinical, radiologic, and autopsy findings of two fatal cases of PRES are described.

Case 1: A 47-year-old female with past medical history of chronic obstructive pulmonary disease, migraines, and drug abuse presented to the emergency department with a two-day history of lethargy and progressive altered mental status. Admission systolic blood pressures were greater than 190mm Hg. A head Computed Tomography (CT) showed bilateral cerebral edema. A head Magnetic Resonance Imaging (MRI) without contrast revealed areas of increased signal involving the gray and white matter of the posterior parietal and occipital lobes. The findings were suggestive of PRES with a superimposed infarction. She was treated for hypertensive emergency that was difficult to manage. On day four, she developed bradycardia, hypotension, posturing, and dilated non-reactive pupils. A head CT revealed transtentorial herniation. Resuscitative efforts were unsuccessful. Autopsy findings of the brain included cerebral edema, most notably in the parietal and occipital regions, with bilateral uncal and tonsillar herniation. Microscopically, acute bilateral cerebral infarcts of the parietal and occipital lobes and parenchymal vasculopathy were identified. Additional findings at autopsy included granular kidneys and diffuse cardiomyocyte hypertrophy, consistent with hypertension.

Case 2: A 27-year-old immunosuppressed female, six months status post-bilateral lung transplantation for cystic fibrosis, was admitted for acute liver and renal failure. Serologic studies were negative for viral and autoimmune hepatitis; a liver biopsy was suggestive of drug-induced liver injury. On hospital day three, she developed altered mental status. A head CT showed signs of vasogenic edema and MRI showed PRES with cerebellar infarction. On day 16, she developed irregular respiration and a large intraparenchymal hemorrhage of the left parietal lobe. Hospital course was further complicated by gastrointestinal hemorrhage and pneumonia with respiratory failure. She was terminally extubated and died on day 21. Brain findings at autopsy included parenchymal hemorrhages in the left parietal and temporal lobes and right caudate nucleus in addition to multiple subacute infarcts in cerebellum and cerebral cortex. Other autopsy findings included microangiopathic renal-glomerular injury suggestive of calcineurin-inhibitor toxicity.

Discussion: While PRES is increasingly recognized clinically, the true incidence is unknown and the pathophysiology remains controversial. The two current main hypotheses include disordered cerebral autoregulation and endothelial dysfunction. The end result of both mechanisms is blood-brain barrier dysfunction with vasogenic edema, particularly in regions supplied by the posterior circulation. Treatment involves removing the inciting factor and supportive measures. Clinical presentation is variable and may include headaches, visual disturbance, seizures, impaired consciousness, and focal neurologic signs. Due to the non-specific clinical manifestations, the differential diagnosis is broad, including status epilepticus, cerebrovascular accident, encephalitis, vasculitis, and cerebral venous-sinus thrombosis. The radiologic findings, which are a key factor diagnostically, are unlikely to be

available in deaths occurring outside the hospital setting. It is important for medical examiners to be aware of risk factors and patterns of injury to suggest PRES as a contributing factor in the cause of death.

PRES, Cerebral Edema, Hypertension

H105 Multiple Symmetric Lipomatosis

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After attending this presentation, attendees will: (1) recognize the features of multiple symmetric lipomatosis, a condition associated with chronic alcoholism; and, (2) understand the possible lethal complications of the disorder.

This presentation will impact the forensic science community by highlighting multiple symmetric lipomatosis, a rare and potentially lethal disorder with a known association to chronic alcoholism.

A significant number of medicolegal deaths involve ethanol. Deaths may be related to the acute, intoxicating effects of ethanol, either in decedents or within persons responsible for causing the deaths of others. Additionally, deaths may be related to chronic alcoholism. A chronic alcoholic may display characteristic external features that allow an observer, such as a forensic pathologist or other physician, to conclude that he/she is probably an alcoholic. Classic external dermatologic stigmata of chronic alcoholism include jaundice, telangiectasias, caput medusa, and palmar erythema.[1] Reported herein are two decedents with a rare condition known as Multiple Symmetric Lipomatosis (MSL), which has a strong correlation to chronic alcoholism. Identification of the peculiar features associated with MSL should prompt the forensic pathologist to consider chronic alcoholism as a probable diagnosis.

Case 1: A 63-year-old man was found dead at home. He had quit drinking approximately two years earlier. He was referred for medicolegal autopsy. At autopsy, he was noted to have a very peculiar appearance, with excessive amounts of subcutaneous adipose tissue involving the proximal upper extremities, upper chest, lower abdomen, and upper thighs, in a bilaterally symmetric pattern. Internal examination revealed hypertensive and atherosclerotic cardiovascular disease, with severe coronary artery atherosclerosis. Toxicology testing was essentially negative.

Case 2: A 57-year-old alcoholic was found dead in his apartment by his landlord, two days after having been involved in a physical altercation with another person. The body was referred for medicolegal autopsy. External examination was notable for the presence of abundant subcutaneous adipose tissue within the proximal upper arms, the chest, the abdomen, and the proximal thighs. Although there was evidence of healing superficial trauma, there were no lethal injuries identified. Internal examination disclosed additional findings of chronic alcoholism, including dilated cardiomyopathy and hepatic steatosis. Also present were pulmonary emphysema and mild to moderate coronary artery atherosclerosis. A postmortem blood ethanol level was 86mg/dL.

MSL has been described using multiple eponyms including Madelung's Disease, Launois-Bensaude Syndrome, and Benign Symmetric Lipomatosis.[2-4] The disorder primarily affects men and can be characterized by fat accumulation around the nape of the neck, upper back, shoulders, and upper arms; the external appearance presents as distinct, well-circumscribed, grossly round masses that protrude from the surface of the body.[5] The disease usually presents in the fourth and fifth decades.[6] The classic presentation depicts a male patient with moderate to high alcohol consumption; alcoholism is described in a majority of patients.[5] Although multiple theories regarding pathogenesis and its relationship to ethanol have been postulated, the underlying cause remains unknown. Known complications include lipomatous infiltration of the mediastinum, somatic and autonomic neuropathy, and malignant transformation, any of which may cause or contribute to death.[6,7]

Although rare, the condition known as multiple symmetric lipomatosis should be considered a manifestation of chronic alcoholism. At autopsy, recognition of rare complications, including mediastinal infiltration/compression by fat, autonomic dysfunction, and malignant transformation, may aid in determination of the cause of death.

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Lipomatosis, Alcoholism, Autopsy

H106 Death Due to a Rare Complication of Colonoscopy and the Potential Medicolegal Implications

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After attending this presentation, attendees will better understand the theorized mechanisms, risk factors, presenting symptoms, and autopsy findings of splenic capsular avulsion. Attendees will also understand the possible medicolegal implications of this and other rare procedural complications.

This presentation will impact the forensic science community by providing an example of a rare life-threatening complication of a colonoscopy procedure and the potential medicolegal implications that could impact a forensic investigation.

A 64-year-old woman was found dead at home after undergoing a screening colonoscopy early that day. At autopsy, 1.9 liters of liquid and coagulated hemorrhage were discovered within her abdominal cavity. There was no evidence of perforation or mesenteric damage throughout the entire colon. The only major abnormality noted was non-traumatic avulsion of the splenic capsule. The underlying granular parenchyma was completely exposed and lacked any lacerations, hematomas, or other evidence of injury. After removal of the spleen, a shriveled capsular remnant was observed attached to the base of the hilum and surrounding the splenic vasculature. This was the only identifiable abnormality capable of causing the severe hemoperitoneum and demise of the patient.

Though rare, splenic capsule avulsion is a recognized complication of colonoscopy. The definitive etiology has not been fully established, but many have theorized that it results from excessive traction on the splenocolic ligament.[1,2,4] This, in turn, results in a tear of the splenic capsule and resultant hemorrhage into the abdominal cavity. Most patients present within the first 24 hours after the procedure with non-specific symptoms. These symptoms can easily be dismissed as common post-procedural sequela, and many patients, such as the one reported here, may not seek medical attention. Numerous risk factors have been proposed, but due to the rarity of this complication, none have been specifically linked to splenic capsular avulsion with any high degree of certainty.

The range of incidence is estimated at approximately 0.001%-0.004%, though these numbers are most likely an underestimation.[3] This may be in part due to the fear of legal reprisal and the desire for mitigation by institutions. In cases of splenic capsular avulsion, evidence of negligence, carelessness, or intentional harm is difficult to prove.[2] The breadth and depth of the medicolegal system does not allow for a simple or concise answer concerning liability in cases of such rare procedural complications. A large subdivision of procedural liability revolves around the laws governing informed consent and, since informed consent has no standardized overarching statutes, the laws in this realm are fluid. Forensic professionals may be called upon to offer expert testimony and having a basic understanding of liability can help better prepare forensic personnel for possible examination. It must also be remembered that care should be taken during legal proceedings to remain impartial and to refrain from speculation or persecution of a clinician's actions.[5]

In conclusion, the paucity of the literature in the area of splenic capsular avulsion after colonoscopy only reinforces the importance of reporting known cases, especially those that end in mortality and, by doing so, raising awareness of this rare but devastating complication of an otherwise beneficial screening procedure.

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H107 Death Due to Bowel Obstruction Secondary to Raw Poppy Seed Ingestion

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After attending this presentation, attendees will understand different methods of poppy seed consumption, the toxicological findings commonly associated with them, the risk factors associated with consuming poppy seed foods, and the potential medicolegal issues surrounding the recreational use of poppy seeds to get high.

This presentation will impact the forensic science community by providing an example of a rare complication of poppy seed consumption and discussing recent literature regarding deaths due to the ingestion of poppy seed tea.

A 54-year-old woman with a history of epilepsy and benzodiazepine dependence was found unresponsive at home. Despite aggressive Cardiopulmonary Resuscitation (CPR), she was pronounced dead at a local emergency room. Family reported she had been experiencing one to two days of intractable vomiting. Her prescriptions included codeine, morphine, and meperidine. At autopsy, brown-black, kidney bean-shaped seeds (1mm) with pitted surfaces were noted around the groin and medial thighs. Internal exam revealed 1,500 ml of identical raw seeds forming a cast-like obstruction extending from her cecum to descending colon. Her postmortem blood morphine level was <10ng/ml. Vitreous fluid was negative for drugs and both blood and vitreous fluid were negative for alcohol. Investigation revealed the decedent had ordered and ingested large quantities of raw poppy seeds days prior to her death.

Seeds from the opium poppy, *Papaver somniferum*, are legally sold and safely consumed around the world in a variety of food preparations; however, recent attempts are being made to consume poppy seeds to achieve an opiate high. Although the poppy seed itself does not naturally contain significant amounts of morphine or codeine, contamination during harvest has been shown to significantly increase the drug concentration on the seeds, ranging from 1.5mg - 294mg morphine per gram of seed and 0.4mg - 294mg codeine per ram of seed.[1,2] The variability in drug level depends on the poppy's variety, geographical location, and specific method of harvest.[1,2]

The normal commercial method of poppy seed use (i.e., baking) has been shown to remove up to 50% of the opiate content on the seed surface, minimizing the concern for opiate toxicity; however, eating the seeds raw, may have the potential to deliver large amounts of opiates, thus increasing the risk.[3] Studies aimed at measuring morphine levels in subjects after eating raw poppy seeds have reported that the seeds are often unpalatable in large quantities, possibly explaining the rarity of cases like the one mentioned above. Although the patient's postmortem morphine level was too low to cause death, it does not eliminate the possibility that she may have experienced a 'high' within hours of consuming the seeds, several days prior to death.

Poppy Seed Tea (PST) has historically been responsible for numerous deaths while being used as a remedy for various medical conditions.[3] More recently, deaths have been attributed to PST in individuals who prepared and consumed the tea with little knowledge or control over its potency.[4] Additionally, these cases often involve concomitant use of other medications, such as benzodiazepines, which potentiate the adverse side effects of opiates and complicate investigation into the exact role that PST played in the death of these individuals.[4]

In conclusion, recent abuse of poppy seeds poses a unique set of challenges for the forensic community. The low cost, legal availability, and oral route of administration makes poppy seeds an accessible alternative for opiate abuse outside of prescription narcotics and heroine and should be considered as a potential cause of death in cases of opiate toxicity without evidence of prescription or street drug abuse.

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Poppy Seed Ingestion, Poppy Seed Tea, Morphine

H108 Dead in a Hot Bathtub: A Singular Case of Heat-Related Death

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The goal of this presentation is to examine the histopathological aspects of a rare case of heat-related death.

This presentation will impact the forensic science community by discussing why there is a necessity for a complete methodological forensic approach by means of autopsy and histopathological examination to diagnose rare cases of heat-related deaths are important.

Deaths from extreme heat are defined as those resulting from a high body temperature or in which heat exposure is recognized as a contributing cause ("heat-related deaths") of death. Heatstroke is a medical emergency characterized by the rapid onset and increase (within minutes) of combined elements (e.g., heat and humidity) on the body. The National Association of Medical Examiners describes that diagnosis may be established from the circumstances of the death, environmental temperature, and/or measured antemortem body temperature at the time of collapse. In cases in which the measured antemortem body temperature at the time of collapse was ≥40.6°C, the cause of death should be certified as heatstroke or hyperthermia. This report describes characteristics, circumstances, radiological, toxicological, and histopathological findings of a heat-related death.

A 26-year-old girl was found dead by her boyfriend in 24cm of water in her bathtub. The house was perfectly tidy, the only significant object was the presence, in the bedroom, of an alprazolam bottle. No other drugs were in the house. The body temperature was 43°C, the bady was dark red and diffuse, and partially vanishing hypostasis and rigor mortis were present. The water temperature in the tub was 27°C five hours after the discovery of the body. The external examination displayed no visible injuries on the body and the autopsy was conducted three days later. Postmortem radiological study with a Computed Tomography (CT) scan was unremarkable. Toxicological exams revealed a blood alcohol content of 1.72gr/L and benzodiazepines were excluded. Macroscopic examination of all organs was unremarkable. The etiopathogenetic definition was outlined by the histological examinations of all organs and skin samples, using Hematoxylin-Eosin (HE) and immunohistochemical staining method. HE-stained skin samples revealed peeling of the epidermidis, subepidermal gap formation, and cyst-like spaces in the epidermidis, indicative findings of heat damage of the skin. Immunohistochemical staining was performed on the skin utilizing heat shock protein antibodies (HSP90, HSP70, and HSP27) and myoglobin antibodies on muscle and kidney samples. The skin displayed an intense positivity to HSP90 and a decreasing positivity to HSP70 and HSP27; kidney samples exhibited myoglobin residues in renal tubules and muscle samples.

Heat-Related Death, Heat Shock, Hyperthermia

H109 Pressure Ulcer-Related Osteomyelitis in a Fatally Neglected Adult

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After attending this presentation, attendees will become more familiar with the assessment and documentation of instances involving malnutrition and negligence, along with methods of assessment and histological sampling of bedsores and osteomyelitis.

This presentation will impact the forensic science community by increasing awareness of neglected adults, and by demonstrating the methods used to document their injuries.

Forensic pathologists have long played a central role in the investigation of child abuse and elder abuse. For most people, dependence on others for care peaks at the extremes of age. The third category of vulnerable individual is not an extreme of age, but rather an extreme of health. These individuals are made medically vulnerable by physical and/or cognitive illness, resulting in partial or total dependency on caregivers to provide the necessities of life (food, water, medication, prevention of harm). In some cases, limited financial resources, decreased access to health-care, or lack of insight into the underlying disease (i.e., the social determinants of health) can further compound this vulnerability.

This report describes the death of a young adult man related to neglect by a caregiver. A traumatic brain injury in early life caused quadriplegia, making him vulnerable to developing infected pressure ulcers (with osteomyelitis) and pneumonia that ultimately caused death in a malnourished state.

A 41-year-old man had suffered a head injury with bilateral subdural hemorrhage at approximately three months of age, which was thought to be related to child abuse. The injury was treated surgically and he was made a ward of the state. The boy was subsequently diagnosed with cognitive impairment, cerebral palsy, and spastic quadriplegia and developed flexion contractures of the extremities. At the time of his death, care had been provided by a relative for several years. The man was discovered unresponsive at home, his sibling notified emergency services, and he was transported to the hospital, where he was found to have agonal respiration and died shortly thereafter.

Postmortem examination revealed a 4'9" (145cm) male, weighing 57 pounds (25.8kg, a Body Mass Index (BMI) of 12.3kg/m^2), with a cachectic appearance, subtotal absence of body fat stores, and flexion contractures. Thirty-three pressure ulcers were identified over bony prominences and in areas of contracture; 18 were stage II, extending into the dermis, while 15 extended into subcutaneous fat, muscle, or bone.

Internal examination revealed acute hypostatic pneumonia, remote subdural hemorrhage, and other evidence of old brain injury. Histological examination of the pressure ulcers revealed necrosis, cellulitis, and granulation tissue formation. Examination of the bone revealed evidence of acute on chronic osteomyelitis.

The cause of death was given as pneumonia, starvation, and infected bedsores in a man incapacitated by an old head injury. The caregiver was charged with murder, found guilty of manslaughter, and sentenced to 20 years in prison.

Elder Abuse, Pressure Ulcer, Neglect

H110 Bifid Cardiac Apex and Sudden Death: An Unusual Case and Review of the Literature

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After attending this presentation, attendees will better understand bifid cardiac apex, which is a very rare morphologic finding in humans and is generally associated with other cardiac anomalies. The goal of this presentation is to contribute additional information regarding the occurrence of this anomaly and its association with other cardiac defects (i.e., Atrial Septal Defect (ASD), multiple Ventricular Septal Defects (VSDs), persistent left superior vena cava, high take-off Right Coronary Artery (RCA)). If one of these diagnoses is made, the pathologist should be prompted to carefully examine the remainder of the heart for other defects.

This presentation will impact the forensic science community by highlighting the essential role of autopsy and histological analysis in order to better understand the cause of sudden cardiac death and be able to identify rare and hidden anatomic anomalies.

Bifid cardiac apex is rarely seen in normal human hearts or in association with congenital heart defects.[1] This malformation has been described in several adult marine mammals, such as the sperm whale (*Physeter macrocephalus*) and manatee (Order: Sirenia); therefore, it is not clear whether the bifid apex is the rule or the exception.[2] In humans, the bifid cardiac apex is normally present during embryonic development prior to completion of ventricular septation.[3] The notch between the two ventricles disappears by the 11th week of gestation, and its postnatal persistence is likely the precursor to a bifid cardiac apex.[4]

This study presents a remarkable case of bifid cardiac apex that was an incidental finding in an 11-year-old boy with sudden unexpected death, followed by review of the literature. The past clinical history of the child was characterized by a diagnosis, at birth, of the atrial septum in the fossa ovalis that underwent spontaneous closure at just over some months of age. Postmortem examination was requested by the emergency department physician and an autopsy was performed ~24h after death. Interestingly the heart (270g) revealed a bifid cardiac apex with a 1.7cm long cleft. All four cardiac valves appeared unremarkable, with no thrombi or vegetation. The fossa ovalis was closed. The coronary arteries presented a high take-off of the right coronary artery (5mm above the sinotubular junction) with a slit-like orifice. Histologically scattered foci of myopericarditis (CD3+) were observed. The chemical analysis report did not detect any toxicological substances. Based on postmortem findings and histopathological report, the final cause of sudden death was opined as fatal arrhythmia in a heart with bifid apex and right coronary high take-off.

With regard to bifid cardiac apex, a review of the literature was conducted and this unique morphologic anomaly has been described previously in eight cases. The characteristics and prognosis of patients, the method used for the diagnosis of bifid apex, and the associated congenital heart diseases have been identified.

In conclusion, through this case and a review of the literature, it is confirmed that the bifid heart is not directly responsible for sudden cardiac death; however, this study identifies the first association between bifid cardiac apex and high take off of right coronary artery.

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Sudden Cardiac Death, Bifid Cardiac Apex, Autopsy

H111 Identity Crisis: The Case of a Wormian Bone Interpreted as a Traumatic Skull Fracture

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After attending this presentation, attendees will: (1) be aware of the importance of correct identification of a wormian bone; (2) understand differentiating features of wormian bones and skull fractures; and, (3) be able to recognize wormian bones.

This presentation will impact the forensic science community by: (1) emphasizing the importance of wormian bone recognition, especially in alleged child abuse situations; and, (2) preventing the misdiagnoses of a wormian bone as a cranial fracture as well as the cranial fracture as a wormian bone.

Wormian bones, also known as intra-sutural bones, are developmental abnormalities of the cranium. They are classified as normal anatomical variants and differ in size, shape, and location. On occasion, they can be mistaken for traumatic fracture in the pediatric population. This distinction has strong consequences for allegations of child abuse, and this case represents an interesting intersection between forensic pathology, hospital imaging, incidental findings, and claims of child abuse.

A 4-month-old Black female was reportedly found unresponsive and subsequently experienced 45 minutes of successful cardiopulmonary resuscitation. Hospital Computed Tomography (CT) revealed a left tibial fracture and an accessory cranial suture; the interpretation of the head CT was then amended to a suspected occipital skull fracture due to its "angularity, location, and presence of tibial fracture." Various consulting physicians labeled the suspected fracture as "non-depressed skull fracture," an accessory suture, and "linear lucency vertically and distally from the middle region of the right lambdoidal suture, possibly reflecting subtle fracture." More detailed radiography of the tibia did not confirm a tibial fracture. The patient was taken off assisted ventilation five weeks after admittance. There were suspicious circumstances concerning the infant's care, including differing histories that she was found between the mattress and wall and found on the ground next to the bed. A preliminary investigation by local police listed the death as a suspected homicide. Criminal charges were made; these charges were dismissed following the conclusions of an autopsy examination.

Wormian bones are commonly located in parietal and occipital bones, mimic linear fracture patterns, lack soft tissue swelling, demonstrate a pattern of sclerotic borders, have no associated diastasis, and merge with adjacent sutures. Meanwhile, fractures are characterized by sharp lucency with non-sclerotic edges on radiography, usually have associated tissue swelling, can cross suture lines, widen when approaching sutures, and can be separated into non-accidental and accidental categories.[1] Lastly, lack of fracture callus cannot be readily relied on to define wormian bones, as fracture callus is not always seen in intramembranous flat bones of the calvaria.[2]

This case, while unusual, is not unique. In fact, at least five case reports have been published in the last ten years of various accessory sutures misinterpreted as abusive fractures.[3] The ability of pathologists and radiologists to differentiate between skull fractures and wormian bones is of critical importance, particularly with allegations of abuse. Wrongful accusations can be devastating for families already experiencing difficult times. This case illustrates a need for further clarification surrounding the pathologic picture of abusive fractures, consistent documentation of wormian bones, and greater emphasis on the absence of soft tissue swelling. Lastly, it encourages caution and thorough consideration of the differential diagnoses of suspected abusive fractures.

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Wormian Bone, Cranial Fracture, Child Abuse

H112 Deaths Due to Post-Intubation Tracheal Stenosis: A Clinical History and Autopsy Findings in Three Cases

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The goals of this presentation are to: (1) recognize how tracheostomies can lead to tracheal stenosis; (2) illustrate gross findings and histologic findings of tracheal stenosis; and, (3) identify clinical history features of tracheal stenosis.

This presentation will impact the forensic science community by reviewing multiple cases, including the literature, which will provide awareness to pathologists and forensic pathologists regarding characteristic history and autopsy findings in post-intubation tracheal stenosis.

Tracheal stenosis is a potentially fatal late complication of a tracheostomy or intubation that is occasionally missed, occasionally mismanaged, and potentially fatal in emergent situations if not diagnosed early in the course of airway management. To diagnose tracheal stenosis while alive requires a high index of suspicion and laryngoscopy. After death, the forensic pathologist should consider the potential for this complication in patients with tracheostomies within the past weeks to months and suggestive clinical histories. Keep this information in mind when deciding to perform an autopsy and during the exam while paying particular attention to the upper airways.

A 26-year-old woman with epilepsy was intubated because of airway management difficulties during her seizures. She ultimately required a tracheostomy. With the tracheostomy, she experienced difficulty breathing, and was believed to be experiencing panic attacks. While at home with family, her breathing difficulties increased and she began having a seizure. Emergency Medical Services (EMS) responded and was not able to establish an airway. At autopsy, she was found to have severe tracheal stenosis, just distal to her tracheostomy tube. A 23-year-old man with Down's Syndrome required a tracheostomy during a hospitalization for pneumonia. Following the removal of his tracheostomy, he was found to have subglottic stenosis. He underwent surgical balloon dilation and was sent home. He developed increasing difficulty breathing and presented to an emergency department with stridor. He could not be intubated and emergent tracheostomy attempts failed. At autopsy, he was found to have severe tracheal stenosis, just proximal to the tracheal bifurcation. A 42-year-old woman underwent a surgical procedure for an abdominal abscess. She required a tracheostomy while hospitalized. Following removal of the tracheostomy, she developed tracheal stenosis. She underwent tracheal balloon dilation, but refused surgical intervention and repeat tracheostomy placement. She died hours following the balloon dilation. At autopsy, she was found to have severe narrowing of her trachea at the presumed tracheostomy site.

Tracheal stenosis is a late complication of a tracheostomy. The incidence has been reported to be anywhere between 0.6% to 21%.[1] Typically, the stenosis is at the level of the stoma, but can be proximal or distal. Clinically significant stenosis occurs when the tracheal lumen is narrowed 50%-60%.[2] Common signs of clinically significant stenosis are a persistent cough, retained secretions, and progressive dyspnea. Stenosis may follow a chronic inflammatory response and the development of excessive granulation tissue after decannulation.[2] A study by Welkoborsky et al. of biopsy samples from 18 patients with tracheal stenosis who underwent tracheal reconstruction demonstrated the most consistent findings were fibrosis and scar formation in all tissue layers and ossific metaplasia of cartilage.[3] Inflammatory findings were typically limited to the mucosa and the inner submucosa[3].

Tracheal stenosis following tracheostomies can lead to death if not recognized by clinicians. Awareness of the condition and the clinical presentation, typically in individuals with significant medical histories, may cause the forensic pathologist to perform an autopsy and direct their examination to a careful evaluation of the upper airways. Identifying tracheal stenosis allows for education of clinicians and the prevention of future deaths.

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Tracheal Stenosis, Intubation, Tracheostomy

H113 Sudden and Unexpected Death During Sexual Activity Due to a Glial Cyst of the Pineal Gland

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This case emphasizes the need for an accurate extensive gross examination as well as a comprehensive brain specimen, to rule out neurological causes of sudden death. After attending this presentation, attendees will better understand pineal region cysts, in which a complete forensic investigation and comprehensive history is essential to ascertain the cause of death. Furthermore, forensic pathologists must take into consideration the physiological modifications induced by sexual activity, which are capable of eliciting fatal neurological events in predisposed patients.

This presentation will impact the forensic science community by demonstrating that a cystic lesion of the pineal gland region may in some instances cause sudden death by interfering with the functions of reticular formation of the brainstem, especially during sexual intercourse. In this case, the intracranial pressure increases, secondary to Valsalva's maneuver during climax which may further aggravate compression on the midbrain and brainstem. This presentation will address a case of sudden and unexpected death during sexual intercourse in a woman with an undiagnosed pineal gland cyst.

Case Report: A 45-year-old woman, in apparently good health, collapsed and died unexpectedly following cardiopulmonary arrest, after reaching orgasm while engaged in sexual intercourse. According to the circumstantial account of relatives, the woman suffered from severe headaches exacerbated by certain types of physical strain, such as sexual activity, although she reportedly had never undergone thorough neurological examination nor diagnostic neuroimaging.

Autopsy Findings: An autopsy was performed 24 hours after her death to ascertain the causes. The external examination was unremarkable and there were no injuries present on gross examination. Lividity was evident and fixed on the dependent regions of her back. No evidence of sexual assault was present.

On internal examination, the patency and elasticity of her coronary arteries appeared normal. The heart weighed 330g and both the myocardium and heart valves revealed no significant gross pathological findings. The brain weighed 1,360g. A cystic lesion of the pineal gland was observed in the midbrain, in close proximity to the quadrigeminal plate. The lesion measured approximately 15mm; however, the anatomy of the ventricular system appeared normal.

Microscopic Examination: Microscopically, the wall of the cyst consisted of a layer of glial tissue, surrounded by a zone of pineal elements. A proliferation of small-sized cells were identified immunohistochemically to be positive for synaptophysin, whereas Epithelial Membrane Antigen (EMA) and Cytokeratin AE1/AE3 were both negative. A focal area of Glial Fibrillary Acidic Protein (GFAP)-positive fibers, substantially devoid of mitotic activity as well as microcalcifications, was also found within the intralesional glial layer. Microscopic examination of the remaining internal organ systems revealed no demonstrable abnormalities.

Discussion and Conclusion: A complete forensic examination corroborates the conclusions that the cause of death was fatal cardio-circulatory failure, resulting from midbrain compression due to a non-neoplastic pineal gland cyst, exacerbated by sexual activity.

The relevance of the case report for the forensic science community consists of demonstrating that a cystic lesion of the pineal gland region may rarely determine sudden death by interfering with the functions of reticular formation of the brainstem, especially during sexual intercourse. In this case, the intracranial pressure increase, secondary to Valsalva's maneuver during climax, may have further aggravated compression on the midbrain and brainstem, thus concurring to cause cardiac arrest.

Cystic lesions of the pineal region are typically found in a small percentage of adult patients. Although their mechanism of development and growth are unknown, they are generally benign, asymptomatic lesions that often go undiagnosed. To date, a mere three cases of fatal outcomes from the effects of a cyst of the pineal gland have been described in the literature. The underlying mechanism, whereby these lesions resulted in death is seldom clear and usually inferred. According to Milroy and Smith, in patients with a history of chronic headache, pituitary cysts should be considered the sole cause of death if there is no other gross or histological evidence of significant pathology.[1]

This case demonstrates the importance of performing appropriate diagnostic tests in patients presenting with chronic headache exacerbated by physical activity and/or sexual intercourse. As regards the forensic perspective, the case emphasizes the need for an accurate and extensive gross examination, including brain specimens, to rule out neurological causes of sudden death.

Upon completing this presentation, attendees will have gained knowledge of pineal region cysts, in which a complete forensic investigation and comprehensive circumstantial history, is essential to ascertain the cause of death. Furthermore, forensic pathologists must take into consideration the physiological modifications induced by sexual activity, which are capable of eliciting fatal neurological events in predisposed patients.

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Pineal Gland Cyst, Sexual Activity, Sudden Death

H114 Sudden Death Secondary to an Undiagnosed B-Cell Lymphoma of the Hypopharynx and Infiltration of the Inferior Constrictor Muscle

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The goal of this presentation is to share a common form of sudden death due to a mechanical obstruction of the hypopharynx by an undiagnosed B-cell lymphoma that was localized and diffusely infiltrating the inferior pharyngeal constrictor muscle.

This presentation will impact the forensic science community by introducing a case that demonstrates once more that in the absence of specific data, a thorough forensic investigation including autopsy, histological examination, and circumstantial data collection is mandatory in order to reach a correct cause of death.

A forensic examination by means of scene investigation, circumstantial data collection, autopsy, and histological and toxicological investigations were performed on biological samples (urine and cardiac blood) taken during the autopsy, and led to the conclusion that the cause of death was asphyxia, correlated with B-cell lymphoma of the hypopharynx.

The victim, in the words of her son, was unaware of any malignant disease but, at the same time was taking medication containing codeine for the hacking cough. This data was confirmed, later, by the analysis of blood and urine, which showed the presence of 48ng/ml of free codeine (therapeutic dose) and excluded the presence of other toxins.

In the presented case, the woman called for rescue but, when the emergency ambulance arrived, was discovered lifeless on her bed with a phone in her hand. The telephone operator reported that the woman had dyspnea and exhibited speech difficulties during the call.

The external examination of the body revealed only cyanosis of the face and the upper part of the chest, as well as the presence of rare bilateral conjuntival petechiae. The autopsy examination highlighted the presence of a wall thickening, infiltrating the circumference, growths and projecting into the hypopharynx lumen with adbundant mucus, of papillomatous aspect. The lungs exhibited congestion and spillage of a foamy material, while the other vital organs display no other disorders.

The histological analysis showed the essential finding of a B-cell lymphoma (CD 20+) of the hypopharynx, diffusely infiltrating the inferior pharyngeal constrictor muscle, in addition to a massive pulmonary emphysema, edema, and abundant congestion of small vassels, coherent with a passive hyperemia. From a clinical point of view, this study highlights how the lymphoma growing through the wall of hypopharynx and diffusing among the fibers of the inferior pharyngeal constrictor muscle, could have shaped an inadequate contractions and a laryngospasm, causing the suffocation.

This type of tumor is extremely rare in this area and literature reports few cases of sudden death, with the occasional autopsy finding of a hypopharyngeal neoformation. According to these cases, the cause of death could start as a possible displacement of the mass leading to asphyxial death or an abnormal contraction of the pharyngeal constrictor muscle.

B-Cell Lymphoma of Hypopharynx, Asphyxia, Sudden Death

H115 Burn Injury: A Case Report and an Experimental Approach

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After attending this presentation, attendees will appreciate the possibility of a simple experimental setting in their day-to-day casework.

This presentation will impact the forensic science community by reporting the effect of a burn injury inflicted in an experimental situation to illustrate the mechanism of a real-world case.

Burn injuries can be difficult to assess in daily forensic casework. There are a few experimental studies suggesting temperature and time frames for different depths of burn injuries, but most studies do not explore etiology and causality, but treatment, and only to a lesser extent they contribute to medicolegal judgments.[1] For obvious ethical reasons, it is not possible to make systematic prospective studies of burn injuries on humans. When dealing with a forensic case involving burn injury, one seldom finds a study, or even case reports, with the same background parameters as a disputed case. Different types of skin (different parts of the body), different injurious materials, and different forces of the heat object are examples of parameters that may differ from those of the disputed case. In suspected child abuse, the assessment of the forensic expert often is one of only a few evidences presented in court, which puts the forensic expert in a difficult situation.

A suspected child abuse case raised the question of whether a dropped fork used as a spatula during panfrying could cause a patterned burn injury with clear, striped, reddened marks with flaky brown epidermis and scabs. The injury was examined by the local general practitioner, photographed, and subsequently sent to the forensic doctor for assessment.

To improve the conditions of an accurate assessment, an experiment mimicking the stated precondition was conducted. An iron frying pan was heated on an electric stove. Apple slices were fried in the pan together with a small amount of olive oil. A stainless steel fork was used as a spatula and, when not used, placed with the convex side of the claws against the bottom of the frying pan. The fork handle was never uncomfortably hot to hold. The subject, a healthy middle-aged female volunteer, exposed the underside of her left forearm to the heated fork, which was placed with a slight pressure immediately against the skin with the convex side of the fork claws (trial 1) for less than one second and later dropped three times with the claw downward from a height of 20cm (trials 2-4). The fork was reheated between the trials as described. Due to pain, the forearm was held under cold running water during several episodes the hour after the trial.

Touching the fork against the forearm (trial 1) resulted in a striped formation indicating pale blisters surrounded by diffuse redness. Dropping the fork on the skin resulted in an irregular diffuse redness (trial 2) and diffuse redness in striped formations (trials 3 and 4). The diffuse redness successively turned sharply demarcated and intensely red during the following hour. After two days, the injuries were still intensely red, sharply demarcated, and one of the blisters collapsed on day two and was replaced with a red scab. The epidermis in the reddened areas successively dried up, turned brown and cracked, the redness faded, and was no longer visible after five days except in the area around the scab (trial 1). Approximately seven days after the exposure, the brownish epidermis started to peel. Even four weeks after the trials, the pale brown discoloration was visible in the previously reddened areas and discrete scarring replaced the blisters and scabs.

In conclusion, taking only the shape and character of the wound into consideration, it was not possible to exclude the suggested cause of the event in this case. This simple experimental setting contributed widely to the conclusion.

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Burn Injury, Child Abuse, Experimental Setting

H116 Optic Nerve Sheath Hemorrhages Associated With Non-Traumatic Subdural and Subarachnoid Hemorrhage: A Case of Undiagnosed Congenital Hydrocephalus

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After attending this presentation, attendees will be able to recognize congenital hydrocephalus and understand the direct relationship between rapidly increased intracranial pressure and ocular and optic nerve sheath hemorrhages.

This presentation will impact the forensic science community by demonstrating the direct relationship between rapidly increased intracranial pressure and intraocular and optic nerve sheath hemorrhages and by reinforcing the need for thoroughness in the investigation and postmortem medical examination of all sudden childhood deaths.

Ocular and optic nerve sheath hemorrhages in children are frequently attributed to inflicted traumatic brain injury; when seen in conjunction with subarachnoid hemorrhage, they are often mistakenly considered pathognomonic of Shaken Baby Syndrome. A case of non-traumatic subarachnoid hemorrhage with optic nerve sheath hemorrhage (Terson Syndrome) from Miami-Dade County, FL is presented.

A 4-month-old, ex-35-week gestation male infant was born by cesarean section on April 1, 2014, as part of a dichorionic, diamniotic twin pregnancy, which was complicated by oliguria and pre-eclampsia. He had episodes of apnea and bradycardia, following birth, and was noted to have a large head. A cranial ultrasound revealed "minimally" dilated lateral ventricles. His pediatrician and neurologist's notes indicate that he was "normocephalic," but his head circumference increased from 34 centimeters at birth to 37 centimeters on April 25, 2014, to 38 centimeters on May 5, 2014, to 40 centimeters on May 12, 2014, and to 41 centimeters on June 2, 2014, which was recorded as a change from the 54th percentile to the 94th percentile from the first to second postnatal month without correction for prematurity. When corrected for prematurity, his head circumference is at the 98th percentile at birth and rises from there. A follow-up ultrasound on May 19, 2014, revealed an increase in the size of the lateral ventricles from 3mm to 5mm.

On July 27, 2014, the child was fussier following a nap, began to have difficulty breathing, and became non-responsive. He was found to have cerebral edema from anoxic/ischemic brain injury and eventually progressed to brain death. The medical notes indicate that this was due to a cardiac arrest, but there is no record of him ever suffering cardiac arrest or a period of hypoxia. Therefore, the only explanation for the anoxic/ischemic brain injury is an intrinsic issue within the brain where the intracranial pressure had increased and reduced cerebral perfusion. He had craniomegaly with progressively increasing hydrocephalus, which when untreated, eventually leads to elevated intracranial pressure and reduction of cerebral blood flow. The finding of retinal hemorrhage was said to be the result of non-accidental trauma, but anything that increased intracranial pressure can cause retinal hemorrhage.

Initially described in the early 20th century, Terson Syndrome referred to vitreous hemorrhage associated with subarachnoid hemorrhage. Today, the definition includes any degree of intraocular hemorrhage associated with intracranial hemorrhage and rapid elevations in intracranial pressure. Although some of the findings in this case mimic those described in cases of inflicted traumatic brain injury, this illustrates the importance of first excluding a natural disease process and thorough examination in all pediatric cases.

Intraocular Hemorrhages, Congenital Hydrocephalus, Terson Syndrome

H117 Hemothorax and Hemopericardium in a Cooked State Due to Burns Secondary to Traumatic Chest Injuries: A Case Report

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After attending this presentation, attendees will better understand fatalities caused by a truck collision resulting in the death of the driver, who was unable to extricate himself for various reasons. This presentation will also discuss internal hemorrhages occurring due to trauma, such as hemothorax or hemoperitoneum, and how they can become altered due to burns if such trucks catch fire after accidents while the driver is trapped inside.

This presentation will impact the forensic science community by addressing a modification of traumatic hematoma due to burns after a collision involving trucks.

In a bizarre accident, two trucks collided on a highway. Both trucks had two passengers: one driver and one cleaner. After the head-on collision, three occupants of the truck quickly rescued themselves; however, one of the truck drivers was unable to extricate himself, as he was stuck in between the driver seat and the steering wheel. As nearby people tried to rescue the truck driver, the truck suddenly caught fire and burned intensely. The unfortunate truck driver could not be rescued and died in the truck. His body was found in a sitting position in charred condition in the truck. The truck was also completely destroyed by the fire.

A medicolegal autopsy was performed on the decedent and it was observed that the truck driver was burned beyond recognition. On internal examination it was observed that he had fractured ribs on both sides and had evidence of a hemothorax; however, the hemothorax was in a burned state with pinkish mass. Similarly, there was evidence of the hemopericardium in burned state. The heart and lungs were lacerated and the other visceral organs in the abdominal cavity were also partially burned. After the accident, the hemorrhagic blood remained within the chest cavity, which also was burned by the intense heat. The finding of a hemorrhage in a burned state in cavities is very rare. Also, such findings indicate that the victim had antemortem trauma before the fire.

This study presents a case report of hemothorax and hemopericardium in a burned state due to the rarity in presentation and also presents the possible indication of their antemortem nature. This presentation will also discuss internal hemorrhages occurring due to trauma such as hemothorax or hemoperitoneum and how they can become altered due to burns.

Hemothorax, Hemopericardium, Burns

H118 Unusual Changes of the Lungs After Inhalation and Chemical Burning With Bleach

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After attending this presentation, attendees will be informed of an unusual fatality of a toddler due to chemical (bleach) burns and the inhalation of bleach fumes.

This presentation will impact the forensic science community by illustrating an unusual pathological change of the lungs as a result of the combined effects of the inhalation of highly concentrated bleach fumes and dehydration due to a large area of skin chemically burned with bleach.

The decedent was a 21-month-old African American male. On the evening of the incident, he and his twin brother were both placed into the same crib. At an unknown time, the twins' aunt "poured" bleach into the crib to clean feces on the victim, pillow, and mattress. At approximately 10:00 p.m. – 10:30 p.m., the aunt left for the evening and her girlfriend arrived at the residence to watch the twins. The girlfriend stated that upon her arrival, the decedent and his twin brother were crying and screaming in the crib. She noticed that the decedent had some discoloration to his skin. She then placed the twins in the bathtub to rinse them off. After the bath, both decedent and his twin brother were given a bottle of whole milk and taken to the living room area of the residence where they seemed to calm down and fell sleep.

The girlfriend reported going to sleep at approximately 2:30 a.m. Around 3:00 a.m., the grandfather and the biological mother of the twins responded to the residence and noticed that the decedent was lifeless. The family called 911 at 5:09 a.m. and CPR was initiated. Law enforcement and medical personnel arrived at the scene and the toddler was pronounced dead at 5:22 a.m.

Investigators identified bleach bottles located under the kitchen and bathroom sinks. Inside the bathtub was a multicolored washcloth and a bleach-faded onesie was recovered from the trashcan. The crib mattress also exhibited damage on the top surface. The decedent, as well as his crib mattress and diaper, were examined utilizing a 400nm - 430nm wavelength alternate light source. Swirl patterns that fluoresced were visualized on the top of the mattress and crystals were present on the bottom of the mattress. Crystals were also identified on the decedent's chin and back

Autopsy Findings: Second-degree burns with scratch/abrasion marks over the chest, abdomen, back, buttock, right arm, and posterior thighs (approximately 65% of the body surface area). Extensive coagulative necrosis of the alveolar walls and empty alveolar spaces in a chicken wire pattern were also found. The lung tissue processing was repeated to rule out the possibility of processing artifacts.

C. vitreous Fluid Analysis: Hypernatremia (sodium: 59mmol/L) and hyperchloremia (chloride: 120 mmol/L). Bleached hair on the back of the head was also found.

Discussion: Bleach-based solution of Sodium Hypochlorite (NaOCL) is one of the most common cleaning agents used in the household. Bleach can cause skin redness, irritation or burning with numbness, pain, blisters, or black dead skin. Ingestion of bleach can cause erosion of the mucosae of the esophagus and stomach as well as hypernatremia, hyperchloremia, and metabolic acidosis. Acute inhalation of high-concentrate bleach can cause non-specific pulmonary changes, such as bronchiolitis, alveolitis to pulmonary edema, as with most other hazardous gases and fumes. In this case, the skin exhibited diffuse dermis necrosis with large areas of sloughing of the dermis as the combined effects of direct exposure to the undiluted bleach and the mechanical scrubbing of the skin with the washcloth. The large area of skin damage will increase systemic toxicity and speed the dehydration of the body. The pathological changes of the lung in the "dry lung" patterns may be the results of high-concentrate bleach fume inhalation combined with dehydration and possible direct aspiration of the bleach as well.

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3. Reisz G.R. Toxic pneumonitis from mixing household cleaners. *Chest*. 1986; 89:49-52.
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Chemical Burns and Inhalation, Bleach, Sodium Hypochlorite

H119 Delayed Post-Hypoxic Leukoencephalopathy (DPHL)

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After attending this presentation, attendees will understand how to conduct the unusual diagnosis, DPHL, and appreciate its causes, differential diagnosis, and neuropathological substrates.

This presentation will impact the forensic science community by enlightening attendees regarding DPHL and by highlighting the unnatural causes of the syndrome.

A 20-month-old infant was taken to a hospital for fever and diarrhea. While being held down on his back, for a rapid Strep swab, he vomited, aspirated, and developed respiratory distress with hypoxia. A chest X-ray revealed dense consolidation in the right upper and middle lobes of the lung. He was intubated and began to require medication to maintain blood pressure. The infant's condition worsened and he developed acute respiratory distress syndrome and cardiac failure. The cardiac function recovered after a few days of extracorporeal membrane oxygenation. Despite the stabilization of his cardiopulmonary status, he had suffered severe cerebral hypo-oxygenation. Following hospital discharge, the infant had a dense left hemiparesis, but had use of his right arm and was improving with therapy. He was beginning to feed himself and was smiling and using several words. Three months later, his neurologic status deteriorated. The Magnetic Resonance Imaging (MRI) of the brain revealed diffusely abnormal white matter. The differential diagnosis included storage diseases, cerebral infections, vanishing white matter disease, and delayed post-hypoxic leukoencephalopathy. Years later, at 5 years of age, his mother fed him through his gastric feeding tube and put him in bed to nap. She later found him unresponsive and not breathing. An autopsy revealed a diagnosis of DPHL.

DPHL is a rare and under-recognized demyelinating syndrome. To date, autopsy and histologic descriptions of this entity are rare. This presentation reports an autopsied case of DPHL and discusses the histologic findings and differential diagnosis.

In the typical, two-stage presentation of DPHL, there is a recovery from a comatose state, followed later by an acute onset of neurologic signs and symptoms. There are no formal criteria for the diagnosis of DPHL, though clinical history and white matter changes are sufficient if other etiologies have been excluded.

DPHL is considered a distinct process in contrast to other direct causes of acute leukoencephalopathy. DPHL was originally described as sequelae of carbon monoxide poisoning and has recently been associated with the use of drugs of abuse. Though its pathophysiology is not understood, carbon monoxide, heroin, and benzodiazepine may be directly myelinotoxic. DPHL from drug exposures is similar to that from other forms of hypoxia; therefore, it is unclear whether neurotoxicity from heroin or other impurities in addition to hypoxia alone is involved. A period of prolonged cerebral anoxia is a common feature of all cases of DPHL. DPHL has also been described in a setting of strangulation and hemorrhagic shock; therefore, cerebral hypo-oxygenation is sufficient to cause DPHL without a specific toxic mechanism.

The gross neuropathological findings include profound widespread degeneration of the white matter with relative sparing of the u-fibers, the gray matter, and the cortical ribbon. The white matter demyelination is associated with the presence of macrophages and reactive astrocytes. It has been proposed that it is damage to glial cells in the white matter that is responsible for the demyelination. It has also been suggested that arylsulfatase A deficiency predisposes susceptible individuals to DPHL and lactic acidosis is the pathogenesis of this disorder. In this case, there was extensive myelin loss in white matter in a severely vacuolated background neuropil and immunohistochemical staining confirmed the diagnosis. The cause of the demyelinating syndrome in this case was pure hypoxia without the presence of drugs of abuse.

Neuropathology, Leukoencephalopathy, Cerebral Hypoxia

H120 Dying to Be Beautiful: Fatal Fat Emboli After Liposuction and Brazilian Butt Lift Procedure

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The goal of this presentation is to illustrate a rare and fatal outcome of cosmetic surgery in a patient with no immediate post-operative complications.

This presentation will impact the forensic science community by demonstrating the sudden death of an otherwise healthy individual following an elective surgical procedure of growing popularity.

Introduction: Cosmetic surgical procedures are on the rise in the United States. In 2015, 15.9 million procedures were performed, including an increased number of buttock augmentations with fat grafting. These procedures are generally considered safe, but, as with any surgery, patients remain at risk for possible adverse effects from bleeding and infection to serious cardiopulmonary complications. While more commonly seen in orthopedic surgeries or trauma, pulmonary fat emboli have been documented in cosmetic procedures.

Materials and Methods: This case involves a 31-year-old Asian female found unresponsive in her hotel room. She had traveled from out of town to undergo an outpatient Brazilian butt lift procedure (abdominal liposuction with fat redistribution to the buttocks), and was recovering in a hotel room. The procedure had gone well, and an exam on post-operative day one revealed no issues. When the decedent failed to arrive at her appointment on post-operative day two and could not be reached via phone, the surgeon initiated a welfare check. A hotel employee found her lying on the bed, fully clothed. Emergency medical services were called, but the decedent was pronounced dead on arrival. An investigation of the room revealed various medications, including diazepam and oxycodone. There was no evidence of a struggle.

Results: External exam revealed multiple 0.25 inch - 0.5 inch surgical incisions on the abdomen, back, buttocks, anterior inguinal region, and right dorsal hand. Yellow-tinged froth was present in and around the nose. Tardieu spots were identified on the lateral right chest. Examination of the deep and subcutaneous fat of the back and abdomen revealed areas of hemorrhage. All organs were grossly unremarkable, but microscopic examination of the lungs demonstrated diffuse, bilateral fat emboli throughout the lung vasculature. Postmortem toxicology demonstrated low levels of her prescribed ketamine and oxycodone in the blood.

Conclusions: Elective cosmetic surgeries are common, but not without risk. Surgical complications can range from minor to fatal. Distortion of tissue boundaries can lead to fat globules entering the vasculature, resulting in fatal emboli. With the increase in popularity of fat redistribution procedures, the safety of these practices must be reviewed in order to prevent unnecessary loss of life.

Brazilian Butt Lift, Fat Redistribution, Fat Emboli

H121 The Cane Sword: A Case Study

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After attending this presentation, attendees will: (1) become more familiar with a unique blade-type weapon, the "cane sword," and the injuries associated with its use; and, (2) understand that, while it is typically impossible to determine with certainty that a particular weapon produced a specific sharp force injury, certain features of a stab wound, including its width and depth, can assist in determining whether or not a suspect blade may have produced the injury.

This presentation will impact the forensic science community by highlighting a unique weapon type that may be encountered in death or crime investigations.

Despite the predominance of firearms-related deaths in the United States and in certain other locales, the most commonly used method of homicide, worldwide, is the use of sharp force.[1] Sharp force injuries are often caused by instruments that contain a blade, also called "blade-type weapons." One of the more unique blade-type weapons is a seemingly innocuous cylindrical "cane" that releases at the handle and slides off to reveal a blade hidden within it, commonly known as a cane sword.[2] This type of weapon allows the user to conceal the blade inconspicuously within the body and hilt of the cane until such a time when the user decides to reveal the blade. Homicides committed using unique weapons such as the cane-sword look very similar to those committed by other blade-type weapons and can be difficult for forensic pathologists, law enforcement officers, emergency medical personnel, and physicians to identify.

This case report provides an overview of the injuries caused by a cane sword. The victim was a 27-year-old Hispanic male who was found unresponsive in an alleyway outside of his ex-girlfriend's house. When law enforcement arrived on the scene, they found the victim with a stab wound in the left mid-back. Cardiopulmonary resuscitation was attempted, but was ultimately unsuccessful. Law enforcement officials began to question surrounding witnesses and eventually were able to obtain the weapon used in the crime: a cane sword. Upon autopsy, the stab wound was found to perforate both the lower and upper lobes of the left lung, resulting in a left hemothorax. The wound track was relatively narrow along its entire course, consistent with having been produced by the long, narrow blade of the cane sword. Additionally, the victim had other blunt force injuries to the face/head, trunk, and extremities. From these findings, the cause of death was determined to be a "stab wound to the back" and the manner of death was "homicide."

The wounds caused by the cane sword in this case included tissue perforation, bleeding, and an eventual pneumo- and hemothorax. These findings are commonly caused by blades in general, not only the cane sword. This case emphasizes the difficulties that can exist when forensic pathologists attempt to determine the exact type of weapon involved in a stabbing injury. While "matching" a particular weapon to a specific wound is not typically possible, certain features of the wound, including items such as track width and length, can assist in determining whether or not a suspect weapon might have caused the injury. Forensic pathologists should always bear in mind the possibility of stab wounds being caused by unique and non-traditional blade-type weapons.

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2. Sword Canes. BudK Worldwide. 2016. http://www.budk.com/Sword-Canes-2893. *Accessed* 25 March 2016.

Cane Sword, Stab Wound, Homicide

H122 Suicide by Trash Compactor

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After attending this presentation, attendees will understand that there are multiple modalities by which one can commit suicide. One of the more unusual modalities is by entering a trash compactor. At the end of the trash chute is a compacting bin that creates devastating, crushing injuries to the decedent. The body can easily lay undiscovered and the compactor contents may be transferred to a distant landfill without the knowledge of law enforcement, friends, or family.

This presentation will impact the forensic science community by sharing this unusual method of committing suicide, and will alert law enforcement and forensic pathologists to consider this possibility when searching for the body of a missing person. Recovery of a body is critical to the grieving process and consideration of the circumstances and injuries helps provide family members with an explanation for the death of their loved one.

Suicide is a significant global public health concern. In the United States, nearly 43,000 people die by suicide every year, with an estimated cost of 44 billion dollars. The annual age-adjusted suicide rate is 12.93 per 100,000 population, and this rate is increasing. Overall, suicide is the tenth leading cause of death. Men are nearly four times more likely to commit suicide than women, and most suicides occur in middle-aged White men. Nearly half of all suicides involve a firearm. Violent and highly lethal methods of suicide are more frequently seen among men.[1] Individuals typically choose a suicide method based upon social acceptability, including cultural factors, and opportunity.[2] A case involving a rare and unusual suicide modality – use of a trash compactor will be presented.

A trash compactor is a machine used to reduce the size of waste material via compaction, using a hydraulic pressure system. Fatalities associated with compactors are usually determined to be accidents, such as those occurring in the workplace or from individuals falling asleep in bins, before being crushed in garbage trucks. Compactors are used in residential apartment buildings to process the large amounts of waste generated, which helps to control rodent infestations in multistory buildings. Compaction ratios, the volume reduction created by a compactor, can be as high as 20 to 1.[3]

A 52-year-old Hispanic man, with a history of depression, traveled from Georgia to New York City to visit his estranged wife in an attempt to reconcile their relationship. He was last seen alive at 9:00 a.m. when his wife went to work. That afternoon, his daughter, who was in the apartment in her bedroom all morning and early afternoon, discovered dried blood droplets in many areas of the apartment. She found blood on the sheets of the mattress in the living room where her father had been sleeping. The daughter also found blood in the open silverware drawer in the kitchen and on the kitchen floor. The daughter called 911 to report her father missing.

The police performed a search of the apartment, hallway, and all public areas, including the perimeter of the apartment building. They identified a blood trail leading from the apartment, along the hallway, and to the trash compactor closet on the same floor. The door to the compactor chute also had blood on it. The police went to the basement, eight floors below, and entered the compacting room, where the body was discovered inside a compacting container.

At autopsy, the decedent was normally developed and well nourished, 5'6" and 147lbs. It was determined from the measurements of the compactor chute door that the decedent was able to fit through the chute if he entered with his shoulders positioned diagonally.

There were multiple blunt impact injuries, including crushing and avulsion of bones and organs. There were multiple fractures of the skull and vertebral column. Every rib was fractured at least once. Multiple compound fractures of the humeri, femurs, tibia, and fibula were noted. There was avulsion of major organs including the brain, lungs, heart, liver, spleen, and kidneys. A horizontal incised wound was observed on the ventral aspect of the left wrist. Adjacent to this incised wound were multiple parallel superficial incised wounds, all consistent with hesitation injury.

Trash compactor fatalities produce blunt impact injuries from the violent crushing motion of the compactor.

This case represents a rare suicidal act by such a method. Individuals more frequently choose suicide modalities such as asphyxia, firearms, or ingestion of pills or poison. A thorough case investigation, including a detailed history, scene investigation and autopsy findings are necessary to determine the manner of death, so that accident or homicide can be excluded. This case highlights the importance of law enforcement performing a thorough search at the scene, including all accessible areas, such as the roof, basement, and surrounding grounds. The thoroughness of law enforcement in this case allowed the decedent to be discovered immediately. Had this modality not been considered, the decedent may have been transported to a distant site where his remains may have gone unnoticed for weeks, months, or even longer.

Reference(s):

1. National Suicide Statistics: http://www.cdc.gov/ViolencePrevention/suicide/statistics/index.html.
2. Methods of suicide: international suicide patterns derived from the WHO mortality database http://www.who.int/bulletin/volumes/86/9/07-043489/en/.
3. The Case for Trash Compactors: http://www.buildings.com/article-details/articleid/14213/title/the-case-for-trash-compactors.aspx.

Suicide, Trash Compactor, Unusual Suicide Modalities

H123 Spontaneous Coronary Artery Dissection as Cause of Death in the Postpartum Period: A Case Report

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After attending this presentation, attendees will better understand the clinical presentation and pathology of this unusual condition, which primarily presents itself as sudden death or myocardial infarction in young women.

This presentation will impact the forensic science community by alerting professionals to this rare condition that, when misdiagnosed, may lead to death and eventually to medical malpractice judicial processes. Since the frequency is small, it seems important to understand common issues in all cases reported so future strategies of early approach and treatment may be implemented.

Presented here is a case of a 37-year-old woman in the postpartum period after her second caesarean, with no complications during pregnancy or childbirth, without any known previous diseases, who was admitted in the emergency room of Vila Nova de Famalicão's Hospital with chest pain radiating to her upper right limb, associated with sudden visual impairment. On admission, she had electrocardiographic changes suggesting an acute coronary syndrome and elevation of the biological markers of myocardial necrosis. Sustained chest pain demanded morphine administration. Because of hemodynamic instability with hypotension, she was provided with lifesaving thrombolysis with alteplase, after what she entered cardiac arrest, irreversible after 90 minutes of advanced life support.

The autopsy revealed endocardial and left ventricular myocardial macroscopic changes, suggesting ischemia, confirmed histologically, and dissection of the common branch, anterior descending branch, and proximal portion of the circumflex branch of the left coronary artery.

Coronary dissection may be classified as primary or may be secondary to aorta dissection, thoracic trauma, or medical procedures such as coronary angiography or angioplasty.

Primary dissections are very infrequent events and usually occur in young people, especially women, and are many times related to pregnancy or puerperium. This is probably due to the hormonal changes that may damage the tunica media, increasing risk of dissection. Hemodynamic stress of labor may also cause tunica intima disruption, followed by true dissection. Spontaneous coronary artery dissection is usually presented as sudden death, so the diagnosis is often made through autopsy. Myocardial infarction also is a common clinical presentation and the diagnosis is made through coronary angiography. Other presentations, such as prolonged angina, are more infrequent. The most commonly affected coronary artery is the anterior descending coronary artery. Treatment is not yet well established and may include cardiac transplantation, emergency coronary artery bypass grafting, thrombolysis, coronary angioplasty, or intensive care, depending on the extension of the dissection and its clinical repercussion.

Forensic Pathology, Coronary Dissection, Postpartum Death

H124 A Selfie Stick Shotgun Suicide

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After attending this presentation, attendees will be familiar with the consequences of possibly using a selfie stick as a shotgun trigger pull in suicide, including inadequate control of direction of fire with subsequent facial destruction, and will review how to manage identification issues following destruction of facial features and postmortem canine depredation.

This presentation will impact the forensic science community by reviewing investigative findings, autopsy findings, recommendations for scientific investigation, gunshot wound path analysis in a death by shotgun involving complicated identification issues, and the probable use of a selfie stick as a trigger pull.

The challenges presented by a disfigured decedent for scientific identification, gunshot wound documentation, and cause of death determination following a novel shotgun triggering method with secondary postmortem animal activity may be overcome through a combination of scene investigation, forensic autopsy, and forensic dentistry.

This presentation reports a death by submental shotgun wound involving a selfie stick and reports the findings from the investigation, autopsy, and forensic dentistry, and discusses the determination of the cause and manner of death.

The decedent was a 20-year-old Caucasian female, married, but living alone with two dogs while her husband was deployed overseas. Friends who knew that she had been going through marital difficulties called police for a well-being check.

A police officer entered the secure residence through a side window. The dogs greeted him without aggression. He located the decedent on the bedroom floor. Her scalp, face, neck, and upper chest above her clothing, were denuded of flesh. Blood spatter was noted across the side of the door, the wall, the ceiling above the decedent, and on a tablet computer and empty glass on the table next to her. Beside her was a shotgun and a selfie stick; blood drip and spatter were noted on both.

A selfie stick is a telescoping device utilized to hold out a personal phone when taking pictures of oneself. This selfie stick was fully extended to approximately three feet. The proximity of the selfie stick to the shotgun and to the decedent's left foot suggested that it had played a role in the event. Investigators theorized that the decedent had utilized the selfie stick to pull the shotgun trigger.

Animal control officers took custody of the dogs, and the decedent was transported to the medical examiner's office. External examination noted tattoos with themes of death and of romantic loss. The area of skin and subcutaneous tissue loss began at the areola of the right breast, involved the upper chest except for areas protected by the decedent's T-shirt, and included the entire anterior and lateral neck and face. The pattern over the torso, neck, and scalp suggested animal activity; on the face, animal activity overlapped ballistic injury. Areas protected by the decedent's thick hair were not involved by animal activity. The central face and jaw were largely absent; investigators submitted a few teeth found on the carpet of the apartment. The calvarium appeared intact.

Tissue loss precluded reconstruction of the actual site of the entrance gunshot wound. There was no skin left to determine characteristics of hard contact. Radiography revealed shotgun pellets within the facial tissue. Facial dissection determined a probable submental entrance wound; the direction of fire was upward. Representative pellets (but no shotgun wad) were retrieved from the soft tissue. There was no pellet entry into the cranial cavity; there were secondary frontal, parietal, and cerebellar contusions, with subarachnoid hemorrhage. The mechanism of death was most likely blood loss from facial injury, with some contribution from concussion and brain injury. Investigators speculated that the direction of fire through the face may have been affected by difficulty in manipulating the shotgun with the selfie stick.

Scientific identification was challenging. There were no fingerprints on file. No usable fingerprints were developed from items at the scene. No X-rays were available for antemortem and postmortem comparison, except

for dental films. Reconstruction of the jaws for dental identification was accomplished by the forensic dentist in cooperation with medical examiner staff utilizing multiple radiographs and reconstructions.

There was no note. The conclusion of investigators was that the manner of death was suicide. Although it could not be demonstrated conclusively that the selfie stick was used to pull the trigger, it appears a reasonable deduction. The outcome for the dogs is unknown.

Selfie Stick, Shotgun Suicide, Postmortem Animal Activity

H125 Acute Exacerbation of Asymptomatic Idiopathic Pulmonary Fibrosis After Radical Resection of Rectal Carcinoma: A Case Report

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The goal of this presentation is to present a rare, unexpected death of a patient who died of respiratory failure due to acute exacerbation of Idiopathic Pulmonary Fibrosis (IPF) after the radical resection of rectal carcinoma.

This presentation will impact the forensic science community by increasing understanding of acute exacerbation of idiopathic pulmonary fibrosis by describing the clinical, radiological, and pathological findings of this case.

Introduction: IPF is a chronic lung disease of unknown etiology, which is histologically characterized by interstitial fibrosis. The cause of IPF-related death is often respiratory failure. Although the disease is chronic, abrupt worsening can occur in some situations, which is called "acute exacerbation" (AE-IPF) or the "terminal complication" of IPF. AE-IPF is a sudden and unexplained worsening of respiratory symptoms accompanied by hypoxemia. The annual incidence of AE-IPF is 10%-15% among IPF patients.

Case Presentation: A 57-year-old Chinese man was admitted to a local hospital with complaints of bloody stools for five months. A 2cm × 3cm mass was found about 2cm away from the anal opening. The rectal biopsy revealed rectal adenocarcinoma. The preoperative chest roentgenogram showed focal and reticular thickening in the bilateral middle and lower lobes. The laboratory examinations showed no inflammatory signs or surgery contraindication. The radical resection of rectal carcinoma was performed under general anesthesia. The patient smoothly recovered from anesthesia after the surgery; however he developed dyspnea, and cyanosis, then became comatose 12 hours after the surgery. Oxygen saturation was reduced to 48%. The oxygen saturation was >90% but unstable by mechanical ventilation. Despite resuscitation attempts, the patient died three days after the onset of symptoms.

Autopsy and Histology Findings: Forensic autopsy was performed because the cause of death was unknown and medical malpractice was suspected. The heart weighed 400g. The thickness of the left and right ventricular walls was 1.10 cm and 0.45 cm, respectively, with slightly dilated right ventricle. Plaque and luminal narrowing were detected in the middle of the left anterior descending artery and right coronary artery, both of which caused <50% stenosis of vessel lumen. The bilateral lungs weighed 1740g and the texture was tough. Histological examination revealed diffuse pulmonary edema, focal emphysema, and medial thickening of pulmonary arteries. In addition, areas of normal lung and interstitial fibrosis changes were concomitantly observed. The interstitial fibrosis changes displayed alveolar wall thickening by excessive collagen, fibroblasts, and slight inflammatory cell infiltration, with reduced alveolar capillary.

Conclusion: Based on the autopsy, histological examination, chest roentgenogram clinical appearance, and acute deterioration with no infections, the leading cause of respiratory failure was identified as AE-IPF. In addition, coronary artery disease had likely contributed to the patient's death. Given the possible mechanism, it was speculated that the inflammatory response to surgical intervention, exposure to high oxygen concentrations, and overdistension of the lung by positive-pressure ventilation was thought to be possible factors that trigger the progression of IPF. Thus, this case highlights that doctors and medicolegalists should pay close attention to AE-IPF, especially in patients without obvious symptoms. Early detection and diagnosis can significantly improve the prognosis.

Acute Exacerbation, Idiopathic Pulmonary Fibrosis, Surgery

H126 Sexual Assaults in Geneva, Switzerland, 2006-2012: Forensic Doctor Usefulness

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After attending this presentation, attendees will understand the main forensic observations noted in cases of sexual assaults in Geneva throughout the 2006-2012 period and the usefulness of the forensic doctor.

This presentation will impact the forensic science community by demonstrating the importance of a multidisciplinary approach, in particular between forensic doctors and gynecologists.

Currently, it is recognized that sexual assaults take on medical importance (physical and psychological) as much as legal and social importance with frequently dramatic long-term consequences for the victims (increase of cardiovascular risks and risk-taking behaviors, such as extreme sports, alcoholism, or smoking).

According to the literature, one woman in six falls victim to sexually-connotative assault throughout her lifetime and one in ten will be victim of rape at least once. This is why quality of care is essential as it allows for the reduction of post-traumatic stress and encourages an increasing number of victims to come forward, as has been the case in Geneva for several years. An increase in the number of cases, from approximately 20 per year in the late 1990s to approximately 100 per year at present was actually noted. Because of this, it was decided to create a protocol, with a rape kit, in order for each victim to be treated in an identical way, by both a gynecologist and a forensic doctor, who can both be consulted during the day, throughout the night, or on public holidays.

The epidemiological studies conducted in both Europe and the United States agree on the fact that the number of victims asking for help is on a constant rise. This is why care dispensed by trained professionals, in particular psychiatrists and psychologists, is systematically proposed to the victims the day after their emergency consultation.

Women who consult after having been sexually assaulted often do so for therapeutic reasons, namely for wound disinfection, detection (and treatment) of sexually transmitted diseases, and for the prevention of non-desired pregnancies. In the majority of cases, they do not file charges and the role of the forensic doctor becomes secondary; however, some victims consult with the goal of discovering evidence (in particular DNA) that would help identify the perpetrator. In this case, the role of the forensic doctor becomes essential. He/she must describe precisely and in detail all observations (bruises, wounds, dermabrasions, bites, etc.) made on the entire body. He/she must ensure that all samples that can be used as evidence are indeed collected (blood, sperm, saliva, clothing, etc.) in search of the offender's DNA. Blood and urine samples must also be taken for eventual toxicological analyses.

Finally, the forensic doctor will need to obtain the victim's consent, whether a complaint has been lodged or not, which will allow him/her to pass on relevant information to the authorities (prosecutor, judge, police). Pictures, also with the victim's consent, will be taken of the face and the body.

Two documents are immediately handed to the victim. The first consists of general information regarding global coverage with, if needed, different telephone numbers. The second consists of a temporary medical attestation containing the main findings, which is useful if the patient wishes to file a complaint.

In conclusion, this study illustrates the different socio-demographic characteristics of the victims, such as age, the location of the assault, the relationship with the aggressor, the number of aggressors, the time of the assault, the time elapsed between the assault and the consultation, as well as other characteristics, such as the results of toxicological analyses, the severity of the injuries observed, and the elements that lead a woman victim of sexual assault to lodge a complaint.

Sexual Assaults, Geneva, Switzerland

H127 The Loss of Dystrophin: An Immunohistochemical Study for Postmortem Diagnosis of Early Myocardial Ischemia

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After attending this presentation, attendees will understand the importance of being informed regarding the detection of immuno-inflammatory and cellular phenomena accompanying cardiac alterations during the early inflammatory phase to aid in the forensic diagnosis of early ischemic damage.

This presentation will impact the forensic science community by providing a new tool to detect early myocardial ischemic damage through the immunohistochemical analysis of dystrophin.

Sudden Cardiac Death (SCD) is an unexpected natural death due to cardiac causes that occurs within a short time period in a person without any prior condition that appears to be fatal. CAD and ischemic cardiac damage are the main causes of SCD. In these casesin which the death occurs within six hours from the onset of ischemic damage, the histological myocardial changes are not specific and cannot provide clear evidence for the postmortem diagnosis.

In recent years, various immunohistochemical studies for the detection of early myocardial infarction were conducted, and various markers were analyzed.[1,2] In fact, following the ischemic injury to the muscle tissue of the heart, the biomarkers released by the damaged cells and the altered cellular and extracellular molecules normally expressed in cardiac tissue can be detected microscopically using immunohistochemical techniques.

This study highlights the utility of dystrophin as a marker of early ischemic damage in a sample of SCDs with macroscopic and microscopic evidences of cardiovascular disease. Dystrophin was isolated in cardiac muscle, tightly associated oligomeric complex of proteins known as dystrophin-glycoprotein complex that plays an important mechanical function in stabilizing the sarcolemma during cardiac contraction and in the transmission of myofibers contraction force. Dystrophin may prove to be a very useful marker in detection of ischemic lesions utilizing immunohistochemical staining of the cardiac tissue, especially in cases in which the conventional histology, with hematoxylin-eosin, fails.

The study reveals that as a result of cellular ischemic damage the progressive loss of sarcolemmal staining of dystrophin occurs. The damage on the tissue is evidenced in microscopic areas with partial loss, such as interrupted sarcolemmal staining of dystrophin in cardiac myocytes, and in microscopic areas with complete loss of staining. These results show a time-dependent depletion of dystrophin; in fact, there is an increase in the area of marker depletion with the increase of post-ischemic time.

In conclusion, the present study demonstrates the usefulness of the dystrophin as marker of early ischemic damage. This marker can be a useful tool to reveal, with the immunohistochemical method, the myocardial damage within six hours of the onset of ischemic injury.

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Dystrophin, Immunohistochemistry, Early Myocardial Ischemia

H128 Changes in Bacterial Community Structure and the Estimation of Long-Term Postmortem Interval (PMI)

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After attending this presentation, attendees will gain a better understanding of the bacterial succession patterns associated with porcine decomposition and their utility in long-term human PMI estimation.

This presentation will impact the forensic science community by providing a new method for long-term PMI estimation.

When human remains are decomposed, investigators ask, "How much time elapsed between the actual death and the discovery of the remains?" Early physiological changes due to autolysis and putrefaction provide some clues and permit reasonable short-term PMI estimations, while insect succession patterns and developmental data allow estimations into the later stages of decomposition. Recent studies on murine, porcine, and human remains have indicated that epinecrotic bacterial succession may provide valuable information regarding elapsed physiological time since death; however, previous studies either had limited replication or were conducted in a laboratory environment. The main goals of this study were to conduct a replicated field experiment on porcine remains for an extended period of time (>1,500 Accumulated Degree Days (ADD), or >60 days), provide information about bacterial changes beginning within a few minutes after death, and develop a method for the estimation of long-term PMI. To accomplish those goals, skin microbial samples were collected from the torsos of the six porcine remains (*Sus scrofa*) every day during the first three days, on alternate days through the second week, and once every week for the remainder of the 60-day period. DNA was extracted using an organic extraction method as described in Zheng et al.[1] Bacterial structure associated with each sample was determined using 16S rDNA MiSeq® sequencing. Sequence data was analyzed using the Mothur pipeline and statistical analyses were performed in R version 3.2.3.[2,3]

Significant changes in bacterial structure were observed across all decomposition stages. Those changes corresponded to changes in the epinecrotic insect communities as well as porcine Total Body Scores (TBS) and Partial Body Scores (PBS). During the initial period of decomposition (from time of death (ADD 0) through the post-bloat stage of decomposition (ADD 149), there was a strong negative linear relationship (R^2=0.7321, y=-2.6411x+8.3845) between bacterial diversity (at 3% genetic distance) and the log 10 of ADD. That initial period of decomposition corresponded with peak adult and larval blow fly activity, as well as with peak Firmicutes and Clostridiaceae abundances; however, across all stages of decomposition, a non-linear relationship was observed (R^2=0.4826, y=1.3968x^2-7.298x+12.156). That weak R^2 value is due, in large part, to shifts in resource availability (namely, the skin being swabbed) and insect activity. The diversity scores between replicates began to diverge between ADD 149 and ADD 267, reconverged by ADD 734, and become more disparate thereafter. Shifts in bacterial diversity after ADD 149 corresponded with major shifts in insect activity and with diminishing amounts of skin and increasing bone exposure.

At the phylum level, an inverse relationship exists between relative abundances of Firmicutes, (increases until ADD 149; then decreases through ADD 1703), and Actinobacteria, which decreases until ADD 149 and then increases through ADD 1,703. Likewise, at the family level, an inverse relationship exists between the relative abundances of Clostridiaceae (increases until ADD 209; then decreases through ADD 1,703) and Enterobacteriaceae (increases until ADD 209; then decreases through ADD 1,703).

In conclusion, this study provides evidence that bacterial succession associated with the skin of decomposing remains has tremendous potential for utilization as an indicator for both short-term and long-term PMI estimations.

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Postmortem Interval, Microbial Ecology, 16S rDNA

H129 Myiasis and Death: Factors and Complications Related to Estimating Time of Colonization After Antemortem Fly Colonization Followed by Death

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After attending this presentation, attendees will be able to assess fly colonization for potential myiasis, determine underlying medical conditions that may predispose a decedent to myiasis, and understand the limitations myiasis may impose on a time-of-colonization estimate for approximating the postmortem interval.

This presentation will impact the forensic science community by illustrating how combining forensic entomology and forensic pathology can help: (1) elucidate the complicated nature of these cases; (2) provide valuable information related to the timing of injuries and the potential of antemortem abuse or neglect; and, (3) unravel unknown medical histories of decedents.

Myiasis, or fly colonization of the tissues of living people, is commonly associated with pre-existing cutaneous lesions resulting from natural disease or wounds or in some cases by accident.[1-4] Fly development is coordinated by temperature, with warm temperatures resulting in faster growth and cooler temperatures resulting in slower growth, with each species responding differentially to a given temperature regime. The procedure for estimating the timing of fly colonization requires some knowledge of the temperatures experienced by the developing larvae. Therefore, in the case of living individuals, body temperature is used to approximate development temperature, whereas in deceased individuals, ambient temperature is used. In cases of myiasis that occur shortly before death, this dichotomy is not so clear. Furthermore, when one considers the medical history and types of cases where myiasis is known to occur (e.g., wounds, known history of gangrenous limbs, decubitus ulcers), normal body temperature may not be an appropriate temperature history to apply to time-of-colonization calculations.

In this presentation, three cases will be used to illustrate how myiasis can be recognized in decedents, what types of medical history might suggest myiasis rather than postmortem colonization, and what limitations this uncertain temperature regime might have on not only time of colonization but postmortem interval estimation. These three cases illustrate that colonization typically occurred when flies had ready access to the body (e.g., ailing decedent found outdoors), that it was observed when the development of the fly larvae was more advanced than would be expected based on the state of, or lack of, decomposition, and occurred in association with medical conditions, such as diabetes mellitus, gangrene, or trauma with sepsis. Cases of myiasis associated with limbs and infections may not be accurately represented by normal body temperature, as in cases of reduced circulation or infection. Perhaps most significant in using the information from the time-of-colonization estimate is the dissociation between the fly colonization event and death, therefore rendering the time-of-colonization interval not representative of postmortem interval but indicative of other events.

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Calliphoridae, Maggots, Wounds

H130 Coupled Microbiome and Insect Evidence in Death Investigations

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After attending this presentation, attendees will understand how human postmortem microbiome profiles can be coupled with entomological evidence to estimate time since death. Attendees will gain a deeper appreciation for microbial evidence and how it compares to other established sources of biological evidence in estimating a minimum Postmortem Interval (minPMI). While several studies have demonstrated the tremendous potential for using microbial community succession during decomposition for estimating a minPMI range, there is much less understanding of how the postmortem microbiome succession compares with other forms of evidence in death investigation. Entomological evidence has been successfully used to estimate the Period of Insect Activity (PIA), an estimate that is usually a close approximation of the minPMI. Thus, comparing how microbial community profile estimates align with associated entomological PIA estimates will inform scientists and investigators of how microbes perform in instances in which the time of death is known.

This presentation will impact the forensic science community by providing: (1) a comparison of microbe and insect evidence collected during a unique case study; and, (2) a critical evaluation of the benefits and challenges of using microbial community evidence in 120 cases from Detroit, MI (referred to as the Human Postmortem Microbiome (HPMM) database). This presentation will evaluate a case study in which both microbial and insect evidence were collected and will compare how the different estimates of minPMI represented the known time since death. Thus, this presentation will also impact the forensic science community by providing an examination of the viability of using microbial evidence from the HPMM database.

The first goal of this study was to evaluate how microbial community (or microbiome) profiles and insect developmental stage estimates compared to a known time of death. The second goal of this study was to compare the postmortem microbiome profile of the case study to similar death circumstances and estimated PMIs of cases that were part of the HPMM database. The last goal of this study was to evaluate the case characteristics of the HPMM database and provide an assessment of the usefulness and limitations of collecting and analyzing the postmortem microbiome during routine death investigation.

In the case study, a cadaver was recovered in Lansing, MI, during the late summer (Lansing Case). It was located in a grassy area under the overhanging trees of a vacant lot in a residential area located between two occupied houses. The decedent was a 27-year-old Black male suspected homicide victim in advanced stages of decomposition. Insect and microbial specimens were collected as described in recent studies and used to make estimates of the PIA and minPMI, respectively.[1,2] For the microbial profiles, samples taken from the buccal cavity were compared to the HPMM database and with estimated PMIs for the following ranges: 1h-24h, 25h-48h, 49h-72h, and >72h.

Insect evidence, based on third instars and presumed pupae of *Phormia regina* (Diptera: Calliphoridae), suggested a PIA range of 13-20 days for the Lansing Case, which was in agreement with the known PMI of 19 days provided by the investigators. When the Lansing Case microbial communities were compared to the larger metropolitan city dataset, there were marked differences in the profiles between cases with estimated PMIs from 1h-72h, but more similarities with those cases with a PMI >73h. For instance, *Sporosarcina* was only found in cases with a PMI >73h, suggesting that this taxon may be an indicator of PMIs of three days or more. Bacteroidia were nearly absent in this time interval and the Lansing Case, whereas they comprised 5%-11% of the communities for all other cases. These results indicate that certain microbial taxa may be indicative of long-term (e.g., >one week) PMIs that may be associated with later developmental stages of blow flies that colonize cadavers; however, additional studies with more cadavers with long-term PMIs are needed to address this important question.

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Postmortem Microbiome, Forensic Entomology, Necrobiome

H131 Analyses of Necrobiome Structure and Function for Utility in Forensic Science

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After attending this presentation, attendees will have a better understanding of how host-associated microbial communities respond following host death.

This presentation will impact the forensic science community by discussing the ways in which the methods outlined and data obtained determine how commensal bacterial populations in host remains translocate, proliferate, and modulate gene expression following death. These data may allow identification of specific microbial taxa or proteins for the potential use in quantifiable measurements of Postmortem Interval (PMI) used in forensic science.

Estimating minimum PMI relies on multiple factors, such as detecting chemical signatures and measuring insect colonization, but a widely used method relies on assessing physical changes a body undergoes during decomposition: fresh (including pallor, algor, rigor, and livor mortis), bloat, active decay, and dry decomposition. Few studies have focused on the dynamics of existing microbial communities located internally and externally of a living individual prior to death, such as in or on the gastrointestinal tract or skin. These microbial communities thrive by utilizing nutrients and surface areas provided by the host, but are usually kept to a specific niche (e.g., stomach, liver) by the host's immune system. Host death renders the immune response relatively inactive, and within 24 hours, bacteria proliferate and translocate to previously sterile spaces inside the body. Determining postmortem microbial community responses to decomposition may prove useful in determining colonization routes correlating to the PMI estimate.

Using a mouse model, this study investigated the postmortem microbial community structure, translocation, and gene expression of the bacterial species present in a controlled setting, minimizing outside microbial influence, focusing only on host-associated bacteria. Immunocompetent mice (n=60) were inoculated intranasally with commensals, *Staphylococcus aureus*-RFP and *Clostridium perfringens*, to introduce controlled aerobic and anaerobic communities, respectively, with the ability to degrade host tissue through protease production. Thirty inoculated mice were immediately surface sterilized with a 10% bleach solution following sacrifice to disrupt the influence of external microbiota. DNA and RNA were isolated and purified from heart, lung, intestine, and bone marrow samples one hour to seven days postmortem, for use in quantitative Polymerase Chain Reaction (qPCR), real-time qPCR, and meta-transcriptomic sequencing. Results of qPCR targeting *S. aureus*-RFP revealed transmigration into many of the tissues in as early as one hour, including previously sterile spaces, such as bone marrow. Additionally, ongoing gene expression analyses show differential expression relative to organ and PMI. Altogether, these early results suggest the bacterial translocation and expression demonstrate promise to be sufficiently predictable for utility in forensic science and the criminal justice system.

Necrobiome, Microbial Community, Gene Expression

H132 Digging Up the Past — An Atypical Medical Examiner's Case

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After attending this presentation, attendees will be familiar with the recovery of historical skeletal remains with evidence of previous postmortem examination.

This presentation will impact the forensic science community by demonstrating how medical examiners and coroners must work closely with anthropologists to determine: (1) if the remains in question are human; (2) if the remains display any trauma; and, (3) if the remains are of contemporary forensic significance or are of historical interest. Attendees will be able to further utilize the information presented from the case summary to promote the participation of the medical examiner or coroner office alongside a forensic anthropology investigation.

This study examines skeletal remains recovered in Edmore, MI, by the Montcalm County Medical Examiner's Office. In August 2015, Michigan state police were dispatched to a private residence where the homeowners had unearthed multiple bones while excavating a portion of their backyard to unclog a sewer line. Upon the arrival of the Montcalm County medical examiner investigator, Michigan State University's Forensic Anthropology Laboratory (MSUFAL) was called in to assist with the recovery. A forensic anthropologist and several graduate students from MSUFAL along with a representative from the Montcalm County Medical Examiner's Office, began mapping, photographing, and excavating the disturbed area identified by the homeowner.

The remains previously uncovered were determined to be human remains from an adult individual. After assessment of the taphonomic signatures and general context, the forensic anthropologist determined the remains were historical, most likely representing a primary interment of an early homesteader of Edmore, dating from the late 1800s to the early 1900s. The general lack of anatomical arrangement and disruption of the overlying soil strata suggest the burial was most likely initially disturbed during construction of the city's sewer line sometime between 1950 and 1960. Due to the scattered disposition of the remains still *in situ*, the MSUFAL decided to continue excavation of the yard following the sewer line, anticipating the recovery of additional bones. The remains of at least one additional individual were eventually recovered.

All of the remains were transported to the Montcalm County Medical Examiner's Office morgue facility for a brief examination by a forensic pathologist, who noted multiple specimens with evidence of sawing in areas atypical for modern autopsy procedures. The remains were then transported to MSUFAL for a more comprehensive analysis. There, a minimum number of individuals (two) was estimated and the postmortem damage to the remains recorded. Multiple cranial fragments from at least one individual evinced cut marks consistent with even striation patterns and several incomplete saw cuts (kerfs). The distribution and location of these cuts are consistent with the separation of the calvarium and a near mid-sagittal section. The observed pattern of these defects was compared to specimens dating to approximately the same period (late 19th to early 20th century) with evidence of postmortem examination. The MSU anthropology laboratory concluded the defects represent evidence of postmortem examination through obsolete autopsy practices rather than anatomical preparations or trauma/clandestine dismemberment. These distinctions are based on the absence of anatomical hardware (including springs, wire, clasps, etc.), the taphonomic signature noted on the remains (including cortical staining, exfoliation, delamination, etc.), and the presence of coffin wear, a phenomenon noted on historical remains consistent with long-term interment within a casket. Based on the totality of evidence, the remains are not considered forensically significant.

The recovery of previously autopsied remains is not common, and the correct interpretation of remains with evidence of postmortem examination is imperative, particularly when the techniques employed do not conform to contemporary autopsy practice. This case demonstrates the importance of a multidisciplinary approach to forensic investigations of skeletal remains that bear evidence of tool marks. The medical examiner's office, the anthropology laboratory, and the various police agencies working together were able to demonstrate the non-forensic nature of the recovered material.

Historical Skeletal Remains, Investigation, Forensic Anthropology

H133 The Evaluation of Acute Alcohol Intoxication as the Primary Cause of Death: A Diagnostic Challenge for Forensic Pathologists

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After attending this presentation, attendees will better understand how to evaluate postmortem blood alcohol concentration in cases in which Acute Alcohol Intoxication (AAI) is considered the primary cause of death.

This presentation will impact the forensic science community by illustrating the characteristics of death scene investigation and autopsy findings, drinking history, and postmortem lethal alcohol concentrations of different biological specimens in deaths from AAI and by demonstrating the significant role forensic pathologists/medical examiners have in improving public health.

Excessive alcohol consumption is a leading preventable cause of death in the world. Deaths caused by AAI remain a major public health issue. Determination of AAI as the primary or direct cause of death continues to present a major challenge to forensic pathologists because postmortem lethal blood alcohol concentration can be affected by many factors. The objective of this study was to evaluate the epidemiological characteristics, death scene and postmortem examination findings, and lethal alcohol concentrations of different biological specimens in the individuals whose primary cause of death was AAI.

This study was a retrospective review of forensic autopsy cases of all deaths due to AAI investigated by the Office of the Chief Medical Examiner (OCME) in the State of Maryland over a nine-year period from 2004 to 2012. During the nine-year period, a total of 35,610 autopsies were conducted by the OCME. Of the 35,610 cases, AAI was listed as primary cause of death in 150 cases. One case was excluded from this study because of prolonged hospitalization. Of the 149 cases, 48.3% were White, 32.9% were Hispanic, 18.1% were Black, and 0.7% were Asian. But the death rate from AAI among Hispanics (10.41 per 100,000 population) was significantly higher than Whites (2.14 per 100,000 population) and all the non-Hispanics combined (1.88 per 100,000 population).

The male and female ratio was 4.5:1. Their age ranged from 16 to 65 years, with a mean age of 37 years. More than 80% (*n*=119) of the individuals had a history of alcohol abuse and 40% were known binge drinkers. Their mean heart weight was 405.87 grams with 13 (8.7%) individuals' heart weight more than 500 grams. The majority (*n*=104, 69.8%) of individuals were overweight with 36 (24.1%) obese. This study observed a strong association between Body Mass Index (BMI) and lethal blood alcohol concentration. The obese group displayed significantly lower blood alcohol concentration than the underweight and normal weight groups. In the obese group, the mean Heart Blood Alcohol Concentration (HBAC) and Peripheral Blood Alcohol Concentration (PBAC) was 0.36% and 0.37%, while in the group with normal BMI, the mean HBAC and PBAC was 0.45% and 0.42%, respectively. Of the 149 cases, 11 individuals had both HBAC and PBAC less than 0.30%. They were relatively younger individuals with a mean age of 26 years, usually binge drinkers (*n*=9), either overweight or obese (*n*=10). Data also demonstrated that intoxicated overweight people were more frequently found in the prone position compared to normal or underweight people. People with a postmortem BAC of <0.3% were mostly overweight or obese with a mean BMI of 31.3 and were more frequently found in a prone position at the time of death. Data suggest that the prone position of intoxicated people, especially when they were overweight or obese, may further compromise respiratory function and lead to death.

This study estimated the phase of alcohol intoxication at the time of death based on the PBAC and Urine Alcohol Concentration (UAC) ratio. Most deaths likely occurred at or close to peak phase (49.6%), followed by post-absorptive phase (31.6%), and absorptive phase (18.8%). The PBAC was significantly lower in the post-absorptive phase when compared with absorptive phase and peak phase. Of the 11 cases with a BAC of <0.3%, nine deaths likely occurred in the post-absorptive phase and two at, or close to, peak phase.

This study indicates that to certify the primary cause of death due to AAI, forensic pathologists/medical examiners should evaluate the postmortem BAC in the light of the individual's age, drinking history, body weight, possible phase of alcohol intoxication at the time of death, and death scene investigation findings, including found position as well.

Acute Alcohol Intoxication, Forensic Toxicology, Forensic Autopsy

H134 Deaths From Combined Methamphetamine and Heroin Use in Denver: 2005-2016

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After attending this presentation, attendees will better understand trends of methamphetamine and heroin use in Denver, CO, some of the possible reasons thereof, and the interpretation of applicable toxicology results.

This presentation will impact the forensic science community by describing emerging trends of methamphetamine and heroin abuse in Denver, a previously unreported subject in the Rocky Mountain region.

Methamphetamine and its metabolite, amphetamine, are strong sympathomimetic and dopaminergic stimulants whose overall overdose mortality in the United States increased nearly two-fold from 1999 to 2009.[1,2] Likewise, there has been an increase in methamphetamine use during the past several years in Denver.[3] Since 1999, deaths from heroin, an opioid with analgesic and sedative properties, increased five-fold nationally.[4] While much is known about the effects of either methamphetamine or heroin alone, comparatively little is known about the two drugs when they are taken concomitantly or in succession.[5,6] Some studies have found a synergistic effect between the two drugs and postulated that one may ameliorate the unwanted side effects of the other.[7] Readiness of availability is another factor.[8] Informal clinical observations in Denver reveal that approximately half of known local injection drug abusers use a combination of methamphetamine taken simultaneously or in succession with heroin, and that this trend is increasing. This study reports mortality data from combined methamphetamine and heroin toxicity using records of the Denver Office of the Medical Examiner (DOME) from 2005-2016.

The DOME maintains a searchable database of all deaths reported to the office using modified Systematized Nomenclature of Medicine-Clinical Terms. The database was queried for deaths in which toxicology results were positive for a combination of methamphetamine and heroin or one or more of its metabolites, 6-monoacetylmorphine (6-MAM) and morphine. Toxicological testing was performed according to internal laboratory protocols with appropriate controls at National Medical Services, Inc., Willow Grove, PA, by means of Gas Chromatography/Mass Spectrometry (GC/MS) and/or Liquid Chromatography/Mass Spectrometry (LC/-MS) on blood samples, and enzyme immunoassay on urine samples. At autopsy, peripheral blood was selected for analysis preferentially, with occasional substitution of heart or cavity blood and the addition of urine, depending upon the circumstances and specimen availability for a given case. Each autopsy report was reviewed by forensic pathologists to ensure that the toxicology results and circumstances surrounding the death were consistent with the use of both methamphetamine and heroin by the decedent. Cases in which drugs were detected but did not cause death directly (e.g., trauma) were excluded.

Sixty-seven total deaths resulting from combined methamphetamine and heroin use were identified since 2005. Deaths from this combination remained stable from 2005 to 2010, and increased steadily from 2010 onward. The absolute increase from 2010 to 2016 was greater than five-fold; the increase was nearly five-fold when corrected for population growth. Methamphetamine was found together with 6-MAM in 46/67 cases (69%). Methamphetamine was found with morphine in the absence of 6-MAM in 21/67 cases (31%).

Frequent detection of methamphetamine with 6-MAM indicates a substantial portion of cases in which methamphetamine and heroin are taken together and cause death rapidly, before either drug can be further metabolized. Detection of methamphetamine with morphine alone suggests acute use of methamphetamine and subacute use of heroin preceding death (i.e., alternating injections). The data are in agreement with clinical observations of drug use behaviors in Denver.

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Methamphetamine, Heroin, Overdose

H135 Forensic Applications of Postmortem Pharmacogenomics

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After attending this presentation, attendees will better appreciate the role of pharmacogenomics testing in the investigation of potential drug-related deaths.

This presentation will impact the forensic science community by promoting the use of pharmacogenomics testing as an adjunct to postmortem drug testing in drug-related deaths to help avoid erroneous certifications of death.

Case 1: A 57-year-old male was found dead in his cell at the Center for Forensic Psychiatry. He was initially asystolic and in cardiac arrest and was pronounced dead shortly after arrival at the emergency room. His past medical history was significant for: hypertension, hyperlipidemia, hypothyroidism, diabetes, alcoholism, depression, schizophrenia, and polydipsia (excessive thirst). His drug count was accurate.

An autopsy demonstrated an abrasion on the submental region. Examination of the anterior portion of the spine revealed an acute fracture and hemorrhage overlying the C6-C7 intervertebral space.

Postmortem toxicological analysis using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) revealed elevated levels of tramadol 3,000ng/mL and the O-desmethyltramadol metabolite at 100ng/mL in iliac blood. The clozapine concentration was 1,100ng/mL and its metabolite, norclozapine, at 680ng/mL in iliac blood. Both drugs were at potentially toxic concentrations. In consideration of the circumstances, autopsy, and toxicology findings, the cause of death was certified as tramadol and clozapine toxicity.

Postmortem genetic testing using Sanger sequencing revealed a heterozygous CYP2D6*4 genotype and indicated that one copy of the CYP2D6 was inactive. This could explain the increased concentration of clozapine, although it is thought that CYP1A2 and 3A4 are responsible for the metabolism of this drug. Tramadol is metabolized by 2D6, 2B6, and 3A4 CYP450 enzymes.

Case 2: An 80-year-old female was admitted to the hospital for biopsy of a pelvic mass. She had a past medical history significant for Chronic Obstructive Pulmonary Disease (COPD), asthma, and arthritis. She was depressed over the new diagnosis of rectal cancer. She was discharged to a nursing home five days later for elder care. Tramadol 50mg/q6 and nortripyline 100mg @ hs were added to her discharge medications.

An autopsy demonstrated a metastatic vagina carcinoma and severe coronary atherosclerosis. Toxicology analysis revealed acetaminophen 120mcg/mL (12-20ug/ml) via High-Performance Liquid Chromatography (HPLC), tramadol 46,000ng/mL, O-desmethyltramadol 500ng/mL (420-720ng/ml) by (LC/MS/MS), nortriptyline 3,800ng/mL (170-380ng/ml) by Gas Chromatography (GC), mirtazapine 240ng/mL (137-225ng/ml) by GC.

Toxicology revealed markedly high levels of tramadol and nortriptyline. The decedent underwent genetic testing using Multiplex Polymerase Chain Reaction (PCR) and Multiplex Allele Specific Extension (ASPE) for CYP2D6 genotype to investigate her ability to metabolize tramadol, nortriptyline, and other drugs. The patient had homozygous mutations, carried two non-functional CYP2D6 alleles, and was a poor metabolizer of tramadol and nortriptyline. The cause of death was certified as tramadol and nortriptyline toxicity due to CYP2D6 poor metabolizer status. The manner of death was accident.

Pharmacogenomics is the study of an individual's genotype and their ability to metabolize foreign compounds. There are some 50 distinct CYP450 enzymes. CYP2D6, CYP2C9, CYPC19, and CYP2D6 account for 75% of drug metabolism by P450 enzymes. Mutations of CYP450 enzymes can result in elevations and decreases in parent drugs and their more potent metabolites: (1) ultrarapid metabolizers: — multiple copies of a gene results in elevated metabolites; (2) extensive metabolizers (wild-type) — single copy of a gene with normal activity; (3) intermediate metabolizers (heterozygotes) — exhibit decreased enzymatic activity; and (4) poor metabolizers (homozygotes/*double heterozygotes*) — have no detectable activity.[1]

Medical examiners and coroners are just beginning to appreciate the role that pharmacogenomics can play as an adjunct for drug death certification. Previous studies have demonstrated the high prevalence of CYP2D6 genetic variations corresponding to intermediate and slow metabolizers.[2]

Death investigators should consider pharmacogenomic testing in the following situations: (1) excessive drug concentrations not consistent with prescribed dosages; (2) deaths occurring shortly after starting a new medication; (3) elevated drug concentrations with diminished or absent metabolites; and, (4) elevated drug concentrations without confirmed history of intentional administration.

Pharmacogenomics has demonstrated a promising role in the interpretation of drug-related toxicity and sensitivity.[3,4]

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Pharmacogenomics, Molecular Autopsy, Drug-Related Deaths

H136 Multi-Site Aspects of Postmortem Redistribution (PMR) and Their Combination With Pathological Findings to Determine Cause of Death (COD) in Suspected Diazepam, Methadone, and Morphine Drug-Related Cases

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After attending this presentation, attendees will understand: (1) that blood as well as other sampling sites (i.e., gastric contents and liver parenchyma) may all contribute to variations of drug blood concentration after death to some degree; and, (2) their combination with pathological findings is required in suspected drug-related deaths.

This presentation will impact the forensic science community by demonstrating that a multi-site approach of PMR in combination with pathological findings is useful in assessing the COD in selected drug-related cases.

There were 24 autopsied cases, sampled as follows: Intracardiac Blood (ICB), Subclavian Blood (SCB), Femoral Blood (FB), Popliteal Blood (PB), Gastric Contents (GC), and Liver Parenchyma (LP). PB was sampled after dissection and clamping of the popliteal vein because of its deep localization in the popliteal fossa, whereas LP was always sampled in the middle part of the liver. Selected substances (diazepam, methadone, and morphine) were sampled in all sites, whereas a complete drug screening was concomitantly performed on FB. GC and LP were homogenized before analysis; LP was also treated with subtilisin. For all sampling sites, morphine and methadone samples were prepared with solid phase extraction and quantified with Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS); diazepam was quantified with High-Performance Liquid Chromatography- Diode Array Detection (HPLC-DAD) after liquid-liquid extraction.

To assess PMR of selected substances, mean concentrations ratios were calculated as follows: ICB/SCB, ICB/FB, ICB/PB, SCB/FB, SCB/PB, and FB/PB. To assess the influence of other sampling sites on PMR, LP/FB and LP/PB as well as the correlation between GC and LP concentrations and the blood mean concentrations were obtained in concerned cases. In addition, for all cases, pathological findings were summarized and compared to toxicological findings in order to determine COD.

Toxicological results indicate that popliteal site is less subject to PMR as suggested by mean concentrations ratios FB/PB (diazepam (N=3) mean ratio = 1.84; methadone (n=15) mean ratio = 1.44; morphine (n=14) mean ratio = 1.86); mean LP/FB and LP/PB ratios are also in accordance (diazepam (n=1): mean LP/FB = 0.05, mean LP/PB=0.07; methadone (n=13): mean LP/FB=26.96, mean LP/PB=36.59; morphine (n=4): mean LP/FB=5.34, mean LP/PB=9.09). Results also suggest that GC and LP concentrations may influence blood concentrations, as seen with the following significant positive correlations observed between methadone GC and ICB, SCB, FB and PB (n=14; r=0.62, r=0.59, r=0.57, r=0.58; $p < 0.05$), as well as between methadone LP and ICB (n=13, r=0.55, $p < 0.05$).

Pathological findings are mostly non-specific in 16 cases in which toxicological findings suggest obvious intoxication to one or more substance(s), and compatible with usual drug-related fatalities findings (pulmonary edema and alveolar hemorrhage (n=14), terminal subendocardial ischemia (n=10), acute hepatic congestion (n=7), acute renal tubular necrosis (n=7)). In four cases in which toxicological findings are equivocal, the non-specific pathological findings may be observed, but considering PB instead of FB concentrations for selected substances may allow clarifying COD (e.g., case #5: methadone FB=240µg/L-PB=103µg/L; case #21: morphine FB=46µg/L-PB=17µg/L). In four cases, COD is natural according to both toxicological and pathological findings (disseminated intravascular coagulation (DIC) and sepsis (n=1), cardiovascular diseases (CVD's) (n=3)), but considering PB instead of FB concentrations still allows helping to determine COD (e.g., case #6: COD=CVD, morphine FB=30µg/L-PB=18µg/L; case #9: COD=DIC and sepsis, methadone FB=265µg/L-PB=123µg/L).

In conclusion, this study is the first to suggest a multi-site approach of the PMR of selected drugs, including popliteal blood sampling in combination with pathological findings, in order to establish COD in drug-related fatalities. These results illustrrate that popliteal vein blood sampling is less prone to PMR, that other sampling sites may significantly influence postmortem blood concentrations, but also that the interpretation of toxicological findings alone, especially with FB instead of PB concentrations, may lead to confusion in determining COD in such cases.

H137 Postmortem Detection of Despropionylfentanyl (DPF) in Drug-Related Deaths

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After attending this presentation, attendees will understand the significance and prevalence of DPF in postmortem toxicology samples.

This presentation will impact the forensic science community by increasing awareness of DPF as a metabolite and synthetic intermediate of fentanyl and its potential significance in toxicologic testing.

DPF is an immediate precursor chemical used in the illicit synthesis of fentanyl, requiring only a single step for final conversion. Incomplete reactivity of this final step, therefore, leaves DPF as a common low-level contaminant in, and a specific marker of, illicitly produced fentanyl. Reports from the Drug Enforcement Administration (DEA) are that an estimated 75% of fentanyl seizures contain DPF as a contaminant at levels up to 2.5% of the tested product. Despite structural similarities, DPF lacks the phenethyl group present in fentanyl and therefore has minimal biological activity; however, because of its use in illicit fentanyl manufacturing, it received Schedule II designation by the DEA in 2010. DPF is also a minor metabolite of fentanyl, though it often doesn't reach detectable levels after administration of therapeutic doses.

The Office of the Chief Medical Examiner (OCME) Toxicology Laboratory initially detected DPF in 2014 in several cases of acetyl fentanyl use. DPF was easily detected in an alkaline drug screen, which involved an alkaline liquid-liquid extraction followed by gas chromatography/nitrogen-phosphorus detection. Confirmation of DPF was performed using solid phase extraction followed by Gas Chromatography/Mass Spectrometry (GC/MS). DPF elutes between ethylmorphine and fentanyl on an HP-5 column and prominent GC/MS ions are 146 and 189. Beginning in March 2016, the laboratory began reporting DPF in postmortem toxicology specimens when several cases concurrently negative for fentanyl and fentanyl analogues were encountered.

To explore this finding, a retrospective analysis of the deaths investigated by the Maryland OCME was conducted for all DPF-positive cases from March 1-July 10, 2016, producing a total of 222 cases. There was a sudden increase in cases between April and May 2016 (27 in April vs. 71 in May), which seemed to have plateaued with 76 cases in June and a projected estimate of 73 cases for July.

The list was then sorted for only those cases in which drug intoxication was the cause of death, leaving 183 for review. The majority of cases, 155, had free morphine detected and similarly, half of the cases, 92, had 6-monoacetylmorphine concurrently detected. These findings suggest that DPF is most often being encountered in the setting of a combined heroin and fentanyl administration.

Additionally, 90 cases had concurrent fentanyl, furanyl fentanyl, or acetyl fentanyl detected. The remaining 93 had no detectable fentanyl or fentanyl analogues identified in any of the tested specimens. The DPF in these cases could reflect one of several possible scenarios: (1) DPF was the predominant chemical in the administered dose (2) an alternative fentanyl analogue that is not detectable with current methods was the active agent; or, (3) DPF may be more slowly metabolized than other metabolites and could, therefore, reflect accumulation from past usage.

Interestingly, in 19 of the 183 cases, either no other illicit drugs or only free morphine at concentrations <20mg/L were detected. Despite the relatively benign toxicologic findings, scene investigation in four of these cases reported that a syringe was found in the immediate proximity of the decedent (in the decedent's arm or under the body), strongly suggesting an acute intoxication as the cause of death.

In summary, the Maryland OCME has seen a recent increase in cases in which DPF is detected in postmortem toxicologic specimens. The majority of these cases seem to reflect concurrent fentanyl and heroin use, although in many of the cases, fentanyl and fentanyl analogues were not present at detectable concentrations. Further, cases which were strongly suspicious for acute drug intoxication by scene investigation have emerged which have been positive for DPF while negative for significant concentrations of all other illicit drugs included in routine toxicology screening. While the exact etiology of the DPF in these specimens remains unclear at this time, it seems evident

that testing for DPF should be included in routine forensic testing to better categorize drug-related deaths and help elucidate the breadth of the fentanyl abuse epidemic.

Despropionylfentanyl, 4-ANPP, Fentanyl

H138 Drop Dead Again: Surge of Deaths Attributed to Fentanyl Analogues in Cook County, Illinois

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After attending this presentation, attendees will understand the importance of the forensic pathologists and toxicologists' role in identifying fentanyl analogues resulting in death, and the necessity of regularly modifying toxicology drug panels to increase the likelihood of the detection of these substances.

This presentation will impact the forensic science community by educating death investigators about a surge in deaths due to fentanyl analogues within a large urban setting, highlighting the importance of regularly modifying existing drug screens to include testing for these and other new substances permeating the community.

The purpose of this study is to review toxicology data from the Cook County Medical Examiner's Office (CCMEO), including decedent demographic information, to educate the forensic community about the presence of fentanyl analogues causing death within the urban setting of Cook County, IL, and to identify trends associated with these deaths.

The Chicagoland region serves as a hub for illicit drug distribution in the Midwest. An epidemic involving fentanyl related deaths occurred from 2005-2007 in Cook County, IL. The term "Drop Dead" emerged because of the swift onset of collapse following injection of the drug. These fentanyl deaths were initially thought to be the result of "tainted heroin"; however, further investigation revealed that fentanyl was being sold as a substitute heroin. The Chicago police investigation traced the distribution to the Mickey Cobra gang, and the production of fentanyl to a laboratory in Toluca, Mexico, which was shut down in 2007. European studies attributed the rise in fentanyl-related deaths in the early 2000s to the disruption of production and distribution of opium in the Middle East. The recent surge in fentanyl- and fentanyl analogue-related deaths may again be associated with this trend.

The Centers for Disease Control and the Drug Enforcement Agency have recently issued health advisories in response to the national increase in fentanyl-related overdose fatalities, including deaths attributed to the presence of fentanyl analogues, most commonly, furanyl fentanyl, acetyl fentanyl, despropionyl fentanyl (4-ANPP), and butyryl fentanyl. The phrase "Drop Dead Again" is coined to describe this new epidemic of fentanyl analogues and fentanyl-related deaths.

In April 2015, the CCMEO's toxicology laboratory began detecting fentanyl and fentanyl analogue deaths. In response to this, the toxicology laboratory revised its short-form drug testing panel, which included testing for alcohol, opiates and cocaine, to include routine testing for fentanyl. Fentanyl analogues screen positive for fentanyl using the Enzyme-Linked Immunosorbent Assay (ELISA); however, further testing by Gas Chromatography/Mass Spectrometry (GC/MS) will be negative for fentanyl because fentanyl analogues will have a distinct chromatographic peak and mass spectrum result.

Since the initial detection, at least 71 deaths have been attributed to fentanyl analogues (+/- additional drugs). The various analogues causing death include acetyl fentanyl (*n*=13), despropionyl fentanyl (4-ANPP) (*n*=52), furanyl fentanyl (*n*=45), and butyryl fentanyl (*n*=1).

For this study, toxicology results and demographic information were reviewed from April 2015 to the present. The decedents ranged in age from 22 to 65 years, with an average age of 41 years. There were 59 male deaths and 12 female deaths. Fentanyl analogue deaths occurred more frequently in association with other drugs and alcohol (55/71 cases). Fentanyl analogues alone were responsible for 16/71 deaths. The most common drugs found in association with fentanyl analogues include heroin (*n*=26/71) and fentanyl (*n*=20/71); cocaine (*n*=13/71) and ethanol (*n*=16/71) were also frequently detected.

One of the many functions of the medical examiner and coroner is protecting the public health and sounding the alarm when new drug trends are detected.

Fentanyl Analogue Intoxication, Sudden Death, Cook County, Illinois

H139 Acute Pulmonary Emphysema (APE) in an Incomplete Hanging

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After attending this presentation, attendees will better understand the occurrence of APE in cases of incomplete hanging and its potential application as a sign of vitality.

This presentation will impact the forensic science community by increasing understanding of pathophysiological mechanisms that lead to death in hanging.

APE has been described in deaths by mechanical asphyxia such as ligature or manual strangulation. In cases of hanging, the role of airway compression in causing death is controversial. Only a few authors have reported APE as a classic sign of hanging, but most studies are based only on standard histological examination of the lung. Morphometric digital analysis of the pulmonary tissue has been proposed to provide objective data on the distention of air spaces more reliably than standard histology.

A recently published study investigated eight cases of complete hanging and eight cases of incomplete hanging, as well as ten cases of freshwater drowning as a positive control group and ten cases of acute external bleeding as a negative control group. Image analysis software (Nikon Elements BR 3.2) was used to detect the alveolar area in histological slides. The results revealed that in incomplete hanging, the Mean Alveolar Area (MAA) was significantly greater (31,522µm²) than observed with complete hanging (21,325µm²) and was similar that one observed in freshwater drowning (33,175µm²). These results suggest that incomplete hanging may cause slower compression of the cervical blood vessels with longer pulmonary distress. Another possible explanation for this observation is that the position of the suspended body causes a greater tensile force on the thoracic cage, thus hindering full expansion and ventilation.

To verify this hypothesis, this study compared eight cases of incomplete hanging where the contact of the body with the ground was minimal (i.e., tiptoes, group A) with eight cases in which the contact was greater (i.e., hanging in a sitting position, group B), using the same morphometric method from the previous study. In order to avoid confusion due to emphysema from another origin, exclusion criteria were: age >65 years, inhalation of blood and/or gastric content, chronic respiratory (in particular chronic emphysema) or cardiac diseases, and cardiopulmonary resuscitation. Postmortem interval at autopsy was <72 hours. Histological slides from each lung lobe were stained with hematoxylin and eosin and examined by optical microscopy (Nikon Eclipse 50i, magnification 10X). The area of every alveolar space was measured using image analysis software (Nikon Elements BR 3.2). The average of the alveolar areas was calculated for each case and the groups were compared.

The results of the present study demonstrated that the MAA of group B (29,581µm²) was significantly higher than that observed in group A (21,652µm²). Furthermore, the MAA observed in group A was similar to that previously observed in the complete hanging group.

These results apparently confirm the hypothesis and suggest that the pathophysiological mechanism leading to death in incomplete hanging with a minimal contact with the ground is similar to complete hanging. In these cases, vascular compression of the neck seems to be more important with possibly less pain and minor respiratory distress. APE is present in incomplete hanging with substantial contact of the body with the ground and could be used as a vital sign.

Emphysema, Hanging, Morphometry

H140 Lethal Masturbation of a Teenager Suffering From a Long QT Syndrome (LQTS) Type 8 With a Newly Discovered CACNA1C Gene Mutation

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After attending this presentation, attendees will be aware of one of the first described lethal cases due to a newly discovered mutation of the Calcium Voltage-Gated Channel Subunit Alpha 1 C (CACNA1C) gene causing LQTS type 8 and that masturbation could be considered as a triggering factor leading to the fatal outcome in such cases.

This presentation will impact the forensic science community by highlighting the need of cardiac genetic investigation of sudden death cases in a forensic context as it could help determine the cause and mode of death. Additionally, it could have major implications for the therapeutic strategy of the family.

Congenital LQTS affects approximately1/2500 people. It can arise from mutation(s) of one of several genes coding for the cardiac ion channels. It involves an abnormal repolarization of the heart that increases the risk of sudden death after severe polymorphic ventricular arrhythmia. Different triggering factors are often described, most of which are hyper adrenergic situations. Their identification is of great importance for clinicians in order to evaluate the best prevention strategy.

This study reports the sudden death case of an 18-year-old male, who was found dead in his bedroom on a summer day at noon, two hours after he came home from taking a school exam. An unsuccessful Cardiopulmonary Resuscitation (CPR) was performed within one hour.

At the scene, he was found with his fly unzipped, with three wet facial tissues near him that tested positive for acid phosphatase, suggesting he had just masturbated. Instruments to roll cigarettes were also found, but no cigarette butts.

Medical background was significant as the male was diagnosed with LQTS (QTc: 480ms) after a syncope at the age of ten years that occurred after swimming. Since then, he had been treated with the usual beta-blocker oral therapy (50mg/m²/day of nadolol). Family screening determined LQTS for his mother and one of his sisters.

The first cardiogenetic analysis was performed after the LQTS diagnosis: KCNQ1 and KCNH2 gene (among 15 genes currently identified) analysis was negative. The availability of the next generation DNA sequencing techniques allowed a deeper analysis of DNA on a postmortem sample and found that he was carrying one of the recently discovered mutations of the CACNA1C gene (cDNA c2573G>A, heterozygous mutation, p.Arg858His), which encodes for the Cav1.2 protein (a1 subunit of cardiac L-type calcium channel).[1] This mutation was shown to be a gain-of-function mutation, highly involved in the prolongation of the duration of the ventricular action potential.

Autopsy found no macroscopic pathological signs except for signs of CPR with epicardial petechiae and unspecific pulmonary edema and congestion. Gross anatomy of the heart and coronary arteries was normal. No sign of Timothy's syndrome was discovered.

The postmortem toxicological analysis found 24.7ng/ml of nadolol in the blood, which is consistent with a therapeutic level. No other drugs or narcotics were found at the toxicology screen, except cotinine and delta-9-tetrahydrocannabinol (0.76ng/ml) and its metabolites (THCCOOH: 2.09ng/ml, 11-OH-THC: 0.65ng/ml), suggesting occasional cannabis smoking with a last smoke within the last 12 hours.

The genetic discovery of this mutation, consistent with the context and background, led investigators to close this case by stating that is was a natural/accidental death by ventricular fibrillation triggered by probable hyper adrenergic context on an LQTS type 8 patient.

Among the possible triggering factors that could be linked to the fatal arrhythmia, masturbation seems to be the leading hypothesis as a physical and emotional exercise, even if the role of the emotional context after the school exam and of the light cannabis intoxication could not be absolutely excluded.

This case is one of the first lethal cases described involving this mutation and could be considered, for forensic purposes, as a natural/accidental form of autoerotic death.

In the medical clinical field, this case highlights that LQTS8 can be associated with a severe phenotype if we consider that sudden death can occur with "moderate" physical exercise, and is resistant to the beta-blocker therapy. This important genetic result will therefore contribute more accuracy in this family LQTS screening.

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Sudden Cardiac Death, Masturbation, Long QT Syndrome

H141 A Rare Case of Fatal Retropharyngeal Hemorrhage in a Patient With Neurofibromatosis Type 1 (NF1)

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After attending this presentation, attendees will be aware that: (1) vascular complications are the most common cause of death after malignancy in patients with NF1; (2) rupture of cervical vessels invaded by neural fibers may result in a large retropharyngeal hematoma and may cause death by acute airway obstruction; and, (3) excessive physical movements may trigger the rupture of vessels infiltrated by neurofibroma.

This presentation will impact the forensic science community by reporting a rare case of lethal cervical hemorrhage associated with NF1. Forensic pathologists should consider vascular complications as a potential cause of death when dealing with sudden death in NF1 patients.

NF1 is one of the most common phacomatoses . It is an autosomal dominant disorder that occurs in approximately 1 in 3,000 newborn infants. The disease is caused by loss-of-function mutations in the gene encoding the tumor suppressor neurofibromin, which lead to the growth of tumors such as neurofibromas.[1] NF1 patients are also at high risk of developing malignant tumors that are a common cause of death in this population. Although rare, vascular complications have also been described as potentially lethal morbidities in NF1.[2]

This presentation reports the case of a 42-year-old woman with NF1 who was admitted to the hospital after complaining of facial and neck swelling while having physiotherapy. On arrival at the emergency room, she presented with an acute respiratory distress and collapsed. Despite an emergency cricothyroidotomy and cardiopulmonary resuscitation, she died a few minutes after her admission.

A whole body Computed Tomography (CT) scan performed before the autopsy revealed an extensive prevertebral hematoma of the neck, associated with a mild kyphoscoliosis of the cervical spine. In addition to the classic hallmarks of neurofibromatosis, external examination revealed facial and cervical swelling as well as scattered petechial hemorrhages on the face. A correctly positioned cricothyroidotomy was also present. Upon internal examination, the major finding was a massive retro-pharyngeal hematoma that markedly deformed the anterior neck structures. Histology revealed a diffuse infiltration of the walls of small cervical blood vessels and of the surrounding soft tissues by a plexiform neurofibroma, with no evidence of malignant transformation.

Death was considered to be due to an acute upper airway obstruction of a large retropharyngeal hematoma caused by an infiltrating neurofibroma. NF1-related vasculopathy is a well recognized but uncommon entity. The intrinsic form, characterized by an accumulation of cells in the intima of the blood vessel, should be distinguished from the invasion of the vessel by a neurofibroma usually growing in a plexiform pattern. Disruption of the vessel is due to weakening of its wall caused by the extensive infiltration by neural fibers.[2]

Vascular complications, such as acute hemorrhages, are the second most common cause of death after malignancy in NF1 patients. Spontaneous hemorrhages, primarily due to the rupture of arterial aneurysms, have been reported in this population in different body locations; however, association of NF1 with neck hemorrhages is rare and is at high risk of lethal upper airway obstruction.[3] As excessive movements of the head have been reported to be sufficient to cause the rupture of cervical vessels previously invaded by the tumor, it can't excluded that physiotherapy may have played a role as a triggering factor in the onset of the hemorrhage in this case.[3]

In conclusion, this case illustrates that, although uncommon, acute hemorrhages are life-threatening complications in NF1 patients. Clinicians should be able to make a prompt diagnosis so that a proper treatment can be undertaken without delay, while forensic pathologists should consider them as a potential cause of sudden death when investigating deaths in patients with NF1.

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Neurofibromatosis Type 1, Neck Hemorrhage, Forensic Pathology

H142 Agenesis of the Thyroid Cartilage Superior Cornua: Implications for Autopsy

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After attending this presentation, attendees will better understand the prevalence of thyroid cartilage superior cornu agenesis in forensic autopsy cases.

This presentation will impact the forensic science community by illustrating the appearance of this variant at autopsy and describing the potential challenges of assessing trauma to the neck structures when one or both cornua are absent.

Examination of the neck for evidence of trauma or disease is a fundamental part of the forensic autopsy. The neck structures are assessed *in situ*, then removed from the body for a more detailed inspection and additional photography. Soft tissue hemorrhage and/or fractures of the hyoid bone or the thyroid and cricoid cartilages suspicious for inflicted trauma may be noted by the forensic pathologist during this portion of the examination. In some cases, developmental variants of the laryngohyoid complex may make it difficult to distinguish between injury, normal morphology, or postmortem artifact. For example, palpation of unilaterally or bilaterally unfused greater hyoid cornua may suggest fracture until further examination reveals the variant. The thyroid cartilage is known anecdotally to present with multiple developmental variants. The prevalence of all thyroid cartilage variants has been estimated to range from 4.8%-29.5% of the population[1]. Yet the literature is lacking in both descriptive and quantitative studies of individual variants, especially as they relate to autopsy cases. One variant observed at autopsy is agenesis of the thyroid cartilage cornua.

The Harris County Institute of Forensic Sciences (HCIFS) Pathology Services Division covers a large jurisdiction, employing 18 forensic pathologists and four doctoral-level forensic anthropologists. As an example of the caseload, in 2015, the HCIFS received 4,644 cases. Of these, 705 received an external examination and 3,939 received a full autopsy. From January 1, 2010, to July 31, 2016, one pathologist performed or supervised 1,230 full autopsies, excluding fetal deaths. A minimal number of these autopsies were of skeletonized remains without preservation of the cartilaginous neck structures. The anthropologists routinely perform laboratory analysis on neck structures when requested by the pathologist of record. The anthropologists examined 209 thyroid cartilage cases between January 1, 2010, and July 31, 2016. A total of 26 cases of thyroid cartilage cornu agenesis were recorded, 19 by the pathologist, and seven by the anthropologists (submitted by five different pathologists).

The majority (15: 58%) of the 26 cases display agenesis of the left cornu, suggesting that the left side may be predilected for an as-yet undetermined reason. Six right cornua were absent and five cases exhibited bilateral agenesis. The sex distribution was 77% male and 23% female. The race/ethnicity distribution was 46% White, 42% Black, and 12% Hispanic. Importantly, males and White and Black individuals are overrepresented within the HCIFS caseload when compared with the demographic composition of Harris County. The manner of death distribution in the 26 cases was 42% natural, 19% homicide, 19% suicide, and 19% accident. Causes of death include strangulation, homicidal violence, asphyxia, blunt force injuries, firearm wounds, toxicity, and cardiovascular disease.

The true incidence of thyroid cartilage superior cornu agenesis in HCIFS cases is not quantified using these data because all cases of agenesis have not yet been collected systematically by the HCIFS pathologists. Additional studies are needed to standardize collection of the data and to examine the possible association of agenesis with variants of the triticeous cartilage.[2] Estimated prevalence of agenesis in this closed cohort of HCIFS cases is 1.8%. Further study may reveal that agenesis of the cornua is not all that rare, and that the left cornu may be absent more frequently. Awareness of the distribution of this normal variant is useful for the internal neck examination at autopsy.

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Forensic Pathology, Thyroid Cartilage, Neck Structure Variants

H143 Pericardial Rupture After Mechanical Resuscitation With the Lund University Cardiopulmonary Assist System, 2nd Generation (LUCAS™2)

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After attending this presentation, attendees will understand how to recognize pericardial rupture after mechanical Cardiopulmonary Resuscitation (CPR) using the LUCAS™2 device, an automatic device for active chest compression and decompression. Attendees will better understand the importance of discerning the complications of CPR and their significance in forensic settings, avoiding misinterpretation of injuries detected at autopsy.

This presentation will impact the forensic science community by highlighting pericardial rupture as a potential complication of mechanical CPR in cases of cardiac tamponade.

Traumatic injuries related to resuscitation maneuvers are well known. After mechanical resuscitation, traumatic injuries are frequent, especially rib fractures; however pericardial rupture has been previously reported as a direct consequence of chest fracture related to CPR, but it is not considered as a typical traumatic complication of CPR. Nevertheless, it has been recently hypothesized that CPR could cause left pericardial tear and hemothorax by the combining stress of pericardial effusion and chest compressions. A brief review of specific literature will be presented.

The Geneva emergency department began using the LUCAS™2 device in 2011. Observation of a new type of traumatic injuries associated with mechanical CPR in bodies that underwent unsuccessful CPR started in the same period in the medicolegal service. A previously published study reports rib fractures were more frequently observed after mechanical CPR. Neither immediate life-threatening injuries nor pericardial tear were observed.

More recently, four cases of pericardial rupture were observed at autopsy after LUCAS™2-CPR. In every case, cardiac tamponade from natural disease was identified as the cause of death (myocardial rupture after infarction or intrapericardial ascending aortic rupture due to dissection or aneurysm). Massive hemothorax was observed each time. Only a small amoount of blood was present in the perforated pericardium. Some cases exhibited concomitant signs of blood loss. All cases presented rib and sternal fractures. In three cases (out of four), rupture was located on the left side. Hemorrhagic infiltration was observed at histology (hematoxylin and eosin staining). Because of these interesting statements, a retrospective study (time period 2011 to 2015) was conducted to better investigate the phenomenon of pericardial rupture in cases of cardiac tamponade. No other pericardial rupture was reported in cases with similar pathology (n=12). Cardiac tamponade was present without any pericardial injury after CPR maneuvers in nine cases (after manual CPR in five cases and mechanical (LUCAS™2) in four cases). Rib fractures were described in both populations, with sternal fractures only present after mechanical CPR.

Cardiac tamponade is associated with high mortality. In such cases, CPR maneuvers are known to be ineffective due to a lack of myocardial load. Referring to previously reported observations, pericardial rupture in cases of pericardial tamponade is to be considered as a potential complication of CPR. During resuscitation, concomitant hemorrhagic shock could therefore occur due to pericardial tear and consecutive hemothorax. In forensic investigation, knowledge of resuscitation artifacts is very important to avoid misinterpretation of trauma injuries described during autopsy procedures. Forensic pathology plays a key role in the description of those lesions.

CPR Trauma Injuries (LUCAS™2), Pericardial Rupture, Cardiac Tamponade

H144 A Staged Hanging: Postmortem Suspension of a Homicide Victim

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After attending this presentation, attendees will better understand the autopsy findings differentiating suicidal hanging from ligature strangulation with a subsequent staged suicide.

This presentation will impact the forensic science community by increasing awareness of homicidal hanging and staged suicide.

Deaths due to asphyxia are a major component of homicidal deaths. The manner of death in hangings is nearly always suicide.[1-3] Although homicidal hangings are generally thought to be highly unusual, a body found in a suspended position also demands consideration of an accidental or homicidal manner of death, as well as the possibility of postmortem suspension. This is a case of a 48-year-old White male who was found within his place of business, partially suspended by a rope looped around an overhead rafter. He had a history of anxiety and had recently spoken to a counselor regarding "bad thoughts." Investigation of his computer revealed recent searches for alprazolam overdose information. He had recently told his wife, "I'm sorry if something bad happens to me."; however, he was not known to have any previous suicidal ideations and no note was recovered.

Scene examination was remarkable for blood present on multiple surfaces, as well as on the hands and face of the decedent. Bloody paper towels were also present in a trash receptacle. The overhead rafter exhibited a pattern of splintering directed away from the decedent. It is unclear whether or not the property was secure upon arrival of law enforcement.

Multiple injuries were present at autopsy, including multiple contusions of the hands and arms, abrasions on the knuckles of the hands, and multiple abrasions about the head. Abrasions on the back of the head were associated with subgaleal hemorrhage. A horizontal, furrowed abrasion was present around the full circumference of the neck. A second, faint impression/furrow with an upward slant was also seen on the left anterolateral neck running to the left ear. Conjunctival and scleral petechiae were present and the hyoid bone was fractured with associated hemorrhage. Layered neck dissection revealed an area of hemorrhage in the right thyrohyoid muscle. Postmortem toxicology was positive for amphetamine and citalopram, both within therapeutic range, and both prescribed to the decedent. No alprazolam was found in the decedent's blood. Based on the findings at autopsy and on scene investigation, this case was certified as a ligature strangulation homicide.

Given that the vast majority of hangings are suicides, consideration of a non-suicidal manner of death is sometimes disregarded at the time of death investigation, and recognizing a homicide with suspension of the victim can be very challenging.[1] In the presented case, an attempt was made to simulate the appearance of suicidal hanging. Clues pointing to homicide in this case were the circumferential and horizontal pattern of the major cervical furrow, the pattern of splintering on the rafter used for suspension, and the non-hanging-related injuries seen on the head and extremities.[2-3] In addition, conjunctival petechiae and fracture of the hyoid bone are unusual findings in hangings.[3] This case is presented to emphasize the importance of critical assessment in the investigation of death related to hanging.

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Homicide, Staged, Asphyxia

H145 An Autopsy Study of Suicidal Deaths — Trends in South India

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After attending this presentation, attendees will better understand the epidemiology and pathology of suicidal deaths in India, their peculiarity, and the changing trends in the patterns of suicide.

This presentation will impact the forensic science community by enhancing knowledge of suicide patterns prevalent in a developing country and how this pattern is different from other parts of the world.

Introduction: Suicide represents a huge human tragedy. Out of 1000 suicides a day in the world, more than 100 occur in the Indian subcontinent. In the span of ten years in India, death due to suicide increased by 62.9%, involving all age groups. Studies indicate that the suicide rates are greatly influenced by several variables, including in age, sex, race, religion, culture, marital status, and social systems. Therefore, this study is an attempt to analyze the recent trends in deliberate self-harm reported in the Manipal region of South India.

Materials and Methods: The present study was retrospective (January 1992-December 2016) over a span of 24 years conducted at the department of Forensic Medicine and Toxicology at Kasturba Medical College Manipal, South India. Relevant data regarding the suicides was gathered from the autopsy files maintained in the department of Forensic Medicine and Toxicology, police inquest reports, and hospital case records.

Results: A total 4,970 cases were autopsied during this period in the department of Forensic Medicine and Toxicology at Kasturba Medical College, of which 894 (18%) were cases of Fatal Deliberate Self-Harm (FDSH) or suicide. The incidence of FDSH was greater in the 21-30-year age group and lowest in the age group of less than 10 years and more than 60 years. Males (66.5%) outnumbered females in this study. The male:female ratio was 2:1. The majority of the suicide victims were married (57.3%). Most of the victims of FDSH belonged to the Hindu religion (86.95%) and 75% of the decedents of FDSH had no any history of illness. Twenty-five percent of the victims of FDSH had a history of physical and mental illness ranging from hypertension, diabetes, and carcinoma to schizophrenia and depression. Despair of life in addition to financial constraints (79.0%) was the most common motive for the victims of FDSH. The most common method used for FDSH was chemical (74.2%). The most common physical method adopted was hanging (15.6%). Among poisonings, organophosphorus compounds were most commonly used and a changing trend of poisoning with paraquate was observed.

Conclusion: The findings of this study are in accordance with various works, not only in India, but in studies conducted worldwide in developing countries.

Forensic Science, Forensic Pathology, Suicide

H146 Talin as a Potential Protein Biomarker in Forensic Investigations

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After attending this presentation, attendees will: (1) understand how to use immunoblotting techniques to demonstrate the correlation between protein integrity and Postmortem Interval (PMI); and, (2) learn methods to assess proteomic degradation in cadaver tissues of from actual criminal cases with times of death between 3-72 hours.

This presentation will impact the forensic science community by providing empirical data from the investigation of PMI-mediated protein degradation in order to provide new insight for formulating an innovative forensic method to determine the time of death.

Changes in brain biochemistry attributable to death can result in altered concentrations of individual proteins in postmortem brain tissues. A recent study investigated the degradation of thanatophagy proteins and revealed that the levels of key proteins involved in postmortem autophagy increased in the brain specimens of actual cadavers from criminal cases. According to the natural order of decomposition, the internal organs in dead bodies decay in a particular order depending on the cause of death, beginning with the intestines and culminating with the brain due to the fact that medial sections of the brain cool more slowly than other tissues. The estimation of PMI is of utmost importance in medicolegal death investigations. There are a number of ways to estimate the PMI; however, the current established methods are susceptible to numerous abiotic and biotic factors. Previously published studies state that protein concentrations in postmortem brain tissues can detect protein changes via immunoblotting and densitometry techniques. There is a paucity of studies that correlate the time since death and cytoskeletal and neuronal protein levels. The objective of the current study was to determine if there is a correlation between protein expression in cadaver tissues and PMI. For this purpose, 18 brain tissues from cadavers from criminal cases were examined to determine how many hours after death the presence of four proteins (talin-1, α-enolase, cofilin-1, and vinculin) are detectable. The cases were divided into three PMI time groups: Group 1, Group 2, and Group 3 (0-24 hours, 24-48 hours, and 48-72 hours, respectively). Talin-1 protein levels steadily decreased with increasing postmortem interval. Interestingly, the study demonstrated that talin-1 protein levels were statistically significant as determined by a one-way Analysis of Variance (ANOVA) test between Group 1 versus Group 2 and between Group 1 versus Group 3. These results provide strong evidence that talin-1 has the potential to be used as a unique biomarker for the establishment of an additional method to estimate the time of death. Future studies would involve mechanistic animal models (i.e., mice and swine) to investigate PMI-mediated protein degradation to provide new insight into formulating an innovative forensic method to determine the time of death.

Talin, Postmortem Interval, Cadaver

H147 A Staged Crime Scene Determination: Correlation of Forensic Evidence

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After attending this presentation, attendees will understand the importance of recognizing crime scene changes or alterations, including manipulation of the body, which is in fact "staging."

This presentation will impact the forensic science community by emphasizing the importance of a careful crime scene examination, especially in cases in which the victim's body or other elements of the scene have been deliberately manipulated.

Geberth in 2006 stated that, "Staging is a conscious criminal action on the part of an offender to thwart an investigation".[1] Chancellor in 2014 classified staged crimes in three types: (1) Primary — a deliberate manipulation of the physical tests or other aspects of the crime scene with the criminal intent of misleading or to diverting a police investigation by adding, removing, or changing the position of evidence; (2) Secondary — the basic intent is to change the placement of the victim in a way to shock or offend society, humiliate or degrade the victim, or enhance any ritual or symbolic meaning that the assailant wants to convey; (3) Tertiary — incidental or innocent changes not performed by the attacker but usually by relatives who alter the crime scene to preserve the dignity of a spouse or to reduce embarrassment and humiliation of the family.[2] Two staged crime scene cases are reported.

Case 1: A young man killed a woman by gunshot for economic reasons in the German countryside, but was not apprehended because the case was declared a suicide. After three years, an "unusual death" of a young woman occurred in southern Italy. The victim was the daughter of the woman killed in Germany three years before. At first, it seemed to be a suicidal event, but the evidence retrieved at the scene, the background of the victim, and meticulous investigation processes eventually connected the two cases. The man was also the culprit of the second murder and he staged the suicide scene of the young daughter. Farewell letters, concealment of clothes, tampering of phone records, and false statements were important evidentiary factors.

Case 2: The culprit was a 33-year-old man with a histrionic personality who liked to woo many women using multiple Facebook® profiles. This case involves a 50-year-old female victim with a submissive personality who had been living with the offender for approximately three years. When the woman decided to end the relationship, she communicated it to friends via Facebook®. The woman was not aware that one of the profiles was a fake created by the man as a means to spy on her. One evening, he went to her apartment but found no one at home. Nevertheless, because he had possession of the apartment keys, he entered and waited for the woman to return. When she came home, the man attacked her verbally, then hit her with little statues found in the house. Subsequently, he strangled her and drowned her in the bathtub. Using her Personal Computer (PC), he attempted to alter the "crime scene" by using the woman's Facebook® profile, sending the message, "Just back home … I met three guys! They'll come here tonight!" to a friend of hers. After sending this message, he left three condoms on the victim's bed. The man was finally identified as the killer. These cases demonstrate that the investigation of a crime must necessarily involve various experts representing several disciplines of the forensic sciences. The results of the autopsy must be correlated with all evidence associated with crime scene, the personal history of the victim, and the ancillary laboratory tests.

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2. Chancellor A. Graham G.D. Staged Crime Scenes: Crime Scene Clues to Suspect Misdirection of the Investigation. *ISJ.* Vol.6, No.1, January 2014.

Crime Scene, Forensic Evidence, Staging

H148 Mimic of Pediatric Head Trauma: Bipartite Parietal Bone in Pediatric Cases

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After attending this presentation, attendees will better understand the anomalous presentation of an accessory suture in the parietal bone (bipartite parietal bone) due to the failure of normal ossification and the clinical pitfalls and complications encountered in the diagnosis. Two recent cases are presented, one an incidental finding on a non-traumatic infant death and the other reported as a suspected traumatic death due to head trauma, which was diagnosed at a children's hospital by a pediatric radiologist as a pediatric non-accidental traumatic skull fracture after review of head Computed Tomography (CT) imaging performed following hospitalization.

This presentation will impact the forensic science community by providing information so attendees can recognize an unusual and rarely reported mimic of pediatric head trauma and by helping prepare attendees for some of the issues that may be raised by conflicting clinical and pathologic diagnoses.

The first case presented is an incidental finding discovered on autopsy in 2015 at the Tarrant County Medical Examiner's Office during a routine examination of an infant death with no other evidence or suspicion of foul play by other investigating agencies. A final determination for the cause of death was sudden unexplained infant death with unsafe sleep environment and a manner of death as undetermined. The second case was reported to the Tarrant County Medical Examiner's Office, also in 2015, shortly after the first case, as a traumatic death due to non-accidental head trauma with a parietal skull fracture after prolonged hospitalization and hospice care. In this case, correlation of the discrepancies between the autopsy finding of an accessory parietal bone suture (bipartite parietal bone) and the clinical finding of a parietal bone fracture was important to further clinical education and proper adjudication of the potential legal proceedings. The final disposition of this case was a ruling of undetermined manner and hypoxic ischemic encephalopathy due to undetermined etiology for the cause of death.

Bipartite parietal bone may be listed under a variety of nomenclature, including divided parietal bone, os parietale partitum, double parietal bone, sutura parietalis transversa, sutura parietalis, anostosis, and os parietale divisum. One or more accessory sutures may be present. A horizontal suture may connect the coronal and lambdoidal sutures or a vertical suture may connect the sagittal and squamosal sutures. The condition is reported in the literature as a rare occurrence.

In conclusion, this presentation will familiarize attendees with accessory cranial sutures, including bipartite parietal bone, an unusual mimic of pediatric head trauma, as well as the development of the cranium that results in this anomaly, the pitfalls associated with the clinical diagnosis, clinical pathologic correlation, and legal ramifications associated with the diagnosis.

Bipartite Parietal Bone, Accessory Suture, Pediatric Head Trauma Mimic

H149 Two Cases of Fatal Pulmonary Tumor Thrombotic Microangiopathy

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After attending this presentation, attendees will have a better appreciation of the value of a thorough autopsy in the diagnosis of this rare cause of progressive respiratory insufficiency and death.

This presentation will impact the forensic science community by emphasizing the importance of complete autopsies in challenging cases of respiratory insufficiency. In particular, this presentation will increase the awareness of diagnosing a rare yet usually fatal cause of pulmonary hypertension through careful correlation of the clinical history with postmortem microscopy and immunohistochemistry.

Pulmonary Tumor Thrombotic Microangiopathy (PTTM) is a rare complication of metastatic cancer that may cause progressive respiratory insufficiency and pulmonary hypertension. PTTM was first described by von Herbay et al. to present with a variety of non-specific clinical manifestations ranging from acute hypoxia from large proximal emboli to pulmonary hypertension developing from microvascular invasion.[1] Other complications include right-sided heart failure and sudden death. PTTM has been reported in 3% to 26% of autopsies conducted on patients with solid tumors; the diagnosis is usually not made until postmortem examination. Generally, there are no distinguishing radiographic findings, and there is no grossly apparent pulmonary tumor metastasis or thromboembolism at autopsy. Microscopically, PTTM is characterized by tumor microemboli, intravascular thrombosis, and fibromuscular thickening of pulmonary small arteries and arterioles.

Vascular Endothelial Growth Factor (VEGF) is an angiogenic and hyperpermeability factor that may mediate the development of pulmonary hypertension in these cases. While the exact underlying mechanism of PTTM is not completely understood, it is theorized that abnormalities in VEGF may be involved in its development; VEGF is expressed more frequently in tumor cells associated with PTTM.

This report presents two cases of PTTM in patients with undiagnosed malignancies (gastric and colon carcinoma) who presented with respiratory distress and subsequent death. Both cases presented with non-specific clinical findings, including cough, hypertension, type 2 diabetes mellitus, and malaise. Chest radiographs were rather non-specific and demonstrated cardiomegaly and vascular congestion. Transthoracic echocardiogram revealed pulmonary hypertension and cardiomegaly. In one case, PE was suspected; however, a ventilation-perfusion scan showed a very low probability of PE and Doppler studies of the legs were negative.

Autopsy disclosed widely metastatic malignancies consisting of poorly differentiated adenocarcinoma. Neither case had grossly apparent pulmonary thromboembolism. Microscopic sections of the lungs found widespread intravascular collections of poorly differentiated carcinoma in small arteries and arterioles. Many of the tumor emboli were seen associated with intravascular fibrin. Arteriolar medial hypertrophy and intimal hyperplasia were present. VEGF-positive gastric adenocarcinoma tumor cells were demonstrated by immunohistochemistry in one of the cases.

These cases illustrate the importance of thorough autopsy, including microscopic studies when indicated, in cases of respiratory insufficiency with subsequent death, since PTTM does not result in large tumor metastases, which would be visible on imaging studies or at gross autopsy examination. Furthermore, antemortem serum analysis of VEGF levels has been suggested as a potential clinical test to aid in diagnosis of this rare complication.

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Tumor Microangiopathy, Dyspnea, Metastatic Complications

H150 Sudden Death Related to Aortic Pathology — A Comprehensive Diagnostic Approach

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After attending this presentation, attendees will better understandSudden Aortic Death (SAD) secondary to aortic rupture, dissection, or perforation. The presentation specifically addresses the diagnosis of SAD, including the importance of postmortem imaging and genetic testing.

This presentation will impact the forensic science community by raising awareness of the underlying pathologies that are related to SAD. Also, in a forensic setting, a comprehensive diagnostic approach is needed to identify underlying (hereditary) diseases, which is of paramount importance for relatives of the deceased.

Aortic ruptures or dissections are common causes of sudden death and are, therefore, regularly observed in forensic pathology practice. These cases could be referred to as SAD, in line with the concept of Sudden Cardiac Death (SCD), which is also a major cause of sudden death at all ages, but with a wide range of different pathophysiological backgrounds.

Recent discoveries demonstrate how SAD is directly related to underlying acquired or genetic diseases, such as Marfan-syndrome and many other newly described syndromes. Still, since the immediate cause of death in SAD (e.g., internal hemorrhage) is often easily recognized during autopsy or postmortem imaging, and the underlying pathophysiology is of less importance for purely medicolegal reasons, SAD is not always investigated in-depth in a forensic setting. This may lead to missed diagnoses, especially for hereditary diseases, which in turn leaves family members of the deceased unnecessarily at risk. The recent developments in postmortem radiological imaging for aortic lesions are promising, and it was suggested that, in some instances, a full autopsy is not necessary.

This study compared two series of SAD, one from a clinical setting and one from a forensic setting. First, this study identified differences in the patient characteristics, the diagnoses, and the diagnostic methods. Second, this study used this information to propose a diagnostic approach for forensic pathologists confronted with SAD.

A total of 59 cases of SAD were included, 46 males and 13 females. Thirty-seven were autopsied in a forensic setting and 22 in a clinical setting. The demography of the two groups and the used diagnostic approach differed considerably. For example, postmortem CT-imaging was systematically performed in the forensic group, often complemented with CT-angiography (56.7%), but the clinical setting never used postmortem imaging. Histological sampling was conducted more extensively in a clinical setting.

Comparison of the diagnoses revealed that in the majority of natural deaths, atherosclerosis and/or Cystic Medial Degeneration (CMD) was the principal pathological substrate for the acute event. Both settings had several cases in which an underlying hereditary disease was suspected. This was confirmed in one case in each setting by genetic testing, which found mutations related to Loeys-Dietz syndrome.

These results underscore the importance of an accurate reconstruction of the mechanism of death in a forensic setting. Therefore, for each case of SAD, a full autopsy and thorough histological examination, generally complemented with postmortem imaging and molecular pathology should be recommended.

Aorta, Sudden Death, Postmortem Genetic Testing

I1 Child Abuse and Neglect Associated With Parental Paranoid Psychotic Disorder

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After attending this presentation, attendees will better understand the importance of deep-rooted childhood experiences in patients with paranoia and psychosis in order to stop the vertical chain of transmission of child abuse and neglect.

This presentation will impact the forensic science community by highlighting how parental psychotic disorders and psychosocial deprivation within the home can result in disturbances of the growth and development of children. This case study demonstrates that rickets can occur from not only genetic or organic causes, but can also be the result of severe child abuse/neglect and poor nutritional status.

An 18-month-old child was hospitalized at the Ospedale Infantile Regina Margherita in Turin, Italy, and examined by staff of the operative unit dedicated to the evaluation of suspected child abuse (Bambi). The male child presented in poor condition with length in the third percentile, weight less than the third percentile, bossing of the forehead, caput quadratum, flared chest, rachitic rosary, widening of wrists, bowed legs, double malleoli, delayed eruption of teeth with enamel hypoplasia, thoracolumbar kyphosis, difficulty in sitting, an inability to walk, and generalized dystrophy. The X-ray and blood tests confirmed the diagnosis of "severe deficiency rickets and poor weight gain resulting in motor delay." A few days later, the child's 2-month-old brother was hospitalized for "poor growth and nutritional status under the normal range." He was generally in poor condition with a growth curve between the 25th and 50th percentile and exhibited sparse subcutaneous fat, a protruding abdomen, and had a poor sucking reflex. The children were two of five sons (the oldest was five years old) of an architect and his wife of Somali origins, residing in Turin for a couple of years. The family was followed for approximately a year by social services because, when the fourth child was one month old, neighbors filed a complaint with the authorities, reporting that the children were tied to radiators. The children had never been visited by a pediatrician. None of them attended nursery school. Hospitalization occurred after a home visit when the pediatric nurse reported that the house was dark and highly malodorous, with shutters closed by chains. The children were playing in small dirty boxes. The mother, by means of a cultural mediator, explained that the 18-month-old child was fed only bread soaked in milk, while the younger brother was fed with cow's milk diluted with water. The third child was underweight because the two older brothers also ate his food. The father was affected for years by a severe form of "paranoid psychotic disorder," usually well compensated by antipsychotic therapy. He convinced his wife, who was extremely dependent on him, that Italy was a dangerous country, with shortages of food and poor health services, and that the children could throw themselves out of the windows if they were not closed. Family history also included a psychiatric disorder of the children's paternal grandmother.

Accumulated evidence consistently demonstrates a relationship between childhood adversity and psychosis in adulthood. Meta-analyses have confirmed that a wide range of adverse experiences in childhood is associated with psychosis.[1] In particular, there is evidence of specific association between insecure attachment/neglect and the development of a paranoid disorder.[2] The fact that the paternal grandmother was suffering from a psychiatric condition may have resulted in her son growing up with poor emotional attachments. The situation may have

contributed to the development of psychotic-paranoid symptoms, subsequently exacerbated by the transfer to a big city in another country where the family was devoid of social relationships.

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2. Sitko .K, Bentall R.P., Shevlin M., O'Sullivan N., Sellwood W. Associations between specific psychotic symptoms and specific childhood adversities are mediated by attachment styles: an analysis of the National Comorbidity Survey. *Psychiatry Research.* 217 (2014) 202-209.

Child Neglect, Paranoid Disorder, Psychosocial Deprivation

I2 Juvenile Perpetrators of Sexual Violence Against Other Minors: In Italy Many Probably Do Not Even Know It Is a Crime

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After attending this presentation, attendees will understand the importance of the research regarding a specific juvenile criminal population.

This presentation will impact the forensic science community by highlighting the fundamental characteristics of this type of juvenile delinquency.

The population of Juvenile Sex Offenders (JSO) is complex and heterogeneous, difficult to define and to empirically describe with validated models. Accurate, validated data are difficult to acquire. In the United States, in 2009, approximately 5% of rape arrests involved juveniles and more than a third (35.6%) of sexual offenses against children were committed by people less than 18 years of age. In Europe, approximately one-third of sexual offenses are committed by teenagers, according to data for England and Germany. In 2015, 845 minors in Italy were reported for sexual crimes, of which 115 were sexual acts against other minors. Altogether, these account for 2.3% of all minors charged at Italian Juvenile Justice Services (data supplied by the Italian Juvenile Justice Center). There have been many attempts at classification, and different explanatory theories have been proposed, but many are, in fact, derived from adult sex offender data.[1] The general delinquency explanation considers JSO to be one of the manifestations of the general antisocial tendency and can, therefore, be explained by the same risk factors and processes that have been used in research on young offenders. Aspects of definition and classification of the JSO become even more complex in relation to different scientific and cultural traditions and also to the different profiles and juridical orders in force in the various nations, and to different contexts. Unlike the forensic psychological and psychiatric standpoint, definitions also vary according to the characteristics of the abuser, the victim, or according to the different means by which the deviant sexual behavior is conducted.[2]

In Italy, the legislative choice to delineate a non-punishment area in relation to sexual activities among consenting minors, albeit exclusively regarding sexual relations between minors over the age of 13 and under 18, implies both that early teenagers have already reached sexual self-determination by 13, and also that those under 17 are able to discern the correctness of their conduct in relation to the age of the partner and to the multiple ways of demonstrating consent or dissent to sexual initiatives. Research conducted in the Apulia region of our country aims to point out, as impact and utility, the obvious inconsistency of the Italian legal system, which recognizes adolescents over 13 the maturity for self-determination of their sexual life, thereby guaranteeing sexual freedom despite the known inability of minors to understand their desires and needs before the age of 14, at least.

The learning objectives of this research are intended to invite reflection among governors, directors, health care personnel, and law personnel. In the current context dominated by the internet and the new communication and interaction (chats, social networks, short message systems) technologies, in which makeup and clothing are increasingly uniform among adolescents and adults, there is a high risk that people less than 17 years of age, who often lack the maturity and necessary experience to escape the error, will begin relationships in which the partner, although appearing older, is below the legal age limit.

Additionally, the delicacy and peculiarities of sexual dynamics that develop in teenage relationships often complicate the difficult psychological and psychiatric forensic investigation of the participants' actual engagement

in the sexual act. The risk is to introduce dangerous subjective indices into the judicial and forensic psychiatric and psychological assessments of the minors' behavior, linked to the perception of sexual acts and the ethical sensitivity of those called upon to judge such acts. This research underlines the need to make changes in the Italian norms regarding sexual violence among minors.

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Children, Sexual Crimes, Punishment

I3 The Implementation of a New Method to Care for Mentally Ill, Incarcerated Clients Who Are Not Competent to Stand Trial

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WITHDRAWN

I4 Internet Crimes Against Children: A Forensic Analysis

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After attending this presentation, attendees will better understand the various internet crimes that can occur against children, the profiles of the offenders from a law enforcement perspective, and the psychiatric evaluation and treatment of the offenders. Attendees will also understand the pervasive role that the internet has played in offenses against children.

This presentation will impact the forensic science community by providing updated information on the diagnoses, treatment, and detection of internet predators of children.

The internet has been used for the consensual exchange of sexually explicit material and sexual exploration among adults, and also, unfortunately, child pornography and other crimes against children. The internet provides perceived anonymity, ease of access, and perceived ability to hide from law enforcement behind the computer. People who misuse the internet to commit sex crimes are not all the same in terms of motivation, goals, background, demographics, psychiatric diagnosis, and risk. Someone who looks at a few images occasionally may be at a different risk for offending against a child than someone who collects thousands of images, different than those who engage in online real-time communicating with a child via chats and webcam, and different than those who solicit to meet a child in person. They likely require different interventions and prevention approaches.

There is a continuum and different risk categorizations and diagnoses of the offenders; however, these different users are often prosecuted and treated the same way. Meanwhile, producers and distributers of pornography are extremely difficult to find and often can't even be identified.

In this presentation, four perspectives on assessing and managing the problem of internet sex crimes against children will be presented. Exploration of challenges of identifying, categorizing, and treating individuals who use the internet to prey on children will be facilitated by forensic psychiatrists to include a history of the problem and case examples. The specific approaches in both the United States and Canada will be outlined by sexual behavior clinic directors. A prevention program in Canada will also be discussed.

A law enforcement profiler of offenders who use the internet as a means of communicating with children found that these pedophiles do not fit the old typology, for example of preferential vs. non-preferential attraction to children. The profiler found that many people who use the internet act differently behind the screen than if they were face to face with a child. The computer allows people who would never approach children to do so in a fantasy context. The profiler developed a typology of people who use the internet to chat with children, who are labeled the Dilettante and the Connoisseur. This presentation will include a description of this typology and its application to the field.

Internet, Predators, Children

I5 Real Child Voices: The Impact of Age and Gender Congruent Voices on Sexual Arousal to Child Sexual Scenarios

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After attending this presentation, attendees will understand the nuances that are important to consider in creating assessment stimuli and understanding assessment results.

This presentation will impact the forensic science community by shedding new light on how the assessment of sexual offenders works and what the results mean.

Multiple factors have been identified that can contribute to an increased risk of child abuse by adult men.[1] Objective measurement of sexual responsivity to depictions of sexual interactions between adults and children is an important and common component in actuarial risk assessment and in the development of treatment plans for men who have sexually offended against children. Penile Plethysmography (PPG), known as the "gold standard" for objective measurement of sexual arousal in men, consists of presenting a variety of test stimuli to the person being tested.[2] During PPG, electronic measurements are made of any changes in penile circumference. Increases in penis diameter after/during presentation of a test stimulus reflect the degree of sexual arousal that the stimulus elicits. The audio depictions in both the United States and Canada have historically used monotonous adult male voices to describe a variety of legal and illegal sexual scenarios.

This study compares two independent stimuli sets on a clinical sample of adult males with pedophilia (n=30) and a sample of healthy controls from the community (n=30). One stimuli set, routinely used at the Sexual Behaviours Clinic (SBC) in Ottawa, Canada, for the past 30 years, uses a monotone male voice describing a variety of sexual scenarios in second-person narrative.[3] The comparison set, created at the Sexual Behaviors Evaluation, Research and Treatment Clinic and Laboratory at the University of South Carolina School of Medicine, uses voices that are age and sex congruent with the storyline and sound effects consistent with the scenarios. Creation of this set, known as Real Child Voices (RCV), includes audio of adult and child actors reading non-sexual scripts in a sound studio that were later professionally spliced together with sound effects to simulate scenarios depicting adult-child sexual scenarios.[3]

Preliminary results of this study indicate that the RCV stimuli set does improve differentiation between clinical and healthy participants. This presentation will review the final results of this study. Based on these results, decisions will be made for changes in standard protocols of the PPG stimuli used for clinical assessment and research purposes. These changes are part of a broader initiative working toward multi-site standardization in the assessment of people with problematic sexual interests and behaviors.

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Sex Offender, Assessment, Paraphilia

I6 A Psychological Formulation Model for the Multi-Agency Intervention: Investigation and Management of Child Sexual Exploitation

Lynsey F. Gozna, PhD, University of Nottingham, Centre for Forensic and Family Psychology, Division of Psychiatry and Applied Psychology, Nottingham, Nottinghamshire NG8 1BB, UNITED KINGDOM*

After attending this presentation, attendees will understand and be able to articulate the critical elements of a psychologically informed approach to responding to cases of Child Sexual Exploitation (CSE). In particular, this takes into account the perpetrator(s), at-risk young people and victims, and the multi-agency team dynamics and practices.

This presentation will impact the forensic science community by considering a breadth of child sexual exploitation targeting and the ways in which these manifest in offending behavior within and across a range of cases. This will be applicable to professionals working across multidisciplinary teams and in multi-agency safeguarding and criminal investigations (including interrogation), community interventions and management, and interventions in correctional and forensic mental health facilities. The formulation model will be readily applicable for professionals to consider in their therapeutic work with children and young people in the community, as well as with offenders in regard to clinical formulation, treatment intervention, and risk assessment.

This study presents the development and validation of a model that assists practitioners in developing a psychological formulation of all parties involved in a particular case and helps focus the decision-making and resources of teams managing disparate interventions and investigations while ensuring a coordinated and coherent approach.

CSE creates manifold challenges for multi-agency practitioners responding to the proliferation of targeting that permeates societal and cyber spaces and results in severe psychological, physical, and sexual harm to children and young people. The psychological model, CAPTIVE, developed for multi-agency, cross-disciplinary teams, enables the critical elements and complexities of victim-offender interactions in the context of CSE and broader sexual offending repertoires to be revealed. CSE research to date has predominantly described multiple *modus operandi* (e.g., party, peer, and trafficking models and risk indicators) while negating the underlying offending outlook and the perspective of the victim. Using an approach comprising personality, mind-set, and motive, the interaction between perpetrator(s) and victim(s) can be placed within the CSE trajectory of offending and harm. This comprises the interaction between perpetrator(s) and victim(s) from the process of initial victim identification and targeting, indoctrination/grooming processes, the nature and severity of victimization, and broader processes to interfere in police investigations and associated witness evidence. The myriad ways in which perpetrators deceive, manipulate and condition victims toward a particular mindset increases the challenges for intervention, whether therapeutic or criminal justice, in addition to the dynamic assessment of future risk. This extends social developmental theoretical approaches and the development and validation of the model has incorporated a mixed methods approach in regard to user logistics and psychological content: (1) practitioner evaluation of the CAPTIVE formulation and assessment of risk; and, (2) police files of CSE cases. Case examples in regard to the interaction of psychological dispositions across a range of victim and perpetrator profiles and interactions were additionally developed and will be presented in regard of the fusion of psychological dispositions across a range of victim and perpetrator profiles and interactions. This enables a consideration of the role of complex, challenging, and destructive characteristics of predatory perpetrators and the mechanisms used to maximize successful victim targeting, online and offline. Practice recommendations will be provided in regard to working with "at risk" and victimized children and young people, investigating and disrupting current known perpetrators, identifying and intervening with "at risk" perpetrators and facilitators of CSE, and case building for prosecution.

Formulation, Child, Sexual Exploitation

I7 Why Do Mothers Abuse and Neglect Their Children?: An Update on 100 New Jersey Cases

Vivian Shnaidman, MD, 10 Vreeland Drive, Ste 103, Skillman, NJ 08558*

After attending this presentation, attendees will understand the primary risk factors leading women to abuse and/or neglect their children and how forensic evaluators should approach these cases.

This presentation will impact the forensic science community by exploring and addressing the critical variables leading toward termination of parental rights in maternal abuse and neglect cases in New Jersey. Solid empirical data will be shared to support the hypothesis.

The goal of this presentation is to explore the four reasons which cause mothers to abuse or neglect their children. This presentation will impact the forensic science community, as well as all forensic evaluators and investigators who work with abused children and abusive parents and adults. By understanding the typography of the abusive mother, assessments and treatment protocols can be formed, which can be recommended to the courts. Likewise, meaningful risk assessments can be performed that will help prevent reunification of children with parents who are highly likely to be abusive again in the future.

This presentation builds on an earlier work from 2009 that examined only 30 cases. This presentation examines 100 cases that will provide much more clinically and statistically significant information.

The popular literature regarding child abuse was examined. While poverty, ignorance, lack of education, and immaturity are often correctly proposed as factors involved in child abuse and neglect, most studies look only at these environmental factors and not at the factors in the mothers themselves. In the presented study, personality, emotional, cognitive, and behavioral factors will be investigated and categorized into diagnostic terms that are easy to understand and to explain. Therefore, a systematic manner of approaching these cases will be proposed. The courts respect and rely upon expert testimony in cases of child abuse and neglect, so a definitive paradigm for understanding risk for child abuse is long overdue.

Forensic evaluations for the family courts in New Jersey were examined. Most psychological and psychiatric reports for the courts have numerous features in common. These include certain demographic data, objective information about the case, the subject's own understanding, and putative or working diagnoses, in addition to specific recommendations. Frequently, the recommendations are not unique. For example, in nearly every case of physical abuse against a child, anger management training was recommended; however, without a solid understanding of the underlying diagnosis and an interdisciplinary approach to treatment, many of these routine interventions become meaningless. Therefore, there is a need to understand the underlying problems that lead to difficulty in controlling the outward expression of emotion and to categorize and treat abusive mothers specifically for their own pathology. In this recent work, the four reasons for child abuse that have emerged are mental illness, mental retardation or other cognitive impairment, substance abuse, and psychopathy.

Correlation coefficients between the existence of abuse and/or neglect to these four causative factors will be investigated and it is hypothesized that as more of these factors are present in an individual mother, the more extensive the abuse and neglect. Additional statistical analysis will be utilized to estimate the significance of having one or more of the specified conditions. In the future, a model will be derived that can be used to estimate the risk of future abuse or neglect against children (similar to risk assessment for violence or sexual violence). The results of the data collection and analysis are expected to fully support the anecdotally observed hypothesis.

Risk assessment for abusive mothers can therefore be understood with a four-pronged approach and this will assist the courts in assessing cases of child abuse and neglect in ways that will protect the children yet preserve the parents' rights to parenthood. This research is seminal in its applicability to various types of abuse against children by their caretakers by helping to conceptualize the reasons for abuse in a systematic way as well as by laying a foundation to derive an actuarial-type instrument that can be utilized in risk assessment and planning for reunification or parental rights termination. The practice of forensic psychology and forensic psychiatry frequently revolves around family law and violence against children by adults. A standard way to approach cases of child abuse, vis-à-vis the courts, can begin not only to explain past behavior but also to predict and prevent future behavior.

Child Abuse, Child Neglect, Termination of Parental Rights

I8 Re-Socialization of the Prisoner Between Psychological Support and Work: The Italian Experience

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After attending this presentation, attendees will better understand one of the most important aspects of detention: the possibility for detainees to follow a societal reintegration and rehabilitation program.

This presentation will impact the forensic science community by contributing to the debate within the legal academic community as to the importance of rehabilitation and working activities in prison, which not only are inmates' rights, but may help them resettle in society and inspire their future observance of the law and the rules of communal life.

Over the past decades, penitentiary psychology has specialized to meet the demands of society to understand and, if possible, prevent deviant behavior; the intervention of the psychologist in prison is therefore driven by the constitutional order that rehabilitation is to enable attitudes, intentions, and behavioral choices of re-socialization and reintegration of the offender and to reduce the risk of recurrence.

In this modern and more constitutionally psychological evaluation, the prisoner's right to work, and rights as a form of alternative measures to detention is also an instrument of knowledge of the concrete possibility of social and economic fulfillment. In that last regard, art. 20 of the Italian Penitentiary Code states clearly that "the organization and methods of prison work must reflect those of employment in free society in order to make the subjects acquire professional training adapted to the normal working conditions to facilitate social reintegration."

We proceed to reach the reaffirmation of prisoners as citizens who can, in parallel with pursuing a path of socio-cultural reconstruction, also be an active part in the global growth of society. The sentence should, therefore, be accompanied by prison treatment that guides energies constructively and by psychological work that facilitates critical review and knowledge about and repair of the damage caused.

In Italy, the activities of public bodies are flanked by many private associations, which in recent years have produced a number of projects regarding work in institutions of confinement. Reeducation is intended as a psychological journey deepening one's own life and re-socialization is the ability to renew one's self to return to being a positive and proactive element in and for civil society. In this psychological journey, the "work" has taken a role of great importance and it has been a source of developmental and cultural growth for Italy.

Through the analysis of official statistical studies, this study indicates that conducting work activities inside prisons significantly reduces the risk of recidivism of prisoners, once released from prison. The socio-psychological assistance of prisoners helps to reduce the percentage of prisoners who, when placed back into society, commit new crimes. Based on this statistical analysis, the Italian government has revolutionized the approach to its execution of sentences.

Reeducation, Psychological Support, Inmates Work

I9 Forensic Psychiatric Assessment of Detainees in the United States Immigration System

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After attending this presentation, attendees will: (1) gain an understanding regarding how forensic psychiatric assessment of immigrant detainees is necessary for the fair and efficient adjudication of removal and asylum proceedings; and, (2) learn of the legal and cultural barriers to forensic assessment of immigration detainees.

This presentation will impact the forensic science community by illustrating the challenges of forensic mental health evaluation of immigrant detainees and identifying best practices for use during removal and asylum proceedings.

Currently, perhaps as many as 1,000 mentally ill detainees are being held with the cost of incarceration increasing daily due to continued delays in processing their cases. The United States immigration system has struggled to keep pace with the increasing number of detainees who have severe mental illness requiring forensic mental health evaluation. Information provided by the government, advocacy groups, and media sources illustrate that, in the growing detention system, providing detainees with adequate legal representation and mental health care pose a daunting challenge. Legal protections for immigrant detainees with severe mental illnesses require a focused emphasis, given the differences in their position compared to those in domestic criminal legal detention. The purpose of this systematic review is to describe the epidemiology, challenges, and best practices relevant to the forensic mental health evaluation of immigrant detainees.

A systematic review of literature indexed in MEDLINE® and PsychInfo® was conducted. The search terms "detainee," "immigrant" and "forensic psychiatry" were entered into a literature search for publications between January 1990 and December 2015. Relevant articles were selected and reviewed. The references for these articles were reviewed to augment the search strategy. The selection criteria for articles was that they contain: (1) peer review articles; (2) United States immigration data; and, (3) forensic assessments.

This study identified 14 articles relevant to this review. The epidemiology of this issue is poorly defined. In addition, there have been few controlled studies identifying best practice interventions. Most articles rely on anecdotal encounters and describe the challenges of working with this difficult and vulnerable population. Several cases, which will be presented, illustrate the difficulty mentally ill detainees face in obtaining fair proceedings. Some of these cases have provided the impetus for recent legal rulings that will impact this process going forward.

The unique requirements for forensic assessment of immigrant detainees require diligent scrutiny from forensic, legal, and advocacy perspectives and make this a crucial area for greater study among forensic psychiatrists and other forensic mental health professionals. A particular need is for mentally ill detainees to be provided counsel and forensic mental health assessment when the need arises. This is especially true given the recent wave of increased undocumented immigrants, including the severely mentally ill.

Immigrant, Forensic Psychiatry Assessment, Mental Illness

I10 The Complicated Relationship Between Fire Setters and Mental Illness

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After attending this presentation, attendees will understand the relationship between mental illness and fire-setting behaviors. Fire-setting behaviors, the crime of arson, and the psychiatric diagnosis of pyromania will be discussed. Attendees will be made aware of the forensic issues related to fire setters, including the role of the fire department and mental health professionals in determining the intentions of the fire setter. Recommendations regarding psycho-education to health care providers and patients will be explored.

This presentation will impact the forensic science community by educating attendees regarding the relationship between fire setters and mental illness.

As documented by the National Fire Protection Association, an estimate of 282,600 intentional fires was reported to the United States fire departments each year during 2007-2011.[1] These fires were associated with annual losses of 420 civilian deaths, 1,360 civilian injuries, and $1.3 billion in direct property damage. Outside or unclassified fires accounted for 75% of these incidents, while 18% involved structures, and 7% were vehicle fires.[2] In 2013 United States fire departments responded to an estimated 29,200 home structure fires that were set intentionally. These fires caused 380 deaths, 880 injuries and $531 million in direct property damage.[2] Fire-setting behaviors are described in the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*), but also have legal implications. Literature surrounding this population has limited guidance for assessing this group. Studies have shown that common motives for fire setting revolve around psychosis (88%), revenge/anger (34%), and suicide (20%).[3]

This discussion will present a case of a middle-aged male who was admitted after a fire that began in his apartment. According to the patient, the fire began in the context of him falling asleep while smoking a cigarette. The patient had a history of being declared not guilty by reason of insanity after he set a fire as a young man, in the context of a psychotic episode. The patient had spent most of his life in state hospitals and was now living in a residential setting. The forensic evidence clarifying how the fire was set is still pending. This presentation will discuss pertinent clinical evidence in assessing fire setters and will discuss the difference between arson and pyromania.[4] Along with clinical evidence, resources used by the fire department in determining whether the fire was set intentionally will be presented. Lastly, this case raises the importance of assessing recidivism in this particular population. Risk factors to recidivism in fire setters will be explored.[5] This presentation seeks to alert the forensic community regarding the complicated relationship between mental health and fire setters and to review current methods of assessing these patients.

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Fire Setting, Mental Illness, Recidivism

I11 Toxicological Findings in Choking of Adult Psychiatric Patients

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After attending this presentation, attendees will be familiar with the toxicology findings in psychiatric patients who died of choking and learn how certain psychiatric medications may cause ingestion issues even when being applied in suggested therapeutic doses.

This presentation will impact the forensic science community by illustrating the importance of toxicology analysis in all cases of choking, especially in psychiatric patients, and raising awareness of the possibility of prevention of such occurrences with timely application of adequate medications.

Choking is a type of obstructive mechanical asphyxiation originating from the blockage of the airway by a foreign body, mostly in the upper larynx, usually with a fatal outcome. Deaths due to choking, whether by aspiration or on a bolus of food, are relatively rare occurrences in the general population; however, an increased incidence of fatal choking among psychiatric patients has been repeatedly postulated. Antipsychotics are often being blamed for ingestion issues and dysphagia through the mechanism of vagal inhibition of the gag reflex. Since severe choking has been associated with higher neuroleptic dosages, the question of this presentation is related to toxicological findings in the fatal choking of psychiatric patients.

During the past 15 years, 12 psychiatric patients (eight men, four women) who died of choking (0.003% of autopsies) were autopsied at the Department of Forensic Medicine, Clinical Centre of Montenegro in Podgorica. A standard full autopsy, followed by Gas Chromatography/Mass Spectrometry (GC/MS) toxicology was performed in every case, in addition to a thorough analysis of medical records and a postmortal interview with relatives, friends, and medical professionals who were taking care of the patient. In addition, special attention was paid to the dental status of the victims, as the most significant factor of the chewing process, with a huge impact on swallowing.

Among those autopsied, four patients had been diagnosed with psychosis (33.3%), three of them were chronic alcoholics (25%), two of them had been diagnosed with schizophrenia (16.7%), one with depression and prescription drug abuse (8.3%) and two were heroin addicts (16.7%). Of four patients who were suffering from psychosis, one was treated with depo neuroleptic, one with the combination of depo neuroleptic and diazepam, one with alprazolam, and one with haloperidol. Of three alcoholics, only one had been diagnosed with chronic alcoholism prior to the autopsy and was undergoing treatment with depo neuroleptic, while the other two, expectedly, didn't receive any treatment. The patients diagnosed with schizophrenia were treated with haloperidol, the patient who suffered from depression was treated with depo neuroleptic, whereas the heroin addicts received methadone treatment. Toxicological findings of all these patients revealed the presence of mentioned drugs in their systems, and their blood concentrations were within therapeutical ranges. Only one patient who was treated with haloperidol for his psychosis had a slightly elevated blood concentration of this drug. The two previously undiagnosed alcoholics had elevated blood alcohol concentrations (2.61% and 1.5%). Most of the patients had a poor dental status (41.7%), with a majority of teeth missing. The dental status of four patients was assessed as medium (with nearly half of the teeth missing). Only one person had a good dental status, with minimal teeth missing.

These results suggest a connection between the use of neuroleptics and choking, demonstrating that it may occur even without exceeding the recommended dosages. Alcohol affects swallowing through the same mechanism, putting patients with acute alcohol intoxication at high risk of fatal choking. The contribution of poor dental status and the direct effect of the disease itself in cases of fatal choking is unquestionable; however, early symptoms of impaired gag reflex and dysphagia should be carefully monitored in psychiatric patients treated with neuroleptics in order to prevent the occurrence of aspiration with the timely application of adequate medications.

Fatal Choking, Toxicology, Psychiatric Patients

I12 Copycat Violence in Psychiatric Patients

Houssam Raai, Bronx Lebanon Medical Center, 1276 Fulton Avenue, 8th Fl, Bronx, NY 10456; Katya Frischer, MD, Bronx Lebanon Hospital Center, 1276 Fulton Avenue, 5th Fl, South Bronx, NY 10583; Amina Ali*, 301 W 45th Street, Apt 6A, New York, NY 10036; and Loveleen Kaur Khehra, BSc, American University of the Caribbean, 901 Ponce de Leon Boulevard, Ste 700, Coral Gables, FL 33134*

After attending this presentation, attendees will be aware of violent behavior and aggression as a copycat phenomenon among patients with psychiatric disorders. This presentation will discuss the cathartic effect and desensitization caused by recurrent exposure to violent media, whether it is fictional or reality based. This presentation will also examine the forensic issues and implications surrounding copycat behavior in mentally ill patients. Recommendations regarding psychoeducation for mental health care providers will be explored, such as assessment for exposure to violent media in evaluation of patients with mental illness.

This presentation will impact the forensic science community by raising awareness regarding the influence of the copycat phenomenon in triggering violent acts and shaping aggressive behavior in patients with mental illness.

This presentation will make a detailed case discussion of a patient who expressed obsessive homicidal thoughts and had a history of multiple psychiatric admissions. During admissions to a community psychiatric hospital, he was diagnosed with major depressive disorder and schizophrenia spectrum disorder. The patient was fascinated with reality-based historical mass murderers, as well as movies and television shows portraying serial killers, such as *Dexter*. A psychological assessment gave no indication of psychopathic traits or antisocial personality disorder in this patient. The patient later committed a murder while being treated in a long-term care psychiatric hospital. The patient in this case exhibited a copycat behavior. While copycat phenomenon is more frequently studied in mass killings, the effect of violent media on single acts of violence, from petty crimes to homicides, is not well studied. It is not clear whether individuals who express an interest in copycat violence have a high rate of mental illness; however, it is possible that having impaired reasoning abilities combined with poor impulse control makes mentally ill patients more vulnerable to suggestibility and imitation of violent acts. Studies and reports warn of the copycat effect after mass killings.[2] It has been indicated that threats to conduct similar attacks tend to increase dramatically in the weeks following a highly publicized mass murder.[3] According to one study, 57% of people expressing these threats and exhibiting copycat behavior had had a contact with psychiatric services; 25% have been diagnosed with behavioral and emotional disorders, 14% with mood disorders, and 6% with schizophrenia group diagnoses.[3] This presentation will submit to the forensic community the potential significance of media violence and copycat effects on mentally ill patients and will examine whether psychiatric patients have a higher likelihood of threatening or committing copycat offenses.

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Copycat Violence, Media Violence, Obsessive Homicidal Thoughts

I13 Posing of the Body and Staging as Other "Causes-of-Death" in Homicides

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WITHDRAWN

I14 Violence Factors and Characteristics of Police Ambush Killers

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After attending this presentation, attendees will understand the manner in which law enforcement characterizes police deaths and the concept of police ambush killings. The characteristics of those who carried out police ambush killings will be reviewed and assessed for violence. The potential risk factors for violence will be reviewed. Recommendations regarding psychoeducation to health care providers, particularly forensic psychiatrists, will be explored.

This presentation will impact the forensic science community by educating attendees about the clinical characteristics of those who have successfully conducted police ambush attacks from 2014 to the present.

On average, 47 felonious police deaths occur annually in the United States. Of these, ten occur annually in the context of police ambush shootings.[1] Police ambush shootings are defined as predatory and sudden attacks on police officers with the intent to kill or injure. The topic of police ambush shootings has garnered increasing significance in the media and society at large, particularly due to recent police killings in Dallas, TX, Baton Rouge, LA, and San Diego, CA.[2] Data from 2010 reveals that alleged offenders in the felonious killings of officers share certain characteristics, including being predominately male, having prior arrests, and being under the influence of controlled substances or alcohol at the time of the incident.[1] Although there is information available on offenders in felonious police deaths, there is little literature analyzing characteristics of offenders in ambush police killings.

Reviewed here are police ambush killings from 2014 to present (16 cases).[1] Using publicly available sources such as the Federal Bureau of Investigation (FBI) websites dedicated to police deaths and media, including newspapers and social media, this presentation will review the assailants in those 23 ambush killings. The goal is to determine if there are any clinical similarities between the perpetrators and consider their risk factors and predispositions for engaging in such acts of violence toward law enforcement. Police ambush killings, while rare, have shown a significant increase in the United States and worldwide. It is poorly understood and infrequently reported in the forensic literature. This study seeks to alert the forensic community about this method of killing police officers as well as to review characteristics of the perpetrators and identify similarities and differences among them. By determining commonalities between these assailants, this presentation will educate health care professionals on these characteristics as well as on the increased incidence of ambush killings in the United States.

Reference(s):

1. https://ucr.fbi.gov/leoka/leoka-2010/officers-feloniously-killed.
2. http://www.cnn.com/2015/08/03/us/police-officers-killed-nationwide/.

Police Ambush Killings, Violence, Mental Illness

I15 The Experience of Revenge: The Patterns, Meanings, and Mindsets of Mentally Disordered Offenders

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After attending this presentation, attendees will be able to interpret and articulate critical elements in the decision-making and behavioral outcomes of revenge-oriented mentally disordered offenders who respond to real or perceived victimization through criminal actions. In particular, the focus of this presentation is to increase the accuracy of offense and behavioral analysis and to consider the potential therapeutic interventions and tailored scenario planning in regard to risk within secure correctional facilities, forensic mental health settings, and upon release into the community. Furthermore, this presentation will address the challenges in predicting harmful revenge-oriented behaviors by individuals who are classified as potentially dangerous persons in the community, but who have yet to act.

This presentation will impact the forensic science community by illustrating the breadth of revenge-oriented activities and the ways in which these manifest in offending behavior within and across a range of offense types. This will be applicable to those working in criminal investigation (including interrogation), community management, and therapeutic interventions in correctional and forensic mental health facilities. The developed revenge model will be readily applicable for practitioners to consider in their work with patients in forensic settings, particularly clinical formulation, treatment intervention, and risk assessment.

The response of individuals to real or perceived victimization can range from avoidance and forgiveness to angry rumination, fantasy, and revenge-oriented acts. The criminal justice system largely neglects consideration of perpetrators who may consider themselves victims and their associated criminality to have been motivated by revenge. As one of the more prevalent motives for interpersonal criminal activity, revenge-oriented acts occur across offense types (e.g., sexual offending, arson, stalking, kidnapping, and homicide) and are influenced by individual differences, including personality, culture, and faith; however, any psychological response that follows a real or perceived slight/victimization includes an individual justification to progress the event toward a subsequent reactionary act of harm, or revenge. This process requires particular understanding in the context of forensic mental health in which the criminal acts emanating from an apparent victimization may be difficult to comprehend or rationalize (for example, due to over-compensatory retaliation, delusional beliefs or conveniently justifiable violence). The experiences of offenders with diagnosed personality disorders and mental illnesses are often characterized by challenges in relationships and interpersonal interactions and, hence, the implications for vengeful acts are apparent. The experience of perceived victimization and associated revenge-motivated actions in a group of mentally disordered offenders, which was conducted using a participant-led approach designed to enable an open dyadic interaction, will be presented.

Participants were in-patients receiving treatment at the mental illness or personality disorder service of a specialist medium-secure forensic mental health unit. The methodological approach adopted intensive interviews using a "Grounded Theory" to fully capture the patient experience of engaging in revenge-oriented acts and to enable theory generation. The focus of this presentation, therefore, will be to present and discuss a theoretical and practice-focused model of the experience of revenge-oriented acts in the context of forensic mental health and to consider the implications of the model as applied to secure and community forensic settings. Case examples will be presented to outline complexities in regard to the "offender-as-victim" perspective and to illustrate the range of meanings emerging from their different perspectives. Conclusions and recommendations will be presented in regard to: (1) the multidisciplinary teams within forensic mental health; (2) indicators of concern for incorporation into tailored therapeutic treatment pathways; (3) the incorporation of an additional item of "revenge" into structured clinical judgement risk measures; (4) implications for collaborative scenario planning for considerations of future risk; and, (5) consideration of a revenge orientation in police investigations and in multi-agency public protection arrangements for the management of offenders in the community.

I16 On the Radicalization Process

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After attending this presentation, attendees will better understand general and specific recruitment tactics among terrorist networks, especially those presently used by the Islamic State of Iraq and Syria (ISIS).

This presentation will impact the forensic science community by providing an in-depth psychological and criminological description of radicalization, brainwashing, and the psychology of the "new terrorism."

The radicalization of young men and women by terrorist organizations, especially the ISIS, has become an overwhelming problem in the world. Recently, and everywhere in the world, young people appear to have been motivated by a complex mix of politics and faith, and their communications illustrate the tactics used to try to recruit other young Europeans and Americans to their cause.[1-3]

For example, the possibility of French citizens returning from Syria as hardened jihadists is the "biggest threat that the country faces in the coming years," said Manuel Valls, the interior minister of France. He added that France and Europe risk being "overwhelmed" by the phenomenon. Mr. Valls estimated that 700 French nationals have either traveled to Syria, have traveled to Syria and returned to France, or are currently in route. Some 21 French nationals have been killed. Shiraz Maher, a senior research fellow at the International Centre for the Study of Radicalisation at King's College London, estimated recently that up to 50 British fighters have already returned home

This study intends to provide an in-depth description of the radicalization process, which is a very important and essential step in terrorist activities. It also proposes a translational analysis of the sociological and psychological processes involved in the terrorist violence. This study is first based on experience in the psychological evaluation of terrorist behavior and, secondly, on an exhaustive review of the current literature. The search terms "terrorism," "radicalization," "social psychology," and "psychopathology" were used to identify relevant studies in the following databases: Scopus, MEDLINE®, Pubcentral, and ScienceDirect.

Because of its importance, understanding the radicalization process should be one of the priorities of behavioral scientists, including forensic psychiatrists, forensic psychologists, and social workers. International and translational studies should be performed with a focus on several aspects, such as radicalization risk factors, brainwashing, cognition modifications, social psychology methods, social network behaviors, the role of the media, and, finally, deradicalization programs.

In terms of perspectives, counter-radicalization programs, such as those run by Saudi Arabia and Sweden, have demonstrated mixed results. The most successful efforts in Britain have been the efforts of the so-called Channel program, which is part of the British government's counter-terrorism strategy to divert young people from extremism. Such efforts, which involve the police, social services, and local authorities working together, draw on methods used to help young people leave gangs.[4-6]

Not all of these people returning from radical countries will have blood on their hands. Governments need to offer a way out for those who realize that they have made a mistake, and Western countries may benefit from an even softer approach. Chastened returning fighters may be the very best people to persuade more young men to forgo the fight. No one yet knows whether today's European jihadists who are fighting for ISIS will become tomorrow's murderers on the streets of Western cities.[4-6]

On a more practical note, it is clear there are currently not enough detailed case studies of terrorists to inform psychological analyses or even to conduct comprehensive reviews of the literature.

Reference(s):

1. Horgan J. Terrorist minds? In: Horgan J, editor. *The Psychology of Terrorism.* 2nd ed rev. and updated. Abington, U.K.: Routledge, 2014;47–77.
2. Post J.M. Terrorisms and terrorist psychologies: an introduction. In: Post J.M., editor. *The mind of the terrorist: the psychology of terrorism from the IRA to al-Qaeda.* New York, NY: St. Martin's Griffin, 2007;1–10.

3. Singer M T. The process of brainwashing, psychological coercion, and thought reform. In: Singer M.T., Lalich J., editors. *Cults in our midst – the hidden menace in our everyday lives.* San Francisco, CA: Jossey-Bass Publishers, 1995;52–83.
4. Kahneman D. The science of availability. In: Kahneman D, editor. *Thinking fast and slow.* Toronto, Canada: Doubleday Canada, 2011;129–45.
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Radicalization, Terrorism, Social Psychology

I17 The Female Psychopath: A Study of a Sample of National Italian Women Offenders

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After attending this presentation, attendees will be able to recognize the characteristics of the female psychopath.

This presentation will impact the forensic science community be helping to identify possible gender-specific factors related to psychopathy.

Background: In Italy, the treatment of mentally ill offenders not guilty by reason of insanity at risk for recidivism (a danger to society) was entrusted to the High Security Hospital (OPG). The Law no. 81 of 2014 ratified the closure of all Italian OPGs from March 31, 2015, and planned the transfer of the ill patients (about 1,500) from the OPGs to small community facilities located in their own regions, so-called Residences for the Execution of Security Measures (REMS).

Until its closure, the OPG of Castiglione delle Stiviere in northern Italy, located in Castiglione's Ghisiola Park in the province of Mantova, was the only one that admitted women; all women who have committed a crime on Italian territory and are at risk for recidivism are sent to the OPG of Castiglione. The search enrolled all the women present at the OPG (86) from February1, 2013, to the end of 2013 to research female psychopathy's prevalence.

Methods and Results: The experimental group consists of 65 mentally ill offenders not guilty by reason of insanity and a danger to society; the control group consists of approximately 20 inmate women offenders without mental disorders. The survey was conducted according to the rules considered by the ethics committees of the respective structures. All women in the study consented and have been subjected to clinical and anamnestic evaluations. They were administered the Structured Clinical Interview for DSM Disorders (SCID) I, SCID II, and other mental tests (Minnesota Multiphasic Personality Inventory® 2 (MMPI-2), MCMI-III, and Repeatable Battery for the Assessment of Neuropsychological Status (R-Bans)) after a period of observation in 2013. A clinical-anamnestic assessment was made to investigate age, marital status, education, personal and family psychiatric history, legal status (infirmity or partial infirmity), type of crime (property or person or drug), pharmacological therapy. The Psychopathy Checklist-Revised (PCL-R) was used to evaluate the index of psychopathy.[1] A PCL-R score considered indicative of psychopathy was a score ≥25, the cut-off recognized at the European level.

Conclusions: Among the significant data was the prevalence of Cluster B personality disorders, the most frequent diagnosis of Borderline, and comorbid substance abuse in 75% of the cases. The most significant results of this research will be discussed.

Reference(s):

1. Hare R.D. (1991). *The Hare Psychopathy Checklist - Revised*. Multi-Health Systems, Toronto, Ontario.

Psychopathy, Female, Gender-Specific Factors

I18 Relationship of Oxytocin (OXT) and the Serotonin Transporter (5-HTT) Single Nucleotide Polymorphisms and Antisocial Behavior

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After attending this presentation, attendees will have gained knowledge about the relationship between Single Nucleotide Polymorphisms (SNPs) associated with genes for oxytocin and the serotonin transporter and specific behavioral traits. Furthermore, attendees will learn about the use of Massive Parallel Sequencing (MPS) in the behavioral genetics and forensic psychiatry field.

This presentation will impact the forensic science community by demonstrating how aggressive and antisocial behavior have become a major problem as the United States currently has the highest incarceration rate in the world. Moreover, these behaviors are some of the leading causes of mental health referrals. The strong heritability and environmental issues surrounding criminal activity indicates that the underlying genetics can help explain at least some features related to these behaviors.

Behavior is a complex process influenced by both genetics and the environment. Some neurotransmitters, including OXT and 5-HT have been associated with social behavioral traits. Certain genes (such as the genes of receptors, transporters, and enzymes involved in metabolic pathways of these neurotransmitters) are associated with OXT and 5-HT. These genes contain polymorphic sites that can be studied to relate or link to certain behavioral traits. SNPs are single base variations located at a specific location on the genome and considered to be the most abundant type of polymorphism in humans. While some associations between SNPs and behavior have been made, many studies have been limited on the number of SNPs due to conventional methods. MPS is a new technology that provides an opportunity to analyze a large number of SNPs simultaneously.

This study analyzed two SNPs located within the intronic region of OXT gene (rs877172 and rs4813625) and three SNPs located within the 5-HTT (rs25531, rs6314 exonic, and rs6311) using Single Base Extension (SBE) and MPS with a custom-designed panel of SNPs linked or related to genes of neurotransmitters. A student sample set (N=100) was genotyped and individuals participated in a survey designed to assess behavioral traits.

Two OXT SNPs were analyzed using both techniques and for all samples, the alleles called were 100% concordant. These results indicate that the custom primer panel may be used to assess a large panel of behavioral markers at once. It was also found that OXT and 5-HT may have an impact on social behavior. Statistically significant associations were found between two SNPs (rs25531 and rs877172) and behavioral traits, including antisocial behavior, drug use/distribution, and property crimes.

The results of this study provide some evidence that OXT and 5-HT can influence behavior. It was found that SNPs associated with 5-HT and OXT influence behaviors, including drug use/distribution, property crimes, and antisocial behavior. Also, MPS may be used in the forensic psychiatry and behavioral sciences field to analyze several SNPs related to multiple behaviors simultaneously. This large panel of behavioral SNPs may be used in early prevention or treatment of psychiatric disorders, which has a large impact on the medical field and criminal justice system. Furthermore, understanding the influence of OXT and 5-HT on behavior may help explain the etiology of aggressive and antisocial behavior.

Single Nucleotide Polymorphism, Oxytocin, Serotonin

I19 Youth Satanism and Forensic Investigation: The Case of the "Beasts of Satan" in Italy

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The goals of this presentation are to examine the phenomenon of juvenile or "acid" Satanism and its relation to youth deviance and to propose an effective approach for the investigation of suspected ritual crimes. After attending this presentation, attendees will develop a further understanding of the characteristics of youth Satanism, coupled with understanding the scientific and forensic investigations of ritual crimes in Italy, including analysis of the famous Italian case of the "Beasts of Satan."

This presentation will impact the forensic science community by providing a better understanding of the differences between Satanism and youth deviance, including discussion of the relevant psychological risk factors.

The "Beasts of Satan" is a very well-known case that occurred in Italy. It dealt with a history of murders and suicides that were intertwined with Satanism and esotericism. The "Beasts of Satan" were a group of serial killings in which crimes were committed between 1998 and 2004. They were unusual crimes committed with great ferocity and utilized such items as satanic symbols, inverted crosses, and the number 666, which is a number that some people relate to the Apocalypse.

The police investigation began in the mountain home of one of the group members, where they found obvious traces of drugs, including cocaine, heroin, benzodiazepines, and books concerning satanic rituals.

This presentation will attempt to highlight factors that can influence the tendency of young people to affiliate with and join such groups, as well as the psychological characteristics of the group members, leading to the different levels of "acid" Satanism. Especially noteworthy is how juveniles in this group were dedicated to the use of significant physical and psychological violence while under the influence of alcohol and drugs, leading to the vicious killing and even suicides of several members of this group. This presentation will also utilize the forensic evidence of these crimes, specifically for the famous murder of Ms. Mariangela Pezzotta, as it provides a deeper look into the interpretation of the satanic symbols used during the crime, which eventually led to recognizing and identifying this group. During this particular investigation, it was discovered that at least three other young men, and likely more, had been murdered at the hands of this group. Attention will also be paid to the psychological risk factors that lead individuals to affiliate with such a group, the irreverent gestures used by these groups during the commission of such heinous crimes, as well as several pieces of forensic evidence, such as fingerprints, DNA, and crime scene analysis. Through this analysis, it is suggested that utilizing an interdisciplinary approach to examine all related factors is the best method to solve such complex crimes. This presentation will demonstrate how the use of criminological factors, anthropological factors, and physical forensic analysis could be very important factors to provide insight into these crimes.

Acid Satanism, Forensic Evidence, Symbols

I20 Fantasy-Reality Confusion: Normal Development or Mental Illness? The Case of the "Slender Man Stabbing"

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After attending this presentation, attendees will have gained knowledge of a criminal case involving juveniles accused of attempted murder that features several forensic mental health elements. Furthermore, attendees will understand the difference between normal developmentally appropriate fantasy and impaired reality-testing, as well as the epidemiology of childhood psychiatric illness that may present with delusions.

This presentation will impact the forensic science community by: (1) presenting a review of the literature regarding the ability to discern reality from fantasy in the course of normal childhood development; and, (2) examining childhood psychiatric illness associated with delusions. These concepts will be related to a nationally known criminal case by exploring relevant forensic mental health issues, such as determinations for juvenile offenders to be tried as adults as well as defense strategies (e.g., the insanity defense and mitigation).

On a Saturday morning in May, 2014, three 12-year-old girls were playing hide-and-seek in a forested park. By the afternoon, one of the girls would be fighting for her life in emergency surgery. A bicyclist had discovered her after she crawled through the forest, having been stabbed 19 times, allegedly by her two playmates. The two girls were arrested the same day, walking near an interstate and carrying bags packed with food, water, and other items, including a bloody kitchen knife. Reportedly, they were on their way to find the mythical mansion of "The Slender Man," a fictional character invented on the internet in 2009. The preteen girls allegedly attempted to murder their friend to prove their loyalty to Slender Man, so as to become his servants and be imbued with supernatural powers. The girls were charged with first-degree attempted murder, which in their state triggers an automatic trial in adult court for any person older than ten years of age.

There are myriad theoretical- and research-based models for normal cognitive development. One of the most prominent of these is Piaget's schema-based cognitive development theory. Cognitive development is the construct used to understand the developing child's ability to differentiate reality from fantasy. Recent research suggests that differentiation between fantasy and reality occurs incrementally, at varying rates based on many factors, and begins at an age much younger than commonly believed. This normal development is in contrast to abnormal psychiatric presentations in children, such as the exceedingly rare childhood-onset schizophrenia, which in pre-adolescents has a prevalence of less than 1 case per 10,000.

A sophisticated understanding of and the ability to discern between developmentally normal fantasy and pathologically impaired reality-testing in children is critical in cases such as the one discussed. For the forensic mental health professional, these concepts are relevant to a range of legal issues, including the determination of criminal culpability and mitigating factors. Moreover, the research findings disrupt several commonly held beliefs among lay people as well as professionals. Given the above, the need for experts in childhood development to be involved in such cases cannot be overstated.

Juvenile, Murder, Mental Health

I21 Resiliency and Trauma: An Interdisciplinary Team Approach to the Evaluation of Individuals in Mass Tort Cases

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The goals of this presentation are to: (1) introduce attendees to an interdisciplinary team approach to mass tort evaluation; (2) familiarize attendees with the concept of resiliency in the forensic evaluation of individuals exposed to stressful and life-threatening events; and, (3) provide attendees with greater awareness into the most common reaction to trauma.

This presentation will impact the forensic science community by informing attendees about the common reactions to life threatening events.

Estimates of the likelihood of developing Posttraumatic Stress Disorder (PTSD) after exposure to a life-threatening event have ranged from 6.6% to 9.7%, with survivors of rape, military combat, and captivity experiencing the highest rates of PTSD, ranging from one-third to more than one-half of those exposed.[1,2] The revised *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) criteria for PTSD broadens the definition of a qualifying trauma and expands and reorganizes the list of accompanying symptoms, which is expected to affect both civil litigation and criminal defense in a wide range of cases that involve trauma. Importantly, available evidence suggests that resilience to life-threatening events is far more common than developing symptoms of PTSD or other psychiatric sequelae. Although definitions of resilience vary in the scientific literature, the concept of resilience is distinct and separate from the process of recovery. One definition of resilience involves the ability to maintain a stable equilibrium based on protective factors, which promote positive outcomes when exposed to stressful and traumatic life events.[3] Resilience to trauma can, therefore, best be understood as the ability to maintain relatively stable, healthy levels of psychological and physical functioning despite exposure to life-threatening events. In children, resilience has been defined as the capacity to negotiate developmental tasks in the face of adversity. Several protective factors are listed as important for the development of resilience in children, such as intelligence, good communication and problem solving skills, the capacity for self-reflection and regulation, and the ability to plan. Similarly, posttraumatic resilience is associated with a cluster of personality traits, including extraversion, high self-esteem, assertiveness, and an internal locus of control.[3,4]

This presentation will begin by reviewing the methodology of the interdisciplinary team approach to conducting forensic evaluations in mass tort cases. A series of case examples will then be presented demonstrating the full range of responses, including resiliency to trauma in a mass tort case evaluated using the interdisciplinary team approach. This presentation will then conclude with a review of the scientific literature on resilience to trauma, including the factors and traits associated with resiliency.

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1. Kessler R.C. et al. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry.* 2005; 62(6): 593-602.
2. Beals J. et al. The Prevalence of Posttraumatic Stress Disorder Among American Indian Vietnam Veterans: Disparities and Context. *Journal of Trauma Stress.* 2002; 15(2): 89–97.
3. Agaibi C. et al. Trauma, PTSD, and Resilience: A Review of the Literature. *Trauma, Violence, & Abuse.* 2005; 6(3): 195-216.
4. Bonanno G. et al. What Predicts Psychological Resilience After Disaster? The Role of Demographics, Resources, and Life Stress. *Journal of Consulting and Clinical Psychology.* 2007; 75(5): 671-682.

Resilience, Trauma, Mass Tort

I22 Medication Madness: Medication As the Basis for the Involuntary Intoxication Defense

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The goals of this presentation are to: (1) provide a summary of the defense of involuntary intoxication as it relates to prescribed medications; (2) review the medications most commonly used by defendants asserting this defense; and, (3) review the pitfalls of the defense to serve as practice pointers for lawyers and forensic mental health evaluators.

This presentation will impact the forensic science community by reviewing the defense of involuntary intoxication as it relates to prescribed medications and by providing practice pointers for those involved in cases in which this defense is raised.

The defense of involuntary intoxication has long been an exception to the general notion that intoxication is not a defense to criminal liability. In most jurisdictions, a criminal defendant may be excused from responsibility if he commits a wrongful act because of involuntary intoxication. This defense is based on the common-law premise that someone who ingests an intoxicant unknowingly, or without awareness of its implications, is not blameworthy.

Defendants may become involuntarily intoxicated through the fault of another; by accidental ingestion, inadvertence, or mistake; or because the defendant has a unique physiological or psychological condition beyond his/her control.[1] Although the term "intoxication" is generally associated with the ingestion of alcohol, the law recognizes that it could include any drug or substance with adverse effects, including prescribed medications.

This presentation will review the results of a recent study on the types of medications most commonly "blamed" by defendants using this defense, as identified in appellate case review.[2] Psychotropic medications are the most common group of medications used by defendants in their claims of involuntary intoxication. This presentation will discuss specific subclasses of medications.

In addition, this presentation will review the key challenges associated with a defendant's use of the defense of involuntary intoxication due to prescribed medication. The major challenge to defendants attempting the defense of involuntary intoxication is one of proof. Although there are jurisdictional variations in the applicability of the defense, most jurisdictions require the defendant to establish intoxication due to the claimed agent, involuntariness, and legal insanity. Given this high standard, defendants seldom meet their burden.

This presentation will provide some instruction for legal counsel and mental health forensic evaluators who are called to assess individuals asserting this defense. Specifically, the following areas will be discussed: (1) establishing ingestion; (2) defendant's misuse of the medication; (3) defendant's concurrent use of alcohol or illicit substances; (4) defendant's prior experience with the medication; and, (5) meeting the insanity standard under these laws.

Psychiatrists have unique skills and expertise to aid in the evaluation of defendants using this defense. Not only do psychiatrists have expertise in psychotropic medications and their anticipated effects, forensic psychiatric evaluators are in a unique position to assist the courts because of their experience in assessing mental state at the time of the crime.

Reference(s):

1. Myers T. Halcion Made Me Do It: New Liability and a New Defense – Fear and Loathing in the Halcion Paper Chase. *62 U. Cin. L. Rev.* 603, 638 (1993).
2. Piel J. The Defense of Involuntary Intoxication by Prescribed Medications: An Appellate Case Review. *J Am Acad Psychiatry Law*. 43:321–28 (2015).

Involuntary Intoxication, Prescribed Medication, Insanity

I23 Vexatious Litigation: Medicolegal Aspect of a Pathological Concept and Its Implication in Canada

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The goal of this presentation is to educate attendees regarding the concept of vexatious litigation and its origin. How this concept impacts the practice of psychiatry and forensic psychiatry will be described as will profiles of the patients who are deemed vexatious litigants.

This presentation will impact the forensic science community by presenting a concept that is not yet broadly used but is useful in managing some difficult patients.

Freedom of expression is a highly regarded social value in many countries; however, without any restriction, some individuals may use this right to create frequent and enduring conflicts. Litigation may become a way of expressing disagreement and contesting authority. The habit of frequently raising conflicts may result from individuals' personality traits, personality disorders or severe mental disorders. In such cases, these constant litigations may be viewed as psychiatric symptoms.

Paranoid personality disorder is the most common disorder in people raising conflicts on a regular basis, primarily because of the misinterpretation of facts. Such individuals often perceive insults and threats where none may exist. Other mental disorders, such as schizophrenia, are also associated with this type of behavior. Indeed, although paranoid ideas drive the need to raise conflict, because of their thought disorganization, the motives of such individuals can be quite vague. In both instances, personality disorder or severe mental disorder, because of the repetitive and baseless nature of the complaints, they may be easily dismissed; however, this may result in increasing the degree of suspiciousness and resentment and can result in individuals escalating their actions. It is therefore important, if possible, to listen to such claims carefully and to respond to them in a way that the complainant is satisfied they have been heard and will therefore not take their actions to a higher level.

As noted previously, complaining may be part of the symptom profile of individuals who suffer with mental disorders. This behavior becomes particularly challenging when it interferes with the therapeutic relationship, which can become a major barrier in rehabilitating the patient. The reasons for the development of such paranoid thinking remain unclear. Institutionalized patients who do not suffer from any major cognitive impairment seem to be more prone to engaging in litigious actions. This and other potential etiological factors will be explored.

As always, when a right is given, some way of controlling this right has to be implemented to ensure that it will not be abused. This is exactly what happens with what are described as vexatious litigations. An individual who is designated a vexatious litigant can see all his/her reporting to legal authorities being dismissed automatically. Designating someone in this manner is a powerful way to restrict his/her rights. This presentation proposes to describe the evolution of the concept of vexatious litigation. The intent is also to bring to attention the Canadian process of making an individual a vexatious litigant and its impact on clinical practice.

This presentation will be focused on the evolutionary concept of vexatious litigation and on describing the psychopathology of the people who are deemed vexatious litigants.

Forensic Psychiatry, Paranoid Traits, Litigation

I24 Controversies in the Assessment of Forensic Parasomnia Episodes

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After attending this presentation, attendees will understand the particular issues related to assessing an episode of criminal behavior in which the defendant alleges the behavior was parasomnic. The legal doctrines are explained and the relevant evidence that will assist the court is identified.

This presentation will impact the forensic science community by aiding attendees in avoiding the pitfalls in this area of forensic science. Attendees will be able to prepare expert reports that are evidence-based but do not stray into policy areas.

It has been increasingly recognized that sleepwalkers are capable of fairly complex behavior, and this has often been raised as a defense in criminal trials. In particular, the potential for sexual behavior in sleep is recognized. The potential for malingering must be considered (although the potential is overstated by some). The expert witness who is cognizant of his/her overriding duty to the court, remains objective, and keeps to proven techniques for assessment, has nothing to fear. This research presents the results of a qualitative study of expert evidence on forensic parasomnias.

There are particular issues for expert witnesses when assessing incapacity due to neuropsychiatric disorders, compared with purely psychiatric conditions. There are well-formulated tests for criminal responsibility for the legally insane (e.g., the M'Naghten Rules or the Model Penal Code test). The same is not true when the defense is based on automatism or unconsciousness. The definitions of automatism rely on poorly defined terms that often smack of dualism, due to the legal concepts of *mens rea* and *actus reus.* In fact, because of the current legal thinking on parasomnias, sleepwalking is essentially a status defense. Diagnosis is crucial, unlike the accused with a mental illness in which the assessment of capacity is key.

The effect of alcohol on sleepwalking is the most contentious issue, particularly in forensic episodes. There are definite policy issues as sleepwalking episodes are difficult for the lay person to distinguish from alcoholic intoxication. Alcoholic blackouts are often a better explanation for amnesia. The literature on this issue is plagued by a tendency for the policy concerns to color the interpretation of the scientific evidence. It is for the jury to decide on issues of fact, not expert witnesses.

Another issue is the provision of measures for social control. This often relies on the insanity verdict or the equivalent. Plain acquittals will not permit mandatory monitoring or treatment. Although in many cases of parasomnia, recurrence of the behavior is extremely unlikely, in some subsets, this is not the case. It should be appreciated that the term "insanity" has no nosological significance. The frequent involvement of non-psychiatrists leads to misunderstandings of the legal meaning of insanity.

Provision of expert evidence in this area requires an appreciation of the legal doctrines to avoid usurping the function of the jury.

Parasomnias, Expert Evidence, Forensic Psychiatry

I25 Incompetently Incompetent: The Use of Forged Forensic Evaluations to Avoid Conviction and Sentencing

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After attending this presentation, attendees will be familiar with: (1) the warning signs that indicate that a forensic psychiatric/psychologic evaluation report may be fabricated; and, (2) steps that can be taken within the forensic system if it is discovered that a forensic evaluation report has been fabricated.

This presentation will impact the forensic science community by enhancing the attendees' abilities to conduct forensic psychiatric/psychologic evaluations by increasing their knowledge of warning signs of fabricated forensic reports and by providing steps that can be taken if they believe a report may be fabricated.

Hypothesis/Proposition: This presentation will use a real-life case to illustrate the detection of fabricated forensic evaluation reports and provide guidance on what to do when such a report is discovered.

Synopsis: This presentation will begin with a brief overview of a criminal case from Fulton County, Atlanta, GA, involving the theft of vehicles left stranded on the roadways during the 2014 snowstorm that left Atlanta paralyzed. Two co-defendants were referred for forensic psychiatric/psychologic evaluations for competency to stand trial. The first defendant was opined incompetent to stand trial, largely based on a previous forensic evaluation report that was provided by his attorney. He was placed in the Fulton County Jail Competency Restoration Program, a unique, jail-based program, but was soon after declared to be not restorable regarding his competency to stand trial. The second defendant was evaluated by a different evaluator who realized there were significant inconsistencies in the previous forensic evaluation report that had been submitted by his attorney as collateral information.

It soon became clear that the previous forensic evaluation report submitted by the defendant's attorney was a forgery, and in comparing it to the previous forensic evaluation report submitted by the first defendant's attorney, it became evident that both evaluations contained numerous statements that were nearly identical and extreme enough that the validity of the reports was dubious. It was confirmed with the alleged authors of the two previous reports that neither had evaluated the defendants in question, let alone authored the forensic reports. This is the first case of forged forensic evaluation reports known in the state of Georgia and involves a fairly high-profile crime in the Atlanta area.

This presentation will educate attendees on signs within a forensic report that may indicate that a report has been fabricated. This presentation will also discuss steps that an evaluator can take to determine validity if an evaluator suspects that a report is forged and, if necessary, to alert the proper authorities.

Forensic Evaluations, Forgery, Competency to Stand Trial

I26 Psychological Assessment in Forensic Context: Detection Deception Within a Mixed Perspective

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The goal of this presentation is to present attendees with an integrative perspective on the procedures to assess deception via psychological assessment in a forensic context. Past and current research have focused mainly on the cognitive-behavioral perspective in order to understand the phenomenon of detection deception. While new methods exist, such as neuroscience, and attempt to provide further understanding, what usually occurs is that they are ignored, which leaves this judgment regarding the subject focus of assessment up to the readers of a psychological report within the criminal justice system.

This presentation will impact the forensic sciences community by providing a link between different perspectives in forensic psychology. Currently, the majority of professionals in this field have been working with psychometric tests or with qualitative tools. Additionally, this presentation will impact the forensic science community by showing that it is possible to link different perspectives and different tests in order to make a psychological report with a more substantial view on the subject focus of assessment, and therefore on the court itself. Reflections regarding guidelines for the field of forensic psychology and future research will also be part of the discussion.

Psychological assessment in the forensic context is one of the most-used tools by professionals in the criminal justice system when there is a need to understand the personality and psychological behavior of victims and defendants. In addition to understanding these behaviors, it is also important, and expected, that the evaluators assess for the possibility of deception. This has led research to focus on the detection deception behaviors within the criminal justice system.

Research in this field has been taking a more individualistic rather than integrative approach; thus, the scope of the research that has been conducted so far is rather simplistic.

This presentation challenges this approach of an individualistic perspective. Psychological reports (n=461), together with the tests used (Minnesota Multiphasic Personality Inventory (MMPI) short version, Symptom Checklist-90-Revised (SCL-90-R), Wechsler Adult Intelligence Scale-Revised (WAIS-R), and Rorschach], were analyzed. These were performed at the Portuguese National Institute of Legal Medicine and Forensic Sciences.

Results identify certain personality traits that were more highly associated with the attempt to deceive during the psychological assessment using personality questionnaires; however, other qualitative tests (e.g., Rorschach) were also performed in order to understand the psychic function of each subject.

The conjunction of these two types of data can provide wider information of the subject's personality and can support an analysis of deceptive behavior. It is also important to take into consideration that the final user of a report, such as a psychological assessment, within the criminal justice system is the court (judges, jurors, prosecutors, and lawyers). Therefore, it is believed that this type of mixed methodology will provide a greater amount of information about subjects instead of only a simple view of some personality traits suggested by psychometric tests.

Finally, there will be reflection regarding possible synergies between different areas of interest related to psychology in an attempt to achieve a greater understanding of the phenomenon of detection deception within the criminal justice system.

Forensic Psychology, Detection Deception, Psychological Assessment

I27 Dress to Impress: A Comprehensive Examination of How Physical and Behavioral Characteristics of Expert Witnesses Can Influence the Trier-of-Fact

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After attending this presentation, attendees will gain new insights into how the trier-of-fact (judge or jury) is influenced by physical and behavioral characteristics displayed by the expert witness. The ability to garner favor and influence others is not based simply on one's expert knowledge. This presentation compiles previous research into a single reference guide for expert witness preparation.

This presentation will impact the forensic science community by providing the expert witness with suggestions on how specific characteristics can be applied to convey one's opinion in a more credible and convincing way.

Expert witnesses in all areas are selected carefully for their level of knowledge, experience, and reputations. It may be perceived that the expert's knowledge of the subject matter is enough to impart credibility; however, expert opinions often require combining objective material with subjective interpretations to arrive at conclusions. As such, forensic psychiatrists serving as expert witnesses must not only possess a high degree of complex medical knowledge, but also the ability to convey their subjective conclusions convincingly. Therefore, knowledge alone is rarely enough to succeed in having the trier-of-fact accept the expert's option.

Research has been conducted on the varying factors that impact perceptions of expert witnesses. This research highlights four key categories of influence: knowledge, confidence, trustworthiness, and likability. As physicians' educational and training experiences are not focused specifically on all of these categories, many expert witnesses in the medical field may find themselves unprepared or unaware of their ability to maximize their courtroom influence. Fortunately, through simple adjustments to the physical and behavioral characteristics of the physician, these categories can be modified.

Most expert witnesses have a basic understanding that some factors, like professional attire and demeanor, can be used to gain favorability, however, some research has gone a step beyond general notions to look at specific characteristics, such as eye contact, posture, speed of speech, specific clothing colors, language, confidence level, and likability, among many others. Many research findings, in fact, may go against what might be commonly believed. For example, the physician may believe that complex language and medical jargon impart intelligence; whereas, in reality, it may be more confusing to the judge and jury. Similarly, the expert witness may use pronouns such as "I" to convey his or her specific expertise; whereas, inclusive terms such as "we" and "us" make the witness more relatable. The ability to ensure that the judge and jury are willing to listen and able to understand the expert witness's opinion is as important as the opinion itself.

This presentation compiles, categorizes, and analyzes previous research into a single reference guide, or checklist, for expert witness preparation. Attendees will learn to gain advantages by incorporating characteristics that can influence the trier-of-fact. In this way, the forensic psychiatrist will be better prepared to meet the specific challenge of conveying complex subjective conclusions in a relatable, confident, and convincing way.

Expert Witness, Perception, Credibility

I28 Killed to Be Saved … A Rare Case of Salvific Matricide From Demoniac Possession

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After attending this presentation, attendees will understand the importance of the existential narrative that underlies the relationship between the perpetrator and the victim, as a means of understanding how rare criminal events can occur.

This presentation will impact the forensic science community by serving as an example of how the individual life path is influenced by an infinite number of more or less pathological relationships.

The "demoniac" theme has not disappeared from the cultural representations of the sacred, the spiritual, the religious, and are understood as attempts to draw causal connections in order to defend man from the unknown, which has always inspired terror. It seems like a paradox, but the more rational society becomes, the more humans seem to need to tap into the universe of the irrational, the sacred, and the supernatural. If this complex cultural and anthropological horizon is combined with psychiatric disease, the scene is set and the risk factors for becoming perpetrators or victims of crime will increase.

This presentation introduces the case of a matricide by a 60-year-old woman, a graduate in psychology with a family history of psychiatric disease and a complex sentimental relationship. The woman was diagnosed with bipolar affective disorder with maniacal symptoms, drug abuse, and had undergone two previous obligatory health treatments. She then began to adopt spiritual therapy, recommended to her by a friend, and suspended traditional drug therapy, using cannabis and alternative therapies. In May 2015, she went to her mother's home for a "therapeutic vocation" (to look after her mother), bringing with her a medicinal syrup recommended by the spiritual therapist for her mother, who suffered from respiratory disease. At the mother's home, she suffered an episode of acute psychotic decompensation in which she became convinced that an evil spell had been cast over the home. This became a fixed idea, and she suffered mood changes and pathological bursts of excitement. On the morning of the homicide, convinced that there were objects evoking negative presences, she created havoc in the home, believing that the devil was concealed inside her mother's throat (because of her respiratory problems). In the throes of an agitated mystical frenzy, she placed a holy card and a rosary into her mother's throat. A second holy card was later found crumpled in her mother's mouth, imprinted with the words, "Satan leave this house, leave my mother and my sister." At the coroner's autopsy, numerous excoriations were seen on the face (due to the violence of the aggression), evident hematomas in the back of the mouth, particularly in the pharyngeal region, blood in the bronchi, as well as areas of pulmonary emphysema alternating with areas of atelectasia; these led the specialist to conclude that death was caused by asphyxia due to obstruction of the upper airways (internal asphyxia).

The choice of homicidal mode is particularly suggestive: suffocation with a "sacred" object, the holy card. The victim had a typical paradoxical psychotic profile; to chase away the demon, to extinguish and definitively silence the demoniac spirit voice, and to save her from evil, the mother was killed with the use of a sacred object. There are an infinite number of more or less "pathological relationships" around us that affect choices, life paths, and chances of fulfilment. Some, definitely abnormal, can certainly have a stronger effect on the life story of an individual; however, crime (and matricide is no exception) is potentially the result of many factors.[1] Identifying the cause as mental disease is not enough; only the analysis of the entire relationship between the perpetrator and the victim can contribute to discerning the background leading to such remarkable, shocking events.[2,3]

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Matricide, Devil, Asphyxia

I29 Family Secrets: When the Horror in the House Has No Obvious Cause and the Need for Unveiling

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After attending this presentation, attendees will understand the importance of both the criminological and anthropological investigation in understanding a crime associated with forensic investigations and forensic psychiatric and psychological investigations.

This presentation will impact the forensic science community by serving as an example of the importance of reconstructing the life experiences of the perpetrator and the victim, in order to unveil the pathway leading to a particularly complex murder.

Particularly violent and brutal homicides, due to their mode of expression, sometimes present a contradictory and paradoxical scenario, complicating the forensic, psychiatric, and psychological forensic reconstructions.[1]

The case is that of a 67-year-old man, a typographer, married with children, whose matrimonial life was apparently marked by quiet harmony until members of the wife's family (sister and mother) intervened in the relationship. After that, the man said his wife had become authoritarian and aggressive, deliberately humiliating him, and their relationship had deteriorated. On the day of the homicide, the woman had, as usual, adopted an authoritarian attitude and started to shout and push her husband. The husband, after having endured this humiliation for many years, suddenly and repeatedly struck her with an axe he was carrying in a bag, inflicting numerous blows that caused significant damage to her head. Immediately after, the man phoned for help.

The man's psychiatric history was negative, and there were no signs of cognitive impairment. For this destructive act directed against his wife, qualifying as abnormal on the psychopathological plane, relevant motivational elements emerged. There were no signs suggesting a temporary psychotic decompensation nor a criminal plan. The scenario depicted a murder of impulse rather than premeditated aggression, bearing in mind the impromptu weapon used (he had not even removed the axe from the bag). In short, it was not an idea that had long been brooded upon and finally implemented (as occurs in cases of delirium), but a deep underlying discomfort that finally erupted as a result of a quarrel (although this was denied), triggered by a contingent event (a verbal provocation) that brought on frustration, followed by an act of physical aggression (a shove) that had an explosive effect on the perpetrator of the murder. From the medical-legal consultation, it emerged that the man had continued relentlessly striking the woman even after she had fallen to the ground, denoting not only that his emotions were completely out of control, but also his wish to "destroy" the victim. The homicidal behavior appeared disorganized, whereas immediately after the murder, he reacted in a usual way, calling for help.

Faced with such a terrible scene, limited information was gained from the forensic psychiatric and psychological assessment conducted on the perpetrator. The man had no criminal record and did not use drugs or alcohol. This elderly person, retired, leading an ordinary and peaceful life, was described by everyone (family, friends, coworkers, jail staff) as a kind person, very polite, and helpful. The only significant finding from information obtained from his children was that the victim had a difficult character, was rigid and complex, and often abused her husband, who had patiently endured it for 26 years. Despite the absence of signs of severe mental illness or important criminal motive, the cause of this murder conducted within the domestic walls of a normal family is to be traced to the relational and historical aspects of the family structure and of the subjects involved in the crime.[2]

The medicolegal, psychiatric, and forensic psychology assessments must include a criminological and relational anthropological analysis, with the goal of establishing a reason for committing such a terrible murder, through close study of the experiences of the subjects involved, and the context in which the murder was committed.[3]

Reference(s):

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Criminalistic, Crime on Impulse, Femicide

I30 The Phenomenon of Suicide by Hanging in Ferrara (1996-2016)

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After attending this presentation, attendees will better understand the evolution of suicide by hanging in Ferrara and the nearby countryside from January 1996 through July 2016.

This presentation will impact the forensic science community by reporting: (1) the evolution of suicidal hanging in Ferrara between January 1996 and July 2016; (2) the incidence of the classic signs of asphyxia; and, (3) the possible correlation between this type of acute violent mechanical asphyxia with individual variables, such as age, housing status, and employment status of the victim, as well as personal, social, and economic changes in the subject's life and the presence of ethanol in the body. The analysis of the seasonal rate of suicides by hanging will also be presented.

The information was extracted from the database of the Institute of Legal Medicine of Ferrara and includes gender, age, employment status of the victim, location of the hanging (city or countryside, closed or open spaces), position of the hanging mark (typical or atypical hanging), presence of the classic signs of asphyxia (petechial hemorrhages, congestion, and edema), signs of pressure on the neck (bruises in the neck muscles, injuries to the hyoid bone or to the larynx), Simon's bleeding, and Amussat's sign.

Between January 1996 and July 2016, 3,521 autopsies were performed at the Institute of Legal Medicine of Ferrara and 317 of these were cases of suicide by hanging. This represents the most frequently used suicide method (60% of suicides). The incidence is higher in males more than 50 years of age and there is a prevalence of unemployed subjects.

This study found an increase in suicides by hanging in the summer and spring and an increase in suicides has been seen in 2001, 2002, and 2013; 99% of suicides by hanging occurred at home or in a nearby business establishment. The incidence of suicides in the countryside is 60%, likely because the population in the countryside is higher than that in the city.

The unusual position of the hanging mark (atypical hanging) has been seen in 75% of the cases. Since only 5% of the cases received a medicolegal examination at the scene, in 95% of the suicides by hanging it was not possible to determine if there was total suspension. Petechial hemorrhages in the skin or conjunctivae and under thoracic serous membrane (such as the pleura or pericardium) were described in 55% of the cases and a transverse laceration of the intimal layer of carotid arteries (Amussat's sign) was described in 22% of the autopsies. Injuries in the neck muscles or injuries to the hyoid bone or to the larynx were identified in only 17% of the cases. Simon's bleeding is extremely rare, described in only 1% of internal examinations. Since 2011, this study has collected the toxicological analyses of 77 cases of suicide by hanging and, in 16 cases, ethanol was present in the body; in ten cases, the concentration was >1.0g/L.

This study demonstrates that hanging is the most common form of suicide in Ferrara. This finding agrees with many studies in different countries. Suicide by hanging is probably so common because the required materials are easily available and the method does not require complicated procedures. Many studies have shown a spike in the number of suicides in the spring and during holidays, while this study's analysis revealed an increase in suicide by hanging during the summer and spring.

This study emphasizes the importance of conducting histological investigations on tissues of the neck, because the classical macroscopic signs of hanging are inconsistent. More data would be helpful in further assessing the relationship between consumption of alcohol and attempting suicide by hanging.

Suicide, Suicidal Hanging, Asphyxia

I31 Epidemiological Study on Suicide Victims in Southern Italy and the Role of Psychoactive Substances

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After attending this presentation, attendees will understand the epidemiological impact of suicides in southern Italy. This presentation will also illustrate the correlation between peri-mortem use of psychoactive substances and suicide.

This presentation will impact the forensic science community by providing new information about suicide, in addition to that pre-existing in literature, which can lead to a new approach in the prevention of suicide. Greater comprehension of the mechanisms that lead to the choice of one method over another will likely help identify subjects with a higher risk of suicide.

Suicide is still one of the most important causes of death in the world. It is a public health issue that affects society and has a continuing ripple effect, dramatically affecting the lives of families, friends, and communities.

A World Health Organization (WHO) report reveals an annual global age-standardized suicide rate of 11.4 per 100,000 of the population (15.0 for males and 8.0 for females) and an estimated number of suicides in Italy (2000-2012) of approximately 3,908, with a prevalence of males over females.[1]

The characteristics and methods change considerably between different communities. Consequently, epidemiological data on suicides is an essential component of national and local suicide prevention efforts. Alcohol and drug use and abuse can substantially influence suicide rates.

With this in mind, suicide victims in Calabria in southern Italy, have been analyzed from 2006 to 2015. The data was limited to victims who received autopsies and various tests, as it was gathered from coroner/medical examiner reports, as well as toxicology and laboratory reports (blood, vitreous humor, and urine analysis). The total number of cases was 159. The cases were analyzed by gender, age, results of toxicological examination, and method of suicide.

Regarding gender, 84% of the suicide victims were males and 16% were females. Our data, according to the international literature, suggest that men, particularly older men, have a higher suicide rate than do women at any age. Females are less prone to suicide and their method of choice is falling from heights. On the other hand, the method of choice for males is either by hanging or guns.

From the analysis of the sample, it was found that the 53% were positive for alcohol and/or drugs. Of these, the majority were men (>90%). Among the males who committed suicide, 39 subjects were positive for alcohol (Blood Alcohol Concentration (BAC) >0.2g/l, 51%) and 10 subjects were positive for polydrug use (13%). Only five females had a BAC above 0.2g/l. No women were positive for the combined use of alcohol and drugs. In the entire sample, the BAC ranged from 0.20g/l to 4.85g/l. The majority of victims who were positive on toxicological investigation were between 30 and 49 years of age.

This study revealed that the combined use of alcohol and drugs can be, especially in males, a risk factor for the choice of the most violent method of suicide (e.g., self-cut throats, rail suicides) as this can lead to an increase in risk taking.

In conclusion, this study emphasizes the importance of postmortem examination and, most of all, the continued reporting of toxicologic findings in suicides. It is useful to monitor patterns of psychoactive substance use as this can help guide suicide prevention in clinical practice and public health policies. Finally, the importance of postmortem "psychological autopsy" studies are stressed to ascertain if the victims were alcohol and/or drugs abusers.

Suicide prevention efforts require coordination and collaboration among multiple sectors of society, including both health and non-health sectors (such as education, justice, law, and the media).

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Suicide, Psychoactive Substances, Prevention

I32 Guidelines for the Evaluation of Sex Offenders and People With Problematic Sexual Interests

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The goals of this presentation are to provide: (1) a history and foundation of jurisprudence governing the evaluation and management of individuals who commit sex crimes; (2) Canadian and American approaches to providing practical, innovative, and effective strategies for the assessment of people with problematic sexual interests and individuals who commit sex crimes; and, (3) an overview of successful treatment strategies used among people with problematic sexual interests and individuals who commit sex crimes.

This presentation will impact the forensic science community by providing education regarding paraphilic interests and sexual offending behaviors from a forensic psychiatry perspective within the context of current legal mandates and oversight. The forensic science community will learn about: (1) the history and foundation of jurisprudence as it applies to the psycho-legal evaluation and management of sex offenders; (2) components of sexual offender assessments conducted in Canadian and American evaluations; (3) innovative techniques used in the assessment of problematic sexual interests; and, (4) the varying approaches to the treatment of problematic interests and evidence that treatment of paraphilias does work.

Social responses to managing individuals who have committed a sexual offense often seek a balance of punishment for the act while also wanting treatment to prevent future offending. A combination of punishment, prevention, and community protection are at the core of criminal justice responses to sexual offending. The history and foundation of jurisprudence governing the evaluation and management of individuals who commit sex crimes will be discussed. This will provide attendees with an understanding of the evolution of public safety laws within the United States and the role of forensic psychiatry in risk determination and prevention of recidivism.

An overview of Canadian and American perspectives on current methods used to assess and treat individuals who commit sexual offenses and people with problematic sexual interests will be provided. First, an American perspective on the assessment protocol for the evaluation of sex offending behavior will be provided. The use of a tripartite approach with the components being clinical review, psychological/psychometric instruments, and physiological assessments will serve as the framework. The physiological element includes Penile Plethysmography (PPG), visual reaction-time measurement, and polygraphy. This will also include a discussion of the development and testing of innovative phallometric stimuli utilizing age- and gender-congruent voices.

The Canadian perspective of the assessment protocol for sex offenders will then be explored. A general overview of the routine and new innovative assessment approaches that are currently being used in the laboratory will be provided. Attendees will be provided with an overview of the Sexual Behaviours Clinic (SBC) of The Royal in Ottawa, Canada. Topics discussed include: referral sources, patient characteristics, objective measures of sexual arousal, as well as psychological measures that are currently being used in the laboratory. New and innovative clinical research projects will also be reviewed, including alternate and complimentary methods for the objective assessment of sexual arousal. These include functional Magnetic Resonance Imaging (fMRI) testing, eye-tracking, visual reaction time, Electroencephalograph (EEG), and objective assessment of female sexual arousal in forensic populations. Improvement upon PPG assessments with the use of novel stimuli sets will also be discussed.

In 2015, The Royal's SBC was awarded the American Psychiatric Association's (APA) prestigious "Gold Award" for a clinical research program of academic excellence. The SBC was unanimously selected from all the psychiatric programs in North America. In its newsletter, the APA noted that "the SBC's innovations have made it a model for treatment of sex disorders worldwide."[1] The successful treatment strategies used within the SBC will be discussed, including group therapy, individual sessions, and pharmacological treatments. Case examples and evidence illustrating that paraphilic disorders can be successfully treated will also be presented.

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Sex Offender, Paraphilia, Evaluation

I33 Motivations to Offend: Hands-On vs. Hands-Off Sex Offenders

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The goals of this presentation are to: (1) identify the motivations behind child molestation and child pornography offenses and to demonstrate that sexual gratification is not the sole motivator for these crimes; (2) compare the motivations of child molesters and child pornography offenders to establish that sex offenders are a heterogeneous group and thus require individualized treatment interventions; and, (3) provide greater insight into the motivations behind child molestation and child pornography offenses, in order to improve risk assessments and design effective treatment strategies for sex offenders.

This presentation will impact the forensic science community by demonstrating that sexual gratification is not always the sole, or even primary, motivation for committing sex offenses against children. In order to effectively treat sex offenders, treatment providers must correctly identify and address the factors that led to the sexual offense. Thus, understanding the subjective motivations behind sexual offenses is necessary, as it is difficult to determine effective individualized treatment strategies for different sex offenders if unaware of their reasons for committing the offense.

Due to the severe and lasting harm caused by sexual offenses against children, treatment programs for sex offenders must be as effective as possible. Consequently, cutting-edge research continues to be required in order to better inform treatment programs and increase treatment efficacy. Since sex offenders are a heterogeneous population, it is essential that treatment providers have a thorough understanding of the many possible reasons for committing sexual offenses. Research has found that the most effective method for reducing sexual recidivism is recognizing and understanding the motivations behind offending.[1] This is logical, since it is unlikely that treatment providers will be able to provide the most effective treatments for sex offenders if the root causes of their behaviors are unknown. For example, a sex offender motivated by pedophilic sexual interests will require different treatment interventions than a non-pedophilic sex offender. It is also important to be aware that child molesters and child pornography offenders can have significantly different motivations for offending and are, therefore, likely to have different risk factors and require different treatments.

To date, most attempts to understand the behaviors of sex offenders have been from an external perspective, where the objective "facts" are considered more meaningful than the offender's subjectively experienced realities; however, attempting to understand the offender's subjective motivations for committing the sexual offense(s) is crucial, as it has been pointed out that "behavior is the product of one's own sense of reality regardless of the degree to which that reality matches the objective facts of that person's life."[2] Determining the subjective motivations of sex offenders requires the use of qualitative methods that permit investigation of factors that may fall outside quantitative frameworks. Thus, the current studies involved a radical shift away from simply describing the characteristics of sex offenders or quantifying what sex offenders do, toward research that will help to identify and explain the subjective experiences behind child molestation and child pornography offenses.

Two studies were conducted using semi-structured interviews with males who were charged with a hands-on sexual offense against a child (study one) or with accessing, possessing, and/or distributing child pornography (study two). These studies employed a novel perspective, focusing on the "why," as opposed to "what" or "how," to explore the motivations that lead men to commit sexual offenses against children. Interviews were analyzed using Interpretative Phenomenological Analysis (IPA), as phenomenology facilitates an understanding of how individuals perceive the world around them and make sense of their lived experiences (a concept often ignored in sex offender research). Child molesters and child pornography offenders were found to share some motivations for offending, such as sexual gratification and difficulties forming or maintaining relationships. Motivations reported exclusively by child molesters included: (1) non-sexual forms of gratification (e.g., seeking intimacy); and, (2) childhood experiences (e.g., personal abuse). Motivations reported exclusively by child pornography offenders included:

(1) different pathways to offending (e.g., development of pedophilic interests); and, (2) mitigating factors (e.g., failure to see harm). The results of this research have the potential to improve the efficacy of risk assessments and treatment strategies for both child molesters and child pornography offenders by understanding and addressing their subjective motivations for offending.

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Sex offenders, Motivations, Treatment

I34 False Accusation of Sexual Abuse Case Study — Psychology of the Lie

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The goal of this presentation is to examine the psychological aspects of fabricated testimony through an unusual case study. The findings derived from this case will be shared with professionals in criminal law, forensic psychiatric medicine, and other behavioral sciences in an effort to provide a more complete understanding of the deleterious effects of false accusations of sexual abuse.

This presentation will impact the forensic science community by challenging forensic psychiatrists and attorneys to analyze their cases with greater sensitivity to the possibility of fabricated testimony and its psychological genesis.

There are several well-known patterns of false accusation of sexual assault. Divorce litigants accuse their estranged spouse in order to gain advantage in either child custody or money settlements. False complainants target wealthy individuals in order to file civil lawsuits against them, again for their money. The present case is unusual because these commonly encountered financial motives were completely absent.

The complainant was 18 years old and living at her parents' home after graduation from high school. One day, she appeared at a sheriff's department substation accompanied by two friends. One of the friends advised that the complainant had been sexually abused by her father; the police received several pages of Facebook® messages in which the complainant described being raped. The messages described unprotected vaginal, oral, and anal penetrations that included ejaculation. This was to have taken place in the family home on a daily basis over a period of one and one-half years.

Interviewed using standardized child abuse investigative techniques, the siblings denied that any abuse or improper touching had occurred. At the home, only one suspected biological stain was located. Collected from an old mattress in the basement, the stain tested negative for seminal fluid.

The prosecution proceeded to preliminary examination. The complainant's testimony was contradictory, but the matter was bound over for trial on several counts of sexual assault. The defense investigation then focused on the details contained in the text messages.

The complainant described being forced to take diazepam and acetaminophen/hydrocodone. Her descriptions of the effects of these drugs included violent seizures and colorful hallucinations. A toxicologist and neuropsychopharmacologist concluded the complainant's descriptions were inconsistent with the known effects of these drugs.

A recurring theme was that the complainant's father would drag her to the basement, lock her in, and sexually assault her. She described being locked in the basement all night. Examination by the defense crime scene investigator revealed that it was impossible to lock a person in the basement. There was no lock, latch, or hook of any type on the door. There was also another door, which led to the yard outside. Again, there was no method by which the complainant could have been locked in.

Reports from the defense experts were furnished to the prosecution. The prosecutor met with the complainant and discussed the findings. The complainant admitted to fabricating the stories and all charges were dismissed. Thanks to the diligence of the prosecutor, a major travesty was avoided. Had the defendant gone to trial, he almost certainly would have been convicted. Notwithstanding that her stories were pure invention, she would have presented as a credible witness.

The psychological question presented in this case is how and why the complainant came to invent the stories of sexual abuse. Another question is why she persisted in her fiction, going so far as to baldly perjure herself at the preliminary examination.

From the extensive emails, a quite detailed psychological portrait emerged. A toxic relationship with a manipulative girlfriend, resentment over her strict religious upbringing, and preoccupation with a series of young adult fiction books about an underground prison had all contributed to the fabricated story.

In summary, the findings derived in this case will be shared with professionals in criminal law, in forensic psychiatric medicine and other behavioral sciences in an effort to provide a more complete understanding of the deleterious effects of false accusations of sexual abuse.

Forensic Psychiatry, Sexual Abuse Allegation, Fabricated Testimony

I35 The Psychology of Drug-Facilitated Sexual Assault: A Case Series From Victoria, Australia

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After attending this presentation, attendees will develop a better understanding of not only the prevalence of drug-facilitated sexual assault, but also victim typology, including an understanding of the role of premorbid mental health conditions and drug and alcohol use.

This presentation will impact the forensic scientific community by widening the understanding of risk factors and contextual factors that may contribute to drug-facilitated sexual assault in the hope that this can inform future prevention strategies.

Despite public perception and media representations that covert "drink spiking" is the primary risk factor for Drug-Facilitated Sexual Assault (DFSA), research suggests that self-administered alcohol and substance use represent a greater risk; however, what remains unclear is the impact of certain combinations of bio-psycho-social factors, such as mental health, age, and socio-economic status, on the development of harmful drug- and alcohol-use behaviors that may increase an individual's vulnerability to DFSA.

Clinical files from the Victorian Institute of Forensic Medicine were reviewed for all cases of alleged DFSA over a three-year period from January 2011 to December 2013 in order to identify any patterns in contextual and victim specific factors associated with different types of DFSA.

A total of 204 suspected DFSA cases occurring over that period that included a forensic examination of the victim and collection and analysis of a toxicological sample were retrospectively reviewed for this study. Nearly all victims (n=190) were female, with a median age of 26 years (range=18-54 years of age). All assailants were male, and 34.4% were considered acquaintances of the victim, while 24.5% were strangers. Assaults typically occurred in private residences (48.1%), and all victims self-reported some form of drug or alcohol consumption prior to the assault occurring. Additionally, while half the victims reported no significant medical or mental health history, 27.9% of the victims reported at least one pre-existing significant mental health diagnosis, an additional 10.3% of the victims reported a pre-existing significant mental health condition and the presence of a physical ailment, and 12.3% reported a pre-existing physical ailment in the absence of mental health issues.

The prevalence of pre-existing mental health conditions in the victims of DFSA was significantly greater than prevalence rates represented in the general population. Given that alcohol and drug use are common maladaptive coping behaviors in individuals with poorly managed mood conditions, these mental health conditions may have increased the victims' likelihood of engaging in risky drug and alcohol use, thus increasing their vulnerability to DFSA.

Sexual Assault, Drink Spiking, Psychology

I36 Enhancing Communication Bolsters Quality and Ethics

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After attending this presentation, attendees will better understand one another's technical terminology and thereby be able to more effectively evaluate the conclusions from forensic work as it continues to come under public scrutiny from origins that have significantly variable validity.

This presentation will impact the forensic science community by enabling attendees to respond to fundamental public criticisms of some of their methods and techniques. Attendees will leave better equipped to assess critical ethical issues arising from recent powerful technical advances, which have the potential to affect the standing of both individuals and organizations within the forensic sciences.

Across their disciplines forensic scientists continue to experience political and professional scrutiny of the validity and reliability of their work. A closely related issue of current importance is an ongoing need for due vigilance regarding the appearance and the reality of professional ethics in the practices of forensic scientists. Recent and current responses on the part of the federal executive branch, as well as the general public to the National Academy of Sciences Report of 2009, seem to indicate that this trend of increasing scrutiny of expert forensic scientists is likely to accelerate. The purpose of this presentation is to promote the value of expanding and strengthening a shared language that will facilitate communication both among forensic scientists and between them and concerned lay onlookers. Enhancing clarity promotes understanding, which in turn serves justice.

Genetics is among the areas in most urgent need of sharing its vocabulary. It is rapidly advancing in its own right as well as in its applications to several of the forensic disciplines. In particular, a great deal is being discovered about both natural and artificial applications of epigenetics, the study of the regulation of genes by means of chemical changes. Diagnostic applications are coming to light, such as an apparent association of addiction with alterations in genetic regulation. There are also apparent correlates with exposure to adverse environmental influences. The same appears to hold for the quality of one's relationships. Another concept valuable for forensic experts to understand is that of Single Nucleotide Polymorphisms (SNPs). These are involved in DNA phenotyping, which can be used to make comparisons among populations instead of individuals. In addition, recent technical advances are greatly simplifying the alteration or "editing" of the very genes themselves *in vivo.* Yet another rapidly emerging development is the genetic study of microbiomes, referring to all the unique microorganisms that populate various bodily surfaces. The techniques being developed have the potential to rule a suspected connection in or out.

The topics discussed here are at relatively early stages of development. As such, they have more to do with investigating crimes than with definitively solving them; with excluding suspects rather than identifying them. They are likely as they progress and mature to give rise to ethics issues and challenges. Finally, the diversity initiative now underway within the American Academy of Forensic Sciences (AAFS) has the potential to engage the means for promoting an enhanced level and quantity of collaboration.

Epigenetics, SNPs, Microbiome

I37 The Dangerous Patient: Is It Ever Ethical Not to Give Informed Consent?

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After attending this presentation, attendees will: (1) understand what informed consent is and why it is important; (2) understand what ethical principles are related to informed consent; (3) understand the ethical model of dialectical principlism and how it can resolve dilemmas; and, (4), appreciate and understand when informed consent may cause significant harm.

This presentation will impact the forensic science community by showcasing an ethical model that can be used in various forensic settings to help the practitioner in resolving complex dilemmas.

The relevance of informed consent may be overlooked within the practice of psychotherapy. Although informed consent is generally thought of in regard to high-risk procedures such as surgeries, it is relevant to any situation in which the patient faces a medical treatment decision. This is true in health care decisions in which the risks or consequences of a treatment may not be as readily recognized. One such area especially important to consider related to patient autonomy in the psychotherapeutic setting is whether to give a patient fully informed consent as it relates to confidentiality and, specifically, the limits of confidentiality.

It could be argued that patients always have a right and ought to know at the outset of treatment the limits of confidentiality. This would inform them as to how their words and the information provided could be shared and, in some instances, used to infringe on their personal freedoms or liberties, such as involuntary hospitalization, gun prohibition, legal and professional consequences, etc. Yet, not giving full informed consent in certain situations may be the most protective action of the patient and/or third parties, thus trumping patient autonomy considerations.

Such scenarios include when patients may be considering dangerous actions, either to themselves or others. Ordinarily, practitioners may want to alert patients to the limits of confidentiality in any setting and respect their autonomy; but, when patients and others' safety is concerned, it can sometimes be of such importance to obtain the information that it warrants not warning patients in ways that might discourage them from sharing the dangerous actions that they are contemplating.

Such situations are complex in that they pit various ethical principles, such as beneficence, non-maleficence, and autonomy, against each other. Dialectical principlism is an ethical model designed to assist in analyzing and resolving such dilemmas. Dialectical principlism operates by laying out all the principles at play, prioritizing and weighing with special consideration of primary versus secondary duties of the particular role, balancing these principles against one another given the specific context, in order to determine how one may act most ethically. This model allows for variability in that not everyone will come to the same conclusion utilizing it.

The psychiatrist is faced with a serious challenge of whether or not to give full informed consent to a patient when there is suspicion that he or she is a danger to either one's self or others. Dialectical principlism provides a framework that the psychiatrist can use to be more equipped to make an informed determination of how to act that is consistent with his or her unique set of values and what is most ethical for the individual.

Ethics, Danger, Informed Consent

I38 The Capacity to Consent to Treatment in Patients With Alzheimer's Disease

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After attending this presentation, attendees will be able to assess the capacity of patients affected by Alzheimer's disease to consent to treatment.

This presentation will impact the forensic science community by providing a framework for assisting patients with Alzheimer's disease to understand their proposed medical treatments.

Informed consent is an essential element in the doctor-patient relationship. In particular, obtaining valid informed consent from patients with neurocognitive diseases is presently a subject undergoing intense study and scrutiny. For this reason, it was decided to look at the factors associated with informed consent in elderly patients with Alzheimer's disease, as defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-V*).

Methods: The experimental group was composed of 70 elderly patients in the Neurodegenerative Disease Unit of the Policlinico of the University of Bari. The control group consisted of approximately 80 elderly patients in the Geriatric Hypertension Clinic Unit at the Policlinico of the University of Bari without clinically relevant neurocognitive problems. The inclusion criteria included: outpatient status, primary school education level at a minimum, conversant in Italian, informed consent, and participation in the study.

The subjects were recruited from the previously mentioned facilities from the outpatient and day hospital programs. Subjects who were hospitalized were excluded.

After acquiring written consent to participate in the research, each participant was subject to ongoing evaluations, as follows: (1) assessment of comprehension sheet; (2) Neuropsychiatric Inventory (NPI) and Assessment Overall Functioning (VGF); (3) neurological evaluation, (4) neuropsychological assessment (mini mental state examination, Frontal Assessment Battery (FAB), Rey Auditory Verbal Learning Test™, token test, verbal fluency test, Boston Naming Test, Stroop Test, Poppelreuter Test, Clock Drawing Test); and, (5) MacArthur Competence Assessment Tool for Treatment (MacCAT-T), a semi-structured interview which explores four dimensions of patients' mental capacities (i.e., understanding (subscale ranges from 0 to 6), appreciating (subscale ranges from 0 to 4), reasoning (subscale ranges from 0 to 8), and expressing a choice (subscale ranges from 0 to 2)). The MacCAT-T does not provide a total score or a cut-off to define patient's mental capacity. The gold standard is an expert's opinion and the semi-structured interview with the caregiver (Consent to Treatment Interview (CTI)).

Results/Conclusions: Many of the patients in this sample did not seem to have the proper cognitive competence to provide valid consent. The present survey intends to analyze the possible qualitative and quantitative correlations between cognitive functioning and the capacity to provide valid consent with respect to the severity of Alzheimer's disease. The results of this study are presented.

Capacity to Consent, Treatment, Alzheimer's Disease

I39 Consent for the Mental Health Treatment of Minors: Who Holds This Decision-Making Authority?

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After attending this presentation, attendees will better understand the different parties who have the legal authority to provide consent for the mental health treatment of minors. While parents or guardians are usually the individuals who grant consent to the mental health treatment for the minor, there are instances when this is not the case. Moreover, a single parent's consent is not always sufficient for proceeding with treatment (i.e., in cases of joint legal custody).

This presentation will impact the forensic science community by raising awareness of the various ethical, legal, and clinical considerations that may occur depending on who provides consent for the minor's mental health treatment. An examination of the laws in several jurisdictions, as well as the ethical guidelines for mental health professionals, will be discussed in relation to the treatment of minors. Additionally, a decision-making model for practitioners to use in deciding whether to treat a minor with or without their parents' or guardians' legal consent will be presented.

When a minor receives mental health services, he/she is usually accompanied to the appointments by his/her parent(s). In fact, in the majority of states, it is generally understood that a minor's parents will be informed of and provide the consent for the child's mental health treatment; however, practitioners may encounter situations in which a parent's legal authority to give consent, or even know that their child is in mental health treatment, is not required, as it is not in the best interest of the child. In addition, there are limited circumstances in which a minor has the legal authority to consent to their own mental health treatment. All of these circumstances will be explored nationally so as to identify the commonalities and the differences in the specific legal criteria for consent among the states.

Knowing who has the authority to give consent, as well as the exceptions to the rule, is critical for the mental health practitioner. The exceptions in which a minor can give consent clearly addresses issues that are out of the ordinary, with particular emphasis on acknowledging that doing so is in the child's best interests. The risk of facing malpractice liability problems, in addition to licensing board complaints, can be a serious issue when treating minors, most notably for those who are unaware of the laws of their state and their professional practice guidelines. Yet, these laws and guidelines are often vague. This presentation is intended to educate the provider regarding situations in which there is a legal exception to obtaining the parents' or guardians' consent. Moreover, case examples illustrating when consent from the minor only is appropriate will be highlighted, as well as suggestions on how to document this in the minor's mental health record.

Minors, Consent, Mental Health Treatment

I40 Glad Tidings About Not Guilty by Reason of Insanity (NGRI): The Benefits of Conditional Release

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After attending this presentation, attendees will be able to: (1) identify which factors are associated with failed or successful Conditional Release (CR); (2) understand procedural components of CR; (3) identify those factors that evaluators give greatest weight in determining readiness for CR; and, (4) identify systemic areas wherein CR procedures can be improved.

This presentation will impact the forensic science community by increasing understanding of procedural changes for conditional release as well as the implications for improved public policy concerning NGRI dispositions in order to optimize the balance of benefits and risks of extended hospitalization versus early release. Those who are involved in making CR decisions and managing NGRI acquittees should find potential areas to improve practices.

The insanity defense is said to be unpopular among defense and prosecuting attorneys alike as well as the general public. When John Hinckley, who attempted to assassinate President Reagan, was found not guilty because of insanity, the oft-repeated refrain was that he was allowed to go "scot-free." There has long been wide-spread concern that an offender who is hospitalized following an NGRI verdict will be released prematurely and commit a horrific crime. Not well appreciated is the fact that improved treatment and management and, in particular, the use of CR has resulted in rates of recidivism for NGRI offenders that are lower than those for convicted criminals after their release from prison.[1] This presentation will explain why CR is effective in reducing the risk of recidivism. Attention will focus on how CR works, what methods are used, which factors are emphasized, and how practitioners define success and failure.[2-5] Empirical data will be presented to illustrate which factors evaluators use in CR decision-making as well as which factors are most effective in managing the CR population.[3] Finally, a brief history of the Psychiatric Security Review Board (PSRB) and CR in Oregon will be explained with data on numbers of individuals released conditionally over the years.[6-8] The effects of recent changes in the law on CR will be discussed.

The idea behind CR is the return of insanity acquittees to their respective communities. To that end, risk assessment evaluations are often utilized to prognosticate the likelihood an insanity acquittee will be able to meet expectations of CR.[1] Yet, risk assessments with insanity acquittees have not undergone vigorous evaluation of their predictive power. This presentation focuses on both strengths and weaknesses of current risk assessment methodologies by evaluating two samples of forensic patients eligible for or on CR. Results from both samples indicate many items ostensibly associated with risk did not predict return from CR or any type of recidivism. The results from this current research call into question the necessity of relying on historical factors of risk assessment, and instead point the clinician to focus on dynamic factors of risk when evaluating insanity acquittees.[2,4]

This presentation will include commentary on the rationale for creation of the PSRB in 1977 by the Oregon state legislature to manage and treat insanity acquittees both in and out of the hospital.[6] Recent data regarding numbers of individuals on CR in Oregon will be reviewed with a particular focus on various living arrangements and how people move between these various living arrangements and the hospital.[7,8] Living arrangements range from highly structured facilities to independent living.[8] The concept of trans-institutionalization in the context of inpatient psychiatry bed reduction strategies will be touched on briefly.[7]

A large number of individuals are acquitted of criminal charges after being found not guilty by reason of insanity; most of these individuals are hospitalized and later seek hospital discharge under the court-ordered provision called CR. Courts rely on opinions from forensic evaluators to determine acquitees' readiness for CR; however, how evaluators make these decisions are unknown.[9-14] This study surveyed 89 CR-readiness evaluators from nine states to understand which factors evaluators prioritize and to understand evaluators' assessment methodologies and their beliefs about the CR process itself. Little uniformity was found among evaluators on any aspect of the decision-making process. Evaluators utilized a wide variety of methodologies when making their decisions on readiness

for CR. Moreover, evaluators' conceptualizations of the CR process itself varied widely. The results highlight the difficulty and confusion evaluators face when conducting CR-readiness evaluations and demonstrate the need for enhanced training, statutory guidance, and standardized evaluation protocols for these evaluations.

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Conditional Release, Insanity Defense, Forensic Security Hospitals

I41 Legal Substance to Become Psychotic?

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The goal of this presentation is to inform attendees regarding the risk of various legal substances. This presentation will describe how people can be clever in avoiding testing positive on a urine test while using various psychoactive substances. Professionals should be aware of the risk of the internet and of the substances when traveling.

This presentation will impact the forensic science community by informing attendees of the risk of new psychoactive substances that are currently on the market.

Psychoactive substances may have a negative impact on people suffering from psychosis. Many substances are well known to be the cause of psychotic relapses, including Tetrahydrocannabinol (THC), cocaine, and crystal methamphetamine, but new products enter the market, such as a synthetic cannabinoid a few years ago, and are often not to detect and, therefore, prohibit.

In the Canadian Review Board system, when an individual is found not criminally responsible for a crime and they are known for using illicit drugs, the Review Board is likely to prohibit them from using any illegal substances, unless this is justified for medical reasons; however, this becomes complicated when individuals use legal substances for their psychoactive properties. Indeed, although they may be prohibited from using any illicit drug, they are allowed to use any substances that are legal in Canada. The impact of these legal psychoactive substances, which can be caffeine or taurine, is unpredictable and depends on the brain's sensitivity.

This presentation reports the case of a 28-year-old man who was previously found not criminally responsible due to a mental disorder. While in the community, this patient looked on the internet to find some substance that would enhance his motivation, concentration, and calmness. As part of his legal disposition, the use of any illicit substance was prohibited; therefore, he sought legal ones, which is how he found kratom. A few hours after using this substance, he became acutely psychotic, with the main symptom being thought disorganization. The decompensation episode lasted for three weeks.

Kratom, whose scientific name is *Mitragyna speciosa,* derives from southeast Asia. Kratom is an opioid receptor agonist and has thus been used in managing chronic pain and opioid withdrawal symptoms. Kratom has been manufactured as a tea and sold from different websites because of its different effects, such as energizing or relaxing the person. Kratom is illegal in many countries and legal or under-controlled in some, such as Canada. Kratom appears to pose a public health risk.

This presentation seeks to describe kratom as a psychoactive substance, in addition to its impact on enhancing psychotic symptoms. This presentation will also address the discrepancy in the legal decisions regarding psychoactive substances among countries.

Legal Substances, Kratom, Morphinic

I42 Using Research Methods and Theory to Solve Crime: A Case Study

Ronald R. Thrasher, PhD, PO Box 2662, Stillwater, OK 74076*

After attending this presentation, attendees will better understand applying social and behavioral theory and research methodology to criminal investigations.

This presentation will impact the forensic science community by providing, in a case study format, an example of how an established social psychological theory and a common research methodology were used to identify an otherwise unknown suspect in a violent rape and murder of a university student case investigated in Oklahoma. This presentation will illustrate how reconsidering behavioral theory from an applied perspective, then employing established research methodologies, can develop innovative investigative techniques for criminal investigations. This presentation should interest academicians in psychology, sociology, criminal justice, political science, and forensics as well as practitioners in criminal/civil investigations, victim services, and the law.

This presentation begins with a brief description of an off-campus rape and murder of an international university student. A victimization profile will be presented. The profile emerges from in-depth interviews with friends of the victim and includes a description of the victim, her social group and setting, and common practices and behaviors. The offense description emphasizes both the physical and behavioral evidence present at the crime scene and how this evidence was used to create a behavioral assessment of the individual responsible for the offense.

This presentation continues with a brief description of the theory of symbolic interaction and explains how this provided additional insight into the possible attitudes, values, and belief systems of the offender and suspect as well as possible suspect-victim interaction. Lacking a suspect through traditional investigative techniques, the use of a questioner is discussed as an investigative technique together with how insights from the behavioral analysis based on symbolic interaction was used to interpret the questioner/survey results.

This case study concludes by discussing how insights from the theory application and research data identified an otherwise unconsidered suspect and, once identified, how this suspect was connected to the crime by available physical evidence.

In conclusion, this presentation will illustrate how a fresh look by academia at theory and research methodology through an applied perspective can yield new and innovative investigative techniques. Operationalization of these techniques can yield investigative evidence that can then support affidavits for search warrants for additional evidence, illustrate further investigative avenues, suggest possible interview/interrogation approaches, suggest testimonial land mines, and provide opportunities for academicians to further contribute to the public good.

Applied Theory, Insightful Interviewing, Applied Behavioral Theory

I43 Measuring Treatment Progress and Outcomes in Forensic Mental Health

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After attending this presentation, attendees will have gained knowledge regarding current impediments to the provision of effective mental health treatment in the forensic setting as well as applicable methods by which to address these through progress and outcome measurement.

This presentation will impact the forensic science community by demonstrating how measurement of progress and outcomes among forensic mental health patients may best be achieved through the use of dynamic measures of risk for violence.

While forensic science has been broadly construed as that branch of the field tasked with informing the legal system in all of its activities, the great majority of that science has focused heavily upon topics germane to offenders' entry into the legal system (e.g., assessment, examination of evidence, etc.); however, at present the United States faces a crisis whereby empirically sound guidance in decisions pertinent to the entry of mentally ill offenders into the criminal justice system vastly outweighs that available for decisions relative to ongoing treatment, placement, and rehabilitation. This presentation addresses one reason for the paucity of knowledge regarding best practices for ongoing offender management through examination of an empirically supported measure of progress or outcomes in this population.

The Mendota Mental Health Institute (MMHI) in Madison, WI, is a state hospital housing male patients committed to the Wisconsin Department of Health Services pursuant to a finding of "not guilty by reason of mental disease or defect" for the commission of a crime. This presentation provides an overview of a system for measuring patient progress and outcomes using measures of risk for violence within the Structured Professional Judgment model embodied by the dynamic (i.e., non-historical, changeable) items of the Historical Clinical Risk Management-20 (HCR-20) and Structured Assessment of Protective Factors (SAPROF) instruments.[1,2] A study at MMHI employed these tools longitudinally and naturalistically by licensed psychologists within multidisciplinary treatment teams following more than 100 male inpatients across one maximum, three medium, and one minimum security units. Measurements were gathered at intervals no longer than once every three months per patient. Data were collected between December of 2014 and May of 2016.

The resulting data reveal that quantification of this measurement, for which raters showed high inter-rater reliability in sample vignettes, yields strong evidence of construct and predictive validity of this method for tracking progress. Patients' scores on the measure were predictive of their movement through different levels of security/privilege at the institute. They were also predictive of behaviors (e.g., future rule infractions) such that lower-scoring patients demonstrated a higher likelihood of "level drops" than higher-scoring patients. In conjunction with data in the literature reporting this metric to be strongly predictive of recidivism over long-term follow-up, these findings are supportive of Structured Professional Judgment as a valid method for measuring progress and outcomes within forensic mental health treatment settings.[3] This presentation discusses both the utility of this tool for ongoing management of offenders within the criminal justice system and also its relevance to informing decisions made about their treatment throughout the duration of their legal interactions.

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Mental Health, Treatment, Progress

J1 Questioned Document Examiner Training: Foundational Readings and Resources

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After attending this presentation, attendees will understand what members of the questioned documents field have relied on for training and education as questioned document examiners.

This presentation will impact the forensic science community by demonstrating the importance of creating systematic and standardized training programs with empirical and measurable benchmarks demonstrating mastery and competence of trainees and by demonstrating methods and procedures that can be used to strengthen and evaluate training in a variety of forensic disciplines.

Current efforts to strengthen the reliability and validity of the methods and conclusions of questioned document examination have included discussions of standardizing training and assessment for trainees. Current training practices include foundational readings and materials that are not assigned consistently across trainers. The purpose of this study is to investigate which readings and materials are considered the most important and are used most frequently among members of the field for education and training purposes.

Efforts such as the National Institute of standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) and working groups such as the NIST/National Institute of Justice (NIJ) Expert Working Group on Human Factors in Handwriting Examination were created to address needs for training, education, research, lab management, and a variety of other issues that are important in moving the field forward and ensuring that current practices are demonstratively the best practices.

Evidence-based practices are those based on significant and reliable evidence derived from empirical evaluation of training methods and procedures. Evidence-based practice is employed classroom settings and policy making to ensure that learners receive the greatest possible benefit from learning experiences.

This paper will present the results of an online survey of the foundational readings and materials used by professional document examiners during training. The survey will be administered via the Survey Monkey online survey service. Participants will be forwarded a link to the survey, which will gather information about materials from an extensive bibliography prepared for use by the NIST/National Institute of Justice (NIJ) Expert Working Group on Human Factors in Handwriting Examination and the OSAC Questioned Documents Subcommittee. Participants will respond to a series of article references by indicating whether they have read the article, whether it was used during their own training, whether they have used it to train others, or whether they would recommend it for training purposes.

Results of this survey will inform the development of training programs that incorporate the identification of relevant knowledge, skills, and abilities; valid and reliable measures of learning; and objective and measurable benchmarks for determining training effectiveness.

Foundational Reading, Training, Measurement

J2 The History and Future of Forensic Document Standards

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The goal of this presentation is to educate forensic document examiners on the past and future of forensic document standards.

This presentation will impact the forensic science community by educating attendees on the past and future of forensic document standards development. Courts of law are relying more on the use of standards in an expert's examinations; a lack of this knowledge may have an impact on whether or not a practitioner is allowed to testify as an expert witness.

Voluntary consensus standards for forensic document examiners had a beginning in the American Society for Testing and Materials (ASTM), with the publication of the Standard Descriptions of Scope of Work Relating to Forensic Document Examiners in 1972. Since then, there have been twenty additional standards published that directly apply to forensic document examination. The sub-disciplines within forensic document examination for which standards have been drafted and published include handwriting, ink examination, typewriting, alterations, and many more. The standards have been published by ASTM (including ASTM International), the Scientific Working Group for Forensic Document Examination (SWGDOC), and in the future will be published by the Academy Standards Board (ASB) as a part of the effort of the Organization of Scientific Area Committees (OSAC).

SWGDOC drafted a large majority, and updated all of the standards that went to ASTM for vetting and publication. When the E30.02 Questioned Documents subcommittee, under the E30 Forensic Science Committee, decided to no longer publish through ASTM and voted to close the subcommittee, ASTM released (eventually) the copyright on the documents that were under the control of E30.02. At that time, SWGDOC took those documents, removed the ASTM language, and posted those documents to the SWGDOC website, making them free for distribution to anyone with an interest in the standards. These standards can still be found at www.swgdoc.org.

In 2015, the National Institute of Standards and Technology (NIST) created the OSAC, with (currently) 25 subcommittees representing different forensic disciplines – forensic document examination being one of them. The OSAC process for drafting, vetting, and publishing standards, guidelines, and best practices will be presented. This process, depending on the type of document being considered, also includes reviews for Human Factors, Legal Analysis, and Quality Infrastructure. The Forensic Science Standards Board will give the final blessing on documents placed in the OSAC registry and that are intended to be labeled as "standards" under the OSAC process. Those that will be labeled as guidelines and best practices need only be approved at the Physics/Pattern Scientific Area Committee level.

Additionally, the American Academy of Forensic Sciences (AAFS) created their own standards development organization, the Academy Standards Board (ASB). The Forensic Document Examination subcommittee at OSAC has decided to publish through the ASB, primarily as the standards will still be available at no cost to stakeholders. The ASB uses a Consensus Body process to vet a proposed draft standard. Once this vetting process is completed the standard will be published as a national standard. The ASB is accredited by the American National Standards Institute (ANSI).

OSAC, ASB, Questioned Document

J3 Contemporaneous Standards in Forensic Document Examination (FDE) — When Is "Close" Close Enough?

Carl R. McClary, BA, 2600 Century Parkway, Ste 410, Atlanta, GA 30345*

After attending this presentation, attendees will better understand what standards are considered contemporaneous based on the condition, age, or other factors of the questioned writing, writer, item, or machine under scrutiny. Writing of the elderly and other cases highlighting contemporaneousness will be demonstrated.

This presentation will impact the forensic science community by providing the ramifications of not having contemporaneous, appropriate standards in certain types of complex cases. Suggestions will be provided for language to be included in this discipline's methods.

Throughout the history of training in Forensic Document Examination (FDE), students have been taught the importance of obtaining adequate exemplars or standards for comparison. The term "adequate" has been construed to encompass the number of comparison documents, comparability (hand printing to hand printing), lack of distortion, and contemporaneousness. This latter caution is not only applicable to handwriting and hand printing examinations, but also to machine-prepared items such as computer printouts, typewriting, rubber stamp impressions, and the like. This fundamental step of examination is based on the effects of continual wear and tear either on the machine, be it the platen, stamp, or other impression device; or the person, such as the hand and motor control performing the bulk of the writing.

In almost every case, the forensic document examiner is tasked with the assessment of comparability of questioned and known items (or questioned to questioned items) with respect to contemporaneousness. Most books on the subject of FDE contain some instruction for obtaining or defining of contemporaneous standards, but most often in a vague manner with no specific guideline. The logical explanation for this lack of specificity is that this factor is largely dependent on the manner and frequency in which the machine has been used or the condition of the individual producing the handwriting in question. By condition, it is meant the physical condition of the individual to include age, sudden increased or decreased use/frequency of handwriting and signatures, physical injuries, illnesses, or even stress. For machines, use normally dictates defects that may come and go (as with repairs). Together, all of these factors are subject to the particular case at hand; but, is there or can there be a consensus on what constitutes contemporaneous writing? Additionally, is there consensus on parameters of time in which to gather contemporaneous exemplars from, for example, a suspect photocopier? Can such a factor be standardized through a published standard on the acquisition of contemporary standards or possibly through additions to the current FDE standards?

This presentation will explore FDE texts for what constitutes contemporaneousness and will contain comments from experienced examiners on what guidelines they employ in their cases to ensure that comparable standards are utilized. Case samples will also be used to illustrate the importance of contemporary standards in particularly complex situations where the lack of such standards could result in inconclusive or erroneous opinions.

Contemporaneous, Standards, Appropriate

J4 Forensic Document Examiner Testimony of Inconclusive Examination Results

Jan Seaman Kelly, BA, 9360 W Flamingo Road, #110-400, Las Vegas, NV 89147*

After attending this presentation, attendees will better understand the occurrence of forensic document examiners providing testimony regarding inconclusive examination results during a legal proceeding.

This presentation will impact the forensic science community by educating attendees that expert testimony has occurred regarding inconclusive results in a variety of legal proceedings.

Kelly and Lindblom explain the standards used by forensic document examiners when providing testimony regarding examination results in legal proceedings.[1] Conclusions follow the American Standards for Testing and Materials® (ASTM) E1658 *Standard Terminology for Expressing Conclusions of Forensic Document Examiners*. This standard, passed in 1995, was based on the 1991 *Journal of Forensic Sciences* letter written by Thomas McAlexander, Jan Beck, and Ronald Dick. The nine-level opinion terminology scale provides a balance between positive and negative opinions that reflect various levels of certainty based on potential limiting factors. The two sides are separated by the "no conclusion" point. The application of the opinion scale is flexible in that it applies to the five-level and seven-level scales. The no-conclusion level is present in all three of the scales.

The term inconclusive is synonymous with no conclusion. Inconclusive is defined by the Cambridge dictionary as "not giving or having a result or decision; uncertain".[2] Inconclusive opinions are issued in a variety of forensic document examinations and occur when the evidence contains at least one limiting factor. An inconclusive result will be based on a limitation that may be based on but not limited to the following factors in handwriting cases: lack of contemporaneous or comparable known standards; brevity of writing in the questioned or known; distortion due to intentional disguise; health, or low writing skill ability; and, lack of significant agreement or differences between the two sets of writings. Even though attorneys desire definitive conclusions, an inconclusive opinion is based on information that may prove beneficial to the trier-of-fact. The issuance of an inconclusive opinion lets the jury and the judge know the evidence was examined and a definitive conclusion could not be issued due to the potential limitations of the evidence.

Testimonies offered by defense witnesses from academia discuss the performance of forensic document examiners on proficiency tests provided by Collaborative Testing Services (CTS). In assessing the error rates in each CTS test, the academic critics stated they did not count inconclusive results because no one offers testimony in court regarding an inconclusive result. This claim has been stated by Risinger, Denbeaux, and Saks in their University of Pennsylvania Law Review article.[3] To determine whether or not this statement was valid, a survey was distributed to forensic document examiners asking the following questions: (1) during the examiner's career, the number of times testimony of an inconclusive result was provided in a legal proceeding; (2) the type of examination; (3) the type of court hearing; and, (4) inconclusive result testimony provided for prosecutor (plaintiff) or defense. The result of this survey revealed examiners provide testimony regarding inconclusive examination results. The presentation will discuss how often this occurs, and the responses to the survey questions.

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Inconclusive, No Conclusion, Survey

J5 Counterfeit Detection E-Learning: Deployment Progress and Content Updates

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After attending this presentation, attendees will better understand how the deployment of counterfeit detection e-learning training at the United States Department of State was accomplished, the benefits and challenges of delivering training on this topic through e-learning, and the task of assessing trainee knowledge retention.

This presentation will impact the forensic science community by providing examples of how training for basic questioned document examination, targeted toward laypersons whose job duties are primarily non-forensic functions, can be delivered at scale to employees of large, complex organizations.

The Department of State recently concluded development of an e-learning primer on counterfeit- and alteration-resistant security features used in documents such as passports, identity cards, visas, birth records, and currency. Instead of training on specific documents, the course focuses on how security feature technologies such as watermarks, micro printing, color shifting inks and optically variable devices are used in similar ways across different document types, and in documents from different issuers. This strategy was chosen because it is impossible for learners to memorize which security features are present in, for example, the hundreds of distinct passports globally in use, but it is possible to understand how to recognize and authenticate the finite pool of security technologies that are used by all security document issuers. The ultimate course objective is for learners to gain the ability to self-train on an unfamiliar document by quickly assessing its security features and drawing conclusions about its authenticity, even in the absence of prior training or experience with that document.

The design and editing processes required for development of e-learning on the topic of counterfeit detection required extensive photography and animations of document manipulation for user interactivity. For example, many of the security feature technologies require transmitted lighting, ultraviolet light, tilt effects, or other specialized viewing conditions to be authenticated, and these effects were simulated in digital animations that provide users with the ability to control lighting conditions and play animations that simulate manipulation of virtual documents. Because the training is deployed over the internet, another important consideration was file size as it relates to data transfer speed and the ability of the course to load quickly, even in locations with suboptimal internet speeds. Furthermore, e-learning can be deployed onto a learning management system (LMS), any mobile device which permits tracking of learner completion and records the results of testing, over the internet as an always-available job aid, or on CD to be used as an instructional tool in classrooms. Accordingly, decisions about the environments in which the course will be used affect the kinds of functionality that can be incorporated into it and will necessitate development of different versions of the course for different applications.

Finally, developing quizzes, review questions, a final exam and a study guide required careful assessment of the specific capabilities and actions that learners are expected to master. Accordingly, the final exam tests understanding of concepts in four primary subject areas: locating security features in documents, risks of counterfeiting and alteration, differentiating between security feature technologies, and techniques for inspecting security features.

Testing utilizes images to measure comprehension, application, and analysis. Passing depends more on the learners' ability to recognize what to do with a document or feature simply by looking at it, instead of recalling abstract information or returning memorized information such as definitions.

Counterfeit, Document, E-Learning

J6 International Cooperation and the International Criminal Police Organization (INTERPOL) E-Learning Program on the Detection of Fraudulent Documents

Daniela Djidrovska, Interpol, 200 Quai Charles de Gaulle, Lyon 69006, FRANCE*

After attending this presentation, attendees will possess background information regarding security and travel documents, fundamental techniques for analyzing security documents, screening and examination processes of identity documents, plus INTERPOL databases and the ID and Travel Documents Reference Center.

This presentation will impact the forensic science community by exploring the "Train the Trainer" and e-learning program developed by the INTERPOL Counterfeit Currency and Security Documents (CCSD) branch that covers the requirements of border control officers and forensic document examiners in member countries, thus ensuring the sustainability of the standardized training modules for the detection of fraudulent documents and adapting this program to the current trends.

The INTERPOL CCSD Branch is responsible for establishing programs that provide forensic support, operational assistance and technical databases in order to assist the 190 member countries of INTERPOL regarding counterfeit currency, security documents and addressing border security issues by improving the integrity of travel and security documents. INTERPOL CCSD also coordinates and supports border-security operations with the goal of disrupting criminals who seek to cross borders using fraudulent documents to hide their true identity.

This presentation will focus on CCSD responsibilities and activities to continuously assess existing measures with the goal of implementing new ones that may provide an "added value" to the international community in addressing counterfeit documents on a global scale. CCSD publications present high-quality products with descriptive text and detailed forensic analyses designed to convey the information in a clear and concise manner. This allows the front-line officer to absorb the information quickly and retain that information for use in the field.

The goal of this program is to foster national forensic capacity on security document examination and to encourage exchange of information/intelligence among front line officials, forensic personnel, and other relevant parties. The goal is to reach out to a wider audience using web-based/e-learning platforms and explore mechanisms which would foster continuing professional development for the beneficiaries in security document examination. The learning objectives focused on the level of awareness of the use of fraudulent documents and methodologies for recognizing the use of genuine documents being used fraudulently. The basic forensic document examination techniques, how documents are falsified, and use of INTERPOL databases, such as INTERPOL ID and Travel Document Reference Center, are the main topics of this eLearning program.

In 2016, training courses were conducted in seven countries (Laos, Ghana, Turkey, Argentina, Cameroon, Malawi, and Cambodia) covering all four INTERPOL statutory regions based on the CCDS "Train the Trainer" program. Border officials and forensic document examiners from 23 different nationalities were trained. All participants were trained to the minimum common standards on the identification of fraudulent documents.

Security, Documents, E-Learning

J7 The Discrimination of Less Frequently Encountered Colored Pen Inks Based on Their Optical Properties

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After attending this presentation, attendees will better understand the most discriminating optical properties of less commonly encountered colored pen inks.

This presentation will impact the forensic science community by providing guidance on the most efficient use of optical techniques to the differentiations of ink samples, given the ink type and color.

Commercially available writing instruments include a wide variety of body styles, ink delivery systems, ink types, and colors. An extensive body of research has been dedicated to investigating multiple methods of ink analyses and differentiation of inks. However, the majority of these studies have focused on the more popular black and blue inks. Less data are available to questioned documents examiners when confronted with less frequently encountered colored inks, such as red, purple, green, turquoise, pink, orange, and maroon. Therefore, this study explored the properties of inks of less common colors of frequently encountered writing instruments such as ballpoint, rollerball, porous-tips, and gel pens. Initially, this study considered the optical properties of the ink samples because these are the first features that questioned document examiners inspect, especially in the context of comparative examinations between unknown specimens and inks from known sources. The goal of this study was to determine the most discriminating conditions for a given set of ink type and color. This was achieved by spanning different illumination techniques that are commonly utilized during questioned document examinations, such as sample exposure to filtered colored light, infrared reflectance, infrared luminescence, and fluorescence by ultraviolet excitation.

A total of 163 colored ink samples of popular brands such as BIC, Zebra, Pentel, Schneider, and Cello were collected from large retailers in the US. They were divided in groups based on their ink type and on their visually perceived colors. The groups consisted of 26 red ballpoints, 6 red rollerballs, 22 red gels, 5 red porous, 23 green ballpoints, 5 green rollerballs, 16 green gels, 5 green porous-tips, 16 purple ballpoints, 4 purple rollerballs, 17 purple gels, 3 purple porous-tips, 2 maroon gels, 2 orange gels, 5 pink ballpoints, 5 pink gels, and 1 pink porous.

Spectral comparisons were carried out to discriminate ink samples within each category. Each sample was observed using an examination matrix where the techniques and filters were selected in the Video Spectral Comparator system including: flood visible light and infrared (IR) reflectance from 530-1000nm; IR fluorescence; and, ultraviolet for 365, 312, and 254nm.

For red ballpoints, 303 of the 325 pairs were differentiated and 189 of the 231 red gel pairs, all ten pairs of the porous pairs, and 3 of the 15 roller ball pairs were also differentiated. The most discriminating illumination type for red pens was absorption starting at 417, 431, 446, 492, or 507 and reflectance up to 612 or 627nm with the IR reflectance band pass filters.

For green ink types, all 253 ballpoints pairs, 120 gel pairs, 10 porous pairs, and 10 rollerball pairs were differentiated. The most discriminating illumination type for green pens was absorption starting at 431, 446, 462, 507, or 522nm, and reflectance up to 702, 732, 717, or 837nm with the IR reflectance band pass filters.

For purple porous pens and rollerballs, all pairs could be differentiated, with the most discriminating illumination type being absorption from 476/482nm, and reflectance up to 732/792nm for purple porous pens using the IR reflectance band pass filters. The most discriminating illumination type for rollerballs was absorption from 462/492nm and reflectance from up to 642/732/792nm. For purple ballpoints and gel pens, all pairs were discriminated; the most discriminating illumination type for both was absorption starting from 431/507nm, and reflectance up to 642 or 792nm using the IR absorption band pass filters.

For pink ballpoints, pink gels, and orange gels no differentiations were observed. However, differentiation of the pair of maroon gels was possible with the most discriminating illumination type being absorption up to 665nm for IR fluorescence filters 485-590 and 485-610nm, and fluorescence up to 725nm using the 515-640nm IR fluorescence filter.

J8 Revealing Hidden Information From the Reverse Side of a Questioned Document

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The goals of this presentation are to: (1) serve as an all-round practice session for practicing document examiners; (2) provide valuable learning experience for newcomers in the profession; (3) highlight the importance of detailed examination of a questioned document that can reveal different kinds of information; and, (4) discuss the circumstances in which the reverse side of the questioned document can provide additional clues to the contents written/printed on the front side of the questioned document.

This presentation will impact the forensic science community by educating attendees how extended repertoire, non-destructive examinations can still be used to clarify specimens that are obscured and complicated. When non-destructive ink differentiation fails on the document's front side, examination options on the reverse side of the same document can lead to the successful detection of multiple writing instruments used in the area of the alleged tampering.

Background: The job of the forensic document examiner is to analyze, compare, and evaluate the questioned document in order to determine genuineness or non-genuineness, to expose forgery, or to decipher alteration using a variety of techniques. When examining a piece of writing for alleged tampering, one of the things the examiner looks for is any difference in the writing instrument/ink used. This will consequently result in one of two findings: (1) that the writing instrument(s) used in the area of the questioned writing is similar/same; or, (2) that more than one (different) writing instruments have been used. In the latter scenario, the interpretation is usually easier; whereas in the former, there may be no tampering or tampering using the same or similar enough writing instrument. The examination may become complicated when the suspected tampered area of writing involves writing instruments having similar enough ink composition to evade differentiation by commonly used non-destructive methods. Such disputed documents prove to be more demanding for the document examiner, and the use of non-conventional techniques accompanied by problem solving approaches become essential.

Method: A few cases are notable in that the question is solved in an unusual and unexpected manner. The case to be discussed required determination of alleged tampering in a questioned cheque. The inks/writing instruments were so similar that the conventional use of light sources and fluorescence filters available in the video spectral comparator (VSC-6000) were unable to differentiate the writings. The examiner was sure that "something" was suspicious about the questioned document, and continued to investigate. At last, by using an unusual and different approach by examining the reverse side of the questioned document, and this approach resulted in the discovery of remarkable evidence that was sufficient to prove alleged tampering in the disputed document. The rear of the document revealed a new dimension for examination of the writing present on the front side. This technique focused not on ink differentiation but towards physical differences in interaction of ink and paper indicating clues about time of writing as well as difference in writing pressure. This approach resulted not only in a number of findings sufficient to prove tampering in the questioned document but also provided probative evidence to the investigation.

Conclusion: Using an extended repertoire, non-destructive examination can still be used to clarify specimens that are obscured and complicated. Where non-destructive ink differentiation fails on the documents front side, examination options on the reverse side of the same documents can lead to successful detection of multiple writing instruments used in the area of alleged tampering.

Forensic Document Examination, Non-Destructive Examination, Tampering

J9 The Evaluation of Instrument Sensitivity and Stability for the Magnetic Flux Measurement Relative to Toner Area as a Screening Tool for Casework Application

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After attending this presentation, attendees will better understand the potential for the use of the Regula® Magmouse 4197 to differentiate between black-and-white toner-printed documents from different sources. Attendees will also understand the magnetic class characteristic groups exhibited by toner-printed documents and how to employ magnetic flux measurement techniques for exclusionary purposes as a precursor to confirmatory techniques.

This presentation will impact the forensic science community by providing a method for quickly screening black-and-white toner-printed documents, thus reducing the need for more time-consuming methodology.

This study was created to address questions raised by previous research into magnetic properties of toners. Specifically, the questions of whether magnetic flux could be positively correlated with toner area, whether the flux value remained constant or showed variation with toner age and degradation, and whether there is a way to standardize the measurement of magnetic flux to account for variations across fonts and characters.

With this goal, a preliminary trial was conducted on 5 samples from the collections of the Université de Lausanne. Measurements were taken from 150 samples in a series of ten trials conducted across a span of one week, from each of 3 different font sizes per sample. The results were compared to the results obtained one year prior for consistency and to check for any possible sample degradation, but were found to be within the expected error intervals indicating no change in magnetic flux behavior. The area of each measurement was determined individually, and the flux per area measurement was checked for correlation. Though there is some difference in the level of intra source variation exhibited by the samples, a positive correlation between area and magnetic flux was observed.

For the second phase of trials, magnetic flux measurements of 150 samples representative of an actual population were collected and considered as a function of their toner area measured individually. Three replicate measurements were collected over the course of one week. The data were analyzed with a two-way ANOVA to determine the thresholds for the class groupings.

For the final phase of verification, the thresholds determined in the previous trials for the number magnetic flux measurements necessary, the class intervals, and exclusion thresholds were tested using a blind trial methodology. Seven samples which had been randomized from the pool of 150 phase two samples were presented for analysis and class identification. The magnetic flux of each unknown was measured three times with varying areas, and the average flux per area was used to determine the class of the sample.

The magnetic flux per area was found to be stable over time, and to exhibit levels of intra and inter source variation which allow for the discernment of class-level characteristics. Exclusions can be made with a high degree of confidence; however failure to exclude or the appearance of consistency necessitates further confirmatory testing. Magnetic flux measurements could be useful as a quick, screening tool to eliminate the need for further testing which may be destructive.

Questioned Documents, Toner, Magnetic Flux

J10 The Determination of Heterogeneous and Homogeneous Line Intersections: Infrared (IR) Versus Polarized Luminescent Techniques

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After attending this presentation, attendees will better understand IR versus polarization techniques on heterogeneous and homogeneous line intersections. The two techniques employ two hand-held devices capable of connecting to a laptop, thus having practical implications in the field. The polarization can provide digital JPEG images for a direct observation of the area of line crossing for a sequential determination. Finally, the device allows ten modes of quantifiable geometrical measurements for an *in situ* comparison, which fulfills the quantifiable requirements recommended by the National Research Council Report in 2009.

This presentation will impact the forensic science community by implementing a novel method of the polarization technique on line intersection determination.

It is hypothesized that the determination of a line intersection is one of the technical challenges in providing evidence where a document is altered. A fraud case where the defendant altered the monetary value was the inspiration of this experimental study. The results of the study may answer the National Research Council's challenge to questioned document examination as being "less scientific" due to a lack of quantifiable measurements.

While a heterogeneous line intersection refers to a line crossing produced by two different writing ink materials (i.e., ballpoint pen vs gel ink pen of the same color), a homogenous line intersection is a line crossing made by the same writing ink material of the same color. It is more difficult to determine the sequence of line intersection in the latter. This study conducts a comparison in the evolution of two methods: IR and polarization. First, infrared luminescence, the main method used to perform such tasks at an affordable cost, indicates certain disadvantages. The IR examination (980 nm) compares a line crossing based on different color indications (quality) and ink concentrations. However, IR technique does not show any color display (reflected image) for ballpoint pen ink – the most common writing medium in the fraud case. Second, when applied to the homogenous line crossing, the color contrast produces an undesirable standard with vague contrast, making a sequential determination much more difficult and uncertain. Finally, current IR technique cannot provide any real-time, quantifiable measurements of stroke width, angle between two strokes, and any ink minutia (skipping, gaps, or holes). On the other hand, the polarization technique (based on the ability of waves to oscillate in more than one direction) enables reduced glare and improved background contrast. Further, the polarization method simultaneously addresses all three of IR's limitations, thus providing a better identification based upon a scientific and objective verification, not just upon a subjective decision of color differentiation and ink minutia.

The sample collection was a simulation of a real fraud case involving an altered number (making $10,000 into $40,000) under a purposive sampling method. The study selected four common types of pens of black color: ballpoint, gel ink, fountain, and permanent marker. A comparison was conducted between the heterogeneous and homogeneous line intersections against each other. The preliminary results indicate some practical differences and three advantages for polarization over IR. First, the polarization technique is a non-destructive method used either before or after the standard examination or verification (as a second opinion). Second, polarization can provide a quantitative measurement of the line intersection and/or other ink minutia in ten geometrical formats, making the examination more reliable and valid. Third, the application of polarization can produce a rapid examination at the scene, in the lab, and even in the courtroom. The results add much needed support for crime scene technicians, examiners, and/or investigators and render this method as sufficient and practical for a real time examination at scenes. The sequence of intersecting lines plays an important role in the forensic examination of a document. It is concluded that if our field conducts widespread testing and adoption of polarization techniques as common practice, our duties and performance on line intersections will be advanced from the past towards a more scientifically sound future.

Questioned Document Exam, Heterogeneous/Homogeneous Line, Polarization Technique

J11 Direct Sample Analysis/Mass Spectrometry (DSA/MS) vs. Separation MS Techniques for the Analysis of Writing Inks

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After attending this presentation, attendees will better understand DSA/MS of writing inks and its comparison to Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectrometry (GC/MS) of the same inks.

This presentation will impact the forensic science community by introducing a new method for the analysis of writing inks and the advantages and disadvantages compared to GC/MS and LC/MS.

Official documents are an essential aspect of almost all legal agreements. Such widespread use of documentation also comes with a large amount of forgery. Criminal acts may include the altering or addition of entries to change the value of the document. Questioned document examinations are therefore required to determine the presence of unoriginal writing within the document. Direct Sample Analysis (DSA) coupled with high resolution, high mass accuracy Time-Of-Flight Mass Spectrometry (TOF/MS) is an emerging technique for the in situ analysis of various substances. DSA is similar to Desorption Electrospray Ionization (DESI) and Direct Analysis in Real Time (DART); however, it uses an atmospheric pressure chemical ionization source for ionization of organic compounds. In this study, DSA was applied to the identification of 80 black and blue writing inks from both ballpoint and non-ballpoint pens. Moreover, the results obtained from DSA analysis were compared with the extraction of the ink from the paper and its analysis by Gas Chromatography/Mass Spectrometry (GC/MS) and nano ultra-high performance Liquid Chromatography-Orbitrap Mass Spectrometry (nLC/MS).

Eighty ink samples were obtained from the United States Secret Service. Approximately 3mm sections of each writing were placed on the DSA sample holder and analyzed in an automated fashion. The holder can analyze 13 samples in a serial manner. Utilizing a high mass accuracy TOF/MS and using internal calibration, mass accuracy of <6 ppm was achieved. DSA analysis was performed on a Perkin Elmer AxION DSA in conjunction with a Perkin Elmer AxION 2 Time-Of-Flight Mass Spectrometer operating at a resolution of ~10,000 at m/z 922. For HPLC analysis, a Thermo Fisher Scientific Easy-nLC 1000 Ultra High Performance Liquid Chromatograph was used in conjunction with a Thermo Fisher Scientific Velos Pro Orbitrap Elite Mass Spectrometer. Two different LC methods were developed one with the analysis time of 30 minutes and the other with the analysis time of 5 minutes. GC/MS analysis was performed on a Perkin Elmer Clarus 680 Gas Chromatograph-Clarus SQ 8C Mass Spectrometer using electron ionization.

Analysis by DSA and LC/MS resulted in the identification of colorants as well as vehicles and additives while analysis by GC/MS mainly resulted in the identification of the non-colorant ingredients. DSA-high resolution MS, as well as Liquid Chromatograph Orbitrap Mass Spectrometry (LC-Orbitrap/MS), provided extensive compositional information. DSA detected more ink related compounds and in more samples than LC/MS. Of the three techniques utilized, DSA provided the greatest number of ink compound identifications and in more samples. Both DSA and LC/MS were able to detect colorants; however, the DSA results were obtained within seconds of mounting the sample while LC/MS analysis took several minutes. In addition to longer analysis time, solubility issues and the elution of small highly charged compounds with the void volume were other main draw backs of LC/MS. Under LC/MS; however, salts are separated from compounds of interest and most compounds are separated from each other, minimizing the suppression effects and simplifying compound identification. In regards to sample preparation, neither method showed a significant advantage over the other. Although LC/MS required an additional extraction step, mounting and aligning samples in the DSA was tedious. Currently, a disadvantage of the DSA method is that samples must be cut out of a document and carefully positioned on the sample stage to be analyzed. GC/MS and LC/MS are indeed informative techniques, but they are also destructive methods, unlike spectroscopic methods such as Attentuated Total Reflectance/Fourier Transform Infrared (ATR-FTIR) and Surface-Enhanced Raman Spectroscopy (SERS); however, the spectroscopic techniques are less informative. GC/MS was shown to be the least informative analysis method for ink compositions, since colorants were mostly not detected and solvents and volatile components detected by GC/MS tend to disappear very rapidly. For profiling purposes, the

use of multiple methods, such as the combination of DSA, LC/MS, and GC/MS as shown in this study, is necessary because no single method detects all components in ink formulations.

Direct Sample Analysis TOF/MS, Nano UHPLC/MS, Ink Chemical Composition

J12 From False Identity and Travel Documents to Forensic Intelligence: Profiling Methods to Support the Detection and Investigation of Organized Crime Groups

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After attending this presentation, attendees will develop and improve their competence and performance in processing forensic case data to provide useful and timely intelligence. Attendees will learn how forensic evidence processed through systematic profiling methods may serve in fighting organized crime groups. This presentation also provides insights to questioned documents examiners as to how they can process their case beyond the usual expertise context.

This presentation will impact the forensic science community by showcasing an innovative and original forensic intelligence approach that will be of interest to forensic researchers, practitioners, and policing partners. This presentation illustrates how forensic science can be used to support the fight against organized crime and also highlights the strength of bridging forensic research, forensic practice, and policing.

The presentation introduces an innovative and effective crime monitoring approach based on forensic intelligence. It is designed to support the proactive detection and investigation of organized crime and terrorist groups that produce, disseminate and/or use forged and counterfeit identity or travel documents. This approach developed at the University of Lausanne, Switzerland, bridges research, forensic practice and policing, and is viewed as a powerful and original source of intelligence in support of crime investigations and national security. It is in the process of being implemented by several police departments in Switzerland and abroad.

In this approach, once a false identity or travel document (e.g., passport, driving license, identity card or visa) is seized by a police or border protection organization, it undergoes a scientific and systematic profiling process that extracts, analyzes and compares the documents material features. The strength of forensic findings is assessed and then integrated with alternative data to support crime analysis and crime intelligence. Such a forensic profiling process provides an objective and measurable mean to highlight links between different cases, to point to prominent *modus operandi* as well as to the production of prolific forgers and forgery workshops. Different profiling methods have been developed to support the process which leverages visual and physical examinations of documents, computer-imaging techniques, or chemical analysis. Some of these methods have already been the subject of scientific publications whereas others are still in the development phase.

The presentation describes the conception, validation and use of these profiling methods using real case data made up of hundreds of false identity and travel documents seized by police and border protection organizations in Europe and North America. It quickly illustrates the databases as well as the data analysis and evaluation tools that have been developed to support the methods. The presentation exposes also how the different profiling methods combine within a common intelligence framework in order to leverage their respective advantages. That framework operates at different levels of generality and may provide intelligence in support of tactical investigations as well as of strategic decision-making.

Specifically, the contribution of the forensic intelligence process is illustrated through a case study concerning a criminal network involved in human trafficking and human exploitation. The approach provided key insights for investigators and for the prevention of further cases. It assisted, for instance, in understanding the organization's *modus operandi*, in locating the forgers, in revealing cross-border connections as well as the extent of the criminal network activity.

The successful implementation of the method in that case and others paves the way for a new approach of detecting and investigating organized crime groups involved in the production, dissemination and/or use of false ID or travel documents for various crime purposes.

Besides false identity and travel documents, the proposed forensic intelligence methodology can be generalized to process forensic case data of any nature. That methodology extends the contribution of forensic science beyond the usual case-by-case expertise context and reveals useful to fighting organized crime.

Forensic Intelligence, Profiling, Documents

J13 An Analysis of Polymers in False Identity Documents: A New Contribution to Forensic Intelligence

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After attending this presentation, attendees will better understand the analysis of polymers in plastic identity documents for intelligence purposes, a field that has not yet been widely explored.

This presentation will impact the forensic science community by presenting a relevant combination of forensic chemistry to document analysis in a new manner. This presentation will also introduce the use of forensic chemistry as a forensic intelligence provider as opposed to it being a primary tool for the court.

The use of a polymeric substrate in the manufacture of travel and identification documents is becoming more and more widespread and popular across the world. Polymers have various characteristics that facilitate the use of many security elements and techniques; however, this does not make these documents forgery- or counterfeit-proof. They are still altered by various criminals and even terrorists to mask their identity and carry out their activities.

The presentation introduces the method and results of a research study with the goal to evaluate the relevance and contribution of polymer analysis in a forensic intelligence framework. Combined with visual examination and description of the documents, non-destructive to destructive analysis methods (such as Fourier transform infrared spectroscopy) were used on sets of genuine and false plastic driving licenses to provide information on the chemical composition of documents (support, printings, imitated security elements). A qualitative High-Performance Liquid Chromatography combined with a quadrupole Time-Of-Flight analyzer (HPLC-qTOF) method was also developed to analyze the additives present in the plastic substrates, hence obtaining a chemical profile of the cards studied.

The results of such forensic analysis methods provide insights and intelligence on the various modus operandi used by criminals to forge documents. It provides as well the ability to link false documents seized at different places and times, which eventually leads to identify criminal networks. It enables also to review and increase detection methods of false documents, and even to guide the design of future documents and their control.

This presentation will assess the contribution of documents chemical profiling by discussing the development of the analytical method in regard to the preliminary results obtained, the comparison with documents visual and physical profiling, whether it is possible and relevant to implement such method in reality, and more.

Analysis of polymers are used here with identity documents but may prove to be as much of interest for counterfeit money-related cases in countries that have plastic currency as well, such as Australia, New Zealand, and Canada.

Polymers, ID Documents, Profiling

J14 Imitation Typewriter Digital Fonts — A Project to Establish a Reference Collection

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After attending this presentation, attendees will better understand the historical origins and development of imitation typewriter fonts, the various reasons for their creation and use, and the concepts for classification of contemporary examples.

This presentation will impact the forensic science community by: (1) raising awareness of the potential for encountering imitation typewriter digital fonts in casework; and, (2) providing information about a research project to establish a reference collection.

Cases involving documents prepared on typewriters might be decreasing, but an increasing number of cases involve computer-generated documents being brought forward as typewritten documents, usually with some excuse for the absence of an "original" document.

The standard guidance for forensic document examiners confronted with copies of apparently typewritten documents is: "[w]hen examining nonoriginal text, determine whether the typestyle and other characteristics are consistent with a reproduction of original typed text or consistent with having been produced by another source (for example, computer generated typestyles that are based on or copied from typewriter typestyles)."[1]

Because such cases often involve documents dated decades before they first surface in the context of a dispute, determination of the technology used in the production of the document or the introductory date of a typestyle can be dispositive. In such cases, the obvious pair of mutually-exclusive hypotheses may be stated as:

> **H_D – (the Digital Hypothesis)**: the document submitted for examination in copy form (or any underlying "original") was created in the virtual reality of a computer screen using a digital font imitating the type design associated with typewriters and requiring one or more technological elements demonstrably not extant at the date of the document; or,
>
> **H_A – (the Analog Hypothesis)**: the underlying original of the document submitted for examination in copy form was prepared using the appropriate analogue impact technology of an actual typewriter potentially extant at the date of the document.

Depending on the context, observations that decisively support the likelihood of H_D relative to H_A can be of considerable probative value to the trier of fact.

Awareness of these issues has prompted a research project that aims to investigate the range of currently available typewriter look-a-like digital fonts and their background information to begin assembling a reference collection of these fonts. In addition to open-source images and digital font files, the project has objectives to gather relevant background information such as date of release, designer, distributor, and character set. When information regarding actual typewriter type designs that were the "inspiration" for the digital fonts is not included with the font files, standard typewriter reference collections will be searched. It is anticipated that this project will be ongoing, with updates as new fonts are released. Differentia for potential use in taxonomic classification of these fonts will be discussed.

A concise overview of the germane historical context will be provided, ranging from imitation typewriter fonts of printers' foundry type in 19th Century print shops through the laser and ink-jet printers in the current century.

A casework example will be presented demonstrating how examination of a digital image revealed an anachronistic typewriter typeface design that was the basis for an even more anachronistic digital font.

Reference(s):

1. SWGDOC Standard for Examination of Typewritten Items §7.4.2; available from SWGDOC.com.

Typewriter, Digital Font, Forensic Document Examination

J15 Quantifying Handwriting Variation

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After attending this presentation, attendees will have a better appreciation of one process that can be used to quantify the range of variation of a writer, which can then be used to discriminate writings during a comparison.

This presentation will impact the forensic science community, in particular forensic handwriting examiners, by adding to the tools available to conduct meaningful examinations.

Forensic handwriting examination requires the evaluation of many features and elements of the writing. Chief among them is the determination of the various ways with which a writer executes his/her letterforms, or glyphs. This has been referred to as variation. It should be noted; however, that not all elements of the handwriting will exhibit the same range of variation in the design and shapes of all letterforms. "Natural variation, then, must be thought of as an attribute of each element in the composition of writing, having some cumulative effect upon the countenance of the total product".[1]

Traditionally, the field of forensic handwriting examination considers this "cumulative effect" or scope of variation of a writer as their "master pattern." This has mostly been a qualitative rather than quantitative assessment, requiring tedious observation on the part of the examiner of each instance of a letterform within the text and within a body of writing for a given writer.

Although there have been computer-based handwriting measurement systems that quantify the variation of certain features, their purpose has been to discriminate handwriting and not to determine the extent of the range of variation.

The intent of this research, initially presented in 2000, was to develop a simple approach to visualize and quantify variation in letterforms. Given sufficient sampling of a writer, it is possible to define those portions of a letterform that are consistent throughout the execution of each instance of a given glyph; this forms the master pattern. Each writer will have an extent of variation around this pattern. An attempt was made to quantify this for each letterform for a given writer.

For this study, a corpus of writing from twenty different writers was used. The writing exemplars for each writer included a portion of the London Letter, as well as a fabricated shopping list. This shopping list was created to ensure specific letter and numeral combinations occurred in the sample. The exemplars were all obtained using original ballpoint pen on paper, and then imaged at 600 pixels per inch resolution.

The protocol to build each glyph histogram for a given writer was the same as used for the initial 2000 study. Individual samples of a letter were overlaid in order to maximize the common area covered. Histograms were subsequently built based on the number of glyphs needed in order to fall below a threshold. A significant difference from the 2000 study was that a more objective approach to superimposition was explored in order to remove subjectivity from the quantification.

Reference(s):

1. Huber, R.A. and A.M. Headrick. Handwriting Identification: Facts and Fundamentals. Boca Raton, Florida: CRC Press, 1999.

Handwriting, Range of Variation, Letterform

J16 A Forensic Examination of Simulations of Illegible Signatures

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After attending this presentation, attendees will better understand various aspects of genuine and forged signatures. Generally, the forensic document expert encounters such cases in order to opine as to whether a given signature is genuine or simulated or whether the disputed signatures match a variable group of signatures.

This presentation will impact the forensic science community by informing forensic document examiners and criminal investigators about various methodologies for the detection of forgery by distinguishing a copied signature from a genuine one.

Signatures are an expression of a living form that is repeated time and again in slightly different form as the writer produces them for various needs. Signatures can be entirely legible or partially legible or they may be completely illegible, that is, where none of the letters are decipherable. Despite the complexity of the nature of signatures, the importance of identification has been recognized. Genuine or forged signatures are generally encountered in forensic documents examination cases. A detailed examination of the genuine signatures of all the three groups, that is, entirely legible (Group I), partially legible (Group II), and completely illegible signatures (Group III) with their respective copied signatures was performed. The writing characteristics, such as line quality, pen-pauses, pen-lifts, retouchings, speed, nature of initial and terminal strokes, alignment, spacing, slant and relative slant, overall size and relative size, rubber stamp effect, legibility, and letter formations were studied in detail. It was found that, slight to considerable variations in all the features were present in the copied signatures of all the three groups. A study of copied signatures has revealed that although the forgers have tried to copy the pictorial appearance and other features of the genuine signatures, there are certain features of the genuine signatures which help to differentiate copied signatures from genuine signatures, for instance, defective line quality in case of copied signatures (i.e., the presence of tremor, pen lifts, and pen pauses at unlikely places and careful retouchings). The presence of blunt starts and ends may also be evident in copied signatures. A rubber stamp effect is found to occur in most of the copied signatures. Also, while copying, the forgers have given more attention towards the letter design of the signature whereas little attention has been given to the alignment, spacing, size, slant, and relative slant. It was observed that due to the complexity in the pattern of signatures and the unfamiliarity with the direction of strokes, the illegible signatures of Group III were most defective in line quality than the legible and partially legible signatures. Although the numbers of signature specimens taken in this study were limited, it is hoped that the observation and conclusion of the study will be helpful in further research and signature examination.

Questioned Documents, Signatures, Simulated Signatures

J17 The Dynamics of Guided-Hand Signatures

Samiah Ibrahim, BSc, 139 Riverdale Avenue, Ottawa, ON K1S 1R1, CANADA*

After attending this presentation, attendees will better appreciate the characteristics of guided-hand signatures and how they differ from the genuine signatures of both the writer and the guider.

This presentation will impact the forensic science community, in particular the Forensic Document Examiners (FDE) community, by providing empirical research in guided-hand signatures, a topic previously covered by more anecdotal research.

In 2008, research designed to study the dynamics of guided-hand signatures was presented at the 66th Annual Conference of the American Society of Questioned Document Examers.[1] Up until that time, little scientific data had been recorded to underpin the claims that guided-hand signatures exhibit certain characteristics as documented in historical literature. Additionally, according to this research, there has not been any considerable research into guided-hand signatures since that time.

Traditionally, the term "guided-hand" referred to those signatures that were executed by an individual with their hand literally guided across the page by another individual. Most commonly this occurred when the writer was frail or in ill health and needed a great deal of assistance with the motor control aspect of executing their signature. The terms "assisted" and "guided" can be a matter of debate because the difference may not be visibly apparent in static images of the completed signatures. Further, there may be portions of the signature that have to be "guided" and others "assisted" during the execution. In effect, the term "guided" has come to infer a deceitful motive. This paper will not debate motives, but rather discuss if it can be determined from dynamic data of the handwriting activity, if a signature can be predicted or determined to have been produced by a writer whose hand was guided.

Prior research focused on observations of the static image of the ink line once the hand had been guided to perform a signature, and then the comparison of this ink line to specimens of both the writer and the guider. This study was conducted to determine if the effects of the guider and the writer could be observed and isolated using dynamic data captured with a digitizing tablet. This data is defined as the spatio-temporal characteristics of the handwriting activity, such as speed, acceleration, deceleration and the amount of time the pen is on and off the paper.

The test protocol defined in the initial 2008 research was adopted. Twenty individuals were tested in both the writer and the guider capacity. These individuals were chosen to include writers of both left and right handedness, writers of various skill including aged and frail writers, and both male and female writers. Each writer was asked to also be the guider during the data collection. The writers were paired to ensure maximal distribution of variables, such that left-handed writers were paired with writers of the same and different handedness, and frail writers were paired with both strong and other frail writers, for example.

Using the same signature capture protocol from the 2008 study, each writer executed six genuine signatures for comparison purposes. Each writer also executed six simulated signatures of a paired-writer. Writers were asked to consciously sign their name while another writer acting as a guider simultaneously attempted to execute the same name. Writers were also asked to hold a pen in a limp hand while the guider attempted to 'sign' their name. This scenario was conducted sequentially so there was some familiarity with the pictorial appearance of the signatures for the writers acting as guiders.

The resultant data was then compiled to demonstrate that the dynamic data from the guided-hand signatures revealed a particular trend that distinguished them from naturally executed writings and from the freehand simulations. The results could not support a predictor model from the dynamic data.

Reference(s):

1. Ibrahim, S. (2008, August). The Dynamics of Guided-Hand Signatures. Paper or poster session presented at the meeting of 66th Annual Conference of the American Society of Questioned Document Examiners. Asheville, NC.

Forensic Document Examination, Guided-Hand, Signature

J18 The Development of the Copybook System in the West Bank/Palestine

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After attending this presentation, attendees will better understand the copybook systems developed and used in Palestine and the West Bank since the inception of handwriting instruction in that state.

This presentation will impact the forensic science community, and in particular the Forensic Document Examiners (FDE) community, by providing insight into the systems of handwriting instruction taught in Palestine and the West Bank.

Arabic ranks as the fifth most-widely spoken language in the world in terms of native speakers; the top five are Chinese, Spanish, English, Hindi, and Arabic. In 1973, Arabic was recognized by the United Nations as an official language alongside Chinese, English, French, Spanish, and Russian.

Arabic is the native tongue of approximately 300 million people, and is the national and official language of some 22 Arab states. Arabic is also spoken and written by non-Arab Muslims around the world as Islam's Holy Book, the Qur'an, is written in this language.

As with other written languages, styles of the written system were documented in order to simplify handwriting instruction for students in the population. The historical importance in forensic document examination of the copybook system is the classification and depiction of what are known as class characteristics. Class characteristics pertain to those features of a writing that are common to a group of individuals; traditionally, this refers to a system of handwriting taught in a school environment and quite typically was created for use in a specific country, nation, or population.

Considering the widespread range of Arabic as a script, it is expected that there are many different copybook systems for the many different Arabic countries and places where Arabic is used in classroom instruction. These different copybook systems will affect the way the Arabic scripts appear, and as a result, there is a possibility to predict the origin of the writer, or at least where the writer's primary education was received, from some of their handwriting characteristics.

In order to identify the class characteristics of the Arabic handwriting taught in Palestinian schools, the copybook systems used over the past 50-60 years were documented for this study. It was found that over three distinct time intervals between 1952 and 2015, three different copybook systems can be recognized. These styles are identified as Riqa, Naskh, and a mix of these two.

Since 2014, the Palestinian Authority, in conjunction with the United Nations Office on Drugs and Crime, has been developing forensic science capacity, and with respect to this research, forensic document examination capability. With this new forensic capability, and as a modern state with a set history of widespread handwriting instruction, there is an opportunity to document the entirety of the copybook systems used in a region. This original research was undertaken to explore and publish as a reference tool for forensic document examiners the copybook systems that have been used in the West Bank and what is known as Palestine, since the beginning of the modern school system that incorporated handwriting instruction for students.

Forensic Document Examination, Arabic Handwriting, Copybook

J19 Features of Elderly Writing in Arabic

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After attending this presentation, attendees will better appreciate the features and elements of elderly writing in Arabic script.

This presentation will impact the forensic science community, specifically the Forensic Document Examiners (FDE) community, by providing information regarding features observed in the writing of elderly Arabic script writers.

Handwriting is a complex human activity that entails an intricate blend of cognitive, kinesthetic and perceptual-motor components. Inevitably, the writing of all persons changes over time; but, how the changes are evidenced and in what time span is quite variable.

While there have been many studies and research conducted over the history of forensic document examination of the writings of the elderly, this cannot be said of all parts of the world and with all scripts. Because handwriting comparison/identification is a considerably new field in Palestine there have not been empirical studies conducted specifically of elderly Arabic writers until now.

The goal of this study is to a preliminary baseline for the state of elderly writing in the region. In this paper, the writings of 47 different writers ranging in age between 65 and 89 years were studied. Participants were asked to complete a questionnaire detailing their name, marital status, age, gender, handedness, level of education, and the geographical area they lived in when they were taught to write. Not only was the information from the questionnaire used in the evaluation of the handwriting, but it served to obtain a representative sample of natural writing from each participant. The questionnaire specifically requested the writer to produce numerals, the characters of the Arabic alphabet, and a text passage on white ruled and un-ruled A4 paper using a ballpoint pen.

Once the data had been gathered, the handwriting samples were classified into age groups as follows: 65-69 years of age (20 writers); 70-79 years of age (19 samples); and, 80-89 years of age (8 samples). All the handwritings were analyzed. Among the features considered were diacritics and punctuation, embellishments, legibility, line quality, overall pressure, size, skill, slant or slope, spacing, speed, terminal strokes, and tremor. The final evaluation, in the discussion portion of this paper, was based on the features of pen pressure, punctuation marks, diacritics, size, legibility, tremor, and baseline alignment; although, other features were peripherally assessed. Factors such as gender (8 females and 39 males) and handedness (45 right-handed, 1 left-handed, 1 declared both) were briefly considered for analytical purposes.

The data from each of these features was then further broken down to show trends across the writing of the elderly for the geographic region of interest. It is noted that the sample size of this study is limited and so it is recommended to expand this study with participants from many more locations of the West Bank and include participants of a fourth age group, that of 90-99 years of age, in order to validate the initial trends outlined in this study.

Forensic Document Examination, Elderly Writing, Arabic Handwriting

J20 Mixed-Script Signatures: A Modern Paradigm for an Age-Old Identifier

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After attending this presentation, attendees will better appreciate a yet-undocumented classification of signatures.

This presentation will impact the forensic science community, and in particular the Forensic Document Examiners (FDE) community, by providing information on an undocumented form of signature that may be encountered in casework, especially with Arabic script writers.

Handwriting has been around since the beginning of civilization and the "signature" or the act of signing a document, has long been accepted by nearly every culture as a form of personal identifier, often signifying one's recognition and agreement on the contents and implications of written words. A naturally developed signature represents the most often reproduced and habitual act of writing, and as such have been studied since the very beginnings of the field of questioned document examination. There is much research, both published and anecdotal, with this objective.

Various signature forms have been identified in the literature such as text-based, stylized and mixed. Discussed here, is a form of signature found in the West Bank and Palestinian Territories that has not been, according to this research, encountered elsewhere.

This paper introduces a new classification or form of the signature, the mixed-script or compound signature. Specifically, this paper will show one such version of the mixed-script signature: the compound Arabic/Latin script which consists of a signature written partially in both the Arabic and English languages (Latin script).

Based on anecdotal observation that the compound signature has not been reported upon elsewhere, this research was undertaken in order to establish how widespread this form of signature is in the West Bank. Moreover, possible reasons for adopting this form of signature are explored: to make the signature more complex and difficult to simulate or forge; to express both Arabic and English/Western cultures in a signature; to show dealings with Western/foreigners for work purposes; as an expression of blended cultures; and, finally for purely aesthetic reasons.

This preliminary study comprised a sample size of 20 writers. Each writer was asked to provide 8 exemplars of their signature using 3 types of writing instrument. Furthermore the exemplars were collected on unlined paper, with ruled paper, and using a constrained space or box on the page.

After collecting the samples they were separated for analysis based on the language with which the writer began their signature. Then, the general aspects and particular characteristics of each signature, such as the layout, regularity/rhythm, slope, and direction of stroke were observed. Internal proportions of the signatures such as the relative heights and sizes of characters of both the Latin-script and Arabic portions of the signature were noted. Certain tendencies were recorded. Specifically, it was noted that for most writers that employed the compound signature the writer wrote one name in one script with the second name in the other script, that the finishing stroke of one script became the initial stroke of the second script, that there were slope differences for the different portions of the signature, that the upper/lower case conventions of Latin-script were not adhered to, and that these signatures appeared embellished and flourished.

This research is presented to show this unusual form of signature. Further research will build upon the small sample size shown here to establish the frequency or prevalence for the mixed-script or compound signature use in the West Bank/Palestine and in other Arabic countries.

Signature, Arabic Script, Latin Script

J21 Analysis and Recognition of Urdu Handwriting

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After attending this presentation, attendees will better understand one of the prestigious languages of Asia. Urdu is the national language of Pakistan and is also spoken and written in India.

This presentation will impact the forensic science community by illustrating the different ways the Urdu languages and alphabets are compared with each other.

Urdu is historically associated with the Muslims of the Indian subcontinent. Apart from its specialized vocabulary, Urdu is mutually intelligible with standard Hindi, which is associated with the Hindu community. The Urdu language received recognition and patronage under British rule when the British replaced both the Persian and local official languages with the Urdu and English languages in the North Indian regions of Jammu and Kashmir in 1846 and Punjab in 1849. Urdu is the national language of Pakistan; it has the same alphabet as Arabic and Persian (Farsi), so it is very important to be able to compare Urdu handwriting and signatures. It is also very interesting that the sounds of the English and the Urdu alphabets are quite similar. This will be a unique presentation and attendees will benefit greatly from it.

This research project is one of the unique research projects of Pakistan and will be of interest to questioned documents experts and students. In this study, data of different people's Urdu handwriting was gathered and analyzed in order to observe the natural variations present in their handwriting and Urdu signatures. The literacy rate of Pakistan is 56%, with most documents being written in Urdu, the national language. Thus, it is very important to learn how these writings compare to questioned documents and signatures.

In this study, a structural method of recognising Urdu handwritten writing characters is proposed. The main problem in the cursive writing identification is the segmentation into characters and into representative strokes. When dividing the cursive parts of the words, it is important to take into account the appropriate properties of the Urdu grammar and the segments connecting the characters with each other along the writing row. The main alphabets of Urdu are quite similar to that of Arabic; however, the problem determined was the detection of disguises and forgeries in the Urdu writing specimens. For this reason the data of different people writing the basic alphabets in Urdu was collected, and the change in the formation of these alphabets over time was observed and compared. In this study, writing character data of similar shapes for more than 20 people was taken into account and then analyzed with their previous writings.

Character Recognition, Cursive Handwriting, Urdu

J22 The Admissibility of Forensic Expert Testimony: 25 Years of Milestones and Impacts

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After attending this presentation, attendees will understand the relationship between impactful events over the past two decades and the admissibility of forensic expert testimony in several areas of forensic practice. These events include judicial decisions in cases such as *Daubert*, *Joiner*, and *Kumho*; revisions in the Federal Rules of Evidence (FRE) 702; the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*; and, other events that are changing the legal landscape for judges and attorneys and the practices of forensic scientists.

This presentation will impact the forensic science community by highlighting important influences on the future direction of forensic sciences, including training, methods, conclusions, certification, and other factors that members of the field are confronting as the requirements for standardization in training, terminology, and other areas of forensic practice continue to evolve.

Scientific facts do not directly represent nature. They contain a social component consisting of human agency, institutions and their norms and values, and the processes of science. The movement of expert testimony from the status of "proffer" to that of "admissible evidence" is a social process in which experts, attorneys, judges, and other entities all participate. It is a negotiated movement from "science," which is itself a social construction, to "legal science," which is mediated by the constructions and deconstructions of attorneys and judges. [1,2]

Judges' interpretations of their gatekeeping responsibilities under the *Daubert* trilogy have imposed more objective and stringent requirements (relevancy, legal sufficiency, and reliability) for the admissibility of some kinds of evidence. Policymakers have also responded to calls from forensic practitioners, academicians, and government agencies by conducting research, increasing funding for studies to establish the reliability and validity of forensic science methods and conclusions, as well as establishing working groups and training to facilitate improvements in the field.

This presentation presents a summary of findings from an empirical content analysis of published judicial decisions concerning cases in which forensic evidence was challenged following the 1993 *Daubert* decision. The purpose of this study of case law is to empirically examine patterns of cases and the variety of factors that judges discuss when describing the reasons for their admissibility decisions, and to investigate how these challenges have occurred in their social, legal, and political context.

Results of a content analysis of published federal district court cases in which the admissibility of expert testimony is at issue will be reported. Prior to coding, the identified cases were evaluated for their coding suitability using guidelines adapted from those developed by Dixon and Gill.[3] Codable cases contained a substantive discussion of the admissibility of proffered testimony that included the rule(s) of evidence relevant to the analysis, and a substantive discussion of how the evidence met or failed to meet the criteria for admissibility. Admissibility will be examined in the context of *Daubert*, *Kumho*, *Joiner*, revision of FRE 702, and the 2009 NAS Report. The unit of analysis is an element of evidence within the opinion. Coding guidelines (e.g., mutually exclusive and exhaustive categories) established by Holsti are employed.[4] All cases were coded, check-coded, and check-verified to increase coder reliability.

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J23 A Review of the Almegciga et al. v. Center for Investigative Reporting, Inc. et al. Decision

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The goal of this presentation is to acquaint attendees to the criteria used in the exclusion of an expert witness in a case involving handwriting. The need for this knowledge by the legal community of training requirements for forensic document examiners will be discussed.

This presentation will impact the forensic science community by addressing the proper training requirements of forensic document examiners and discussing how document examination meets the *Daubert* criteria.

This presentation is a case study of the judicial decision in Erica Almegciga against Center for Investigative Reporting, Inc., Univision Communications, Inc., Univision Noticias, Bruce Livesay, Josiah Hooper issued on May 6, 2016 by the Honorable Jed S. Rakoff in the United States District Court Southern District New York. Judge Rakoff's ruling in the *Daubert* Hearing excluded the testimony of the plaintiff's handwriting expert. This presentation will discuss the events that occurred in this case as well as testimony given by plaintiff and defense witnesses.

The *Daubert* Hearing was subsumed with the defendant's Rule 11 motion to determine whether the plaintiff's handwriting expert should be allowed to testify in trial. The purpose of a *Daubert* Hearing is to determine: (1) Whether the technique or theory can be tested (falsification); (2) Whether there are existence and maintenance of standards; (3) Error rate; (4) Peer review; and, (5) General acceptance and other factors determined by the Court.

A *Daubert* Hearing grants the judge flexibility to determine whether the science meets Rule 702 requirements of reliability and relevance ("fit"). To reach a proper decision, testimony is given to explain how the science meets each of the *Daubert* criteria. In this case, testimony evidence was not provided by the plaintiff to establish how forensic document examination satisfies each of the five prongs of *Daubert*. The defense produced opposing expert testimony from a witness who discounted the claims of forensic document examination as a reliable and relevant science. The result of the testimony left a very one-sided view of the science of forensic document examination. Since a judge is to consider the evidence provided in court, Judge Rakoff was left to base his decision only on the testimony of the defense witness and a Plaintiff's witness whose qualifications appeared not to be contested by the parties, and, therefore, not scrutinized by the court.

The defense expert is a college professor and an attorney. He states he has conducted a thorough reading of the forensic document expertise and has concluded it is baseless. From his readings, he testified to the shortcomings of forensic document examination and believes it should not be allowed in the courtroom. This presentation will explore the accuracies of the main points proffered by the defense expert.

Forensic document examination (also referred to as Questioned Documents) has been a section of the American Academy of Forensic Sciences since the inception of the organization. Criteria for membership to the Questioned Documents Section are in alignment with educational and training requirements listed in ASTM E2388-11 *Standard Guide for Minimum Training Requirements for Forensic Document Examiners*. The accepted methodology for the examination of handwriting is also published in ASTM E2290-07a *Standard Guide for Examination of Handwritten Items*. Practitioners who meet the criteria for membership in the Questioned Documents Section conduct examinations using methodologies that meet each of the five *Daubert* criteria. This presentation will also discuss the numerous research projects conducted by members of academia, either separately or in partnership with an examiner, who have established forensic document examination, as well as the practitioners who satisfy AAFS membership requirements meet the *Daubert* standards.

***Daubert* Criteria, Admissibility, Handwriting Evidence**

J24 The Background, Training, and Experience of Questioned Document Examiners: Present Practice and Future Directions

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After attending this presentation, attendees will understand some of the principles of evidence-based learning as it relates to creating valid and reliable training in the field of questioned documents. Attendees will learn: (1) how to identify knowledge, skills, and abilities relevant to the field; (2) how to set training goals and objectives; (3) how to design training exercises that produce measurable results; (4) how to create assessments that demonstrate trainee success; and, (5) how to create benchmarks for determining competency.

This presentation will impact the forensic science community by demonstrating the importance of creating systematic and standardized training programs with empirical and measurable benchmarks demonstrating mastery and competence of trainees and by demonstrating methods and procedures that can be used to strengthen and evaluate training in a variety of forensic disciplines.

Current efforts to strengthen the reliability and validity of the methods and conclusions of various forms of pattern evidence and to validate the training methods of forensic practitioners have led to attempts to create standardized training programs that measure trainee progress and mastery, and are of consistent content, quality, and length. Efforts such as the National Institute of standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) and working groups such as the NIST/National Institute of Justice (NIJ) Expert Working Group on Human Factors in Handwriting Examination were created to address needs for training, education, research, lab management, and a variety of other issues that are important in moving the field forward and ensuring that current practices are demonstratively the best practices.

Evidence-based practices are those based on significant and reliable evidence derived from empirical evaluation of training methods and procedures. Evidence-based practice is employed in classroom settings and policy making to ensure that learners receive the greatest possible benefit from learning experiences.

This paper will present the results of a recent survey of the education and training background of 97 professional document examiners, highlighting their views on current training and suggestions for improvement. The current movement toward an educational model of training standards and practices will be discussed.

Further discussion will center around creating training programs that incorporate the identification of relevant knowledge, skills, and abilities; creating measurable and objective course goals; specifying learning objectives that incorporate introductory, intermediate, and mastery level goals; creating valid and reliable measures of learning; and, creating objective and measurable benchmarks for determining training effectiveness. This discussion will include information about identifying constructs to be measured, how to measure the reliability and validity of assessment techniques and constructing standardized tests and measures.

Additionally, information is presented about designing evaluation research to investigate the extent of success of training programs. This discussion will include the importance of properly-conducted evaluation research which will provide both feedback to forensic trainers and empirical data to inform evidence-based practice.

During this presentation, there will be an interactive discussion with attendees to demonstrate how to create training module objectives, to establish trainee learning objectives, to identify various skills and training techniques, to create assessment measures which will operationalize learning outcomes, and to identify and operationalize observable and measureable benchmarks to demonstrate trainee proficiency on training tasks.

Finally, a discussion will be facilitated among the attendees about the strengths and weaknesses of time-based compared to competency-based training programs.

Training, Evaluation, Measurement

J25 Minimizing Cognitive Bias in Forensic Document Examination

Jane A. Lewis, MFS, 544 E Ogden Avenue, Ste 700-289, Milwaukee, WI 53202*

After attending this presentation, attendees will better understand how to effectively minimize cognitive bias in forensic document examination and will be aware of practical methods to implement prevention of receiving biasing information in everyday casework.

This presentation will impact the forensic science community by: (1) focusing awareness on how attorneys and investigators inadvertently transfer biasing case information to the forensic document examiners with whom they interact; and, (2) determining whether or not this information adversely affects case results. The forensic science community will be made aware of previous flawed studies that used small sample sizes and college students "trained" to be forensic document examiners. The results of these flawed studies have been applied to the work of forensic document examiners.

Bias in forensic sciences is the topic *du jour*. Critics of forensic science suggest that forensic scientists risk mistakes in their work due to cognitive bias. A brief literature review of bias in forensic document examination will be summarized. Cognitive bias results when people steadily depart from rational decisions. Psychological studies have found that cognitive bias can be unconscious and forensic scientists may not be immune. Forensic document examination is the application of science to questions concerning documents. Documents that typically come into question include: wills, trusts, partnership agreements, personal guarantees, loan documents, change of beneficiary forms for life insurance policies and retirement accounts, checks, bank robbery notes, suicide notes, nomination papers, and threatening letters. Forensic document examiners wish to minimize cognitive bias in their work. First, the problem needs comprehension. Minimizing cognitive bias in forensic document examination is sensible and has already been addressed in at least one government laboratory in Australia. Government laboratories can introduce policies to limit contextual information, like a suspect's confession in a police report. Changes to the case submission forms have also been established to limit biasing case information. These procedures may come at a cost of time and money to the laboratories, but can have a valuable impact on potential bias. Suggestions for minimizing cognitive bias in civil cases will be described in detail. In forensic document examination, this can be accomplished with a few useful remedies easily incorporated in the everyday work of the expert. Professional societies' codes of ethics can be amended to include changes forbidding attorneys and investigators who submit the majority of civil and criminal cases from sharing biasing information. Phone scripts and impartiality memos can also be used to caution attorneys and case submitters to refrain from conveying potentially biasing case information to the expert with whom they work. Training in cognitive bias should be part of the general training for investigators, attorneys, and not only forensic document examiners but all forensic practitioners.

Cognitive Bias, Forensic Document Examination, Impartiality

J26 Forensic Document Examination in International Humanitarian and Human Rights Cases

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After attending this presentation, attendees will better understand, via examples, how forensic document examination can play a role in international humanitarian and human rights investigations. Attendees will also be made aware of some of the operational challenges that may be faced in such work and how these may be mitigated.

This presentation will impact the forensic science community by increasing attendee awareness of the role that forensic document examination has in such work. Forensic document examination is not always thought to play a role in such investigations/cases and this presentation is meant to inform those who may not have considered this previously.

Forensic document examination may not be the first forensic science that comes to mind in matters of international humanitarian law (IHL) and human rights investigations. Anthropology, forensic pathology, odontology, scenes of crime, DNA, and related fields are sometimes thought to be the only specializations that have relevance to such investigations.

Yet even a limited review of the jurisprudence (judgements and trial transcripts) of international tribunals and courts such as the International Criminal Tribunal for Rwanda (ICTR), the International Criminal Tribunal for the former Yugoslavia (ICTY), the Special Court for Sierra Leone (SCSL), and the International Criminal Court (ICC) disclose many examples of disputed documents.

These disputed documents cover the range of forensic document evidence including handwriting, signatures, stamp impressions, and other issues. Forensic document examination may assist in addressing the question of command or superior responsibility, for example who did or did not order or command persons to carry out certain activities. Documents are often involved; either directly in the activities that may constitute serious violations of international law, or indirectly by purporting to account for events. Possibly complicating the situation is that disputed documents are not usually restricted to a crime scene or scenes unlike some forensic evidence which may be concentrated in one locale.

There is also the possibility that document examination has a role to play in the reparation phase of international justice. As an example, the ICC has a mechanism whereby individuals found to be criminally responsible for crimes in the ICC jurisdiction may be ordered to make reparations to the victims (individuals or groups) of the crimes. In this regard, the issue of asset recovery and tracing may be an avenue where document examination has a contribution to make to international justice.

Some of the challenges of conducting document examination in international investigations will be explained with suggestions on mitigating these challenges. Language is an obvious limitation when the script of the questioned documents is one that the document examiner is not familiar with. Language challenges are also present if any forensic report has to be translated into another language, not to mention interpretation done at trial. Given that many of the disputed documents are in the custody of entities that will not provide the originals to the laboratory, copy quality then becomes a significant impediment to many examinations. Against all of this are the general challenges of logistics and security which are intertwined with the all of the above. The suggestions offered may serve as a reference for document examiners called upon for such work.

Document Examination, Humanitarian Law/Investigation, Human Rights Law/Investigation

K1 Alcohol and Psychotropic Drugs in Traffic Accident Fatalities in Calabria, Italy, From 2006 to 2015

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After attending this presentation, attendees will better understand the epidemiological impact of Motor Vehicle Crashes (MVCs) in southern Italy and will be aware of the characteristics of traffic accident victims and the link between fatal crashes and alcohol/psychotropic drug consumption (including the role of drug-alcohol and drug-drug combinations in the risk of accidents).

This presentation will impact the forensic science community by illustrating the role of alcohol/psychotropic substances in causing traffic accidents in order to guide driver education programs and improve public safety.

MVCs due to impaired driving are a leading cause of preventable injury and death. Alcohol is involved in approximately 1/3 of serious MVCs resulting in severe injury or death. In the United States, the 2007 National Roadside Survey found that the percentage of drivers who were using drugs (14%) was greater than the percentage who were using alcohol (12%).[1]

Alcohol and drugs are significant risk factors for traffic deaths in Italy. In 2012, 1.8% of checked drivers were found to be impaired by alcohol or drugs. The current Blood Alcohol Content (BAC) limit in Italy, which became law in 2002, is 0.5g/l. Since July 2010, there is zero tolerance for young drivers, novice drivers, and professional drivers for whom the BAC limit is equal to 0.0 g/l. In 2008, ISTAT (National Institute for Statistic) indicated that 2% of traffic fatalities were due to drunk driving. However, this figure is probably underestimated, due to the difficulty involved in collecting this information at the crash scene and also because the autopsy is not required in all cases of fatal crashes.[2]

In this paper, we analyzed the characteristics of road crash victims, paying special attention to their toxicological findings. We have explored the relationship of drunk and drugged driving and the two combined in fatal MVCs. To accomplish this, a population represented by subjects who died in road accidents in the southern Italy (Calabria) from 2006 to 2015 was analyzed.

Data were limited to fatally injured drivers that were tested with autopsy and toxicology tests, using a tiered approach. Out of the 185 fatally injured drivers with toxicological tests information, about 33.5% tested positive for drugs and/or alcohol (n=62). In this study, a three "level alcohol" scale was built based on the classification found in the article n.186 of the Italian Traffic Code: mild (0.5> BAC <0.8 g/L); moderate (0.8>BAC<1.5g/L); and, severe (BAC>1.5g/L). Thirty-nine subjects were positive having BAC values over the legal limit: mild-level-14.5%; moderate-19%; and, severe-29%. Thirteen subjects tested positive for psychotropic substances such as drugs (cocaine, THC, heroin, amphetamines, opiates, benzodiazepines, barbiturates) and their metabolites. Finally, ten victims had high levels of both alcohol and psychotropic substances.

Cases were also analyzed by age and gender to capture different age-based patterns of drug/alcohol consumption as well as gender variations in risk taking. The proportion of men with positive toxicological analysis exceeded that of women: 90% of the victims were males and 10% were females. Furthermore, the results were highlighted to the most frequently represented age group among the road victims, dividing the sample in positive and negative toxicology tests. In conclusion, alcohol and psychotropic drugs indicate a significant risk factors for MVCs.

According to the results of the study, it is appropriate that the existing alcohol-related traffic laws in Italy become stricter.

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Fatal Crashes, Toxicology, Public Health

K2 An Analysis of Cannabinoids in Whole Blood

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After attending this presentation, attendees will better understand how to effectively use protein precipitation and 2D Ultra High-Performance Liquid Chromatography/Mass Spectrometry (2D-UHPLC/MS) for the analysis of Δ^9-Tetrahydrocannabinol (THC), 11-OH-Δ^9-Tetrahydrocannabinol (THC-OH), and 11-nor-9-carboxy-Δ^9-Tetrahydrocannabinol (THC-COOH) in whole blood.

This presentation will impact the forensic science community by illustrating how combining a very simple sample preparation with a 2D-LC analytic approach in UHPLC allows for reaching the required sensitivity cut-offs with high-throughput capacity and robustness. This method claims to be a very interesting substitute to a classic technique based on Gas Chromatography/Mass Spectrometry (GC/MS) for the quantitation on cannabinoids in whole blood.

Hypothesis: Protein precipitation and 2D-UHPLC-MS provides effective clean-up of whole blood to analyze THC, THC-OH, and THC-COOH in a semi-automated way.

Methods: Sample Preparation – 100µL of whole blood (or calibrators, quality controls and unknowns) are mixed with 20µL of internal standard mixture in methanol (containing deuterated analytes). The protein precipitation step is done by the addition of 200µL of cold acetonitrile, 0.1% formic acid. After a ten minute incubation in the freezer, samples were centrifuged for five minutes at 13,000rpm. Next 200µL of supernatant were mixed with 200µL of water (0.1% formic acid) and were analyzed. The injection volume for each sample was defined at 100µL. **UHPLC Conditions –** Chromatographic separation was achieved using two Kinetex™ columns (PFP, 5µm and XB-C18, 5 µm). A Sciex 4500 QTRAP® was used for the MRM detection with an electrospray interface in positive/negative ionization mode. The run time of 2D-UHPLC program with equilibration was optimized at ten minutes using a solvent gradient of water 0.1% formic acid and methanol 0.1% formic acid.

Results: The sample preparation consists of a simple pre-treatment step using precipitation of whole blood samples, followed by dilution and injection. UHPLC methodology consists of a water/methanol mobile phase with an efficient 2D-LC gradient on Kinetex™ columns. Limits of quantitation were obtained below 0.5ng/mL for THC and its metabolites. Real samples were analyzed by this workflow. Results from a negative and a positive sample will be shown. Results are based on spiked standard curves in drug-free whole blood. The calculated concentrations for the positive sample were: 1.4ng/mL for THC, <0.5ng/ml for THC-OH, and 3ng/mL for THC-COOH.

Conclusion: Cannabinoids in whole blood analysis has been demonstrated on a 2D-UHPLC system, coupled to the SCIEX 4500 QTRAP® Mass spectrometer. Analysis is sensitive to detect concentration below 0.5ng/mL for each compound with Signal to Noise (S/N) greater than 10:1 (based on 3SD of the noise).

This method uses only 100µL of whole blood without offline solid phase extraction or liquid-liquid extraction. The short runtime allows for a high throughput whilst maintaining chromatographic performance batch after batch. No cross contamination was observed between the highest sample concentrations to the negative samples.

2D-UHPLC/MS, THC, Whole Blood

K3 The Distribution of 11-Nor-9-Carboxy-Tetrahydrocannabinol (THC-COOH) in Korean Drug Abusers' Hair

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After attending this presentation, attendees will better understand the recent trend of cannabis abuse in Korea and the distribution of THC-COOH in drug abusers' hair.

This presentation will impact the forensic science community by providing statistics of the distribution of THC-COOH in drug abusers' hair that help explain the current state of cannabis abuse of Korea via forensic toxicology.

Introduction: Cannabis is the second most commonly abused drug in Korea following methamphetamine. Δ^9-Tetrahydrocannabonol (THC) is the primary psychoactive constituent of cannabis. The metabolite of THC, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) can be detected in hair of cannabis users. The National Forensic Service (NFS) has performed THCCOOH analysis in the hair of drug abusers since November 2009; resulting in about 1,500 hair samples submitted every year for cannabis analysis to NFS. In this study, the distribution of THCCOOH in the head hair of Korean drug abusers during the years 2014 and 2015 was investigated.

Method: Possible contaminants on the surface of hair samples were eliminated by washing twice with 2mL methanol and distilled water, sequentially. Hair samples (about 20 mg) were then digested with 1M NaOH, extracted with mixed organic solvents (n-hexane:ethyl acetate), and subsequently analyzed by LC-MS/MS. THCCOOH-d_3 was used as internal standard. Identification and quantification of THCCOOH and THCCOOH-d_3 were made in multiple reaction monitoring (MRM) mode at m/z 245, 191, and m/z 248, respectively (quantifier ions are underlined). The limit of detection (LOD) was 0.05pg/mg and the limit of quantification (LOQ) was 0.10pg/mg.

Results: A total of 2,932 cases were submitted to NFS for cannabis analysis in hair (including body hair) of drug abusers from 2014 to 2015. Among them, 851 cases (30% of total cases) were positive for THCCOOH. The concentrations of THCCOOH in head hair (no. of cases=776, no. of segments=837) ranged from 0.10pg/mg to 235.9pg/mg (average=3.34pg/mg, median=1.05pg/mg). Of these cases, 746 cases (96%) were males and 30 cases (4%) were females between 16 and 68 years of age (average=42). Using statistical analyses, concentrations of THCCOOH in 776 samples of head hair were classified as low, medium, or high ranges (i.e., 0.10pg/mg-0.42pg/mg, 0.42pg/mg-3.25pg/mg, and 3.25pg/mg-235.9pg/mg, respectively). The concentrations of THCCOOH in both the head and pubic hair from the same individuals were compared in 15 cases. In 13 of these cases, the THCCOOH concentration in pubic hair was on average 5.3 times higher than that in head hair. Segmental analysis of each hair was performed at 3 cm intervals in 19 cases (with hair more than 9cm in length), and concentrations of THCCOOH gradually decreased from the root to the shaft in 12 cases.

Conclusions: Within the last two years, the detection rate of THCCOOH in the hair of drug abusers was as high as 30%, proving that cannabis abuse in Korea is just as serious as methamphetamine. Further investigation of the distribution of THCCOOH in hair will be useful in understanding patterns of cannabis abuse in Korean society.

THC-COOH, Hair Analysis, LC/MS/MS

K4 A Study of an Active-State Cannabinoids 1 (CB1) Receptor Model and Synthetic Cannabinoid Interactions

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After attending this presentation, attendees will better understand the interactions of select classes of synthetic cannabinoids and their metabolites with a CB1 receptor model.

This presentation will impact the forensic science community by contributing to the understanding of essential interactions between specific substituents of different classes of synthetic cannabinoids with specific CB1 receptor residues through molecular modeling.

Synthetic cannabinoids have emerged onto the drug scene as an alternative to marijuana.[1] Similar to Δ^9-Tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana, synthetic cannabinoids interact with G-coupled protein receptors found in the brain, immune system and peripheral organs.[2] Two cannabinoid receptor subtypes have been identified, CB1 and CB2. The binding of THC and synthetic cannabinoids to the CB1 receptor is believed to be the cause of the psychoactive effects due to the CB1 receptor's location in the brain.

In this study, an active-state CB1 receptor model proposed by the Doerksen lab was used to compare the ligand-receptor interactions between the CB1 receptor and the various families of synthetic cannabinoids and the THC compound. This study was done using Schrodinger's Maestro molecular modeling program. Synthetic cannabinoids from the different classes were selected based on their affinity to bind to the CB1 receptor. The docking of the ligands to the receptor took place after both the synthetic cannabinoid ligands and CB1 receptor model were prepped for docking and a grid of the active site was generated. In order to increase understanding of the interactions between cannabinoids and the CB1 receptor, parameters were set to give the five best possible poses, or positions, for the ligand interacting with the receptor. Once the ligands were docked to the CB1 receptor model, the interactions were analyzed. The information collected from this study included the amino acid residue interaction with the ligands and the bond distances of these interactions, the docking score of each ligand and each pose, and ligand Ki values. This study was also able to show more specific information pertaining to these interactions such as the presence of Pi-Pi stacking, hydrophobic residue interactions, charged or polar residues, and solvent exposure.

Results from this study show the potential of revealing key residue interactions with the CB1 receptor and how the interactions vary by class and chemical structure within classes. Identifying the key interactions between the synthetic cannabinoids and the CB1 receptor has the potential for a better understanding of the effects of these drugs, including toxicity and potential for abuse. A computer program database could be developed to help predict new structures and different classes of synthetic cannabinoids that have not previously been identified. Future research will include studying more classes of synthetic cannabinoids and other synthetic drugs along with the metabolites of these substances.

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Synthetic Cannabinoid, CB1 Receptor, Molecular Modeling

K5 An Evaluation of Cannabinoid 2 Receptor and Endogenous Cannabinoid 2-Arachidonylglycerol in the Central Nervous System

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After attending this presentation, attendees will better understand conclusions drawn during drug and toxicology-based testing. Cannabinoids (CBs) are constituents of marijuana (phytocannabinoids) and marijuana-like compounds, which are endogenously or synthetically produced.

This presentation will impact the forensic science community by the elucidation of cannabinoid receptor 2 (CB2R) brain expression as revealed by the interdisciplinary approaches of immunocytochemistry, Western blot, and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

This presentation evaluates results generated in an *in vitro* study regarding analysis of endogenously produced cannabinoids and CB2R expression in the astrocyte using Western and liquid chromatography tandem mass spectrometry LC/MS/MS analysis. This research could impact the forensic science community in relation to evidence of CB2R's upregulation and potential targeting in the central nervous system. Cannabinoids (CBs) are constituents of marijuana (phytocannabinoids) and marijuana-like compounds which may be endogenous, or synthetically produced.[1,2] When CBs bind to cannabinoid receptors, they are able to produce their effects. Cannabinoid receptor 1 (CB1R) as opposed CB2R, generates negative psychoactive manifestations such as short-term memory loss, hallucinations, and impaired motor function.

Botanicals, including marijuana, contain over 460 known compounds. Approximately 60 of these compounds are unique substances to cannabis named cannabinoids.[3] Evidence shows that these compounds are helpful during neurodegenerative disease states which impair the normal functions of neuronal activity. In the brain, astrocytes provide neurotrophic and metabolic support for neurons facilitating their ability to function properly. Therefore, the study of astrocytes is important to ensure proper protection of neurons in disease states. Astrocytes take on the morphological change represented in fibrous astrocytes when immersed in an inflammatory state named reactive astrogliosis.[4] During this period of reactivity, the astrocyte produces a number of pro-inflammatory cytokines and anti-inflammatory cytokines. These cytokines both help and at times cause damage to the cell in an attempt to return to its homeostatic state. It is unclear whether astroglial cells undergoing gliosis produce anti-inflammatory responses that are expressed via a CB2R-mediated pathway. Astroglial-mediated neuroprotection would be potentially invaluable since astroglial cells outnumber neurons and microglia in brain. Up-regulation of existing CB2R in this type of cell population would enhance the neuroprotection that is desired. This study aimed to solidify the expression of CB2R in astrocytes to establish its potential use with naturally occurring cannabinoids while avoiding psychoactivity, caused by activation of CB1R.

Sprague-Dawley rats were harvested at one to three days old and their brains utilized for cell culture. Astrocytes were then isolated from microglia and neurons then plated in pure astrocyte cultures in 35mm and 100mm dishes. Subculture of astrocytes up to passage three (p3) was performed before experimentation. Lipopolysaccharide (LPS) was added to samples at 0, 0.010µg/mL, 0.10µg/mL, 1.00µg/mL, and 10.0µg/mL. Quantitative analysis of CB2R was implemented using Western blot. LC/MS/MS quantitative experimentation was executed following liquid-liquid extraction of lipids from our homogenized in vitro samples to detect endocannabinoid 2-arachidonoylglycerol (2-AG) concentrations. Concentrations of 2-AG increased dose-dependently by 12% at 1.0µg/mL of LPS. CB1R was identified but there was no change in concentration compared to the control. CB2R was detected at a dose-dependent 45% increase in rat cortical astrocytes (RCAs).

These findings imply that astrocytes express CB2R protein and endogenous cannabinoid 2-AG during inflammation in astrocytes. 2-AG was readily profiled using LC/MS/MS analysis. Quantitative analysis of both CB2R and 2-AG lead to implications that ligand-receptor binding of the cannabinoid system, specifically via CB2R signaling, can be explored in the astrocyte. Therefore, it is pertinent that in prospective studies specification of

CB2R is targeted. Despite the controversial recent findings of CB2R in astrocytes, we have shown that they are, in fact, present in RCAs. This is very beneficial to brain tissue that has undergone any type of traumatic insult regarding its functionality. It is potentially an implication for isolating modulation of CB2R so that its anti-inflammatory properties could be taken advantage of, providing a novel route to anti-inflammation. Moreover, cannabinoid ligands are an ideal route of administration in that they bypass the psychoactive side effects that are characteristic of CB1R binding and activation.

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Astrocyte, Cannabinoid Receptor, Endogenous Cannabinoid

K6 The Results of Analysis of 6-Monoacetyl Morphine (6-MAM) in Operating Under the Influence (OUI) Drug and Driving Cases and Drug-Facilitated Crimes (DFC) in the Commonwealth of Massachusetts During a Five-Year Period

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After attending this presentation, attendees will better understand the extraction and analysis method for 6-MAM from opiate-positive urine cases involving both drug/driving and drug-facilitated cases employing commercially available Solid Phase Extraction (SPE) cartridges, then analyzing the samples by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

This presentation will impact the forensic science community by offering analysts working in forensic facilities information regarding the extraction and analysis of 6-MAM in urine samples obtained in OUI cases and comparing the results with those obtained from DFC cases using SPE and LC/MS/MS. This compound is used as a confirmatory biomarker for recent heroin (diacetyl morphine) use (i.e., within one hour of administration). It is known that heroin is quickly metabolized in the human body to morphine and its glucuronides via the production of 6-MAM. The forensic toxicology community recognizes that 6-MAM may not be detected in whole blood samples, while it is able to be confirmed in urine samples in the same case. The information presented will allow analysts to differentiate opiate use (morphine, codeine) from heroin administration in populations of OUI and DFC cases.

Method: Prior to analysis, samples of patient urine (>10mL) were collected at local hospitals by medical staff using forensically sealable containers. The samples were transferred under chain of custody via police agencies to the laboratory where they were stored in unpreserved conditions at 4°C. For analysis, 1.0 mL samples of urine (calibrators, controls, and test samples each containing deuterated internal standard (6-MAM-d_3)) were diluted with pH 6 buffer. The samples were each applied to conditioned mixed mode SPE columns. Each SPE column was washed with DI H_2O, aqueous acetic acid, and methanol, then dried. Each SPE column was eluted with of a solution consisting of methylene chloride-isopropanol-ammonium hydroxide (78-20-2) and the eluates were dried under a gentle stream of nitrogen. The dried residues were dissolved in 100µL of mobile phase for analysis by LC-MS/MS. Tandem mass spectrometry was performed in positive multiple reaction monitoring mode (MRM). Liquid chromatography was performed using a polyaromatic column in gradient mode with a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid at a flowrate of 0.55mL/minute. The following MRM transitions were monitored (quantification transition ions underlined): 6-MAM (328.1 to <u>165.1</u> and 211.1), 6-MAM-d_3 (331.2 to <u>165.0</u> and 211.3), respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of 6-MAM in both OUI and DFC cases.

Results: The limits of detection/quantification for this method were determined to be 0.5ng/mL and 1.0ng/mL for 6-MAM. The method was found to be linear from 1.0ng/mL to 1,000ng/mL (r^2>0.999) for 6-MAM. The analyte recoveries were found to be >95% for 6-MAM and 6-MAM-d_3. Interday/Intraday variation of the method was found to be <8% and <10%, respectively. Matrix effects were determined to be <6%. Details regarding the concentrations of 6-MAM found in five years' worth of genuine OUI cases ranged from: 178 Males: mean age=42 years (45ng/mL-365ng/mL: median=275ng/mL); 82 Females: mean age=29 years (23ng/mL-137ng/mL: median=82ng/mL). With respect to DFC cases, 31 Males: mean age=35 years (11ng/mL-93ng/mL: median=45ng/mL); 130 Females: mean age-24 years (5ng/mL-67ng/mL: median=18ng/mL).

Conclusion: The data obtained in this study compares the concentrations of the biomarker of heroin use (6-MAM) found in the urine of opiate positive cases obtained from two populations of samples often presented to forensic toxicology laboratories. The information obtained from the analysis of these antemortem samples (in terms of 6-MAM concentrations) can offer analysts involved with OUI and DFC cases valuable information regarding the previous administration of heroin (diacetylmorphine) by the subjects vs regular opiates (morphine/codeine). It is accepted by the forensic toxicology community that the presence of 6-MAM is an indicator of heroin being used by subjects within a short period of time of administration. This information enables the toxicology analysts to offer submitting agencies more appropriate interpretation regarding the ingestion of heroin.

Heroin, LC/MS/MS, SPE

K7 The Identification of the Pyrolytic Products of Two Drugs of Abuse: Heroin and Fentanyl

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After attending this presentation, attendees will: (1) better understand the developed procedure for identifying pyrolytic products; (2) be aware of new analytes for heroin and fentanyl to be used in analysis; and, (3) have an insight into the possibility of exploration into the toxicity of pyrolytic products.

This presentation will impact the forensic science community by providing additional target analytes of heroin and fentanyl while providing insight into possible additional origins of toxicity.

Overdoses due to heroin and fentanyl have increased in recent years. Heroin is an opioid pain killer commonly abused for its euphoric effects. Side effects of heroin include difficulty breathing, nausea, vomiting, and decreased heart rate. Fentanyl is also an opioid used to treat severe pain, typically after surgery or during chemotherapy. Heroin is approximately ten times as potent as morphine while fentanyl is approximately ten times as potent as heroin. As a result, fentanyl overdoses can occur at much smaller doses than with heroin. Recently, cases have been reported in which heroin has been cut with fentanyl. This poses danger to both experienced and naïve users as they are unaware of this combination. Susceptibility to overdose is increased as they ingest a nonlethal dosage of heroin, but are unknowingly ingesting a lethal dose of fentanyl.

There are several modes of ingestion in which people abuse these drugs, but when inhaled, increased risk to the users may arise. Smoking delivers the drug directly into circulation without first-pass metabolism, which increases the risk of sudden death. In addition, the smoking process itself could produce acutely toxic products that have yet to be identified as the parent compounds thermally degrade into pyrolytic products that often differ from the metabolites. The toxicity of these unique pyrolytic products is not well understood. Accordingly, the goal of this project was to characterize the pyrolytic products of heroin and fentanyl, compare to literature findings, and determine if there are any unique products that are formed in the presence of both compounds.

A previously developed procedure was utilized to pyrolyze heroin and fentanyl individually and combined. Samples were collected using a methanolic extraction from the residue and glassware. Samples were evaporated over nitrogen gas, reconstituted in methanol, and analyzed using gas chromatography/mass spectrometry. The identification of observed products was confirmed with reference standards where available, and any remaining products were tentatively identified via a NIST library search. To date, the following compounds have been confirmed as pyrolytic products of fentanyl: N-phenyl-propanamide, 4-(2-phenylethyl)-pyridine, and quinolone. As for heroin, the confirmed products are as follows: morphine, morphine-3-acetate, 6-monoacetylmorphine, 1-naphthalenol, and quinolone. Pyrolysis of the mixture produced a combination of these products, but no unique compounds were observed. Some of the products are also metabolites which could have important implications for estimation of doses from metabolic products.

Those who view this poster will be able to understand the developed procedure for identifying pyrolytic products, learn of new markers of heroin and fentanyl use to include in analytical procedures, and have an insight into the possibility of exploration into the toxicity of pyrolytic products.

Pyrolysis, Heroin, Fentanyl

K8 Occupational Lethal Asphyxiation by Toxic Gas in Unconfined Spaces: Hydrogen Sulphide (H_2S) Poisoning

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After attending this presentation, attendees will better understand specific aspects of H_2S poisoning, an important cause of work-related deaths. The goal of this presentation is to recount the story of the deaths of two men who, while working in a truck tank which transported leachate, were poisoned by hydrogen sulfide in open air. Variations in pathological and histological findings, coupled with toxicological results and crime scene investigations will be illustrated.

This presentation will impact the forensic science community by emphasizing the fact that H_2S is a harmful and lethal chemical, and accidents may occur with exposure to its natural gaseous state in various work environments.

Introduction: Hydrogen sulphide (H_2S) is a toxic gas generated by non-specific and anaerobic bacterial reduction of sulphates and sulphur-containing organic compounds at temperatures >20°C. Severe or fatal H_2S intoxications are very uncommon in locations which lack the characteristics of confined spaces.

Goals: An accidental death of two workers during the filling of a truck tank with leachate. The first employee (P.R.) at the time of the opening of the hatch of the tank was hit by the gust of gas and fell to the ground outside the tank. A fellow worker (L.F.), went to his aid but also lost consciousness and crashed to the ground.

Methods: Environmental analyses were performed on the air outside the tank with both closed and open porthole; inside the tank at various depths, on liquid samples of the tank, and during the collection of the leachate.

Air monitoring was performed at two hours, twenty days, and seventy days after the incident by using Gastec Color Dosimeter Tubes. Elemental analysis, gas chromatography/mass-spectrometry (GC/MS) analysis and potentiometric titration were performed on the liquid sample. Toxicological analyses on biological samples were performed about two months after the autopsies. Thiosulfate (H_2S metabolite) was quantified using a GC/MS technique after derivatization with pentafluorobenzyl bromide.

Results: Air was collected outside the tanker at various times after the accident and revealed the following gas concentrations: two hours: H_2S=102 ppm and 153ppm; CO=37ppm; flammable vapors: absent; twenty days: H_2S=6 and 19ppm; seventy days after the incident: at closed hatch=35ppm; at open hatch=105ppm. Air monitoring inside the tanker seventy days after the incident detected: H_2S=700ppm and 750ppm, 2-propanethiol, and 2-butanethiol. Analyses performed on several specimens that were taken from the tanker confirmed total sulfur with an almost neutral pH (7.9).

Toxicological Findings: Postmortem blood specimens obtained from the two workers showed:

P.R. (next to the hatch): CO=3.7%; cyanides - negative; alcohol <0.05g/l; thiosulfate venous blood: 4.5mg/l; thiosulfate arterial<0.1mg/l; toxic volatile, non-volatile, and drugs were negative.

L.F. (away to the hatch): CO=2.1%; cyanides - negative; alcohol: <0.05g/l; thiosulfate venous blood: 1.21mg/l; thiosulfate arterial <0.1mg/l; toxic volatile, non-volatile, and drugs: negative.

Conclusions: Despite the possible loss of H_2S from the tanker during rescue operations and the time elapsed from the accident (seventy days), there was still a lethal concentration of the gas present inside the tanker: 700 ppm and 750 ppm.

Prolonged inhalation of this concentration causes sudden loss of consciousness that can lead to fatal respiratory paralysis if rescue operations are not rapid. These environmental findings were confirmed by toxicological analyses through the measurement of thiosulfate, which is one of the main H_2S metabolites.

H2S, Work-Related, Analyses

K9 Arsenic-Fed Piglets: Assessing Heavy Metal Levels in Decomposing Pig Tissues and Soil Samples

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After attending this presentation, attendees will better understand the arsenic levels found in decomposing tissue and affiliated soil samples.

This presentation will impact the forensic science community by developing standards by which arsenic levels can successfully be detected in tissue during decomposition.

The goal of this study is to develop standards by which arsenic levels can be successfully detected in pig tissue during decomposition. An essential goal of a forensic toxicologist is the analysis of biological matrices for the presence of drugs, metals, and other toxins as they assist investigations associated with the potential use of these materials acutely prior to homicidal-death. Currently, there are several methods available for the assessment of heavy metals such as arsenic in human tissue collected during a typical autopsy.[1] However, when one considers examining decomposing tissues these tests are limited in their ability to detect heavy metals like arsenic. A method has been developed that allows the detection of arsenic levels in decomposing pig tissue (hair, skin, muscle, and bone) as well as from soil and insects. The purpose of the experiment is to set minimal expectations of arsenic levels in these tissues. Little data specific to how arsenic might degrade during decomposition is available. Understanding this process would help assess acute-toxic antemortem dosages specific to death events.

Conducting this research and presenting this work begins a conversation specific to heavy metal findings in human tissues that will improve the ability to meet the *Daubert* standards. The experiment began with the feeding of arsenic (potassium arsenate) to four piglets; the feeding of arsenic was at sub-lethal concentrations over a three-day period. The piglets received a total of 2.8mg/kg of arsenic during this time. On the fourth day the pigs were euthanized. The housing and feeding of the pigs during this stage of the experiment was in accordance with Texas Tech University IUCAC procedures. Two additional piglets were not fed arsenic. After euthanization, all six piglets were set out for surface decomposition. In doing so, each pig was placed in a separate cage.

Soil samples were taken before placement and acted as control samples to evaluate if an increase in arsenic levels could be detected in the soil. Tissue samples were also taken from the pigs at the start of this stage of the experiment. Additional tissue samples were taken after ten days, seventeen days, thirty days, sixty-six days, and ninety days. Toxic assessment of these tissue samples began shortly after they were taken. The samples were prepared using standard methods for digesting tissue prior to assessment using a nitric acid/hydrogen peroxide (3:1 ratio) bath. The tissue samples were analyzed in a Thermo FS95 GF95 graphite furnace.[2,3]

Decomposition took place rather quickly due to the arid summer environment in which the pig carcasses were subjected to. By the tenth day, several pigs showed skeletal material denude of soft tissue, specifically at the limbs.[4] The pigs that were dosed with arsenic displayed a faster decomposition rate in comparison to the control pigs. Entomological activity was observed in all carcasses starting on the first day, but by the end of the assessment period the arsenic-dosed pigs showed great evidence of insect activity. Toxicological assessment shows that considerable levels of arsenic concentration were identifiable in all tissue samples. Soil samples also showed an increase in arsenic concentrations. Pre-carcass decomposition soil samples averaged 1.69ppm of arsenic. The average amount of arsenic found in the soils on day ten was 2.57ppm. This observation might be due to soil acidity activity drawing the arsenic from the pig carcass into the soil.[5] There was no significant change in the arsenic concentrations from the soil under the control pigs (Table 1).

Table 1. Soil Samples and Arsenic Levels

Day	n	Range	Mean	SD
Day 1	6	1.46-1.86	1.69	0.159
Day 10	4	1.94-2.95	2.57	0.400

Arsenic concentrations are measured in ppm.

Table 2. Tissue Samples and Arsenic Levels for Day 10

	n	Range	Mean	SD
Control Pigs	2	0.003-0.024	0.013	0.014
Arsenic-Fed Pigs	4	0.017-0.0648	0.037	0.021

Arsenic concentrations are measured in ppm.

This research provides evidence that arsenic concentrations are identifiable in decomposing tissues (Table 2). The data obtained from this study demonstrate that arsenic concentrations will accumulate in the soil beneath a decomposing carcass. In conclusion, this research helps set standard expectations for analyzing arsenic concentrations in decomposing tissues and soil samples.

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Arsenic, Decomposition, Toxicology

K10 Postmortem Identification and Quantitation of Loperamide in the Human Brain

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After attending this presentation, attendees will understand the significance of P-glycoprotein (P-gp) and the potential for toxic Central Nervous System (CNS) effects as they relate to loperamide.

This presentation will impact the forensic science community by providing proof that loperamide does in fact cross the human Blood-Brain Barrier (BBB) when taken at supratherapeutic doses.

Loperamide has been accepted as an effective, over-the-counter anti-diarrheal drug with low abuse potential. It is a synthetic opioid that lacks CNS activity at prescribed doses and originally identified safe because of its inability to access the brain. Several older publications note that loperamide is peripherally-acting and does not cross the BBB. The North Carolina Office of the Chief Medical Examiner (NC OCME) has encountered over 20 cases where loperamide contributed to death by drug overdose.[1] A 34-year-old female found unresponsive in her bed was suspected to be a loperamide-related death. Statements made by the family recognize that she had found an article claiming that Loperamide could possibly help alleviate back pain. One week prior to her death she was taking 40 tablets per day.

When taken in excess, the organic base extraction with gas chromatography-mass spectrometry/nitrogen phosphorus detector (GC-MS/NPD) screening method identifies loperamide followed by a separate technique to confirm and quantify the drug by liquid chromatography-tandem mass spectrometry (LC/MS/MS). While brain is not a specimen routinely obtained at autopsy, the possibility of detecting loperamide in brain may help explain the CNS effects. The quantitation of loperamide in brain was accomplished using a matrix-spiked specimen comparison.

Specimen	Loperamide Concentration
Central Blood (Heart)	Present
Peripheral Blood (Iliac)	0.19mg/L
Vitreous Humor	0.034mg/L
Urine	1.3mg/L
Liver	2.3mg/kg
Brain (medulla)	0.081mg/kg
Brain (other)	0.093mg/kg

Other Drugs	Peripheral Blood (mg/L)	Liver (mg/kg)
Alprazolam	0.070	-
Diphenhydramine	1.5	5.7
Duloxetine	0.24	2.0

Loperamide is a known substrate for the efflux protein P-gp, which appears to be instrumental to loperamide behaving as a centrally-active substance. P-gp is expressed at the BBB, resulting in the ability to translocate potentially toxic substances out of the brain. While taking an excess of loperamide may overwhelm the efflux transporter at a large enough concentration, taking it in combination with a drug that inhibits its action will heighten the likelihood of adverse events, such as overdose or death. In this case study, the decedent was also taking duloxetine, a known P-gp inhibitor.

The case was ruled an accidental drug poisoning from loperamide, alprazolam, duloxetine, and diphenhydramine intoxication. While several animal studies have suggested the possibility of loperamide crossing the human BBB, the postmortem brain tissue from this decedent suggests that loperamide does in fact act on the central nervous system, contrary to early literature studies.

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Loperamide, Human Brain, Postmortem

K11 Suicide After 25C-N-Methoxybenzyl (25C-NBOMe) and 25H-N-Methoxybenzyl (25H-NBOMe) Consumption

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After attending this presentation, attendees will understand the importance of developing new methods for the determination of New Psychoactive Substances (NPS) in biological matrices as the use of these drugs of abuse may lead to tragic consequences.

This presentation will impact the forensic science community by providing information on NBOMes and on NPS intoxication in general that represents an emerging problem; as such, forensic toxicology laboratories should improve routine methods for the detection of these new drugs of abuse. NPS in biological matrices should be routinely determined in forensic toxicology investigations, since the use of these drugs of abuse may lead to tragic consequences.

A 16-year-old male was found dead in a waterway after he was seen jumping into the water stream. The boy was seen deeply agitated and confused after having attended a party with friends. At the party location, policemen seized several doses of marijuana as well as pieces of blotter paper. A complete autopsy and a histological evaluation of the main tissues were performed; though the death occurred by drowning, toxicological exams were requested by the prosecutor in order to evaluate the potential role of drugs of abuse in this death. Blood (peripheral and central) and urine samples as well as blotter papers were collected and analyzed as follows.

The blotter paper was soaked into 500µL methanol and sonicated up to five minutes. An aliquot of the solution was directly injected into a gas chromatograph coupled with mass spectrometer (GC/MS), while 50µL of the same solution were taken to dryness and, after derivatization with pentafluoropropionic anhydride (PFPA), reconstituted in ethylacetate and injected into the GC/MS. The two analyses of the blotter paper revealed the presence of substances belonging to the 25-NBOMe family. The N-methoxybenzyl (NBOMe) derivatives are emerging psychedelic drugs, with severe hallucinogenic effects that can occur even after an intake of 50µg-250µg of the substance.

A liquid chromatography tandem mass spectrometric (LC/MS/MS) system was used to identify and quantify five different 25-NBOMes (namely: 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25H-NBOMe, and 25I-NBOMe) in blood and urine. 25E-NBOMe was used as internal standard, since at the time of the death, this NBOMe was not yet available. 1mL of urine and 1mL of blood (peripheral and cardiac) were diluted in 2mL phosphate buffer containing IS and purified on a solid phase extraction (SPE) cartridge. The elution solvent was dried under nitrogen stream and reconstituted in 200µL mobile phase. The chromatographic separation was performed on a C18 column in gradient elution. Two Multiple Reaction Monitoring (MRM) transitions were monitored for each compound, in positive polarization. Calibration curves ranged from 0.2-5.0ng/mL with LOD and LOQ for the five 25-NBOMes calculated at 0.05ng/mL and 0.1ng/mL respectively. Linearity, accuracy, precision, ion suppression, carry over, and recovery were tested and fulfilled the laboratory's acceptance criteria.

Blood and urine provided positive results for 25C-NBOMe and 25H-NBOMe are as follows: 2.80ng/mL and 0.29ng/mL in peripheral blood; 1.43ng/mL and 0.13ng/mL in central blood; and finally 0.94ng/mL and 0.14ng/mL in urine respectively. Eventually, the seized blotter papers were tested with LC-MS/MS and the presence of both 25C- and 25H-NBOMe was confirmed. THC and THCCOOH were also found in the biological fluids: 15.5ng/mL

and 56.0ng/mL in peripheral blood, 9.9ng/mL and 8.5ng/mL in central blood respectively, and urine THCCOOH (9.5ng/mL).

Though the boy was certainly under the influence of THC at the time of his death, the concentration of cannabinoids in the biological fluids is not consistent with the symptoms (agitation, hallucinations, and confusion) just before the incident. On the contrary, NBOMes can produce severe hallucinations even at very low doses, and 25C-NBOMe is considered potentially toxic at the levels measured in this boy's blood.

This presentation will impact the forensic science community by improving information on NBOMes and generally on NPS intoxication that represent an emerging problem. Therefore, forensic toxicology laboratories should improve routine methods for the detection of these new drugs of abuse.

Forensic Toxicology, 25C-NBOMe, 25H-NBOMe

K12 An Analysis of Illicit Substances From Postmortem Samples Using Biocompatible Solid-Phase Microextraction (BioSPME)

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After attending this presentation, attendees will have a better understanding of how BioSPME can be used as an extraction method for the detection of illicit substances from postmortem samples such as blood.

This presentation will impact the forensic science community by providing a new procedure for postmortem toxicological testing that is faster than current analytical methods. The use of BioSPME coupled with Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) allows for minimal sample collection, preparation, and analysis for illicit substances in a shorter period of time.

Forensic pathologists are responsible for collecting postmortem samples for toxicological testing. These samples may include bile, vitreous humor, urine, blood, liver, gastric contents, brain, and kidney. The forensic toxicology laboratory is then responsible for analyzing the samples for common drugs and poisons, which for a criminal investigation may be time consuming and could cause analytical backlogs. Over the past couple of years, the application of *in vivo* SPME has grown due to its ability to be directly injected into a biological matrix without the removal of sample. BioSPME fibers have been developed to absorb any drugs present without the interference of macromolecules that are present in a biological sample. The application of the BioSPME fiber to postmortem samples will allow for faster analysis time.[1-3]

In this study, a BioSPME extraction and LC/MS/MS method have been developed to analyze 6-monoacetylmorphine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone, and oxymorphone in a blood matrix. Two types of BioSPME fibers have been explored when developing this method, C-18 and mixed-mode coated fibers. These fibers are conditioned, directly injected into a biological matrix (blood) for extraction of possible drugs, desorbed into solution, screened by GC/MS, and finally analyzed by LC/MS/MS. Extraction variables included extraction time, desorption time and volume, drying time, and reconstitution volume and solvents. This procedure utilized a screening method comprised of an HP 6890 Series GC system coupled with an HP 5973 Mass Selective Detector using splitless injection. Gas chromatography was performed using a Rxi-5Sil MS (29.0m x 0.25mm, 0.25µm). The quantitation method was comprised of AB SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer with an electrospray ionization (ESI) source operated in the positive ion mode. Liquid chromatography was performed on a Shimadzu® LC system using an Ascentis® Express Biphenyl Column (50cm x 2.1mm, 2.7µm) with the weak mobile phase consisting of 0.1% (v/v) formic acid in water and the strong mobile phase consisting of 0.1% (v/v) formic acid in acetonitrile. The flow rate was 0.3mL/min, column temperature was set to 30ºC, and injection volume was 1µL. A gradient curve was used over a run time of five minutes per sample. This method is being first optimized using bovine blood and then being applied to postmortem blood samples provided by the Lehigh County Coroner's Office.

In conclusion, the use of BioSPME in an extraction procedure allows for minimal postmortem sample preparation and collection. BioSPME coupled with the use of the LC/MS/MS would require less amount of time to analyze for drugs that may be present. Forensic toxicology laboratories would benefit by employing a method that would be able to decrease the problem of long extraction and analysis.

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Forensic Toxicology, BioSPME, Postmortem

K13 The Detection and Quantitation of Insulin Analogs by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS) in Postmortem Vitreous Humor

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After attending this presentation, attendees will be able to describe the chemistry of insulin and the challenges surrounding its analysis by LC/MS/MS in forensic samples. In addition, attendees will be able to implement a forensically validated LC/MS/MS method.

This presentation will impact the forensic science community by providing a novel approach for the simultaneous detection and quantification of human insulin and five pharmaceutical analogs as well as describing this approach's application in a series of forensic death investigations.

Insulin is a 51-amino acid peptide hormone produced and released by beta cells in the pancreas in response to rising blood glucose levels. Insulin plays a vital role in glucose metabolism, allowing glucose to enter cells where it can be used to drive cellular activity. The analysis of biological specimens for the presence of exogenous insulin is of special interest in select postmortem investigations. Like other drugs and chemical agents, these compounds may be implicated or suspected in the cause of a death. Toxicological analysis, however, is challenging due to the limited stability of insulin in whole blood and complexities associated with sample preparation and instrumental testing. As a consequence, the determination of insulin in postmortem cases is not routinely performed or offered by forensic laboratories.

Several novel LC/MS/MS based methodologies have been published combining low flow chromatography (microbore or nanobore), anti-insulin antibody immunopurification, as well as ion mobility mass spectrometry. While these approaches have seen some success, no single approach has successfully integrated a straightforward, high throughput, preparation method with clear, unambiguous, discrimination between insulin and the recombinant analogs. The work described here enables unambiguous differentiation of human insulin as well as five pharmaceutical analogs including insulin aspart, glulisine, glargine, lispro, and detemir through the use of high sensitivity liquid chromatography tandem mass spectrometry.

Analysis was performed from 500µL of human vitreous humor with porcine insulin as an internal standard. Insulins were extracted from 500µL of human vitreous via a protein crash (1:1 acetonitrile) followed by solid phase extraction. Purified extracts were evaporated to dryness and re-solubilized in Tris (2-carboxyethyl)phosphine hydrochloride to liberate the alpha and beta chains. Analysis was carried out on an Agilent 6495 triple quadrupole mass spectrometer coupled with a 1290 infinity UHPLC. Separation was performed on a stepwise gradient over 15 minutes on an AdvanceBio Peptide Mapping 2.1mm x 100mm superficially porous column. Validation has been performed in accordance with SWGTOX guidelines including an assessment of within and between day accuracy and precision, limits of detection (60pg/mL) and quantitation (500pg/mL), interference, and stability. Finally, the approach has been successfully applied to an authentic postmortem case.

Insulin, LC/MS/MS, Intact Protein

K14 An Analysis of Stimulants and Metabolites From Dried Blood Spots (DBS) Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will gain knowledge and insight into the advantages of the usage of DBS as a sample collection technique for Driving Under the Influence of Drugs (DUID) cases as well as an appropriate procedure that allows for the extraction and analysis of stimulant-type drugs from DBS.

This presentation will impact the forensic science community by providing new information and methods pertaining to the field of drug testing and forensic toxicology. This proposed method reveals multiple advantages for the use of DBS compared to typical DUID sample collection procedures and could ultimately be adapted as a means of roadside sampling during these investigations. These DBS samples allow for a less invasive sample collection, lower amounts of blood needed, and an easier means of storage and transportation.[1]

The majority of DUID investigations rely on blood testing to determine the types and quantities of drugs present in a sample due to the fact that blood allows for the analysis of parent compounds as well as their metabolites.[2] During a typical DUID process, blood samples are collected from the individual once the stop is completed and the person is transported to the location of sample collection. Often times there is a long time in between the time of the stop and the time the sample is collected, which typically involves large volumes of blood being collected. This large time span can cause decreased levels of the drugs present in the sample, and it is possible for these samples to fall below screening cut-off values.[3]

In this report, a selective liquid chromatography tandem mass spectrometry (LC/MS/MS) method was developed in order to analyze stimulant type drugs that have been extracted from DBS. FTA DMPK-C blood cards were used as the medium to collect and store the blood samples. The extracts from 30µL of blood deposited on blood cards were analyzed using a Shimadzu LC system connected to an ABI Sciex 3200 QTRAP triple quadrupole mass spectrometer operating in positive-ion mode. The liquid chromatographic separation of the compounds was completed and optimized using a Restek Ultra® C_{18} column (5.0cm x 2.1mm, 3.0µm). The HPLC method used a binary mobile phase system consisting of a 0.1% formic acid weak phase and a 0.1% formic acid in acetonitrile strong phase and the total run time was 6.5 minutes. A retention time versus temperature optimization study provided the most favorable separation conditions at 25°C. Optimum MS conditions (Q1 and Q3 ions, collision energy, declustering potential) were determined for each of the compounds as well as their internal standards. Amphetamine, benzoylecgonine, cocaethylene, cocaine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methamphetamine were the seven stimulants chosen as the focus for this study. The extraction procedure for the DBS was optimized through testing multiple extraction solvents, mechanical mixing techniques, blood spot sizes, and drying down techniques. The optimized method was used to analyze authentic blood samples from toxicology laboratories to test its fit for purpose.

In conclusion, this developed method has potential to be used in forensic toxicology testing during DUID investigation. This method could allow for roadside sampling onto the DBS cards which will decrease the time between the DUID stop and sample collection, allowing for more accurate levels of the drug present in these samples to be determined during the forensic testing.

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Stimulants, Dried Blood Spots, LC/MS/MS

K15 Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) -Based Analysis of *In Vitro* Covalent Modifications of Glutathione (GSH) and Peptide Thiols by Drugs of Abuse

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After attending this presentation, attendees will better understand whether reactive drug metabolites are likely to form adducts with GSH and other thiol-containing peptides. Attendees will also understand the LC/MS/MS analytical approach required for detection of these adducts.

This presentation will impact the forensic science community by demonstrating the potential of important drugs of abuse and/or their reactive metabolites to covalently bind to thiol-containing peptides in an in vitro assay, as models for in vivo protein adduction.

The human blood proteins hemoglobin (Hb) and serum albumin (SA) have free thiol groups that can be covalently modified by reactive chemicals or their metabolites. Such reaction products ("adducts") are stable entities that accumulate during chronic exposure. The possible application of protein adducts as long-term exposure markers to facilitate forensic toxicological detection of drugs of abuse has not been explored. This work examined the capability of various abused drugs, including morphine, cocaine (COC), methamphetamine, naltrexone (NAL), methylenedioxymethamphetamine (MDMA), Δ^9-tetrahydrocannabinol (THC), buprenorphine, and methylenedioxypyrovalerone to form adducts with GSH *in vitro*. In addition to GSH, an N-acetylated thiol-containing peptide (Ac-PAACAA) was used as a model to confirm the potential of these drugs and/or metabolites to covalently bind to protein thiol residues. Acetaminophen (APAP) and clozapine (CLZ), both of which have been previously reported to covalently adduct proteins *in vitro* and *in vivo*, were also included as positive controls.

For the metabolism/adduction assay, each drug was added to a plastic vial with residual solvent removed via vacuum centrifuge. Human liver microsomes (HLM) were combined with NADPH in the presence of a regeneration system containing glucose-6-phosphate and glucose-6 phosphate dehydrogenase, in sodium phosphate buffer (pH 7.4). Contents were pre-incubated for 15 minutes at 37°C, followed by addition of GSH or peptide. The vial was reincubated at 37°C for 3 hours and then centrifuged. A 100µL aliquot of supernatant was removed and added to a clean LC/MS vial for analysis.

Instrumental analysis of GSH assay products was performed using negative ESI on an Agilent 6460 LC/QqQ/MS and an Agilent Zorbax Rapid Resolution HD Eclipse Plus C18 column was used for separation. The mobile phases used were as follows: (a) water with 0.1% acetic acid; and (b) 95% acetonitrile, 4.9% water, 0.1% acetic acid. The total run time was 14 minutes with a 2-minute post-run for column re-equilibration. Instrumental analysis of peptide assay products was performed by flow injection analysis (FIA) using positive ESI on an Agilent 6460 LC/QqQ/MS.

MS analysis successfully identified multiple stable metabolites and GSH adducts of the test drugs. Several previously unreported peaks were identified as adducts of GSH with metabolites of the test drugs, including *m/z* 482 for APAP, 470 and 481 for MDMA, and 648 and 616 for CLZ. The GSH adducts for several drugs, including COC, NAL, and THC detected in this study are the first such reported for these drugs. Covalent adduction to the Ac-PAACAA peptide was observed for the majority of drugs tested, with eight of the drugs showing high potential for adduct formation. MS/MS data confirmed the identity of the major peak for each drug as the drug-peptide adduct. The mass difference between the adducted and unadducted peptide corresponded to the molecular mass of the drug or a metabolite, minus a proton lost by the bond formed between the drug and the cysteine thiol.

Demonstration of the capability of these drugs to covalently bind to thiol residues *in vitro* represents a critical first step in assessing their protein binding capabilities. Further studies are underway to determine if such adducts can be detected in human blood protein *in vivo* and therefore employed as long-term biomarkers for exposure to drugs of abuse.

Toxicology, Metabolite Adducts, LC/MS/MS

K16 The Development and Validation of a Method for Cocaine/Crack Cocaine Biomarkers in Human Oral Fluid, Urine, and Plasma by Liquid Chromatography/Mass Spectrometry (LC/MS) and Its Application in Drug Users

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After attending this presentation, attendees will be able to implement a method for cocaine/crack cocaine biomarkers in three biological matrices and assess analytical results from the analysis of plasma, urine, and oral fluid within a population of drug users.

This presentation will impact the forensic science community by providing simpler and faster extraction techniques made possible with LC/MS, which will provide evidence of analytical methods capable of quantifying the target compounds and also provide data on the temporal trends of cocaine/crack cocaine use within this population.

Drugs of abuse, including cocaine, are responsible for many social and economic problems worldwide. Within a global context, Brazil has a negative role in the cocaine market and is known as a trafficking country, besides having a high rate of cocaine and crack/cocaine users. Liquid chromatography coupled to mass detector (LC/MS) has some notable differences from gas chromatography coupled to mass spectrometry (GC/MS) such as its capacity to analyze polar, non-volatile, and thermally labile compounds.[1,2] There are methods in the literature for the analyses of the pyrolysis products of cocaine anhydroecgonine methyl ester (AEME) and anhydroecgonine (AEC) in oral fluid, urine, and plasma by GC/MS and LC/MS/MS but there are no methods that use a single-stage LC/MS.[3,4] The goal of this research was to develop, validate, and apply three bioanalytical methods in the analysis of cocaine (COC), benzoylecgonine (BZE), cocaethylene (CE), AEME, and AEC in oral fluid, urine, and plasma by LC/MS prioritizing speed of analysis, robustness, and low-cost.

Validation experiments were performed on an Agilent LC 1260 coupled to an Agilent 6120B mass spectrometer operating in ESI+ mode using a Kinetex HILIC column Phenomenex (150mm x 4.6mm, 2.6mm) at 30°C. Isocratic elution of ACN:MeOH:CH_3COONH_4, 13mm, pH 6.0 (55:10:35) as mobile phase, flow as set to 0.8mL/min with a total run of 13 minutes and injection volume was 10µL. Samples were prepared by buffer dilution (oral fluid) and protein precipitation using acetonitrile (urine and plasma), followed by centrifugation, filtration, and injection. The methods were validated following the guidelines set forth by the RDC 27/2012 (ANVISA) and supplemented by SWGTOX and FDA guidelines.

Cocaine and/or crack cocaine users (*n*=124) were recruited at service centers specializing in drug addiction in the city of Porto Alegre, Brazil. The study received institutional review approval for human subject studies. Data collection was obtained through interviews conducted within the first 24 hours after admission. Subjects were asked to donate samples of blood, urine, and oral fluid. All biological samples were collected at the same time range (mean interval of 1 hour ± 20min). Samples were stored at -80 ± 2°C until analysis.

Calibration curves were linear in the range of 4.5ng/mL to 544ng/mL (oral fluid) and 5ng/mL to 320ng/mL (urine and plasma) and the experimental detection limit ranged from 1ng/mL to 3.4ng/mL for all analytes. The between run variability ranged from 0.9% to 8.5% for the low control, and 0.6% to 13.5% for the high control. For the low and high controls respectively, accuracy ranged from 0.7 to 13.6% and 0.1% to 12.8%. The method was free from carryover, interferences from matrix effects, and interferences from commonly encountered related analytes (*n*=10).

The sample comprised of 118 males and 6 females (mean age 34 ± 9, 47.6% Caucasians, 56.5% unmarried). Subjects were mostly low-educated (62.9% with less than eight years of schooling) and unemployed (58%). Regarding substance abuse, the majority of the subjects (56.5%) reported daily use of cocaine in the previous three months. COC was detected in 93 subjects, BZE in 94, CE in 33, AEME in 13 and AEC in 70 subjects. In 37.6% of the cases where COC was detected, its use was confirmed in all three matrices. BZE was detected in three matrices in almost half of the total samples (45.7%). The prevalence decreases in crack metabolites (AEME and AEC) and CE, which could be detected more frequently only in urine.

The methods were successfully validated and proved to be suitable to detect and quantify cocaine in oral fluid, urine and plasma, in all types of utilization: salt form (COC and BZE biomarkers), alkaline form (AEME and AEC biomarkers), and concomitant use with ethanol (CE).

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Cocaine, LC/MS, Drug Users

K17 The Separation of Chemically Similar and Isobaric Novel Psychoactive Substances (NPS) Using 2D-Liquid Chromatography (2D-LC)

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After attending this presentation, attendees will understand the principles of 2D-LC and its application for the separation of isobaric NPS that would otherwise co-elute in conventional 1D-Liquid Chromatography (1D-LC) separations.

This presentation will impact the forensic science community by demonstrating how 2D-LC can be a useful technique for the separation of NPS, including those from the cannabinoid class of drugs, which represents a large area of interest for detection and identification in forensic toxicology.

The constant emergence of NPS provides a significant challenge to detecting and identifying compounds of interest. New compounds are often isomers or analogs of existing NPS, and since such compounds are often structurally related, there is a risk of co-elution during 1D chromatographic analyses. If the coeluting compounds are unknown or previously unreported, a problem could arise in identification if the compounds are indistinguishable using mass spectral data alone (e.g., isobaric derivatives). To solve this problem, a better separation approach must be used to ensure that all compounds are resolved prior to identification.

Two-dimensional liquid chromatography (2D-LC) has been proposed as a method to improve separation and resolution of co-eluting and isobaric compounds prior to further mass spectral analysis. 2D-LC uses two orthogonal separation systems, or dimensions, to improve the resolving power of the overall separation by combining the power of each dimension.

This research focused on development of a comprehensive 2D-LC method in which two reverse-phase columns were used, one in each dimension, with the entire eluent from the first dimension transferred to the second dimension. An Agilent Poroshell 120 Bonus-RP column (2.1mm x 150mm, 2.7µm) was used in the first dimension, and a Supelco Ascentis Express Biphenyl column (2.1mm x 100mm, 2.7µm) was used in the second. An Agilent Infinity 1290 Diode Array Detector was used between the first and second dimensions, and an Agilent 6530 Quadrupole Time-of-Flight (qTOF) mass spectrometer was used after the second dimension. Two simple NPS mixes were created in methanol for use in development of the 2D-LC method. These mixes, called co-elution (CE) mixes, contained five co-eluting compounds each—CE Mix 1 contained isobaric compounds from the JWH-019 family of cannabinoids and CE Mix 2 contained non-isobaric compounds from other cannabinoid families that co-eluted in 1D-LC separations.

It was hypothesized that a 2D-LC system would enable separation of all five components in each mix in a single, continuous analytical run. Method development involved the optimization of each of the two dimensions, first as traditional 1D systems, then together as a continuous 2D system. Once optimized, each of the complete mixes and individual mix components were analyzed using the 2D methods. The first dimension was operated with a very narrow gradient and slow flow rate over 25 minutes. The second dimension was operated under a much faster flow rate using a shifted gradient to promote separation of co-eluting compounds after the first dimension analysis.

Initial 1D analysis using the bonus-RP column demonstrated that only three components of the five-component CE Mixes could be separated, with minimal resolution. In contrast, using the 2D-LC method, full separation of all five compounds in both CE Mixes 1 and 2 was achieved with good resolution. The separated and resolved peaks could then be analyzed by mass spectrometry. These studies confirmed that the use of 2D-LC can be a powerful technique when attempting to separate co-eluting and isobaric NPS.

Work is continuing to increase the number of compounds contained in each mix that can be successfully separated via 2D-LC. The method will also be further optimized so that separation of co-eluting and isobaric compounds from additional classes of NPS can be achieved.

2D-LC, Cannabinoids, NPS

K18 The Evaluation of Mono-, Di-, and Trivalent Cations for the Optimized Surface-Enhanced Raman Spectroscopy (SERS) Enhancement to Detect Synthetic Cannabinoids in Biological Samples

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After attending this presentation, attendees will understand: (1) the principles of SERS; (2) how the use of mono-, di-, and trivalent cations as aggregating agents with gold nanoparticles can affect SERS enhancement and detection of synthetic cannabinoids; (3) how SERS can be used to create a low limit of detection for synthetic cannabinoids; and, (4) how SERS can be a fast and easy analysis for drug detection in toxicological samples.

This presentation will impact the forensic science community by demonstrating the application of SERS as a useful procedure for detecting trace levels of various JWH and PINACA compounds and their metabolites in solution that is rapid, sensitive, and applicable to a variety of biological matrices.

The use and abuse of synthetic cannabinoids has become a global issue due to their easy access and growing popularity in young adults. This popularity has led to an increase in emergency room visits due to synthetic cannabinoid intoxication in recent years. As more of these drugs become illegal, new synthetic legal versions of these compounds are made, which presents problems for the forensic scientist as standard methods may not detect the target drug.

The most common method of screening for drugs of abuse in biological samples is the immunoassay. However, this method presents some disadvantages, particularly for newly synthesized compounds which may not respond to the test. Other problems include cross-reactivity between different synthetic cannabinoids, hook effects, and high cut-off values for determining if the drug is present. More advanced methods, such as GC/MS, have also been used; however, these procedures involve complex sample preparation and long run times. A potential solution to this issue is surface enhanced Raman spectroscopy.

Raman spectroscopy is an under-utilized technique for the detection and identification of drugs due to its perceived low sensitivity. However, when Raman spectroscopy is performed in the presence of metallic nanoparticles, the signal can be enhanced by several orders of magnitude due to localized metallic plasmon field effects. This process is known as Surface Enhanced Raman Spectroscopy (SERS). The addition of aggregating agents, generally ionic salts, further increases this signal due to the creation of hot-spots resulting from nanoparticle interactions. This method has recently been confirmed to work for the toxicological detection of benzodiazepines with limits of detection ranging from 1ng/mL - 200ng/mL.

In this project, gold nanoparticles were prepared using a sodium citrate reduction with mono, di, or trivalent cation aggregating agents. The concentration, absorbance, size, and zeta potential of the nanoparticles was analyzed before and after the addition of the aggregating agents (KCl, NaCl, $MgCl_2$, $CaCl_2$, $AlCl_3$, and $RuCl_3$) to assess the effect on the SERS enhancement. It was experimentally determined that the SERS enhancement for mono and divalent cations was due to the chloride anion interaction with the nanoparticle. However, the trivalent cations produced SERS enhancement via the cations interaction with the surface of the nanoparticle to cause aggregation.

Experiments were performed using a variety of synthetic cannabinoids including JWH-018, JWH-073, JWH-081, JWH-122, APINACA (AKB48), AB-PINACA, ADB-PINACA, AB-CHMINACA, and several of their metabolites. The six different chloride aggregating agents were examined at 0.0167M. From this analysis, 0.017M of $MgCl_2$ was determined to be the optimal aggregating agent. Then, varying concentrations of $MgCl_2$ were examined to optimize sensitivity of detection for a bench top and portable Raman system. While 0.017M $MgCl_2$ was the optimal concentration for the bench top Raman, a portable Raman system required a 2-fold increase in concentration of $MgCl_2$ for optimal detection. Using this SERS method, synthetic cannabinoids could be detected at concentrations as low as 18ng/mL. Spiked urine samples at physiological concentrations were next screened using a supported liquid extraction involving an ammonium acetate buffer followed by dichloromethane solvent system.

These results demonstrate that SERS can be a useful and more comprehensive alternative to immunoassays in the screening of synthetic cannabinoids in urine.

SERS, Synthetic Cannabinoids, Toxicology

K19 A Comparison of Two High-Resolution Mass Spectrometry Data Acquisition Methods for the Screening, Quantitation, and Confirmation of Compounds in Postmortem Blood

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After attending this presentation, attendees will understand two high-resolution accurate-mass mass spectrometric methods for detecting drugs of abuse in postmortem blood and will be able to compare them for suitability in their laboratories.

This presentation will impact the forensic science community by providing forensic toxicologists with the tools to correctly identify, quantify, and confirm a large panel of compounds, including benzodiazepines, opiates/opioids, and more in a single analytical run with minimal sample preparation, thereby saving time and other resources.

Background/Introduction: Forensic toxicologists need to quantitate target compounds and screen for many more in as little time as possible. In the past, samples were screened either by GC/MS or immunoassay, both of which have significant limitations. GC/MS requires labor-intensive sample preparation, including derivatization. Multiple immunoassays must be used to cover different compound classes, and immunoassays are not specific to a particular compound. LC/MS techniques allow for simpler sample preparation and identify individual compounds, not just classes.

Methods: A single point calibrator (1ng/mL-1000ng/mL, compound dependent), two QCs (one at half and one at double the calibrator concentration), and five unknown post mortem blood samples were processed by a collaborating laboratory. Protein precipitation with a solution containing six internal standards was followed by evaporation of the supernatant and reconstitution with phosphate buffer. The calibrator and QCs contained 21 compounds including benzodiazepines, opiates/opioids, cocaine metabolite, gabapentin and pregabalin to evaluate method performance. Processed samples were subject to reversed-phase chromatographic separation followed by detection on a hybrid quadrupole-Orbitrap™ mass spectrometer. Data was collected using two methods. In the first, the mass spectrometer collected high-resolution full-scan spectra at a resolution of 70k (FWHM at 200*m/z*) along with data-dependent fragmentation spectra (FS-ddMS2) for any masses detected from a target list of over 400 compounds. In the second, full-scan spectra were again collected, followed by all-ion fragmentation (FS-AIF). Targeted compounds were identified using retention times and accurate mass *m/z* within 5ppm mass accuracy from the full-scan data. Confirmation was accomplished either by matching the MS2 spectra to a spectral library or by presence of known fragments in the AIF data. Detection limits were evaluated using the 21 representative compounds in the calibrator and QCs. Quantitation was performed on the full-scan extracted ion chromatographic peak using the single point calibrator and linear-through-zero calibration curves. Method performance was evaluated by analyzing the calibrator, QCs, and unknown blood samples previously analyzed by the collaborating laboratory and comparing the two sets of results.

Results: Desired limits of detection (0.75ng/mL-500ng/mL) were achieved for all 21 evaluation compounds in the calibrator and QC sample. All QC compounds that had deuterated analogs as internal standards were within 20% of nominal concentration. Accuracies for some of the compounds that did not have deuterated analogs were outside of the 20% range, suggesting that analogs are needed if rigorous quantitation is required. These data agreed with the results obtained by the collaborating laboratory (data not shown).

For screening of the five unknown samples, quantitative results were obtained for many of the 21 evaluated compounds. Qualitative results were obtained for compounds in the screening database of over 400 compounds. Results were reported for any peak that was both detected and confirmed. These values and results again agreed with those obtained by the collaborating laboratory.

FS-ddMS2 and FS-AIF performed equally well for confirmation of compounds within the concentration range of the QCs.

Conclusion/Discussion: The developed methods were able to both quantitate a target set of compounds and detect unknown compounds in post-mortem blood samples. Both methods performed similarly and met common industry requirements for sensitivity. The FS-ddMS2 data still offers the strongest identification since the fragmentation spectra ”fingerprint” is collected for a specific precursor. AIF data is less specific since the

fragments are generated by all ions eluting at the same time. The advantage of collecting AIF data is the ability to conduct confident retrospective data analysis using fragmentation data. Compounds from many classes of drugs were successfully and specifically screened in a single analytical run.

Mass Spectrometry, Screening, Drugs of Abuse

K20 Screening, Confirmation, and Quantitation of Synthetic Cathinones and Cannabinoids in Urine by High-Resolution Accurate-Mass Mass Spectrometry

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After attending this presentation, attendees will understand the capabilities of a high-resolution, accurate-mass mass spectrometer for the screening and confirmation of synthetic cathinones and cannabinoids. Attendees will see the impact the chromatographic method can have on the mass spectrometer method performance.

This presentation will impact the forensic science community by providing tools to detect and confirm current novel psychoactive compounds.

Introduction: Forensic laboratories need reliable, flexible methods for detecting novel psychoactive compounds such as synthetic cathinones and cannabinoids. The methods need to be easily modifiable to include new compounds. LC/MS is ideally suited for this type of application; it can easily detect different classes of compounds in a single analytical run.

Objective: To demonstrate the performance of high-resolution mass spectrometry for identification, confirmation, and quantitation of synthetic cathinones and cannabinoids in urine.

Methods: A single point calibrator at cutoff concentration (25ng/mL-500ng/mL, compound dependent) and two quality controls (QC) one each at 50% and 150% of the calibrator concentration were prepared by fortifying blank urine with 32 synthetic cathinones and cannabinoids. The calibrator, QCs and an unknown sample were processed by protein precipitation followed by dilution. Processed samples were subject to HPLC separation followed by detection on a hybrid quadrupole-Orbitrap™ mass spectrometer. Two chromatographic gradients were used. The first was a two-minute elution intended for screening that provided limited chromatographic separation of isobaric compounds. The second was a nine-minute elution used for confirmation. The mass spectrometer collected full-scan (FS) spectra at a resolution of 70k (FWHM at 200*m/z*) along with data-dependent fragmentation spectra (ddMS2) for masses on the target list. Compounds were identified using retention time and accurate *m/z* (5ppm mass window) from the full-scan data. Semi-quantitation was performed on the FS extracted-ion chromatographic peak using the single point calibrator and linear-through-zero calibration curves. Confirmation was accomplished by spectral library matching with the MS2 spectra. Isotopic pattern matching was added to the longer method. To assess method performance, the calibrator and each QC sample were injected ten times with each method to determine mass accuracy, peak area precision, and quantitative performance. The unknown sample previously analyzed by a collaborating laboratory was injected three times with each method to determine identification accuracy.

Results: Data from the short method showed mass accuracies within 1 ppm for all but one compound which was within 2.2ppm. The long method, run several days after the short method and near the end of the recommended instrument calibration period, showed mass accuracies within 3ppm except for the same single compound, which was within 4.2ppm. Peak area precisions for all compounds and all concentrations were better than 13.9% and 8.1% for the short and long methods, respectively. Calculated concentration precisions across all compounds and all concentrations were better than 9.8 % and 8.5% for the short and long methods, respectively.

The short method was intended only to screen for compounds using retention time and accurate *m/z* from the FS data as identifiers. The longer method, which provided better chromatographic separation, included isotopic pattern matching and fragment spectral matching for confirmation. Fragment spectral matching was included in the short screening method to determine if it could be of any utility. Three compounds (MDPV, mephedrone and methylone) were identified and confirmed in the unknown sample using both methods. The longer method gave higher library matching scores and isotopic patterns. A fourth compound was identified as Methedrone by *m/z*, retention time, and isotopic pattern matching. However, it failed confirmation by spectral matching. It was suspected that this unconfirmed compound might be a metabolite of mephedrone. A literature search revealed a possible match, which was confirmed with a theoretical fragmentation spectra match.

Conclusion: The developed methods accomplished their goals of identifying, confirming and quantifying 32 synthetic cathinones and cannabinoids in urine. The short method was intended as a screening-only method not requiring definitive confirmation. It surpassed that goal by also providing confirmation through library matching

of fragmentation spectra. The longer method provided better confirmation and greater quantitative precision. Its higher quality fragmentation spectra resulted in higher library matching scores. The addition, isotopic pattern recognition also contributed to more confident confirmation.

Mass Spectrometry, Novel Psychoactive Substances, Confirmation

K21 A Biochip Array Screening of Blood and Urine Samples for the Recommended Drugs Associated With Driving Under the Influence of Drugs (DUID)

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After attending this presentation, attendees will better understand the application of biochip array technology to the simultaneous screening of drugs associated with DUID. The goal of this presentation is to describe how matrix-specific tests can be developed to adhere to guidelines for both urine and blood.

This study will impact the forensic science community by providing the results of two biochip arrays that allow the simultaneous determination of drugs associated with DUID and included in Tier 1 and Tier 2 under reported recommendations. Twenty immunoassays arrayed on each biochip surface allow this multi-analytical screening from a single whole blood or urine sample. This leads to test consolidation and an increase in the screening capacity in test settings.

Biochip array technology enables the simultaneous detection of multiple analytes from a single sample. As drug impaired driving is becoming a major problem in the United States and worldwide, recommendations for the toxicological investigation of drug-impaired driving and motor vehicle fatalities were reported. These recommendations focused on a two-tier approach of drug analysis. Tier 1 consisted of the most prevalent drugs found in the United States impaired driving population and Tier 2 drugs being less frequently encountered, with regional significance and/or beyond the routine analytical capabilities of some laboratories. Tier 1 drugs should be the minimum testing that should be completed in drug driving casework.[1] Recommended cut offs have been stated suitable for the matrix of interest such as blood and urine. This study reports the applicability of a biochip array to the simultaneous screening of Tier 1 and Tier 2 drugs in whole blood and a second biochip array suitable for urine. This leads to test consolidation and an increase in the screening capacity, which is relevant in test settings.

Competitive chemiluminescent biochip-based immunoassays were employed. Ligands were immobilized and stabilized to the biochip surface defining an array of twenty discrete test sites (15 Tier 1 assays and 5 Tier 2 assays). The signal output is inversely proportional to the concentration of drug in the sample.

Tier 1 assays included were: Amphetamine (AMPH), Methamphetamine (MAMP), Barbiturate (BARB), Benzodiazepine Class 1 (BENZ1), Benzodiazepine Class 2 (BENZ2), Cannabinoids (THC), Cocaine/Benzoylecgonine (BZG), Hydromorphone (OPDS), Meprobamate (MPB), Methadone (MDONE), Opiates (OPIAT), Oxycodone (OXYC1 and OXYC2), Phencyclidine (PCP), and Zolpidem (ZOL). Tier 2 assays included: Buprenorphine (BUP), Dextromethorphan (DMP), Fentanyl (FENT), Tramadol (TRM), and Tricyclic antidepressants (TCAs). Two panels were developed so that the desired cut off were achieved in each matrix and that the relevant parent and metabolite compounds were detected in the whole blood and urine respectively. The assays are semi-quantitative and applicable to both the fully automated Evidence Analyser and the semi-automated analyser Evidence Investigator. The systems have dedicated software to process, report, and archive the data produced. The sample volume required is 60µl of whole blood (diluted 1 in 4) and 10µl of neat urine.

In this initial evaluation, the limit of detection was determined by running 20 negative urine samples and 20 negative blood samples. The resultant mean concentration +3STDEV was less than 50% of the cut off required. The cut off values were further validated by assessing inter assay precision. Blood and urine samples were spiked with the appropriate drug compound 50% below, at the cut off, and 50% above the recommended cut off. Three replicates were assessed over five separate runs and the inter assay precision calculated to be less than 20% for all assays across both blood and urine panels.

In conclusion, the results indicate applicability of biochip array technology to the simultaneous screening of drugs associated with DUID in Tier 1 and Tier 2 under reported recommendations. The twenty immunoassays arrayed on each biochip surface presented both the desired sensitivity and reproducibility required to achieve

screening at the recommended cut offs. This methodology allows for multi-analytical screening of samples, leading to test consolidation and increased screening capacity in test settings.

Reference(s):

1. Logan, B.K. et al. Recommendations for toxicological investigation of drug-impaired driving and motor vehicle fatalities. *Journal of Analytical Toxicology.* 2013:37(8):552-558.

DUID, Biochip Array, Tier 1

K22 An Assessment of the Stability and Degradation of Mephedrone in Solvents and Biological Matrices

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After attending this presentation, attendees will better understand the matrices and storage conditions in which mephedrone is most stable. This will be of benefit in the interpretation of toxicological samples suspected of containing this drug.

This presentation will impact the forensic science community by providing stability information resulting from a quantitative 30-day study evaluating how long mephedrone can be detected in its parent form in working solutions, blood, and urine at three storage temperatures. The results of this study will allow toxicologists to more accurately analyze both clinical and postmortem samples containing mephedrone.

Synthetic cathinones are structural analogs of cathinone, the active substance in the *Catha edulis* (Khat) shrub that is indigenous to the Middle East and East Africa. Although once marketed as "legal highs," many countries passed legislation restricting the usage of synthetic cathinones. However, they continue to appear in forensic casework, so their accurate analysis in case samples is critical. Current literature recognizes the instability of some synthetic cathinones and even cathinone itself but offers little information about degradation products and pathways as well as long-term stability in working solutions and biological matrices. This research investigates the stability and degradation of mephedrone (4-methylmethcathinone, 4-MMC) in methanol, acetonitrile, human whole blood, and urine at 21°C (room temperature), 4°C (refrigerator), and -20°C (freezer) temperatures over 30 days.

Solutions of mephedrone in each of the four matrices (1mg/L) were divided into aliquots (100µL, solvents; 1.2mL, biological matrices) and stored in triplicate at each temperature for extraction and full-scan GC/MS analysis on days 0, 3, 7, 14, and 30. At room temperature, mephedrone in samples decreased in concentration by over 30% by day 3 and by 88% by day 30. At 4°C, over 50% of mephedrone experienced degradation from its parent form in methanol. In contrast, samples stored in acetonitrile suffered a 30% loss by day 30 at room temperature, demonstrating significantly higher stability than in methanol. In human whole blood, samples lost over 95% of their original concentration at room temperature and approximately 25% in the refrigerator and freezer. In urine, samples lost over 40% of their concentration by day 30. GC/MS and LC/TOF were used to elucidate the structures of possible degradation products.

This information regarding stability significantly influences the toxicological analysis of forensic case samples in the context of postmortem samples as well as clinical samples involving suspected driving under the influence (DUI) and drug-facilitated sexual assault (DFSA) using mephedrone. Because working solutions and biological samples experience varying rates of degradation, it is crucial to take the stability of mephedrone in both contexts into consideration when reporting concentrations and drawing conclusions from data resulting from analysis of case samples. This stability information also allows more accurate interpretation of samples retroactively tested in cases where mephedrone was not initially a drug of interest.

Mephedrone, Stability, Degradation

K23 SWATH™ Acquisition vs. Information-Dependent Data Acquisition (IDA) With Application to Broad-Based Drug Screening

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After attending this presentation, attendees will understand the use of two powerful data acquisition modes as they pertain to laboratory screening for drugs of abuse by Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS). Attendees will be able to evaluate the capabilities and limitations of each and determine which mode is best suited to specific laboratory applications.

This presentation will impact the forensic science community by providing an assessment of data-rich LC/TOF acquisition modes and their effectiveness in solving complex drug-screening problems.

LC/TOF mass spectrometry is increasingly being utilized in laboratories across the country due to the ability to perform sensitive, highly specific, rapid screening for large numbers of compounds in short run times. The fast electronics of these systems allow the use of various data acquisition modes that trade-off file size and data complexity with sensitivity and various degrees of analytical specificity. We describe a comparison of two such data acquisition modes for an application focused on analysis of human blood samples for recreational and novel psychoactive drugs.

SWATH™ acquisition is a data-independent acquisition mode for use in broad-based drug screening that collects high resolution accurate mass data on all fragments of all parent masses throughout the chromatographic run. Complimentary to this method, the system offers a data-dependent acquisition mode, known as information-dependent data acquisition (IDA) that collects data only when triggered by criteria set within the analytical method. Both acquisition modes generate accurate mass data that can be searched against accurate mass extracted ion chromatogram (XIC) lists and accurate mass spectral library databases. This study employed the use of SWATH™ acquisition and IDA to blood samples injected in duplicate on opposing methods.

As part of a larger Institutional Review Board approved study, blood samples were collected from participants at an electronic dance music festival in Miami, FL. Blood samples were collected by a trained phlebotomist following explanation of study design and completion of informed consent. In total, 139 blood samples were collected over a three-year period, of which 40 samples were chosen at random for analysis in this study.

Screening analysis was performed at the Center for Forensic Science Research and Education (Willow Grove, PA). Blood samples (0.5mL) were extracted using Borax buffer (0.1M, pH 10.4) and n-butyl chloride/ethyl acetate (70:30). Instrumental analysis was performed using a TripleTOF™ 5600^{+} mass spectrometer (Sciex, Ontario, Canada) coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (Shimadzu, Kyoto, Japan). A reverse phase gradient of ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) was used to create chromatographic separation on a Phenomenex® Kinetex C18 analytical column (50mm x 3.0mm, 2.6µm) at a flow rate of 0.4mL min^{-1}. Following positive electrospray ionization, precursor ions were acquired by TOF MS scan and isolated based on overlapping mass range windows (SWATH™ acquisition) or traditional unit mass isolation (IDA). Fragmentation was achieved using a rolling collision energy of 35±15eV. Data processing was performed using PeakView Software (Version 2.2) under identical criteria as previously determined and verified through method development and validation.

Of the 40 blood samples chosen at random, 30 (75%) screened negative by both SWATH™ acquisition and IDA. The remaining 10 samples were sent to NMS Labs (Willow Grove, PA) for appropriate confirmatory testing based on the screening results from both acquisition modes. Of these 10 samples, SWATH™ acquisition positively identified all compounds present in 9 (90%) samples, with 1 (10%) false positive. IDA positively identified all compounds present in 6 (60%) samples, with 1 (10%) false positive. Three (30%) false negative samples occurred during IDA screening for the following analytes: MDMA, oxymorphone, and levamisole. All three of the false negatives for IDA were attributed to failed library criteria.

In conclusion, SWATH™ acquisition and IDA were successfully used for the acquisition of accurate mass data from blood samples taken from human subjects. Both acquisition modes were able to identify compounds present in the blood samples, but SWATH™ acquisition more accurately identified compounds in relation to confirmatory testing. SWATH™ acquisition and IDA have distinct features and characteristics, but the data acquired in this study shows that SWATH™ acquisition could be preferred over IDA based on more reliable positive screening results.

LC/qTOF, SWATH™, SCIEX™

K24 A Rapid Quantitative Analysis of Stimulants by Ultra High-Pressure Liquid Chromatography-Mass Spectrometry (UHPLC-MS)

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After attending this presentation, attendees will have a deeper understanding of a UHPLC-Tandem Mass Spectrometric (MS/MS) method for analyzing blood specimens for common stimulants, including prevalent drugs of abuse, such as amphetamine, methamphetamine, 3,4 Methylenedioxymethamphetamine (MDMA), Phencyclidine (PCP), and cocaine.

This presentation will impact the forensic science community by providing a quick, comprehensive method for stimulant confirmation performed on UHPLC-MS/MS. This method is capable of analyzing compounds often analyzed in separate methods and creates a green waste stream.

The presented assay is used for the quantitative analysis of amphetamine, methamphetamine, MDMA, MDA, MDEA, phentermine, phenylpropanolamine, pseudoephedrine, ephedrine, cocaine, benzoylecgonine, and PCP. These drugs all exhibit stimulating effects on the central nervous system, such as euphoria, excitation, feelings of strength, hallucinations, and feelings of well-being or calmness.[1-3] All of these drugs are classified as stimulants with the exception of PCP, which is a hallucinogen (all drugs of interest will be referred to as "stimulants" for convenience throughout this presentation).

Stimulants have been used to treat a variety of medical ailments over time, including asthma, obesity, narcolepsy, ADHD, and as an anesthetic. As stimulant use grew, the abuse of these drugs grew as well and their medical use decreased to only a few specific cases.[4] Stimulants are seen in forensic toxicology as one of the most prevalent impairing compound groups, with an estimated 1.7 million users of one or more of the drugs of interest in 2012.[5,6] The Substance Abuse and Mental Health Services Administration estimates approximately 3.6 million users of nonmedical stimulants, methamphetamine, MDMA, cocaine, and PCP, with trends of steady or slowly declining usage over the last 10-15 years. The 2015 Drug Enforcement Agency National Drug Threat Assessment shows methamphetamine and cocaine as readily available in most areas.[7,8] The need for accurate and efficient analysis of these drugs as well as other stimulants will persist for the foreseeable future.

Blood specimens analyzed using this method are prepared using solid phase extraction on SPEware Trace-B columns. The columns are conditioned prior to sample addition, and washed with water, dilute acetic acid, methanol and ethyl acetate. Ethyl acetate, ammonium hydroxide, and isopropanol are used for the elution. This method produces a green waste stream as halogenated solvents are not used and only 9mL total are necessary to condition and wash the columns.

This assay utilizes an Agilent 1290 UHPLC equipped with an Agilent phenyl hexyl 2.5µm X 100mm analytical column and phenyl hexyl guard column interfaced with an Agilent 6410 triple quadrupole mass spectrometer, equipped with a electrospray ionization source operated in the positive mode. The method achieves chromatographic separation of all 12 analytes over a 10 minute analytical run time. Two MS^2 fragmentation transitions are monitored for mass spectral identification of each analyte. This method proposes linear quantification ranges of 5ng/mL-500ng/mL for all analytes except cocaine and benzoylecgonine, which will be 10ng/mL-1000ng/mL.

A method has been presented that provides quantitative analysis of 12 stimulant analytes via UPLC/MS^2. This method provides clear chromatographic separation of all compounds, including isomers pseudoephedrine and ephedrine, and large linear dynamic ranges. This assay combines drugs that are normally analyzed separately, which saves time and materials allowing for rapid analysis of samples. By utilizing UPLC/MS^2 this method removes the need for derivatization, which again minimizes prep time and also minimizes loss of these volatile compounds through the heating process. Validation of this method will follow SWGTOX guidelines to ensure the quality of data obtained.

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Stimulants, UHPLC-MS/MS, Amphetamines

K25 An Evaluation of Proposed Metabolites in Authentic Blood and Urine Specimens After the Ingestion of W-18

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WITHDRAWN

K26 Screening for Fentanyl and Its Analogues in Postmortem Specimens Using the OraSure Fentanyl Enzyme-Linked Immuno-Sorbent Assay (ELISA) Direct Kit Assay

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After attending this presentation, attendees will better understand the performance characteristics of a commercially available fentanyl immunoassay screening test for the routine screening of postmortem specimens for a range of both structurally related and structurally unrelated compounds.

Fentanyl is a synthetic opioid that was first introduced into medicinal practice in 1963. Unfortunately, use and abuse of a range of synthetic opioids, including fentanyl, has contributed to an increase in fatalities across the United States of America. Within the New York City Office of Chief Medical Examiner, several structurally-related drugs have been identified in addition to illicit fentanyl, including acetyl fentanyl, furanyl fentanyl, and 4-ANPP. U-47700 has also been identified in a number of cases and although it is a synthetic opioid, it is structurally unrelated to fentanyl. The challenge for all forensic toxicology laboratories is to ensure their testing protocols are sufficiently robust to identify new and emerging drugs and adapt their protocols as necessary to meet this requirement.

This presentation will impact the forensic science community by providing cross-reactivity data for new synthetic opioids and the assay efficiency for the detection of different synthetic opioids in postmortem casework.

The OraSure Fentanyl ELISA direct kit assay was evaluated using the Dynex DSX automated microplate analyzer. The target analyte was fentanyl and in-house positive controls were prepared by fortifying drug-free matrices (blood, serum, and urine) at 0.5ng/mL, 1.0ng/mL, and 1.5ng/mL and 0.5 ng/g, 1.0ng/g, and 1.5ng/g for tissues. Each of the synthetic opioids (acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, isobutyryl fentanyl, norfentanil, parafluorobutyryl fentanyl, sulfentanil, valeryl fentanyl, 3-methyl fentanyl, 4-methoxy butyryl fentanyl, U-4770, MT-45, and W-18) were prepared at 0.5ng/mL, 1.0ng/mL, 2.0ng/mL, and 10ng/mL to evaluate cross-reactivity in comparison to the target compound fentanyl. Postmortem specimens were diluted using forensic diluents provided with the kit at 1:5 for blood and serum, 1:3, 1:5, and 1:10 for tissues, and 1:60 for urine. A total of 183 postmortem cases were screened using ELISA and confirmed for the presence or absence of synthetic opioids using a combination of gas chromatography-mass spectrometry (GC/MS), liquid chromatrography-time of flight (LC/TOF) and liquid chromatography-tandem mass spectrometry (LC/MS/MS).

The cross-reactivities for the following drugs were close to or greater than 100%: acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, parafluorobutyryl fentanyl, 4-methoxybutyryl fentanyl, valeryl fentanyl, isobutyryl fentanyl, and 3-methyl fentanyl. Norfentanyl, sufentanil, W-18, U-47700, and MT-45 did not cross-react. Of the 183 cases screened by ELISA and confirmed by mass spectrometry, there were three false negative and five false positive with an overall efficiency of 95%.

Synthetic Opioids, ELISA, Cross-Reactivity

K27 A Rapid Analysis of Pharmaceuticals in Human Tissues by 2D-Liquid Chromatography/ Tandem Mass Spectrometry (2D-LC/MS/MS) Technology

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After attending this presentation, attendees will better understand a rapid sample preparation method for use when extracting drugs from human tissues prior to analysis via LC/MS/MS.

This presentation will impact the forensic science community by showcasing a quick sample prep method of a complex matrix without any evaporation-to-dryness step as well as the advantages of using multidimensional chromatography.

Introduction: In postmortem forensic toxicology, tissues such as heart, lung, liver, spleen, kidney, brain, and stomach muscle are often utilized for testing due to the state of human remains or lack of available blood or urine. However, detection and quantitation of drugs in complex matrices, such as these tissues, is challenging due to time-consuming extraction processes and at times the inability to detect an analyte at trace concentrations. Additionally, an analytical method capable of screening a large number of compounds is time-consuming to develop and difficult to optimize for every compound.

Objective: A robust extraction and clean-up methodology, in which a homogenization step precedes extraction, is required to efficiently extract drugs from complex matrices, to reach a target Limit Of Detection (LOD), and to maintain instrumental performance. Traditional solid phase extraction techniques require a lengthy evaporation step, which can take hours. The objective of this study was to develop a micro extraction protocol combined with multi-dimension chromatography to decrease sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques.

Method: For this study, in collaboration with the Federal Aviation Administration, de-identified human tissue samples consisting of brain, heart, lung, kidney, liver, spleen, and muscle were analyzed. The method described includes 21 compounds and metabolites including: zolpidem, citalopram, norbuprenorphine, oxycodone, normeperidine, dextrorphan, dextromethorphan, diazepam, diltiazem, quetiapine, diphenhydramine, buprenorphine, promethazine, dihydrocodeine, doxylamine, flecainide, hydromorphone, nordiazepam, temazepam, n-desmethylcitalopram, and oxazepam. Samples were homogenized using a Precellys Evolution tissue homogenizer (Bertin Technologies, Montingny-le-Bretonneux, France) incorporating a mixed mode reversed-phase/ion exchange sorbent. The use of a 2D-LC/MS/MS technology eliminated the need for a lengthy evaporation step in the extraction method as eluents were transferred directly to the LC vials for analysis. The chosen 2D-LC/MS/MS (Acquity 2D UPLC with Xevo TQD, Waters, Milford, MA, USA) used in this application was selected using a 6x6 automated method development protocol.

Results: The manual extraction of tissue samples was completed in less than 30 minutes. The analysis was performed using 100uL of the final organic solvent (methanol or acetonitrile) extracts. The Limit Of Quantitation (LOQ) for all drugs was measured at 100ng/g from a 1g sample mass. The limit of detection LOD for most drugs was determined to be 10ng/g (10ppt) with some as low as 1ng/g (1ppt).

Conclusion: The micro extraction protocol combined with multi-dimension chromatography used in this study decreased sample preparation time significantly without sacrificing the quality seen with current single dimension chromatography techniques. The procedure developed in this study can be utilized on various human tissues and completed in less than 30 minutes before injection of 100uL final extract into the 2D-LC/MS/MS system.

LC/MS/MS, Multidimensional Chromatograph, Pharmaceuticals

K28 Techniques for Screening and Confirmation of U-47700 and Flubromazepam in Two Non-Fatal Cases

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After attending this presentation, attendees will understand the need for extensive screening and confirmation capabilities in cases involving U-47700 and flubromazepam.

This presentation will impact the forensic science community by emphasizing the importance of using advanced instrumentation to detect emerging drugs of abuse when routine analyses are unremarkable.

A 21-year-old male (Case #1) was reported as acting erratic and had elevated vital signs when examined by a medical officer. He eventually confessed to using synthetic cannabinoids and nutritional supplements. The subject attributed his symptoms to an over-the-counter stimulant, but a search of his room found evidence of steroid use. A second 21-year-old male (Case #2) was reported as acting erratically and "shaking," but further investigative information was lacking. Specimens from both cases were subjected to volatile analysis by headspace/gas chromatography (HS/GC) equipped with a flame ionization detector (FID), immunoassay analysis (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, oxycodone, phencyclidine, 6-acetylmorphine, LSD), and a full scan GC/MS (GC/FS-MS) alkaline drug screen. Based on case history, further testing of specimens was performed by liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/qTOF). Presumptive positive samples were confirmed by GC/MS and LC/MS/MS. Testing results are listed in Table 1.

Table #1. Analytical Results	**Case #1**	**Case #2**
Volatiles (GC/FID)	None Detected	None Detected
Urine Immunoassay	+ Benzodiazepines	+ Benzodiazepines
Blood/Urine Benzodiazepine Confirmation (GC/MS)	None Detected	None Detected
Urine Basic Drug Screen (GC/FS-MS)	Chlorpheniramine Dextromethorphan	Diphenhydramine
LC/QTOF Drug Screen (Case #1: Blood) (Case #2: Urine)	U-47700 Flubromazepam Chlorpheniramine/Dextromethorphan	U-47700 Diphenhydramine
Urine Basic Drug Confirmation (LC/MS/MS)	63ng/mL U-47700 127ng/mL Flubromazepam 419ng/mL Chlorpheniramine 2330ng/mL Dextromethorphan	127ng/mL U-47700 5ng/mL Flubromazepam 130ng/mL Diphenhydramine
Blood Basic Drug Confirmation (LC/MS/MS)	36ng/mL U-47700 450ng/mL Flubromazepam 120ng/mL Dextromethorphan	20ng/mL U-47700 50ng/mL Flubromazepam
Blood Synthetic Cannabinoid Screen (LC/MS/MS)	None Detected	Not analyzed – insufficient history

These cases highlight the importance of using advanced instrumentation to compliment traditional analytical techniques. Based on in-house data, U-47700 has not shown cross-reactivity with immunoassay kits in fortified specimens below 10,000ng/mL. Flubromazepam will cross-react with benzodiazepine kits between 75ng/mL-100ng/mL. It is possible that flubromazepam metabolites are what caused the immunoassay benzodiazepine positive in Case #1. With a panel of mostly therapeutic analytes, the flubromazepam would be missed during the confirmation step. In these two cases, GC/FS-MS did not detect U-47700 or flubromazepam. Large, unidentified peaks were observed that were eventually attributed to U-47700 metabolites. Based on the case histories and initial testing results, additional testing was assigned to the LC/qTOF. By utilizing lower detection limits, expanded spectral libraries, and the benefits of LC over GC, U-47700 was detected in both cases, and flubromazepam was detected in Case #1. Both cases were then confirmed by LC/MS/MS, operating in multiple reaction monitoring (MRM) mode. Due to the increased sensitivity of the LC/MS/MS, urine from case #1 was analyzed even with limited volume left after all other testing had been completed.

These cases highlight difficulties that "designer" drugs can pose to traditional screening and confirmation techniques in the field of toxicology. As their popularity grows, laboratories need to utilize advanced instrumentation such as LC/qTOF and LC/MS/MS to detect these compounds. Comprehensive instrumentation and methodologies are vital in keeping up with emerging drug trends, sample volume limitations, and lower detection limits often required for these compound classes.

U-47700, Flubromazepam, LC/QTOF

K29 A Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Quantitative Analysis of Cocaine and Its Metabolites in Urine, Oral Fluids, and Blood for Forensic Toxicology

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After attending this presentation, attendees will be able to describe simple sample preparation in urine, oral fluid, and blood and will understand the method development and optimization criteria that include Limits Of Quantification (LOQ), Limits Of Detection (LOD), extraction efficiency, matrix effect, accuracy, and imprecision for an LC/MS/MS method for the specific detection and characterization of cocaine and its metabolites in three different biological matrices.

This presentation will impact the forensic science community by describing an analytical method that offers simple sample preparation of three different biological matrices and the specific, focused chromatographic separation and mass spectrometric detection of cocaine and its metabolites in urine, oral fluid, and blood. Investigators need LC/MS/MS methodologies that are specific and focused for the identification and quantification of compounds such as cocaine that are robust, offer accurate results with enhanced sensitivity and specificity, are fast and easy to use, and applicable in the laboratory without the risk of false positives or negatives.

Introduction: LC triple mass spectrometry is suited for rapid analysis of multiple analytes of similar and different structures and physico-chemical properties. A highly sensitive and specific LC/MS/MS analytical method has been developed and optimized for the specific quantitation of cocaine and its metabolites by LC/MS/MS as they have similar physico-chemical structures and properties to achieve the best and most accurate results and include Cocaine (COC), Benzoylecgonine (BZE), Anhydroecgonine Methyl Ester (AME), Cocaethylene (COE), Cocaine-N-Oxide (CNO), Ecgonine Methyl Ester (EME), Ecgonine (ECG), Hydroxybenzoylecgonine (OH-BZE), Norcocaine (N-COC), and Fluorotropacocaine (FTC) and their corresponding internal standards when possible.[1] Simple sample preparation techniques were used that included 1:10 and 1:5 dilution in urine, respectively, and oral fluid and 1:2 acetonitrile protein crash in blood. One-dimensional (1D) chromatographic configurations achieved the required specificity and sensitivity allowing quantitating of these specific analytes over their relevant dynamic range. Therefore, the purpose of this method was to design, develop, and optimize a simple and accurate quantitative method to specifically measure cocaine and its metabolites for forensic toxicology by LC/MS/MS to achieve the most accurate and robust results of these compounds of similar properties.

Method: A Thermo Fisher Scientific™ TSQ Endura MS/MS in positive electrospray mode and a Thermo Scientific™ Vanquish™ High-Performance Liquid Chromatography (HPLC) system were utilized for this analysis. Aliquots of 100µl of urine, oral fluid, and blood were used for the analysis of cocaine and its metabolites. Various core shell columns were evaluated due to their low back pressure and robustness with biological matrices and included the RP-MS, AQ C-18, Phenyl-Hexyl, PFP, and C18 columns. Different organic and aqueous phases with different combinations of modifiers that included formic acid, ammonium formate, acetic acid, etc. were evaluated. A Thermo Scientific™ Accucore™ C18 100mm x 2.1mm, 2.6µm column was chosen with a water:methanol mixture containing 0.01% formic acid and 5mM ammonium formate gradient that achieved baseline chromatographic separation in a five- minute run time. Quantitative analysis was performed using Selective Reaction Monitoring (SRM) transition pairs for each analyte and internal standard in positive mode due to the compounds' chemistries and structures. The accuracy of the method was verified using reference materials from UTAK Laboratories as well as urine, oral fluid, and blood samples from various individuals.

Compound ng/ml	LLOD Urine	LLOQ Urine	LLOD OF	LLOQ OF	LLOD Blood	LLOQ Blood
COC	0.05	0.1	0.1	0.25	0.05	0.1
BZE	0.1	0.25	0.25	0.5	0.1	0.25
AME	1	2.5	2.5	5	1	2.5
COA	0.25	0.5	0.5	1	0.25	0.5
CNO	2.5	5	5	10	2.5	5
EME	0.5	1	1	2.5	0.5	1
ECG	1	2.5	2.5	5	1	2.5
OH-BZE	2.5	5	5	10	5	10
N-COC	1	2.5	2.5	5	1	2.5

Results: Good linearity and reproducibility were obtained with the concentration range from 1ng/ml to 1,000ng/ml for the respective cocaine and its metabolites with a coefficient of determination R^2>0.98 or better for all drugs in the various matrices. The Lower Limits Of Detection (LLOD) and Lower Limit Of Quantitation (LLOQ) for each compound (except Fluorotropacocaine (FTC)) in each matrix were determined as shown in the table. Excellent reproducibility and accuracy were observed for all compounds within the reference materials and subject samples (CV<10%) for all configurations in all matrices.

Conclusion: A sensitive, simple, specific, and accurate LC/MS/MS method was developed, optimized, and verified for the measurement of cocaine and its metabolites. The sample preparation techniques are quick and easily applied for high throughput analysis in urine, oral fluids, and blood for forensic toxicology. The method was developed to achieve the best and most sensitive results possible for the analysis of these compounds without having to compromise the sensitivity or specificity of the method due to the presence of other compounds that do not have similar physico-chemical properties.

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Cocaine, LC/MS/MS, Biological Matrices

K30 A Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Quantitative Analysis of Hallucinogenic Drugs in Urine, Oral Fluids, and Blood for Forensic Toxicology

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After attending this presentation, attendees will be able to describe simple sample preparation in urine, oral fluid, and blood matrices and will understand the method development and optimization criteria that include Limits Of Quantification (LOQ) and Limits Of Detection (LOD), extraction efficiency, matrix effect, accuracy, and imprecision for an LC/MS/MS method for the specific detection and characterization of hallucinogenic drugs as a whole and as subgroups in three different biological matrices.

This presentation will impact the forensic science community by describing analytical methods that offer simple sample preparation of three different biological matrices and the specific and focused chromatographic separation and mass spectrometric detection of hallucinogenic drugs in urine, oral fluid, and blood, both together as a complete analytical group and separately based on their individual drug class. Investigators need LC/MS/MS methodologies that are specific and focused for the identification and quantification of compounds such as hallucinogenic drugs that are robust, offer accurate results with enhanced sensitivity and specificity, are fast and easy to use, and applicable in the laboratory without the risk of false positives or negatives.

Introduction: LC triple quadrupole mass spectrometry is suited for rapid analysis of multiple analytes of similar and different structures and physicochemical properties. Hallucinogens are a diverse group of pharmacological agents that directly affect behavior, are abused, and include dissociative drugs such as benocyclidine, dextromethorphan, dextrorphan, dehydronorketamine, methoxetamine, 3-methoxyphencyclidine, 4-methoxyphencyclidine, norketamine, and phencyclidine; LSD drugs such as lysergic acid diethylamide, lysergic acid n,n-methylpropylamide, and 2-oxo-3-hydroxy-LSD; NBOMe analog drugs such as 25B-NBOMe, 25C-NBOMe, 25I-NBOMe, 25I-NBOH, 25I-NBF, and 25I-NBMD; plant-based hallucinogenic drugs such as cathinone, 7-hydroxymitragynine, mescaline, mitragynine, Salvinorin A, and Salvinorin B; and tryptamines such as bufotenine, N,N-Dimethyltryptamine, 5-MeO-DALT, 5-MeO-MiPT, 5-MeO-DiPT, 5-MeO-DMT, 5-MeO-AMT, psilocybin, and psilocin.[1-6] Highly sensitive and specific LC/MS/MS analytical methods were developed for the quantitation of hallucinogens together and as separate sub-groups. Simple sample preparation techniques were used that included 1:10 and 1:5 dilution of urine and oral fluid and 1:2 acetonitrile protein crash of blood. One-dimensional (1D) chromatographic configurations achieved the required sensitivity, allowing quantitation over the analytes' relevant dynamic range. Therefore, the purpose of this method was to design, develop, and optimize simple and accurate analytical methods to measure hallucinogens as a whole and as subgroups for forensic toxicology in matrix by LC/MS/MS to achieve the most accurate and robust results while demonstrating the analytical compromises involved with multiple compounds of different structures and physicochemical properties.

Method: A Thermo Scientific™ TSQ Endura MS/MS in positive and negative electrospray mode and a Thermo Scientific™ Vanquish™ High-Performance Liquid Chromatography (HPLC) system were utilized. Aliquots of 100µl of urine, oral fluid, and blood were used for the analysis of hallucinogens. Various core shell columns including RP-MS, AQ C-18, Phenyl-Hexyl, PFP, and C18 were evaluated due to their low back pressure and robustness with biological matrices. Different organic and aqueous phases along with different combinations of modifiers, including formic acid, ammonium formate, etc., were evaluated for each method. A Thermo Scientific™ Accucore™ C18 100mm x 2.1mm, 2.6µm column with a water:acetonitrile mixture containing 0.1% formic acid and 5mM ammonium formate gradient was used for all methods achieving baseline chromatographic separation in six-minute run times for all six methods.[6] Quantitative analysis was performed using Selective Reaction Monitoring (SRM) transition pairs for each analyte and internal standard in positive and negative mode due to the compounds' different chemistries and structures. The accuracy of these methods was verified using reference materials from UTAK Laboratories as well as urine, oral fluid, and blood samples from various individuals.

Results: Good linearity and reproducibility were obtained with the concentration range from 1ng/ml to 1,000ng/ml for the hallucinogens with a coefficient of determination R^2 >0.98 or better for all drugs in the various matrices. The Lower Limits Of Detection (LLOD) and Lower Limit Of Quantitation (LLOQ) were determined for

the hallucinogens and methods to demonstrate the differences in sensitivity due to the compromising of the method parameters and varied considerably. Excellent reproducibility was observed for these compounds (CV<10%) for all configurations in all matrices.

Conclusion: Sensitive, simple, specific, and accurate LC/MS/MS methods were developed and verified for the simultaneous measurement of hallucinogens as a whole and as subgroups. The sample preparation techniques are quick and easily applied for high throughput analysis in urine, oral fluids, and blood for forensic toxicology. The methods were developed to achieve the best and most sensitive results possible for the analysis of hallucinogens without compromising the sensitivity or specificity of the methods due to the drugs' different physicochemical and structural properties.

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Hallucinogens, LC/MS/MS, Biological Matrix

K31 The Chinese Influence on the Novel Psychoactive Substances (NPS) Movement in the United States

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After attending this presentation, attendees will be able to describe the influence that legislation both within and outside the United States has on NPS popularity, availability, and positivity in forensic casework.

This presentation will impact the forensic science community by detailing the change in popularity of NPS in reaction to legislation, which underscores the need for a comprehensive and current scope of toxicological testing and increased awareness by investigators, forensic scientists, and legislators.

One of the hallmarks of the NPS movement is the ebb and flow of the drugs. The active ingredients in products such as K2 and bath salts sold for recreational abuse are constantly changing, creating a significant challenge for the forensic science community. The changing landscape of NPS has been heavily influenced by legislation in the United States (for example, the rapid disappearance from circulation and forensic casework in 2013 of the stimulant 1,3-Dimethylamylamine (DMAA) after pressure was placed on supplement manufacturers by the Food and Drug Administration (FDA) to reformulate their products after a series of deaths). Changes in legislation generally precipitate changes in positivity in tests that cover NPS, which constantly require updates to their scope. It appears that recent Chinese legislation has also had an impact on the drug landscape in the United States, as an October 2015 ban of 116 chemicals by the Chinese Food and Drug Administration was followed by a change in positivity for NPS in the United States, as many of these substances were originating from China and being sold as bulk research chemicals over the internet.

As soon as the Chinese ban went into effect, web forums dedicated to the use of NPS detailed what substances were no longer available and recommended alternatives. The list of banned substances included stimulants, psychedelics, sedatives, and synthetic cannabinoids. While positivity for some substances declined sharply, the ban also created opportunities for others to emerge.

The change in popularity for the use of alpha pyrrolidinovalerophenone (alpha PVP) was probably the most dramatic example of the influence the Chinese legislation had on the United States drug market. Alpha PVP quickly gained popularity as an alternative to Methylenedioxypyrovalerone (MDPV) after its ban through the United States' Synthetic Drug Abuse Prevention Act of 2012. From 2013 to 2015, alpha PVP was the most popular synthetic stimulant detected in an expansive toxicological screen for NPS. In 2013 and 2014, alpha PVP accounted for roughly a quarter of all positive analytes. Use in 2015 peaked, when it accounted for 34.7% of positives, but has dropped drastically so far in 2016, accounting for seven positives in nearly 400 cases.

Ethylone was also specifically outlawed in the Chinese legislation. Ethylone gained popularity as an alternative to methylone, which was also banned in 2012. Methylone's use prevailed in 2013, accounting for 26.3% of the positive results; however, in 2014, the positivity for methylone dropped to 13.8%, which coincided with a rise in ethylone, which increased approximately 16% between 2013 and 2014. In 2015, ethylone accounted for 24.7% of the positives, but has only been confirmed in five cases so far in 2016. Similar trends were seen in samples collected from attendees at electronic dance music festivals in the United States.

Several synthetic cannabinoids were covered by the Chinese ban, including AB-FUBINACA, AB-CHMINACA, and 5F-AMB. In October 2015, AB-CHMINACA accounted for roughly 20% of the positive toxicology findings. By June of 2016, AB-CHMINACA was only detected in approximately 3% of positive synthetic cannabinoid cases. Many of these synthetic cannabinoids continue to maintain some kind of presence in the United States; this could be explained by multiple source countries.

A number of fentanyl analogues were also outlawed in the ban; these include acetyl-, butyryl-, and β-hydroxythiofentanyl. This ban has possibly played a role in the rise in popularity of other fentanyl analogues that are causing increasing numbers of fatal and non-fatal overdoses in the United States, with many states reporting findings of analogues, including furanyl fentanyl, para-fluorofentanyl, and other designer opioids, including U-47700. The earliest known reports of these substances are from November/December 2015.

The continuously changing landscape of NPS is a challenge for the forensic community as it requires an enormous amount of resources to stay abreast of the drug trends as they change in reaction to legislation at home and abroad.

NPS, Legislation, China

K32 The Prevalence of New Psychoactive Substances (NPS) in Northeast Asia From 2007 to 2015

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After attending this presentation, attendees will understand the prevalence of NPS in northeast Asia (China, Japan, Korea, and Taiwan). As this is the first study of NPS in four countries, it will provide an opportunity to develop a comprehensive and sensitive mechanism for early detection of NPS in northeast Asia.

This presentation will impact the forensic science community by providing information concerning the NPS situation in northeast Asia, especially to those who study the toxicological aspects of NPS in other parts of the world.

Recently, NPS have been a global trend in drug abuse and the regulation of these substances has been a worldwide concern.[1,2] In order for effective regulation of NPS, it is necessary to share information related to these emerging substances around the globe. Although many studies have been published on the prevalence of NPS in western countries, there is not enough information available for certain countries in Asia. Therefore, it is important to investigate the current status of NPS abuse in these countries and to provide information on NPS in the interest of effective regulation.

This study was performed using data provided by the Korea Food and Drug Administration (KFDA), the Data Search System for New Psychoactive Substances by National Institute of Health Sciences in Japan, the Embassy of the People's Republic of China in India, the China Food and Drug Administration, and the Taiwan Food and Drug Administration (TFDA) from 2007 to 2015.[3-6] Each substance was categorized in nine groups according to the classification used in the United Nations Office on Drugs and Crime (UNODC).[7]

It has been shown that a total of 978 NPS were reported in northeast Asia from 2007 to 2015. Among the 978 NPS, 882 substances are legally restricted in at least one country (90%) and 96 substances were not currently controlled (10%) in any country. The number of controlled NPS that are currently controlled in all four countries was only 25 out of 882 NPS. It was found that Japan is the most proactive country in terms of the NPS regulation, with 37% of the total number of controlled NPS, followed by South Korea (25%), China (20%), and Taiwan (9%).

Comparing the number of NPS newly regulated in each country every year, NPS has been widely scheduled in 2011 and the number of scheduled NPS has dramatically increased from 2013 to 2015. The most commonly controlled NPS were synthetic cannabinoids, synthetic cathinones, and phenethylamines, as shown in a global trend reported by the UNODC annual announcement.

This research was performed to study the prevalence of NPS and to provide information on the current status of NPS in northeast Asia. It has shown that northeast Asia is also in danger from these emerging NPS and that effective regulation and information-sharing across countries is important for the prevention of the negative social effects of NPS use and abuse. This study will also raise NPS awareness in local law enforcement.

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New Psychoactive Substances, Northeast Asia, Prevalence

K33 The Evaluation of Temporal Changes of Novel Psychoactive Substances (NPS) Use Within an Electronic Dance Music (EDM) Population Over a Three-Year Period

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After attending this presentation, attendees will be able to better evaluate the toxicological results of blood, urine, and oral fluid samples collected at an EDM festival. The forensic science community will be able to compare the results from this sample collection to samples collected in the past two years to evaluate changes in NPS use within this population.

This presentation will impact the forensic science community by providing information on the most recent NPS being used within this EDM population. Using these results, laboratories will be able to expand their scope of testing to include emerging NPS, if current testing does not already include these compounds.

EDM festivals have gained recent media attention due to drug-related deaths and hospitalizations. In June 2016, at the Sunset Methylenedioxymethamphetamine (MDMA). There were a further 57 hospitalizations and many arrests and citations made at the festival due to involvement with MDMA, LSD, and marijuana. This population can provide key insights into determining new trends in NPS in the recreational drug market.

This study was approved by an Institutional Review Board for the participation of human subjects. Blood, urine, and oral fluid samples were collected at an EDM festival in Miami, FL, in three successive years (2014, 2015, and 2016). Blood samples were collected by a certified phlebotomist in gray-topped collection tubes, urine samples were self-collected using sterile collection cups, and oral fluid samples were self-collected using a Quantisal™ oral fluid collection device.

In 2016, a total of 248 subjects completed the donation of blood, urine, and/or oral fluid samples. Ninety-four of those subjects also provided survey information with respect to their age, gender, and recent drug use. Of these 94 participants, 13 provided blood samples, 50 provided urine samples, and 86 provided oral fluid. The average age of participants was 23 (±4.7) years of age. Seventy-four (79%) of the subjects indicated they had used a recreational or medical substance within the past week. Alcohol was the top response (48%), followed by marijuana (31%), and Molly/MDMA/Ecstasy (15%).

Based on the 2016 blood sample (n=12) analysis, Tetrahydrocannabinol (THC) (n=3) was the most commonly encountered drug, followed by MDMA/MDA (n=2), LSD (n=1), and amphetamine (n=1). For the 2016 urine samples (n=50), carboxy-THC (n=11) was the most commonly encountered drug/metabolite, followed by amphetamine (n=4) and MDMA/MDA (n=3). One urine sample was positive for dibutylone, an emerging NPS detected for the first time in this population during this sample collection, and butylone, a possible metabolite of dibutylone and also an emerging NPS detected in samples collected in 2014. For the 2016 oral fluid samples (n=244), THC (n=79, 32%) was the most commonly encountered drug, followed by MDMA (n=28, 11%), cocaine (n=21, 9%), and amphetamine (n=16, 7%). Other NPS, including dibutylone, butylone, ethylone, and 4-FA, were also detected.

Over the three-year sample collection period, there have been significant year-to-year changes in the NPS drugs present. During the 2014 sample collection, the most commonly encountered NPS was methylone, followed by alpha-PVP, MDMA, dimethylone, ethylone, butylone, and 4-FA. During the 2015 sample collection, the only NPS detected was ethylone. During the 2016 sample collection, MDMA was the most commonly encountered drug, showing a trend back to traditional NPS. Emerging NPS, like dibutylone, were detected for the first time during analysis of 2016 samples, demonstrating that the designer drug market is continuing to evolve.

These results stress the importance of updating laboratory methods for the detection of emerging drugs, the ability to distinguish between isomeric NPS, and the usefulness of this target population for monitoring trends.

NPS, MDMA, Oral Fluid

K34 A Death Due to the Use of the Novel Psychoactive Substances (NPS) (2-Aminopropryl) Benzofuran (APB) and N-Methyl Derivative (MAPB)

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After attending this presentation, attendees will be informed of the potential need to reevaluate previously collected mass spectrometry data as new designer drug data becomes available. The forensic science community will also become educated regarding the effects of APB and MAPB and their potential contributions to death.

This presentation will impact the forensic science community by alerting the community to a relatively new designer drug in use across the nation and around the world.

New designer drugs that have been developed with slight chemical modifications to known and/or controlled drug structures are being synthesized and abused on a continuing basis. The challenge to stay ahead of the curve of detecting these novel drugs is an ongoing problem in forensic pathology and toxicology. This presentation discusses the novel psychoactive drugs, APB and MAPB, so that they may be accurately sought after and detected in a case of drug abuse or overdose in which suspected drugs are not immediately identified by routine drug screening.

APB and MAPB are novel psychoactive benzofurans that share structural and psychoactive properties with Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) and also with amphetamine and methamphetamine. They are classified as NPS and were first noted to be used in 2010 in the United Kingdom. Due to several deaths in the United Kingdom and other countries, these drugs are currently illegal in some countries. Though they are not scheduled under the United States. Controlled Substances Act, they fall into the Federal Analogue Act. The Federal Analogue Act allows any chemical with a high potential for abuse that is "substantially similar" to a Schedule I or II drug to be treated as such under the law. The effects of these drugs are classic for stimulants and nearly identical to that of MDMA, including tachycardia, hypertension, hyperthermia, hallucinations, seizures, insomnia, and anxiety. The pharmacologic mechanism of these drugs is via inhibition of dopamine, noradrenaline, and 5-Hydroxytryptamine (5-HT) serotonin transporters. They also act as ligands of 5-HT2A and 5-HT2B. Due to the novelty and possible lack of identification of these drugs, deaths in the United States associated with the use of APB and MAPB are sparse in the literature.

This case study highlights the unfortunate death of a 15-year-old Caucasian female with a prior history of alprazolam and marijuana use who was observed snorting/inhaling an unknown powdered drug with subsequent hallucinations and seizure activity prior to becoming unresponsive. She was transported to the hospital where she was pronounced deceased.

At autopsy, pulmonary edema and petechiae of the epicardial aspect of the heart and thymus were identified. An edematous brain was identified, with no evidence of herniation. A preliminary drug screen performed on heart blood revealed the presence of lidocaine, acetaminophen, and amphetamines. No amphetamines were determined upon confirmation testing; no bath salts, cannabinoids, or other stimulant designer drugs were identified on select panels. While ethanol was not detected, the presence of lidocaine and naloxone are likely attributed to resuscitation efforts. Although acetaminophen is not typical of resuscitative efforts, it can be seen with opiate use. No opiates were identified in this case.

Given the history of drug use immediately followed by hallucinations, seizures, and death, and negative confirmatory toxicology, the cause of death was originally listed as "complications of inhalation of an unknown drug" and manner of death was accident.

Nearly a year later, the toxicology results were reviewed due to a law enforcement request for laboratory records on the case. The case was reevaluated utilizing the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Gas Chromatography/Mass Spectrometry (GC/MS) electronic library that contains spectra for hundreds of legally confiscated drugs. Two peaks matching APB and MAPB were identified and later confirmed by tandem mass spectrometry. The cause of death was amended to be APB and MAPB intoxication. The structural

similarities of APB and MAPB to amphetamine and methamphetamine explained the preliminary positive drug screen for amphetamines. This case highlights the importance of keeping GC/MS and Liquid Chromatography/ Mass Spectrometry (LC/MS) libraries up to date, so that when a novel drug is suspected, a timely detection can be made. This can also help keep the forensic pathologist up to date with the proper classification of drug-related deaths.

APB, MAPB, Amphetamine

K35 Demonstrating the Need for Complete Testing Methods When Screening for Novel Psychoactive Substances (NPS)

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After attending this presentation, attendees will understand the need to consider the metabolism of known, infrequently detected compounds when screening for NPS.

This presentation will impact the forensic science community by increasing awareness that complete analytical testing profiles must be used when interpreting data regarding NPS. Sample data from two case samples will be used to demonstrate this point.

NPS use has been a main focus of forensic and clinical toxicology for the past several years. The Department of Defense (DoD) and its Drug Demand and Reduction Program (DDRP) have utilized surveillance testing to keep pace with emerging drug trends in service member urine samples. The Armed Forces Medical Examiner System (AFMES) Division of Forensic Toxicology uses Liquid Chromatography/quadrupole Time-of-Flight/Mass Spectrometry (LC/qTOF/MS) for a portion of their surveillance testing, as well as for select human performance and postmortem casework.

Toxicology analysis is often directed based on initial screening results and case history. Initial screens are typically based on enzyme immunoassay and Gas Chromatography/Mass Spectrometry (GC/MS) techniques. These approaches, while powerful, have known limitations in scope. New immunoassay kits are often unable to stay current with NPS trends. GC/MS screening methods may not yield satisfactory performance for compounds that are thermally labile or have poor volatility. New instrumentation, including LC/qTOF, is important in addressing the limitations of routine toxicology screening techniques and was used to investigate potential NPS detection in the following cases.

Case 1: Urine from a routine human performance case was screened by an alkaline GC/MS method. Ethcathinone (N-ethylcathinone), cathinone, cathine, phentermine, and trace amounts of diethylpropion were presumptively identified. An unidentified cathinone-related peak was also observed in the GC/MS chromatogram. LC/qTOF was used to confirm the previous findings, as well as propose reduced metabolites for ethcathinone and diethylpropion.

Diethylpropion (diethylcathinone, amfepramone) is a Schedule IV anorectic not commonly observed in DoD samples. Known metabolites include reductions and N-dealkylations (ethcathinone, cathinone). Inclusion of diethylpropion to screening techniques and NPS panels, particularly ones including cathinones, is vital to accurately interpret ethcathinone detection as a result of diethylpropion and not illicit NPS use.

Case 2: Hospital urine from a postmortem case was screened using a routine alkaline GC/MS method. Lamotrigine and a large, unidentified peak were detected. The unknown mass spectrum resembled a phenethylamine and was suspected as a possible NPS. Lamotrigine was confirmed by GC/MS. Midazolam and 1-hydroxymidazolam were also confirmed by GC/MS as a result of routine immunoassay screening. LC/qTOF was used to investigate the suspected NPS.

Labetalol was detected by LC/qTOF in addition to the previous case findings. Labetalol is an antihypertensive with mixed α- and β-adrenergic receptor antagonist activity. It is not commonly detected in DoD samples. After further review of the LC/qTOF data, the potential NPS was proposed as 3-Amino-1-Phenylbutane (APB), a previously reported labetalol metabolite and isomer of methamphetamine. Both the GC/MS library and LC/qTOF databases were updated using a certified reference standard for future casework.

The cases described in this presentation serve as a reminder that positive detections of “designer drugs” and suspected NPS cannot be interpreted in a vacuum; complete analytical profiles must be available to the laboratory. In certain cases, detection of these compounds may be due to metabolism from infrequently encountered, legitimately

used prescription medications. Expanded testing methods, such as LC/qTOF and regularly updated GC/MS libraries, greatly aid in properly assessing NPS-related positives.

NPS, QTOF, Screening

K36 Clinical Manifestations of U-47700 and Flubromazepam Intoxication

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After attending this presentation, attendees will be able to describe the pharmacological effects observed in two individuals exposed to opioid and benzodiazepine Novel Psychoactive Substances (NPS).

This presentation will impact the forensic science community by comparing behavioral and toxicological findings in two NPS intoxication cases. This information is useful in directing efforts toward additional screening when an investigation indicates drug use, but initial tests are unremarkable.

Pharmaceutical companies and academic researchers continue to develop new alternatives to traditional licit and illicit drugs. These advances led to the creation of compounds now sold as designer drugs or NPS. NPS, such as synthetic cannabinoids, cathinones, phenethylamines, tryptamines, opioids, and benzodiazepines, may be pursued as "legal highs" for individuals attempting to evade legal repercussions.

This presentation reports two non-fatal cases of NPS intoxication. The first involved a 21-year-old male exhibiting flushed skin, nausea, confusion, and unpredictable behavior. On several occasions during the incident, he became combative with others and would lose consciousness once seated. He was transported to the hospital, administered resuscitation measures, and responded positively to naloxone. The initial clinical laboratory urine toxicology screen was positive for benzodiazepines only. Additional hospital urine toxicology found chlorpheniramine, dextromethorphan, dextrorphan, and diphenhydramine. Investigation of his belongings revealed syringes, estrogen blockers, and supplements. He admitted to using synthetic cannabinoids and to purchasing several vials of U-47700 from the internet for back pain. After release from the hospital, he was observed sleeping for 19 to 24 hours. The second case involved a 21-year-old male with a history of cocaine use who was shaking and acting erratically and reported using pre-workout supplements.

Venous blood and urine from both cases were submitted for routine toxicological analysis. Positive findings are reported in Table 1.

Table 1. Toxicology Results

Case 1	Blood	Urine
Case 1	36ng/mL U-47700 450ng/mL Flubromazepam 120ng/mL Dextromethorphan	U-47700 Flubromazepam Dextromethorphan Chlorpheniramine
Case 2	20ng/mL U-47700 50ng/mL Flubromazepam	U-47700 Flubromazepam Diphenhydramine

Two NPS, U-47700 and flubromazepam, were detected in both cases, with low U-47700 concentrations quantified in the blood. U-47700 is an N-substituted cyclohexyl benzamide synthetic μ-opioid receptor agonist with 7.5 times the potency of morphine. AH-7921, a structurally similar yet less active opioid analgesic, first appeared four years ago. This signals a progression toward the use of stronger alternatives to prescription opioids and heroin. Patents that describe this atypical opioid class also contain derivatives expected to possess similar activity as U-47700 at μ-opioid receptors.

Flubromazepam is a designer benzodiazepine reported to have an extended duration of action and induce excessive sedation and fatigue. Higher flubromazepam concentrations were found in Case 1, in which prolonged sleep was indicated by case history. Although flubromazepam and U-47700 have not been well-characterized in human subjects, opioids and benzodiazepines in combination may elicit profound Central Nervous System (CNS) depression and require medical intervention, as observed in Case 1. Published fatalities describe U-47700 in combination with fentanyl, antidepressants, antipsychotics, and other NPS. Reported here is the first non-fatal multi-drug intoxication cases of U-47700 in combination with flubromazepam and U-47700 is highlighted as a potent opioid difficult to detect by routine toxicological analysis.

U-47700, Flubromazepam, NPS

K37 Synthetic Cathinone Stability in Blood Using Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS)

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After attending this presentation, attendees will better understand the stability of synthetic cathinones in blood stored at various temperatures over a period of 30 days.

This presentation will impact the forensic science community by increasing attendees' knowledge regarding the stability of cathinone designer drugs in blood.

Understanding drug stability under various storage conditions is crucial to toxicological data interpretation. While the stability of many drugs of abuse are known, information pertaining to the stability of cathinone designer drugs is relatively limited. Previous literature has described stability for a select few cathinones. This research presents a systematic evaluation of the stability of 22 synthetic cathinones in blood stored at four temperatures over a period of 30 days.

Solid phase extraction and LC/qTOF/MS equipped with a Poroshell 120 EC-C18 column were used to identify and quantify 22 synthetic cathinones. Blood was fortified with analytes at high (1,000ng/mL) and low (100ng/mL) concentrations and stored at four temperatures (32°C, 20°C, 4°C, and -20°C) for up to 30 days. Quantitative analyses were performed at various time points (hours, days, and weeks) throughout the course of the study. The following cathinones were included in the study: methcathinone, ethcathinone, pentedrone, buphedrone, 3-Fluoromethcathinone (3-FMC), 4-Fluoromethcathinone (4-FMC), 4-Methylethcathinone (4-MEC), 4-Ethylmethcathinone (4-EMC), mephedrone, methedrone, 3,4-Dimethylmethcathinone (3,4-DMMC), ethylone, methylone, butylone, pentylone, eutylone, Methylenedioxypyrovalerone (MDPV), 4-Methylpyrrolidinobutiophenone (MPBP), 3,4-Methylenedioxypyrrolidinobutiophenone (MDPBP), alpha-Pyrrolidinopentiophenone (alpha-PVP), pyrovalerone, and naphyrone. A total of nine deuterated internal standards were used. The LC/qTOF/MS analytical procedure was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation. Limits of detection and quantitation ranged from 1ng/mL to 5ng/mL. Accuracy ranged from 94%-111% and intra-/inter-assay precision ranged from 0-9% and 3%-7%, respectively. Cathinones were considered stable if concentrations were within 20% of the expected concentration.

The stability of synthetic cathinones was highly temperature dependent. Considerable degradation was observed in blood stored at 32°C and 20°C (60%-100% loss at 32°C for all cathinones). The ring substituted and unsubstituted secondary amine cathinones were the least stable, with a select few reaching 100% loss within 30 days at 32°C. Among the secondary amine, 3-FMC was the least stable and was undetectable within the first 48 hours at 32°C and 20°C. The methylenedioxy-pyrrolidine type synthetic cathinone (MDPBP and MDPV) were the most stable. Although MDPBP and MDPV experienced a 60% and 84% loss at 32°C, no significant degradation was observed at or below 20°C.

Synthetic cathinone stability in blood was highly dependent on temperature and drug structure (secondary/tertiary amines and benzylic substituents). Some drugs within this class experienced dramatic losses within hours at room or elevated temperatures. Conditions under which samples have been transported (to the laboratory) or stored must be considered if quantitative results are to be reliably interpreted.

Synthetic Cathinones, Stability, LC/qTOF/MS

K38 Method Validation Using Multiple Compound Mixtures for Screening/Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)

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After attending this presentation, attendees will be aware of the ability to use mixtures, rather than running individual compounds, to validate a screening/conformational method for detecting NPS in biological fluids using LC/QqQ/MS. The goal of this presentation is to include more than 800 NPS in the final validated method for screening. The 800 NPS that will be included in this method will cover a wide variety of drug classes with a focus on synthetic cannabinoids due to their current importance in many cases entering toxicology laboratories.

This presentation will impact the forensic science community by introducing the development of a validated, comprehensive screening method for NPS, which would help with the screening of potential drug abusers. This proposed method will help with the detection of NPS so that a higher number can be detected, thus decreasing the number of false negatives. The goals of this presentation are to be able to use one comprehensive method to screen for the majority of NPS, which may not be detected through current screening methods, and to present an alternative to current screening methods, as this method is capable of detecting a higher number of compounds.

This presentation is intended to demonstrate the use of standard mixtures rather than individual compounds to validate a screening/confirmatory method for detecting NPS in biological fluids using LC/QqQ/MS). The ultimate goal is to include more than 800 NPS in a final validated method. The NPS that are included in the method cover a wide variety of NPS drug classes and metabolites, with a focus on synthetic stimulants and cannabinoids, due to their current importance in forensic toxicology casework. NPS have become a major issue in toxicology laboratories because of their potentially high potency, their ability to remain undetected by many current screening methods, and their rapid development in attempts to avoid current scheduling laws. The proposed method is anticipated to allow the comprehensive detection of the majority of NPS in a single run, with concomitant reduction in the number of false negatives.

An Agilent® 1290 Infinity High-Performance Liquid Chromatography (HPLC) system and Agilent® 6460 QqQ/MS with Jet Stream Technology Electrospray Ion Source (ESI) was used for this research. A total of 826 compounds to be included in the final method were analyzed using both Flow Injection Analysis (FIA) and Agilent® Optimizer software for optimizing fragment transitions in order to create a triggered Multiple Reaction Monitoring (tMRM) method. Once the tMRM method was created, all compounds were run through an Agilent® ZORBAX® Rapid Resolution HD Eclipse Plus C18 column (3.0mm x 100mm; 1.8µm particle size) in order to obtain retention times. Retention times were collected with a gradient of 95% A (5mM ammonium formate in HPLC water with 0.1% formic acid) and 5% B (methanol with 0.1% formic acid) from 0min-1min, increasing to 95% B over 1min-9.5min, then 98% B for the remainder of the run. All retention times were used to create the final method for validation.

In order to fully validate the method, calibration curves must be created for each drug standard. Since completing individual calibration curves for each of the 826 NPS included would be extremely time-consuming and inefficient, an approach using a series of standard calibration mixes was explored. In order to validate the proposed method for 826 compounds, mixes were created to evaluate multiple compounds at a time. Mixes contained 29 to 37 different compounds, leading to 25 total mixes encompassing all 826 NPS. Criteria for individual mixes included the presence of unique transitions for each component, no co-eluting compounds, and a minimum of 0.2min between compound peaks in the same mixture. Seven different calibration levels were chosen for method validation as follows: 1ng/mL, 2ng/mL, 5ng/mL, 10ng/mL, 20ng/mL, 50ng/mL, and 100ng/mL. All calibrators also incorporated an internal standard "supermix" composed of 22 deuterated standards representing multiple drug classes. Calibrations were performed with both methanol-based and spiked matrix (urine) mixtures for method optimization. For urine, calibrations were completed using a "dilute and shoot" approach, with a 1:5 dilution injected directly into the instrument.

To date, individual calibration curves have been created for eight different NPS mixtures representing 240 of the 826 proposed compounds for the final validated method. The majority of compounds exceeded the required R^2 value for validation using six replicates at each level. LC chromatograms were analyzed using the Find-by-MRM software algorithm, which identifies each compound in the mixture based on its targeted transitions. This approach was capable of identifying all compounds in each mixture. The results of these experiments clearly demonstrate the value of using standard mixes for method validation in comprehensive toxicological analysis. Work is continuing to create calibration curves for the remaining compounds, with the goal of using mixtures containing a maximum number of compounds in order to limit the number of mixtures needed for full validation.

LC/QqQ/MS, Method Validation, Novel Psychoactive Substances

K39 Characteristics of Drug Use Among Drivers in Houston, Texas

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After attending this presentation, attendees will better understand the prevalence and concentrations of drugs found in blood samples of Houston drivers involved in traffic accidents or suspected of impaired driving.

This presentation will impact the forensic science community by providing important regional information so attendees can better understand the demographic profile of drug-impaired or suspected impaired drivers in Houston, TX. The long-term objective is to help design and implement regulations and prevention methods that will lead to a reduction in drug-impaired driving, an urgent traffic safety concern.

Commonly abused drugs, including amphetamines, benzodiazepines, cannabis, hypnotics, and opioids, have been associated with increased road traffic crash risks due to their psychoactive properties that can impact driving-related functions.[1] According to the 2014 National Survey on Drug Use and Health (NSDUH), ten million people, aged 12 years or older, reported driving under the influence of illicit drugs during the past year. That is an increase from the 9.9 million people who reported similar activity in the 2013 NSDUH survey. The survey additionally found the rate of driving under the influence of illicit drugs to be highest among young adults 18 to 25 years of age. Houston is the most populous city in Texas and the fourth most populous in the United States. Hence, evaluation of the Houston dataset can also provide insight into drug use patterns among drivers in other major metropolitan areas.

This research includes cases of driving while intoxicated or driving under influence of drugs, both fatal and non-fatal, occurring in 2014 or 2015 that were analyzed by the Houston Forensic Science Center in 2015 for common drugs of abuse in blood samples. These samples were collected from drivers and submitted by the Houston Police Department. The data evaluated includes drug concentrations and detection rates in addition to demographics, including age, sex, and race/ethnicity. These samples are routinely analyzed for blood alcohol first. All fatality cases and those with a blood alcohol concentration of <0.010g/100mL proceeded to further toxicological analysis. The blood samples were screened by immunoassay for benzodiazepines, benzoylecgonine, cannabinoids, methamphetamine, opiates, and phencyclidine. After October 19, 2015, an additional six immunoassay kits (amphetamine, barbiturates, carisoprodol/meprobamate, methadone, oxycodone/oxymorphone, and zolpidem) were added to the testing panel. Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Mass Spectrometry (LC/MS) systems are used as a secondary analysis to confirm a positive screening. An external laboratory completed the majority of the confirmatory analyses.

In 2015, the laboratory tested 425 impaired driving or traffic accident cases for common drugs of abuse in blood. Of those cases, 34% tested positive for cannabinoids, 27% for alprazolam, 12% for cocaine/metabolites, 12% for hydrocodone, 10% for phencyclidine, and 6% for carisoprodol/meprobamate. The mean blood concentrations of Δ^9-tetrahydrocannabinol, alprazolam, cocaine, hydrocodone, phencyclidine, and carisoprodol/meprobamate were 3.8ng/mL (median 2.9; range 0.5-17), 67 ng/mL (49; 5.6-450), 56ng/mL (38; 15-120), 65ng/mL (45; 6.4-311), 44 ng/mL (39; 7.3-92), and 4.5/21 µg/mL (4.6/17; 0.5/3-17/55), respectively. Males accounted for 79% of the cases and Whites for 60%. Blacks accounted for 30%, Asians for 4%, and Hispanics for 3%. The mean age was 33 years (median 31; range 17-83).

The list of prevalent drugs found among suspected impaired drivers in Houston or involved in a traffic accident was similar to those observed in other laboratories.[2] Since cannabis is the most widely abused illicit substance, it is not surprising that it is also the most frequently detected drug (other than alcohol) in this study and others.[3,4] The number of illicit drug users has been steadily rising nationally since 2002, making risk assessment and prevention of drug-impaired driving increasingly important.

Reference(s):

1. Gjerde et al. Driving under the influence of non-alcohol drugs – an update. Part I: e pidemiological studies. *Forensic Sci Rev.* 2015;27:89-113.
2. Logan et al. Recommendations for toxicological investigation of drug-impaired driving and motor vehicle fatalities. *J Anal Toxicol.* 2013;37:552-558.

3. Wilson et al. Fatal crashes from drivers testing positive for drugs in the U.S., 1993-2010. *Public Health Rep.* 2014;129:342-50.
4. Legrand et al. Alcohol and drugs in seriously injured drivers in six European Countries. *Drug Test Anal.* 2013;5:156-165.

Drugs, Toxicology, Impaired Driving

K40 Driving Under the Influence (DUI) of Methamphetamine/Amphetamine (mAMP/AMP) and Cannabinoids in the City and County of San Francisco

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After attending this presentation, attendees will possess the necessary knowledge to interpret mAMP/AMP and cannabinoid blood concentrations in DUI cases based on a seven-year review of such cases in San Francisco, CA.

This presentation will impact the forensic science community by demonstrating the need for and usefulness of comprehensive drug testing in DUI cases, irrespective of the driver's alcohol concentration.

In the City and County of San Francisco, the American Board of Forensic Toxicologists (ABFT) -accredited Forensic Laboratory Division (FLD) of the Office of the Chief Medical Examiner (OCME) performs all forensic toxicology testing, including postmortem as well as human performance forensic toxicology cases. A retrospective review of all suspected DUI cases submitted to the FLD between January 2, 2009, and January 28, 2016, was undertaken in order to better understand and characterize the incidence of mAmp/Amp in DUI cases in San Francisco.

Prior to August 1, 2014, DUI specimens were only screened for drugs by immunoassay and/or gas chromatography/mass spectrometry if drug testing was specifically requested by the police or if the blood ethanol was ≤0.10% weight by volume (w/v). Since August 1, 2014, all DUI specimens are screened for drugs regardless of the driver's blood alcohol concentration, and the testing protocol used is in compliance with *Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities*.[1]

Examination of digital and physical records revealed that in the seven-year period of interest, the FLD has performed analyses in 5,715 DUI cases (562 felonies; 5153 misdemeanors). Of these, 75 cases (1.3%) had mAmp/Amp and cannabinoids in blood and within this group, mAmp/Amp and cannabinoids were the only drugs found in 42 blood specimens; 59 of the 75 drivers with mAmp/Amp and cannabinoids were male (78.6%) and 34 of the 42 drivers with only mAmp/Amp and cannabinoids in their blood were male (80.9%). Whites, Hispanics, and Blacks comprised the three most common race groups among the 75 drivers with mAmp/Amp in their blood and among the 42 drivers with only mAmp/Amp and cannabinoids in their blood. The mean and median age of the 75 drivers with blood mAmp/Amp and cannabinoids was 36 and 33 years, respectively, but in the 42 cases with only mAmp/Amp and cannabinoids in their blood, they were 39 and 35 years, respectively.

In the 75 cases with blood mAmp/Amp and cannabinoids, the mean and median concentrations were as presented below:

(ng/mL)	mAMP	AMP	THC	THC-COOH	THC-OH
Mean	266	49	3.2	43	3.1
Median	190	37	2	19	2

In the 42 cases with only mAmp/Amp and cannabinoids in their blood, the mean and median concentrations were as presented below:

(ng/mL)	mAMP	AMP	THC	THC-COOH	THC-OH
Mean	297	47	3.3	40	1.75
Median	245	40	2	26	1.5

In the 33 cases with other substances detected, the most common were benzodiazepines (*n*=13; 17.3%), ethanol (*n*=11; 14.6%), and cocaine and/or its metabolites (*n*=7; 9.3%). Other compounds detected included MDMA/MDA, methadone, morphine/codeine/6-MAM, hydrocodone, oxycodone, zolpidem, and mirtazapine.

Analysis of Variance demonstrated statistically significant differences ($p<0.05$) when the concentrations of mAMP found in the male drivers (458ng/mL, *n*=54) was compared to that found in the female drivers (228ng/mL, *n*=12).

Initial incident times and time of blood draw were provided in 37 of the 75 cases and yielded mean and median times between the two events of 130.8 minutes and 120 minutes, respectively.

This retrospective review of mAmp/Amp and cannabinoid DUI cases in San Francisco clearly demonstrates that polysubstance DUIs remain an issue in this jurisdiction and comprehensive drug screening beyond alcohol in DUI cases is justified in the interest of public safety.

Reference(s):

1. Barry K. Logan, Kayla J. Lowrie, Jennifer L. Turri, Jillian K. Yeakel, Jennifer F. Limoges, Amy K. Miles, Colleen E. Scarneo, Sarah Kerrigan, Laurel J. Farrell. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. *Journal of Analytical Toxicology.* 2013;37:552–558 doi:10.1093/jat/bkt059.

mAMP/AMP Cannabinoid, Toxicology, DUID

K41 Driving Under the Influence of Drugs (DUID) "Per Se" Laws: Practical Application and the Inclusion of Inactive Metabolites

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After attending this presentation, attendees will better understand the application of DUID "per se" laws in Nevada and the strategies used to prosecute cases when only inactive metabolites are reported.

This presentation will impact the forensic science community by discussing the practical application of DUID "per se" laws. The evaluation of casework experience over an extended period of time may also be useful for states considering DUID legislation.

Driving under the influence of alcohol "per se" laws have existed in the United States for more than a century and they remain a condition in order for states to receive federal highway tax funding. Although no federal requirement currently exists regarding drugs and driving, 22 of the 50 states have passed some form of zero tolerance or "per se" DUID legislation.[1] After strong lobbying by victim advocates, DUID "per se" laws were added to the Nevada Revised Statutes (NRS) in 1999.[2] This legislation equates to a prohibited substances law in which a person cannot legally operate a motor vehicle when they have a prohibited substance in their blood or urine above a defined threshold. Prohibited substances listed in the NRS are Schedule I or II drugs and metabolites for which a person does not have a valid prescription.

In Nevada, DUID cases are typically pursued in one of two manners. The first approach requires the prosecution to demonstrate that an individual was operating a motor vehicle while under the influence of a controlled substance. Results from standardized field sobriety tests performed during a traffic stop can be coupled with a supporting toxicology report to prove impairment. The second approach requires the prosecution to demonstrate that a person was operating a motor vehicle with a prohibited substance in their system as defined by "per se" legislation. Blood or urine taken from a person cited for DUID is analyzed and the results of the chemical test are admitted as evidence. Because of the challenges with proving the impairment of a suspected drugged driver, the second approach is often utilized.

A landmark case occurred in 2000 when a motorist drove into the median on Interstate 15 and ran into a group of teenagers working along the roadside as part of a juvenile rehabilitation program (*Williams v. State*, 2002).[3] Six teenagers lost their lives on that tragic afternoon. Although the jury was not convinced that the driver of the vehicle was impaired, the driver was found guilty of having prohibitive substances in her system and was sentenced to prison. Nevada DUID "per se" laws continue to be used in the prosecution of drugged drivers to this day. The types of cases range from the routine DUID case with no accident or injury to high-profile incidents involving substantial bodily harm, including death.

There has been much discussion regarding Nevada DUID "per se" legislation. The list of urine drug levels, the difficulty relating blood drug concentration to impairment, the pharmacokinetic variances between different individuals, and the inclusion of inactive metabolites are all talking points that hold merit. Arguments supporting the law include the frequency of finding physiologically active compounds in a person's system when the case involves rapidly metabolized drugs, such as cocaine and marijuana. For DUID blood cases analyzed by the Las Vegas Metropolitan Police Department from 2006 through 2015, Cocaine (COC) was reported 20.2% of the time when Benzoylecgonine (BZE) was present (n=1,692; mean COC concentration=102.6ng/mL, COC range: 50ng/mL-3,000ng/mL; mean BZE concentration=704.4ng/mL, BZE range: 50ng/mL-6,400ng/mL) and Δ^9-Tetrahydrocannabinol (THC) was reported in 77.0% of cases when 11-nor-9-carboxy-THC (THCA) was present (n=7,757; mean THC concentration=8.9ng/mL, THC range: 2.0ng/mL-141.9ng/mL; mean THCA concentration=64.3ng/mL, THCA range: 5.0ng/mL-837.6ng/mL).

DUID "per se" laws are a valuable resource for prosecutors in Nevada. From a legal perspective, having a defined threshold for prohibited substances represents a clear standard of permissive inference; however, it is important to point out that the prosecution of DUID cases is not the only concern. This legislation has further-reaching effects as it facilitates the goal of making the roadways in Nevada a safer place. Moreover, the application of DUID "per se" laws helps victims and families gain closure after a disastrous event.

Reference(s):

1. Hedlund, James. *Drug Impaired Driving: A Guide For What States Can Do.* (2015). Retrieved from the Governors Highway Safety Association Website: http://www.ghsa.org/html/publications/2015drugged.html (accessed May 17th, 2016). Nev. Rev. Stat. § 484C.110.
2. *Williams v. State*, 118 Nev. 536, 50 P.3d 1116 (2002).

DUID, Forensic Toxicology, Per Se

K42 The Incidence of Drugs and Alcohol in More Than 18,000 Drivers Using the Recommendations of a 2013 Consensus Study

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After attending this presentation, attendees will better understand the incidence and concentration of impairing drugs detected in Driving Under the Influence of Drugs (DUID) cases and the frequency of mixed drug and alcohol use.

This presentation will impact the forensic science community by providing an assessment of the current incidences and concentrations of impairing drugs in a large driving population based on a consensus-derived scope. In addition, recommendations for additional analytes are suggested, based on expanded testing.

Introduction: In 2013, a report was published that examined the capabilities of laboratories performing toxicological investigations of drug-impaired driving and motor vehicle fatalities cases.[1] This report included a consensus scope of analytes to be tested as well as proposed screening and confirmation cut-off concentrations. NMS Labs adopted these analytes in its ProofPOSITIVE® DUID/Drug Recognition Expert (DRE) drug testing panels. The results of more than 18,000 cases performed in the last year ending June 30, 2016, are described.

Methods: Drug screening was performed using standard Enzyme-Linked Immuno-Sorbent Assay (ELISA) protocols for the following drug classes and cutoff concentrations: (1) 500ng/mL — carisoprodol/meprobamate; (2) 40ng/mL — barbiturates; (3) 25ng/mL — methadone; (4) 20ng/mL — amphetamine/methamphetamine, benzodiazepines, cocaine/metabolites, and opiates; (5) 10ng/mL — oxycodone and phencyclidine; and, (6) 5ng/mL — zolpidem.

Panels could be ordered with or without a two-column Gas Chromatography/Flame Ionization Detector (GC/FID) headspace volatile panel (ethyl alcohol cut-off: 0.02g%). Confirmatory testing was performed either by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) or Gas Chromatography/Mass Spectrometry (GC/MS). Volatile confirmations were performed by headspace GC/FID using a second aliquot.

Results and Conclusions: A total of 18,321 cases were analyzed of which 10,776 were run with the volatile component. A summary of positive findings are noted in the table below.

ANALYTE	INCIDENCE	RL	MEAN	MEDIAN	RANGE
	% Reported +	NG/ML			
THC-COOH	43.9	5.0	45	29	5 - 1600
THC	40.7	0.5	5.8	3.7	0.5 - 110
11-OH-THC	24.9	1.0	3.4	2.5	1 - 38
ALPRAZOLAM	12.4	5.0	63	42	5 - 880
MORPHINE	11.1	5.0	54	30	5 - 3800
AMPHETAMINE	8.5	5.0	64	38	5 - 2500
BENZOYLECGONINE	8	50	651	360	50 - 5200
CLONAZEPAM	6.8	2.0	27	19	2 - 510
OXCODONE	6.8	5.0	69	38	5 - 1000
7-AMINOCLONAZEPAM.	6.7	5.0	38	26	5 - 490
METHAMPHETAMINE	5.2	5.0	263	150	5.2 - 3600
CODEINE	3.8	5.0	22	8.1	5 - 820
COCAINE	3.7	20	84	55	20 - 1000
NORDIAZEPAM	3.3	20	252	110	20 - 3300
6-ACETYLMORPHINE	3.2	1.0	6.1	2.3	1 - 760
METHADONE	2.9	20	264	210	20 - 2000
LORAZEPAM	2.7	5.0	60	35	5 - 920

*Presenting Author

DIAZEPAM	2.6	20	267	130	20 - 5900
HYDROCODONE	2.5	5.0	39	24	5 - 630
OXYMORPHONE	2.4	1.0	5.1	2.8	1 - 65
EDDP	1.8	20	54	45	20 - 330
MEPROBAMATE	1.5	1000	12719	10000	1000 - 49000
ZOLPIDEM	1.5	4.0	196	120	4.3 - 1400
CARISOPRODOL	1.4	200	4414	3400	220 - 19000
COCAETHYLENE	1.2	20	44	37	20 - 210
ALPHA-OH ALPRAZOLAM	0.96	5.0	8.9	7.5	5 -29
TEMAZEPAM	0.86	20	167	51	20 - 2500
OXAZEPAM	0.66	20	149	43	20 - 4400
HYDROMOPRHONE	0.62	1.0	5.5	2.1	1 - 83
PCP	0.62	5.0	48	46	8.1 - 120
DIHYDROCODEINE	0.51	5.0	9.2	7.8	5 - 27

Table 1: Positive Tier 1 analytes (with an incidence exceeding 0.50%)

Ethanol was detected in 62% of cases tested with mean and median concentrations of 0.16g% and 0.15g%, respectively (range: 0.02g%-0.44g%). At least one drug was present in addition to alcohol in 56% of alcohol-positive cases. The majority of drug-positive cases were positive for more than one drug as shown in Table 2. Some of the most popular combinations will be presented.

# DRUGS	N	%
1	6545	35.7
2	2608	14.2
3	1789	9.8
4	1075	5.9
5	539	2.9
6	286	1,6
7	134	0.73
8	78	0.42
9+	42	0.23

Table 2: Summary of the number of drugs or metabolites present in all drug-positive cases, excluding alcohol (Cannabinoids were considered a single analyte.)

A total of 1,853 cases were tested for an expanded panel of nearly 200 drugs by Liquid Chromatography/Time-Of-Flight (LC/TOF). Of these, excluding caffeine, the most common drugs detected were buprenorphine (*N*=121, 6.5%) and fentanyl (*N*=106, 5.7%). These results suggest that consideration be given to add these compounds in future Tier 1 scopes, particularly due to the increase in fentanyl and designer fentanyl substitution to the current heroin supply.

Reference(s):

1. Logan et al. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. Journal of Analytical Toxicology 37: 552-8, 2013.

DUID, Drug-Impaired Driving, Incidence of Drugs

K43 The Results of the National Safety Council's Alcohol, Drugs and Impairment Division (NSC ADID) Survey of Drug Testing in Driving Under the Influence of Drugs (DUID) and Traffic Fatality Investigations

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After attending this presentation, attendees will be able to compare their laboratory practices for toxicological testing in drug-impaired driving and traffic fatality cases and evaluate their cutoff limits for screening and confirmation of commonly encountered drugs to other United States and Canadian laboratories.

This presentation will impact the forensic science community by providing results from a survey conducted under the NSC ADID regarding laboratory practices to update the current guidelines and recommendations for laboratory testing in DUID and traffic fatality investigations to improve standardization.

The purpose of this project is to provide toxicology laboratories with a list of commonly encountered analytes and appropriate screening and confirmation thresholds in DUID cases and motor vehicle fatalities. Standardization of analytical testing addresses concerns highlighted in the 2009 National Academy of Sciences (NAS) Report.[1] Additionally, having a standardized approach will improve the quality of statistics reported for DUID and motor vehicle fatality cases.

Toxicology laboratories were surveyed about their drug testing practices, specifically with respect to the matrices tested, scope of testing, cutoff concentrations for screening and confirmation, and whether they are in compliance with the 2013 guidelines and recommendations.[2] Changes in drug trends and improvement in testing technologies and capabilities of forensic toxicology laboratories were also addressed. The survey was sent via SurveyMonkey® to individuals who confirmed their participation, and ultimately 70 completed surveys were included in the data analysis.

Of the responding laboratories, 90% test blood samples, 68% test urine samples, and 1% test oral fluid samples in DUID casework. Screening methods for blood include Enzyme-Linked Immuno-Sorbent Assay (ELISA) (71%), Gas Chromatography/Mass Spectrometry (GC/MS) (50%), Liquid Chromatography/Mass Spectrometry (LC/MS) (34%), Enzyme-Multiplied Immunoassay Technique (EMIT) (11%), and Liquid Chromatography/Time-Of-Flight (LC/TOF) (9%). Urine screening included ELISA (46%), GC/MS (37%), EMIT (27%), LC/MS (26%), and LC/TOF (6%). Confirmatory methods were 87% GC/MS and 73% LC/MS for blood samples, and 77% GC/MS and 46% LC/MS for urine samples. A total of 34% of respondents reported unconfirmed screen results, with many commenting that the report states the result is not confirmed. Reasons for reporting unconfirmed results included legal time constraints, confirmatory testing not available, relevance of the drug, or poly-drug case policy.

Compliance with the scope of testing and cutoff limits from the 2013 recommendations revealed that 15% of the laboratories met or exceeded the recommendations, while 47% are currently changing their methods in order to meet them. For blood, screening for opiates and confirmation of cannabinoids and opiates were the most frequent categories for which the recommendations were not met.

Updates to the 2013 cutoffs and recommended test menu will be determined at a consensus meeting of the participating laboratories in November, with distribution by NSC ADID in early 2017.

Reference(s):

1. Committee on Identifying the Needs of the Forensic Science Community. *Strengthening Forensic Science in the United States: A Path Forward*. National Research Council of the National Academies, 2009.
2. Logan B.K., Lowrie K.J., Turri J.L., Yeakel J.K., Limoges J.F., Miles A.K., Scarneo C.E., Kerrigan S.B.A., Farrell L.J. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. *Journal of Analytical Toxicology.* 2013 Aug; 37(8): 552-558.

DUID, Cutoffs, Guidelines

K44 Alere™ DDS®2 Mobile Test System Screening for Delta-9-Tetrahydrocannabinol (THC) With Oral Fluid Confirmation by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will understand the use of the Alere™ DDS®2 Mobile Test System as it pertains to field screening for THC in comparison to concentrations determined by confirmation using LC/MS/MS in oral fluid samples collected from human subjects at a music festival.

This presentation will impact the forensic science community by assessing the feasibility of using a field-deployable testing device for the detection of THC. On-site testing devices may be employed for roadside drug screening in subjects suspected of impaired driving; therefore, reliable results are imperative for the employment and use of these devices.

Oral Fluid (OF) is increasingly used as an alternative to blood or urine testing for determination of drug use in impairment cases. OF is advantageous in comparison to blood and urine due to non-invasive collection procedures and the ability for the sample to be collected at the time of incident. The collection and analysis of in the field has become possible with the DDS®2 Mobile Test System.

The objective of this study was to compare the field results for the DDS®2 Mobile Test System to a laboratory-based LC/MS/MS confirmatory analysis with respect to detection of THC in human subjects. As part of a larger Institutional Review Board (IRB) -approved study, two OF samples were collected from participants at a music festival in Miami, FL. One OF sample was field tested using the DDS®2, and a confirmatory OF sample was collected using Quantisal™ oral fluid collection devices. The DDS®2 OF sample was field tested for drugs of abuse including amphetamine, methamphetamine, benzodiazepines, cocaine, opiates, and, specifically with respect to this study, cannabis (THC), at a cutoff of 25ng/mL.

LC/MS/MS confirmatory analysis was performed using an Agilent® 1100 series liquid chromatograph coupled to an Agilent® 6430 tandem mass spectrometer. Calibration and control samples were prepared in pooled, expectorated, THC-free, OF and diluted in Quantisal™ buffer to match the composition of field-collected OF samples. The method was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for quantitative methods. The LC/MS/MS Limit Of Quantitation (LOQ) was 2ng/mL and the Limit Of Detection (LOD) was 1ng/mL.

Of the 628 participants who provided Quantisal™ OF samples, THC was detected in 231 subjects (36.8%), with THC being over the LOQ (2ng/mL) in 190 subjects (30.3%). The mean and median OF THC concentrations were 57.1 and 10.8 ng/mL, respectively, and ranged from 1.0ng/mL to 1,473ng/mL; 144 (22.9%) samples were positive for THC between 1ng/mL, the LC/MS/MS LOD, and 25ng/mL, the cutoff of the DDS®2; 125 participants provided DDS®2 OF samples that were field tested and the results were as follows: 27 (22.1%) field tested positive for THC, 92 (75.4%) field tested negative for THC, and 6 (2.5%) field tested invalid for THC.

Positive field-test results were confirmed by LC/MS/MS, with 27 (100%) being positive for THC at an LC/MS/MS threshold of 5ng/mL, 23 (85.2%) at 10ng/mL, 21 (77.8%) at 15ng/mL, and 18 (66.7%) at 25ng/mL. Of the 92 DDS®2 THC negative results, 89 (96.7%) were confirmed negative, with THC concentrations less than the DDS®2 cutoff of 25ng/mL. Of the 89 confirmed negatives, 65 (73.0%) were negative below a confirmation threshold of 1ng/mL, while 24 of the screened negative results tested positive for THC between the LOD of 1ng/mL and the DDS®2 cutoff of 25ng/mL. Three samples (3.4%) were determined to be false negatives, with DDS®2 field tests producing a negative result, but with LC/MS/MS confirmation results being greater than 25ng/mL (25.4ng/mL, 26.6ng/mL, and 42.3ng/mL).

For detection of THC in this sample population, the DDS®2 displayed sensitivity, specificity, Positive Predictive Value (PPV), and accuracy as displayed in Table 1 at LC/MS/MS thresholds of 25ng/mL (the DDS®2 published

threshold) and 1ng/mL (the LC/MS/MS LOD). Using a cutoff concentration of 25ng/mL resulted in fewer of the cannabis-using subjects being identified (18% positivity vs. 45% positivity at 1ng/mL).

Table 1	**25ng/mL LC/MS/MS Cutoff**	**1ng/mL LC/MS/MS Cutoff**
Positivity Rate (n=119)	18% (n=21)	45% (n=54)
DDS®2 Sensitivity	90%	50%
DDS®2 Specificity	100%	100%
DDS®2 PPV	100%	100%
DDS®2 Accuracy	98%	77%

These results from cannabis-using subjects demonstrate the value of field-based OF testing and illustrate the significance of the cutoff concentration with respect to performance evaluation and detection of drug use.

Oral Fluid, THC, Alere™ DDS®2

K45 Detecting Drugged Driving in Wisconsin: A Comparison of Whole Blood and Roadside Oral Fluid Specimens

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The goal of this presentation is to introduce attendees to the application of a portable, roadside oral fluid instrument to evaluate the frequency of individuals Driving Under the Influence of Drugs (DUID) and compare the results to evidentiary whole blood specimens.

This presentation will impact the forensic science community by providing valuable information regarding the efficacy of using oral fluid screening as a screening tool to detect and arrest drugged drivers.

A pilot study was conducted in Dane County, WI, combining resources from the Wisconsin Department of Transportation's Bureau of Transportation Safety, Dane County Sheriff's Department, local law enforcement, and the Wisconsin State Laboratory of Hygiene (WSLH) to compare evidentiary whole blood specimens collected from subjects arrested for allegedly Operating (a motor vehicle) While Intoxicated (OWI) to oral fluid results.

The objectives of the study were to evaluate: the utility of the oral fluid detection system as a tool for law enforcement, the prevalence of OWI, specifically the number of individuals driving under the influence of both alcohol and drugs, the differences between detecting drugs in oral fluid and in whole blood, and the effectiveness of the WSLH drug cancellation policy when Blood Alcohol Concentrations (BAC) exceed 0.10g/100 mL.

Following a traffic stop and collection of legal blood specimens, subjects were asked to voluntarily participate in the pilot study and provide an oral fluid specimen. Participation in the study did not influence the outcome of the subject's OWI offense. Immediately following the legal blood draw, oral fluid was tested by law enforcement with an Alere DDS®2 oral fluid testing instrument following collection with the system's collection cartridge. Four portable instruments were deployed at various locations throughout the county with the capability of detecting six drug classes (target cutoff concentration), including amphetamine (50ng/mL), benzodiazepines (temazepam=20ng/mL), cocaine (benzoylecgonine=30ng/mL), methamphetamine (50ng/mL), opiates (morphine=40ng/mL), and cannabis (THC) (Δ^9-THC=25ng/mL). Oral fluid results were forwarded to the WSLH, paired with the subject's blood specimen, and subsequently de-identified following the University of Wisconsin-Madison protocol.

A total of 117 subjects aged 18 to72 years volunteered to participate in the study, yielding a final number of 104 (*n*=22 female, *n*=82 male) valid oral fluid specimens. Evidentiary whole blood specimens were analyzed for alcohol and drugs following routine protocols: Gas Chromatography (GC) -headspace/ Flame Ionization Detector (FID) for volatiles, Enzyme Immunoassay (EIA) for barbiturates (secobarbital=100ng/mL), buprenorphine (100ng/mL), benzodiazepines (lorazepam=40ng/mL), cocaine (benzoylecgonine=50ng/mL), opiates (morphine=40ng/mL), and THC (carboxy-THC=10ng/mL), and finally an alkaline basic drug screen. To compensate for differences in drug categories between the EIA screen and oral fluid panel, EIA and alkaline basic drug screen results were combined when comparing whole blood to oral fluid.

Seventy-six of 104 (73%) subjects suspected of OWI were driving under the influence of alcohol and 71 of those 76 (93%) had BACs exceeding 0.10g/100 mL. In cases in which BAC exceeded 0.10g/100mL, drugs were detected in both oral fluid and blood for 28 and 29 subjects, respectively. Fifty of 104 arrests (48%) occurred between the hours of midnight and 6:00 a.m., 22/104 (21%) between the hours of 12:00 p.m. and 6:00 p.m., and 29/104 (28%) between the hours of 6:00 p.m. and 10:00 p.m.

In general, oral fluid results were consistent with results obtained from evidentiary blood specimens. THC was the most frequently detected drug category in both oral fluid (*n*=46) and whole blood (*n*=44). Benzodiazepines were detected more frequently in blood (*n*=11) than in oral fluid (*n*=6). In contrast, amphetamine and methamphetamine were detected in 14 oral fluid specimens; however, they were reportable in only two blood specimens. Data discussed is preliminary in nature and detailed analysis is ongoing.

Driving, Intoxicated, Drugs

K46 Blood and Oral Fluid Cannabinoids' Pharmacokinetics and the Evaluation of Two On-Site Oral Fluid (OF) Screening Devices for the Prediction of Δ^9-Tetrahydrocannabinol (THC) in Oral Fluid and Blood Following Edible Cannabis Administration

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After attending this presentation, attendees will understand the relationship between blood and OF THC pharmacokinetics following controlled edible cannabis administration to frequent and occasional cannabis smokers. Additionally, attendees will understand the utility of on-site OF screening devices for predicting the presence of THC in OF and blood.

This presentation will impact the forensic science community by filling an important knowledge gap in cannabinoid pharmacokinetics following ingestion of cannabis-containing edibles and by improving interpretation of screening and confirmatory cannabinoid tests.

OF is an attractive testing matrix for driving under the influence of drugs settings. Its utility on the roadside is increased with on-site screening devices. In addition to testing for the presence of OF THC, on-site results may help predict THC in blood. This was previously demonstrated with specimens collected roadside and following controlled inhaled cannabis, but never following edible cannabis. To properly interpret test results, additional characterization of the relationship between blood and OF cannabinoid pharmacokinetics following ingestion of cannabis-containing edibles is required.

Nine frequent (≥5x/week) and seven occasional (≥2x/month, but <3x/week) cannabis smokers provided written informed consent to participate in this National Institute on Drug Abuse Institutional Review Board, Food and Drug Administration (FDA), and Drug Enforcement Administration (DEA) -approved study. On the morning of dosing, participants consumed a cannabis-containing brownie (6.9% THC, ~50.1mg) in 10min. Blood and OF were collected before and up to 48h post-dose and analyzed for THC. Confirmatory OF specimens were collected with the Quantisal™ device. The Draeger DrugTest® 5000 (DT5000) or Alere™ DDS®2 (DDS2) on-site screening devices were randomly assigned to individual participants. OF specimens were collected for 5min or until the volume-adequacy indicator turned blue. Pharmacokinetic differences between smoking groups were evaluated by independent samples *t*-tests (two-tailed $p < 0.05$ significance threshold). OF/blood THC ratios were calculated for all paired samples when analytes ≥ limit of quantification (0.5µg/L blood, 0.2µg/L OF). Effects of time and smoking group on observed OF/blood THC ratios were evaluated by repeated measures analysis of variance; post hoc tests were conducted with a Bonferroni correction. For on-site device performance evaluation, qualitative DT5000 and DDS2 results were compared to quantitative OF and blood THC results at various confirmatory cutoffs and sensitivity, specificity, and efficiency were determined.

There were no significant differences in mean (range) OF THC maximum concentrations (C_{max}) between frequent (573 (39.3-2,111) µg/L) and occasional (362 (115-696) µg/L) cannabis users, or in time of C_{max} (t_{max}, 0.33h); however, there was a significant difference in time of last positive (t_{last}) THC OF results between frequent (39 (20->48) h) and occasional (23 (20-26) h) users. Significant differences in blood THC C_{max} between frequent (17.7 (8.0-36.1) µg/L) and occasional (8.2 (3.2-14.3) µg/L) users, and in t_{last} (>48h and 17 (8.0-38.0) h, respectively) were observed. OF/blood THC ratios were initially large and variable 0.5h post-dose, and concentrations were not significantly correlated. Ratios from 1h to 5h post-dose were significantly smaller than those at 0.5h. Performance criteria for the DT5000 and DDS2 were >80% overall (both groups) with a confirmatory OF THC cutoff ≥5µg/L; no true positive result was observed by 8h with either device at this cutoff. Performance criteria were >80% with a blood THC ≥5µg/L cutoff for occasional smokers with the DT5000 and for frequent smokers with the DDS2.

Differences observed between blood and OF THC pharmacokinetics (partially due to initially large oromucosal contamination) contribute to the lack of a significant correlation within the first 5h after oral cannabis. While a reliable conversion between blood and OF THC concentrations does not exist, OF can predict the presence of THC

in blood. These data will aid and improve cannabinoid screening and result interpretations.

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Oral Fluid/Blood THC Ratios, Oral Fluid Screening, Edibles

K47 Direct Tissue Sampling of Diazepam and Amitriptyline Using Mixed-Mode Solid-Phase Microextraction (SPME) Fibers: A Feasibility Study

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After attending this presentation, attendees will understand the use of mixed-mode SPME fibers as direct tissue sampling tools to determine tissue concentrations of neutral and cationic compounds.

This presentation will impact the forensic science community by providing data on direct tissue sampling of diazepam and amitriptyline using mixed-mode SPME fibers.

Recent work with SPME fibers *in vivo* has shown that this technique is easily applied directly in semi-solid tissues; however, at this time, data on tissue sampling are still very limited, and adequate models to study sorption from tissue are lacking. Furthermore, quantification of actual tissue concentrations remains a challenge in the application of SPME in tissue.

The goal of this research is to evaluate the applicability of the C18/Strong Cation Exchange (SCX) -coated SPME fiber as a direct tissue sampling tool. The C18/SCX (mixed-mode) fiber coating consists of hydrophobic C18 chains and SCX groups, made up of propylsulfonic acid.

Diazepam and amitriptyline were used as test compounds to demonstrate that this fiber can efficiently extract both neutral and cationic compounds. Diazepam is neutral, and its behavior is predictable based on the octanol-water partition coefficient (K_{ow}). Amitriptyline is >99% positively charged at pH 7.4 and is likely to behave differently in both agarose gel and tissue compared to neutral compounds. Agarose gel was used as a tissue surrogate to mimic changes in matrix tortuosity as expected in tissue. Pork muscle was used as tissue source and was loaded with the analytes of interest using 24-hour incubation in spiked Phosphate-Buffered Saline (PBS).

Linear sampling isotherms were observed for agarose gel. The results with tissue were more complex as the cubes of muscle meat were difficult to equilibrate to a homogeneous loading concentration in the applied test systems. This influenced the sampling kinetics and extraction linearity with unknown uncertainty. Still, the C18/SCX fiber extracted both diazepam and amitriptyline from the muscle tissue and, when diazepam concentrations were higher in tissue, similar high levels were determined via microextraction.

Sorption affinity of both diazepam and amitriptyline is decreased (±1 log unit) when sampling from agarose gel compared to PBS due to the presence of different binding groups in agarose. When comparing sorption affinities between agarose gel and tissue, sorption affinity from tissue is ±1 log unit lower for diazepam, while this is ±2 log units for amitriptyline. This indicates that tissue contains even more binding sites for these compounds compared to agarose gel. Interestingly, for both compounds, equilibration in tissue occurred faster than equilibration in agarose gel or PBS, most likely caused by direct fiber contact or through facilitated transport.

The proposed SPME method yielded detectable fiber concentrations after direct sampling in agarose gel and loaded tissue, including short sampling times and different loading concentrations in tissue. Although more research is needed to obtain good quantitative results, these results illustrate that the C18/SCX fiber is a sensitive tool to determine tissue concentrations of neutral and cationic compounds.

In future research, such quantitative measurements must be pursued to apply the current SPME methodology in forensic toxicology. An example of this can be studying postmortem drug redistribution. SPME fibers can be placed directly *in situ* in tissue or blood without removal of these matrices. As shown here, only a short time interval is needed to obtain detectable fiber concentrations. Furthermore, SPME does not disturb the existing equilibrium in the body as only very small amounts are extracted. This would allow for repeated sampling in the same system for a prolonged period. This could eliminate the current difficulties in studying postmortem redistribution as a kinetic study can be executed in a single postmortem case.

Solid-Phase Microextraction, Tissue Sampling, Agarose Gel

K48 The Quantification of Loperamide by Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will be able to: (1) understand the abuse potential of loperamide; (2) explain the loperamide method-validation study summary; and, (3) describe loperamide abuse cases in Alabama.

This presentation will impact the forensic science community by providing additional analytical options to laboratories and increase awareness of loperamide as a drug of abuse.

Hypothesis: GC/MS may be used to quantitate loperamide when it is abused in postmortem toxicology cases.

Statement of Content/Methods: Loperamide is a member of the opioid drug classification. At therapeutic concentrations (≤5ng/mL), loperamide is restricted to the gastrointestinal tract where it functions as an anti-diarrheal. Loperamide is abused as a replacement for legally controlled opioids, to mitigate withdrawal symptoms, and in monitored known abusers. In this validation, loperamide was quantitated in whole blood by GC/MS following a basic drug extraction (liquid-liquid extraction) using n-butyl chloride. Loperamide (m/z 239, 72, 266) and loperamide-d_6 (m/z 245, 78) ions were monitored and data was collected using both Selected Ion Monitoring (SIM) and scan modes in a 10-minute method. In accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines, validation studies included: selectivity, reproducibility, specificity, stability, Limit Of Detection (LOD), regression model analysis, and matrix enhancement/suppression.

Summary of Results: Method selectivity was evaluated through inter- and intra-day accuracy, precision, Coefficient of Variation (CV), and reproducibility. Inter-day accuracy, precision, and CV were measured at three concentrations (200ng/mL, 400ng/mL, and 650ng/mL) over the course of the validation (seven batches). Result ratios were calculated by dividing the measured result by the intended result and then averaged. The inter-day average result ratio was 1.05 ± 0.09 with CV=8.87%. The intra-day accuracy and precision were determined at three concentrations in replicates of three over three days with an average result ratio of 1.03 ± 0.09 with CV=8.53%. Reproducibility of the method was evaluated through standard addition and comparisons to previous results. The reproducibility result ratio was 0.95 ± 0.10 with a CV=10.84% across ten standard addition samples. Specificity assessment included analysis of ten different blank matrices, 60 commonly encountered drugs, and the individual analysis of loperamide (4,000ng/mL) and deuterated loperamide at high concentrations (1,000ng/mL). No interference was observed. Stability of the extracts was evaluated at room temperature over the course of five days. The loperamide to loperamide-d_6 ratio remained consistent between days one to five with a CV=6.59%. The method was determined to be transferable across two analysts. The LOD was set at 100ng/mL. A linear range from 100ng/mL to 1,000ng/mL was determined through regression analysis. The regression analysis was calculated from analyte area responses for both loperamide and loperamide-d_6. Matrix enhancement, suppression, and recovery were evaluated through a modified Matuszewski study. Neither matrix enhancement nor suppression was observed for loperamide at 750ng/mL and loperamide-d_6 at 300ng/mL (-6.5% and -4.2%); however, both analytes at 300ng/mL exhibited some suppression (-57% and -55%). Recovery ranged from 31% to 36% for all concentrations of loperamide and loperamide-d_6.

Conclusion: Results of this validation demonstrate that the designed method is highly selective and precise, <10% CV and specific, and no interference was detected. The LOD and Limit Of Quantitation (LOQ) linear range are suitable for the seven loperamide abuse deaths that have been observed in Alabama (concentration range from 130ng/mL to 1,400ng/mL) in the last two years. Using the loperamide to loperamide-d_6 ratio corrected for matrix suppression and recovery. Per this research, the presented method sufficiently meets the needs of postmortem toxicology laboratories.

GC/MS, Loperamide, Overdose

K49 The Quantitation of Mitragynine in Human Whole Blood by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) and Its Application to the Analysis of Toxicological Samples

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After attending this presentation, attendees will be able to describe the pharmacology and toxicology of the psychoactive drug mitragynine and will be able to implement a quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for testing mitragynine in biological samples.

This presentation will impact the forensic science community by providing insight into the analysis of the naturally occurring, emerging psychoactive substance mitragynine, the challenges associated with its analysis and interpretation, and the quantitative results from authentic casework with associated case histories.

Mitragynine is an indole alkaloid and the major constituent found in *Mitragyna Speciosa*, a tropical plant of the Rubiaceae family known as "kratom" in Thailand and "biak-biak" or "ketum" in Malaysia. The leaves of the plant contain a variety of alkaloids and are used recreationally for their stimulatory and narcotic analgesic effects during manual labor. Medicinally, kratom is used as an opioid substitute for drug withdrawal or to treat pain and diarrhea. Its use has recently been implicated in deaths following its use; however, there are few reports of quantitative results from these cases. The Center for Disease Control (CDC) has reported that the exposure-related calls about kratom have increased tenfold from 2010 to 2015 and the Drug Enforcement Administration (DEA) includes it on its Drugs of Concern list. Kratom use appears to be increasing in the United States and is considered an emerging public health threat; to be able to better differentiate concentrations associated with adverse effects compared to fatalities, a quantitative method for the toxicological determination of mitragynine was needed. This presentation describes the development and validation of a quantitative analytical method for mitragynine and its application to testing blood samples from forensic investigations.

A liquid-liquid extraction method was developed to recover mitragynine from human whole blood. Blood, 0.5mL, was extracted using borax buffer (0.1M, pH 4.0) and n-butyl chloride/ethyl acetate (70:30). Extracts were evaporated to dryness and reconstituted in mobile phase for analysis by LC/MS/MS. Analysis was performed using a UPLC-Tandem Quadrupole Detection (TQD) in positive electrospray ionization mode utilizing a gradient of ammonium formate (pH 4.0) and acetonitrile (10:90) isocratically on a silica column. D_3-Mitragynine was used as internal standard. Validation of the method followed the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines and included assessment of precision, accuracy, limits of detection (LOD) and Quantitation (LOQ), recovery, ion suppression, interference, and stability. The method has an LOD of 0.16ng/mL, LOQ of 5.0ng/mL, and Upper Limit of Quantitation (ULOQ) of 500ng/mL. Precision of the controls revealed a maximum % CV of 4.9% and accuracy was limited to a 5.4% difference.

Samples from forensic investigation cases were analyzed using the validated method. The method successfully resolved mitragynine from three other inactive alkaloids (speciogynine, mitracillatine, and speciocillantine) present in the kratom plant and detected in authentic toxicological samples.

The method successfully passed validation, and 19 cases submitted for forensic toxicology analysis that had qualitatively screened positive by Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) using an independently validated method were analyzed using the procedure as described. Mitragynine was not confirmed in four cases, which could be attributed to stability. Of the 15 remaining cases, mean and median mitragynine concentrations were 59ng/mL and 25ng/mL, respectively, with a range of 10ng/mL to 220ng/mL; these values are consistent with concentrations of mitragynine reported in fatalities, which ranged from 20ng/mL to 600 ng/mL.

Kratom, Mitragynine, UPLC-MS/MS

K50 The Detection of Cocaine and Its Major Metabolites in Rodent Bone Following Outdoor Decomposition After Chronic Cocaine Administration Using 2D-Liquid Chromatography/Tandem Mass Spectrometry (2D-LC/MS/MS)

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After attending this presentation, attendees will better understand how to perform an extraction of a drug of abuse from bone in approximately one hour. Additionally, attendees will understand the background and utility of multidimensional chromatography.

This presentation will impact the forensic science community by showcasing a procedure offering quick sample preparation and short extraction time of a very complex matrix (bone), which can be applied to any laboratory using LC/MS/MS to analyze and quantify drugs of abuse in any matrix.

Objective: In the field of forensic toxicology, several challenges exist with quantification of cocaine and metabolites in postmortem samples. Cocaine can prove difficult to detect and quantify in blood, urine, and soft tissues following extensive decomposition. Alternative matrices, such as hair, nails, and bone, could prove useful in detecting chronic drug use in postmortem toxicology cases. Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes and the inability to detect an analyte at trace levels. Further, analysis of drugs in hard tissues, such as hair and bone, has only been attempted in recent years. Even fewer studies have investigated the detection of drugs following the decomposition of remains, specifically outdoor decomposition. The objective of this study was to develop a robust extraction and clean-up methodology with preceding homogenization to efficiently extract drugs from complex matrices, to reach a target Limit Of Detection (LOD), and to maintain instrumental performance.

Method: In this study, a method analyzing cocaine and its major metabolites benzoylecgonine and ecgonine methyl ester was developed. All rat specimens used for this study underwent 10-12 weeks of chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before euthanization. Average daily dosages for each rat fell within a range of 13mg/kg-19mg/kg. Fourteen cocaine-positive rats were placed outside and above ground in a gated facility for a period of 12 months. All recoverable pelt and skeletal samples were collected for testing. A second group consisting of 16 cocaine-positive rats was placed outside and above ground in a gated facility for one week. A group of four cocaine-positive rats were removed for testing on the second week and every week following. All recoverable skeletal samples were collected for testing. Drug-free control rat bones were also acquired by placing drug-free rats outdoors, above ground, until full decomposition occurred.

After homogenization of whole bones, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent, which yields two eluting fractions — one with neutral and acidic entities and a second one with basic analytes. The use of a 2D-LC/MS/MS technology eliminates the need for a lengthy evaporation step in the extraction method. The chosen 2D-LC/MS/MS used in this application was identified using a 6x6 automated method development protocol. The manual extraction of the bone samples was completed in less than one hour. The analysis was performed using 100µL of the final organic solvent (MeOH) extracts.

Results: The Limit Of Quantitation (LOQ) for cocaine and its metabolites was measured at 100ng/g sample material. The response factor of analytes was high enough that the LOD was estimated at 10ng/g (10ppt).

Conclusion: The micro extraction protocol combined with multidimensional chromatography decreased sample preparation time without sacrificing the quality seen with current single-dimension chromatography techniques. The procedure developed in this study can be utilized on bone and completed in less than one hour before injection of 100µl final extract into the 2D/LC/MS/MS system.

LC/MS/MS, Multidimensional Chromatograph, Bone

K51 Capsule Phase Microextraction (CPME): A Powerful New Arsenal in Analytical and Forensic Sample Preparation

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After attending this presentation, attendees will have a thorough understanding of the fabrication, working principle, and advantages of CPME in preparing different analytical, environmental, toxicological, pharmaceutical, food, and forensic samples for chromatographic separation and identification.

This presentation will impact the forensic science community and scientists interested in analyzing trace organic analytes in various sample matrices by potentially offering a paradigm-shift approach in sample preparation by the total elimination of sample pre-treatment steps such as filtration, protein precipitation, centrifugation, etc. from the sample preparation workflow, which is notoriously time consuming, error prone, and labor intensive.

Due to the ever-increasing demands from the public and regulators wishing to establish the principle of Green Analytical Chemistry (GAC) in all aspects of the analytical process, the current trend in sample preparation inevitably favors miniaturization of the extraction device, reduced sample volume, reduced organic solvent consumption, and a minimized amount of waste generated in the sample preparation process. Owing to the high consumption of toxic and hazardous organic solvent and other shortcomings of major sample preparation techniques, including Liquid-Liquid Extraction (LLE) and Solid Phase Extraction (SPE), a number of miniaturized and green sample preparation techniques, such as Solid-Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE), Thin Film Microextraction (TFME), Microextraction by Packed Sorbent (MEPS), and Fabric Phase Sorptive Extraction (FPSE), have emerged during the past few decades.[1-5] These techniques are desirable as environment friendly, require smaller sample volume, and are faster, more sensitive, and efficient.

Although the new generation of sample preparation techniques represents improvements, most of these techniques cannot directly handle real-life analytical, environmental, toxicological, pharmaceutical, food, and forensic samples, which often contain high volumes of particulates, debris, biomasses, and other matrix interferents. Sample pretreatment steps, such as filtration, centrifugation, protein precipitation, etc., are often needed, leading to significant analyte loss with serious ramifications on forensic evidence.

CPME has been developed to focus on the majority of the shortcomings not adequately addressed by other sample preparation techniques.[6] CPME completely eliminates the sample pretreatment/clean-up step from the sample preparation workflow. CPME utilizes a porous tubular polypropylene membrane capsule with a 0.2µm pore size and 1.8mm internal diameter to encapsulate sol-gel hybrid organic-inorganic sorbent in the form of monolithic bed or spherical particles. The porous membrane capsule allows easy permeation of aqueous sample containing the target analyte(s) while protecting the sorbent from being contaminated by matrix interferents. A magnetic metal rod embedded in the microextraction capsule allows for spinning the device when placed on a magnetic stirrer and diffuses the sample matrix for fast analyte-sorbent interaction and rapid extraction equilibrium. High loading of sol-gel sorbent provides excessive sample capacity for target analyte(s) and fast extraction kinetic due to the sponge-like porous architecture of the sol-gel sorbent. Protection of the sorbent from contamination via encapsulation into a porous tubular membrane capsule has made CPME an impressive and robust sample preparation technique. After the extraction, a small volume of organic solvent can be used to desorb the accumulated analyte(s). Due to the high pre-concentration factor achieved in CPME, no solvent evaporation and sample reconstitution is required. The prepared sample can be analyzed by gas chromatography, liquid chromatography, or capillary electro chromatography to obtain complimentary information if a mutual solvent equally compatible with these chromatographic techniques is chosen.

Analytical data obtained from a number of real-life applications of CPME, including illicit drug residues and their metabolites in urine and blood samples, will be presented to showcase its advantages, extraction characteristics, performance superiority, and analytical figures of merit.

Reference(s):

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Capsule Phase Microextraction, Sample Preparation, Green Analytical Chemistry

K52 The Screening of Biofluids for the Detection of Traditional Illicit Drugs, New Psychoactive Substances (NPS), and Pharmaceuticals by Automated Online Extraction Using Turbulent Flow Chromatography and High Resolution Accurate Mass-Hybrid Quadrupole-Orbitrap-Mass Spectrometry (HRAM/Q-OT/MS)

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After attending this presentation, attendees will understand the advantages of performing toxicological screening of biofluids using automated online extraction coupled with HRAM/Q-OT/MS for the detection of traditional illicit drugs, NPS, and pharmaceuticals of toxicological interest.

This presentation will impact the forensic science community by providing and demonstrating the applicability of a workflow for a targeted screening of blood, urine, and hair samples for forensic toxicology purposes.

Clinical and forensic toxicology laboratories worldwide are analytically challenged by the unceasing spread of traditional illicit drugs, the increasing number of NPS, and the misuse of prescription medications. Therefore, these laboratories are increasingly required to provide fast, comprehensive, and highly sensitive screening protocols for identifying a large number of drugs of toxicological interest and/or their metabolites in biological fluids. Hence, these laboratories need to evolve from classic, yet time-consuming, off-line Liquid-Liquid Extraction (LLE) or Solid-Phase Extraction (SPE) sample workup procedures to automated procedures. Moreover, in addition to traditional chromatographic and mass spectrometric techniques (Gas Chromatography/Mass Spectrometry (GC/MS), Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS/MS)), advanced MS techniques, such as LC coupled with HRAM-Q-OT/MS, may be employed, allowing for simple, fast, and sensitive untargeted or targeted screening procedures.

The purpose of this presentation consists in the description of the workflow developed at an Italian laboratory for a targeted screening of blood, urine, and hair samples for forensic toxicology purposes using an automated on-line extraction system based on turbulent flow chromatography coupled to a new-generation HRAM-Q-OT/MS system.

After the addition of deuterated internal standards and a simple deproteinization step (blood and urine), or external decontamination, pulverization, and solvent extraction (hair), samples were processed on-line by a Transcend™ II turbulent flow chromatography (TurboFlow) system. Analytes were separated on an UltiMate® 3000 Ultra-High-Pressure Liquid Chromatography (UHPLC) system equipped with an Accucore™ Phenyl-Hexyl analytical column, and detected by an Q Exactive™ Focus HRAM-Q-OT/MS system, equipped with a Heated Electrospray Ionization (HESI) -II source. MS acquisition was performed using positive/negative switching in full scan mode at a resolution of 35,000 and subsequent Data-Dependent Acquisition (DDA) mode, performing High-energy Collisional Dissociation (HCD) experiments at a resolution of 17,500 according to dynamic exclusion and inclusion lists on the masses of interest. Identification of analytes was based on accurate mass measurements of their MH^+ ESI-generated ions in full scan conditions, evaluation of MH^+ isotopic patterns, detection of accurate masses of MH^+ collision-induced product ions, and comparison with full HR-MS/MS library spectra.

The developed workflow proved to be fast, reliable, and highly sensitive. Total run time per sample, including minimal sample pretreatment, TurboFlow processing, and HRAM-Q-OT/MS analysis, ranged from 20-30min (urine, blood) to 40min (hair, barring solvent extraction). This workflow was applied to detect traditional illicit drugs, NPS (mainly phenethylamines, tryptamines, piperazines, cathinones, synthetic cannabinoids), and pharmaceuticals of toxicological interest and/or their metabolites in blood, urine, and hair samples for applications in the fields of Driving Under the Influence (DUI) of drugs, Workplace Drug Testing (WDT), drug-facilitated crimes, and Postmortem

(PM) toxicology. Representative analytical findings from selected forensic toxicology cases will be presented and discussed (e.g., DUI cases in which THC and metabolites (Case 1) or cocaine, oxycodone, venlafaxine, quetiapine, diazepam, and/or their metabolites (Case 2) have been detected/quantified in urine and blood) demonstrating the usefulness of the described analytical approach in replacing immunoassay screening. Discussion will include a fatality case in which heroin, cocaine, levamisole (cocaine adulterant), fluoxetine, promazine, diazepam, and/or their metabolites were detected/quantified in PM urine, blood, and vitreous humor and a driving licence regranting case in which trazodone and its metabolite m-chlorophenylpiperazine were detected in hair collected from a driver wrongly charged with DUI of amphetamines merely on the basis of a positive immunoassay urine screening.

Forensic Toxicology, Biofluids Screening, High Resolution MS

K53 The Direct Detection and Ultrafast Quantification of Drugs of Abuse in Serum by Probe Electrospray Ionization/Tandem Mass Spectrometry (PESI/MS/MS)

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After attending this presentation, attendees will understand the basic principles of PESI, a novel ambient ionization technique, and its use as an ultrafast and direct detection method of drugs of abuse in serum without sample preparation.

This presentation will impact the forensic science community by describing a highly sensitive and non-chromatographic analytical method capable of simultaneous sampling and ionization and ultrafast detection and quantification of drugs of abuse in serum without sample pretreatment using PESI/MS/MS.

PESI is a unique ionization technique that uses a probe needle that enables simultaneous sampling and ionization. Its high ionization efficiency is comparable to that of nano-ESI. Previous studies have reported on the effectiveness of PESI/MS/MS in the intact analysis of endogenous compounds.[1,2] The objective of this study was to evaluate the applicability of PESI/MS/MS to the direct analysis of drugs of abuse in serum without sample preparation. Quantitation was also investigated using the Internal Standard (IS) method.

PESI/MS/MS was performed using a Shimadzu LCMS-8040 equipped with PESI ion source in the Multiple Reaction Monitoring (MRM) mode using either one quantifier transition or two transitions (quantifier and qualifier) for each analyte. Serum samples were spiked with reference standards of drugs of abuse of various drug classifications. A 15µL aliquot of the spiked serum containing 50ng/mL of IS compounds were pipetted onto a sample plate. Sampling time was set at 0.30 minutes.

All target drugs selected for this study were detected by PESI/MS/MS, exhibiting that the present method can detect a wide variety of drug types. Qualitative investigation demonstrated that the majority of the drugs could be detected as low as 1ng/mL in serum, while zolpidem could be detected in serum as low as 0.1ng/mL, demonstrating the highly sensitive nature of the method. Preliminary evaluation of the present method with acetyl fentanyl, MDMA, methamphetamine, oxycodone, haloperidol, ketamine, risperidone, zolpidem, and diazepam with their respective stable-isotope-labeled analogs as IS compounds demonstrated linearity of the calibration curves over the specified range (1ng/mL-100ng/mL) with an R^2 value of 0.99. Calculated Limit of Detection (LOD) and Limit of Quantitation (LOQ) values ranged between 0.12ng/mL-0.49ng/mL and 0.33ng/mL 1.5ng/mL, respectively. Intra- and inter-day accuracy (% relative error) ranges were 0.1%-13% and 0.2%-14%, respectively. Repeatability (%RSD, n=5) ranged between 0.6%-14% for all target compounds. Application to real autopsy samples was tested and it was verified that the present method is suitable for practical use. Results demonstrated that PESI, having high ionization efficiency superior to that of conventional ESI, allows for increased sensitivity with sub-nanogram level detection.

The simple, rapid, and highly sensitive direct detection method for drugs of abuse in serum by PESI-MS/MS allowed for the attainment of the ideal scenario in forensic toxicology in which no sample preparation is required for sample analysis. Samples collected at autopsy or a crime scene can be subjected to immediate and rapid drug testing. Since no chromatographic separation is required, the process of sample set-up to qualitative and quantitative MS analyses can be completed in less than one minute. Carry over between samples is negligible owing to the disposable sample plates and needles used as the ionization probe. Finally, the present method has a high potential to be applied to other biological specimens (whole blood, urine, tissue) as well as seized drugs, providing a powerful tool in the analysis of biological specimens for drug detection.

Through this research, a novel drug detection and analysis method is introduced. This method presents application possibilities in various fields such as forensic toxicology, clinical toxicology, and Therapeutic Drug Monitoring (TDM).

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PESI/MS/MS, Drugs of Abuse, Direct Detection

K54 False Positive Enzymatic Alcohol Results: Two Cases From 2014

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After attending this presentation, attendees will understand the chemistry behind Alcohol Dehydrogenase (ADH) -based assays and the potential for false positive results.

This presentation will impact the forensic science community by increasing awareness regarding this phenomenon and thus decreasing the potential for the release of false positive ethanol results.

Presented are two medical examiner cases from North Carolina where different hospitals reported positive ethanol results in children using ADH-based assays, while another laboratory's (headspace Gas Chromatography (GC)) ethanol results were negative. It has been reported in the literature that false positives from ADH-based assays can occur in combination with high levels of endogenous lactate and Lactate Dehydrogenase (LDH), in the presence of the excess NAD+ in the assay, thus producing NADH and a false-positive "ethanol" result. While presumed to be rare, in 2014, conflicting ethanol results occurred in two decedents with drastically different medical conditions. In both instances, there was a significant impact of the findings on the families, as social services became involved and accusations were made regarding family members and/or friends potentially administering alcohol to children.

By understanding the chemistry behind hospital ethanol testing, this presentation will provide the toxicology community with the knowledge of how enzymatic assays can produce false positive results. After reviewing the two cases, toxicologists will be able to better recognize the types of presentations that potentially will manifest such erroneous results.

Case	#1	#2
Age/race/sex	6/B/M	2/B/F
Hospital Ethanol Result	76mg/dL 6/14/14 1557 52mg/dL 6/14/14 1704 39mg/dL 6/15/14 0359	53mg/dL 6/6/14 ~18:15
Abnormal Lab Results	pH= <6.80 (range: 7.35-7.45) Lactate >20.0 (range 0.6-1.8 mmol/L) % methemoglobin 1.9 (range 0.4-1.5%)	Anion Gap: 23 (range 3-15 mmol/L) ALT: 1404 (range 10-32IU/L) AST: 2322 (range 18-36IU/L) Potassium: 13.2 (range 3.5-5.1mmol/L) Sodium: 169 (range 134-143mmol/L)
Patient Timeline	Deceased ~15 hours after admission	Deceased in ER 6/6/14 17:57 ~1 hour after EMS was called
OCME Ethanol	Antemortem blood (6/14/14) & Urine (catheter): None Detected	Postmortem blood: None Detected
Cause of Death	Drowning	Bacteremia/sepsis

It is important that clinical laboratories utilizing enzymatic alcohol assays be aware of abnormal clinical lab tests that may result in, or contribute to, false positive or falsely-elevated ethanol results (such as either elevated lactate and LDH, or in the absence of a direct measurement, an anion gap or case circumstances at least minimally

consistent with a significantly elevated lactate concentration). More conclusive testing methodologies, such as dual column headspace gas chromatography, should be considered for confirmation of ethanol results that may have significant forensic or legal consequences.

ADH-Based Assays, Lactate Dehydrogenase (LDH), Forensic Toxicology

K55 The Increased Prevalence of Illicit Synthetic Opioids in Postmortem Casework at the New York City Office of Chief Medical Examiner (NYC OCME) From January to June, 2016

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After attending this presentation, attendees will better understand the prevalence of illicit synthetic opioids in postmortem casework at the NYC OCME through the first six months of 2016.

This presentation will impact the forensic science community by providing a detailed overview of the cases submitted to the NYC OCME Forensic Toxicology Laboratory where synthetic opioids were identified. This presentation will also help attendees inform their laboratories of the scope of testing and highlight the limitations of current testing protocols. This will be achieved with the use of specific case examples typifying the cases where historical or scene information provides critical information for the toxicological investigation, to cases where key information was not readily available.

The Forensic Toxicology Laboratory at NYC OCME receives between 5,000 and 6,000 postmortem cases each year from the five city boroughs (The Bronx, Brooklyn, Manhattan, Queens, and Staten Island) with an estimated combined population of 8.5 million. Forensic toxicology and drug-testing laboratories across North America and Europe have reported an increased prevalence of illicit synthetic opioids resulting in accidental overdose, predominantly involving fentanyl. A number of structurally related "fentanyls" have also been reported, including furanyl fentanyl, butyrl fentanyl, acetyl fentanyl, and other structurally unrelated synthetic opioids, including U-47700 (3,4-dichloro-N-[(1R,2R)-2-(dimethylamino)cyclohexyl]-N-methylbenzamide), MT-45 (1-Cyclohexyl-4-(1,2-diphenylethyl)piperazine), and W-18 (4-chloro-N-[1-[2-(4-nitrophenyl)ethyl]-2-piperidinylidene]-benzenesulfonamide).

A total of 306 cases were identified as containing an illicit synthetic opioid equating to approximately 11% of all cases submitted for toxicological investigation. Fentanyl was most commonly identified (90.8%), followed by norfentanyl (54.2%), acetyl fentanyl (8.8%), furanyl fentanyl (6.5%), 4-ANPP (4-anilino-N-phenethylpiperidine or despropionyl fentanyl) (5.2%), and U-47700 (2.6%).

Multi-drug intoxication was prevalent throughout the 306 cases and the most common drugs found in combination with the above synthetic opioids were heroin metabolites (46.7%), cocaine and metabolites (37.6%), other opioids (35.3%), benzodiazepines (31.4%), alcohol (26.1%), and cannabinoids (19.9%). Amphetamines (including synthetic cathinones) were detected in 4.9% of cases, while etizolam and PCP were identified in 2.6% and 2.3% of the cases, respectively. Numerous other prescription medications were identified but not collated for this study.

Twenty-four cases were identified with either furanyl fentanyl, 4-ANPP or U-47700, or a combination. Eleven of these cases were identified in the month of June alone, raising concerns that the prevalence of the newer illicit synthetic opioids would continue to increase through the remainder of 2016.

The synthetic opioids in this study were identified through a combination of Enzyme-Linked Immuno-Sorbent Assay (ELISA) as an initial screening tool only, Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS), and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), using commercially available reference standards.

Synthetic Opioids, Fentanyl, Postmortem Forensic Toxicology

K56 The Use of Liquid Chromatography/Time-Of-Flight (LC/TOF) Data-Mining Techniques to Evaluate Evidence of Use of Dipyrone and Levamisole in Conjunction With Fentanyl and Other Illicit Recreational Drugs

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After attending this presentation, attendees will be familiar with the popular cutting agent dipyrone, its pharmacology and metabolism, and will be able to assess the significance of its presence in addition to certain popular drugs of abuse.

This presentation will impact the forensic science community by highlighting the use of potentially toxic drugs mixed with certain street drugs and narcotics.

Dipyrone is an antipyretic and non-narcotic analgesic drug not available in the United States. It has been associated with a variety of toxicities, including hematologic toxicity (blood dyscrasias), and has been associated with bronchospasm and anaphylaxis in asthmatics. Similar to another popular cocaine cutting agent, levamisole, the reported side effects of dipyrone use include agranulocytosis, aplastic anemia, hypersensitivity reactions, toxic epidermal necrolysis, and porphyria. Like other bulk white powders, such as diltiazem, phenacetin and levamisole, dipyrone has been reportedly used as a cutting agent with illicit drugs. This study evaluated five months of postmortem toxicology screening data between February and June 2016, to assess which illicit drugs were found in combination with dipyrone and levamisole.

Methods: Cases submitted to NMS Labs for drug testing in postmortem investigations between February and June 2016 were analyzed by LC/TOF, using an Agilent® 1290 Infinity® High-Performance Liquid Chromatography (HPLC) coupled with an Agilent® 6230 Time-Of-Flight/Liquid Chromatography/Mass Spectrometry (TOF/LC/MS). Retrospective reprocessing of data was performed to identify cases in which dipyrone (based on the presence of its metabolites and breakdown products noramidopyrone, 4-formylaminoantipyrine, and 4-acetylaminoantipyrine) and other popular cutting agents, such as levamisole and diltiazem, were present. Also evaluated were drugs subject to abuse (cocaine, Benzoylecgonine (BZE), morphine, 6-monoacetylmorphine (6-MAM), fentanyl, norfentanyl, acetyl fentanyl, despropionyl fentanyl, butyryl fentanyl, furanyl fentanyl, β-hydroxythiofentanyl, and 3-methylfentanyl).

Results: Data from a total of 13,268 runs were evaluated. The drugs of interest were found as follows: fentanyl/norfentanyl 1758 (13.2%); acetyl fentanyl 257 (1.9%); butyryl fentanyl 16 (0.1%); furanyl fentanyl 229 (1.7%); morphine/6-MAM 2528 (19.0%); and cocaine/BZE 1220 (9.2%).

Evaluating combinations of the above drugs in the 1,758 cases in which fentanyl was detected revealed it was present alone in 675 cases (38%), while in 386 cases (22%), it was present with morphine/6-MAM, in 139 (7.9%) cases it was present with cocaine/BZE, and in 62 cases (3.5%), it was present with acetyl fentanyl. Fentanyl was detected with butyryl fentanyl and furanyl fentanyl in less than ten cases.

Table 1, below, represents the positivity rate of drugs discussed here with common cutting agents dipyrone, levamisole, and diltiazem. Cases in which fentanyl and norfentanyl were present were removed to avoid confounding from dipyrone associated with fentanyl.

Drug	Positives (%)	Dipyrone present	Diltiazem present	Levamisole present
Fentanyl/norfentanyl	1758 (13.2%)	13.2%	3.3%	16.9%†
Acetyl fentanyl*	31 (0.2%)	3.2%	3.2%	16.1%†
Butyryl fentanyl*	14 (0.1%)	0%	7.1%	35.7%†
Furanyl fentanyl*	202 (1.5%)	1.5%	0%	24.3%†
Morphine/6-MAM*	1812 (13.6%)	1.6%	3.3%	11.5%†
Oxycodone*	1386 (10.4%)	0.4%	3.0%	11.5%†
Cocaine*	1087 (8.2%)	1.3%	2.2%	62.9%

**Excludes cases in which fentanyl or norfentanyl were present. † Excludes cases in which cocaine was present.*

Levamisole was the most commonly observed cutting agent and was most frequently found in combination with cocaine (62.9%) and furanyl fentanyl (24.3%) and less frequently with other drugs (11-17%). Dipyrone was present most frequently in cases involving fentanyl (13.2%), followed by acetyl fentanyl (3.2%), and seen less than 2% of the time in combination with the other drugs. Diltiazem was seen infrequently (3.3% or less) in any combination. There are limitations to the data, including the fact that all the compounds have different half-lives, so residual cutting agents from previous ingestions cannot be ruled out as a source. In the case of diltiazem, it may have been ingested therapeutically. Nonetheless, the data are beneficial in identifying the high prevalence of levamisole and dipyrone, compounds that may contribute to the toxicity of these street drugs in drug user deaths.

Dipyrone, Levamisole, Drug-Related Deaths

K57 The Use of a Forensic Case Management System to Deliver Evidence-Based Science and Research

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After attending this presentation, attendees will better understand how extracting information from a case management system can inform interpretation in medicolegal casework.

This presentation will impact the forensic science community by informing attendees of the value of producing evidence-based opinions in casework.

The Victorian Institute of Forensic Medicine (VIFM) provides forensic medical and scientific services for medicolegal death investigations, convictions within the judicial system, and the health and safety of Australians as well as a variety of overseas communities. VIFM operates under two main streams, Forensic Services and Academic Programs. Forensic services include forensic pathology, sciences (toxicology, molecular biology and histology), and clinical forensic medicine.

VIFM services are reliant on a highly developed integrated Case Management System (iCMS). This flexible system has been modified and improved over a 20-year period and can be readily adapted to any forensic or clinical setting to provide organizational efficiency and effectiveness in managing casework. This system allows all specialties within the forensic investigation pipeline to have real-time access to case information. This includes case history, analytical results, uploading of supporting documents and images, case communications, and other investigative information. The system provides key indicators and other operational metrics that can be used to monitor performance. In addition, iCMS offers a reporting mechanism to determine the number of cases received, per case type, time taken to complete a case, and produces reports with statistical analysis. With its unique design, this web-based system can be installed in large or small organizations using a modular approach; its design permits incremental evolution in the system to support organizational change and operational service demands.

Finally, the iCMS provides a mechanism to capture case information that can be readily accessed for research purposes, as it offers a database of invaluable forensic information. For example, captured drug and alcohol data sourced from the iCMS has facilitated national and international research projects, such as understanding the prevalence of drugs in fatality drivers and identifying emerging trends in unexpected drug deaths.[1,2]

From case initiation to the production of evidence-based research, the iCMS provides an innovative and seamless approach to forensic operations.

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Evidence-Based, Databases, Research

K58 Fluoxetine Toxicity — Pharmacogenomics, Drug Interactions, and Dosage Converge to Create the Perfect Storm: A Case Report

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After attending this presentation, attendees will better understand the importance of contributing factors to toxic concentrations of fluoxetine, such as polymorphism of metabolizing enzymes, drug interactions, and drug dosage.

This presentation will impact the forensic science community by providing toxicologists, pathologists, and the medical community with an increased awareness of pharmacogenomics as plausible causes contributing to lethal drug levels, especially in conjunction with drugs taken concomitantly at high dosages.

Introduction: An 18-year-old, non-verbal, White male diagnosed with autism was found dead in his room. Lethal levels of fluoxetine were found in the blood. The medical examiner believed the deceased may have ingested the fluoxetine intentionally. Investigation revealed the parents of the young man administered the correct amount of medication. They believed the capsules may have been the wrong strength.

Method: The capsules were analyzed and found to contain the correct dosage of fluoxetine. Based upon the high levels of fluoxetine found in the blood and liver, pharmacogenomics testing was performed. The postmortem blood was extracted, purified, and genotyped. The amplicon-based targeted sequencing method was chosen to achieve results. Targeted regions were amplified using a SmartChip Polymerase Chain Reaction (PCR) system and sequenced in parallel with deep coverage on MiSeq® platform. The sensitivity and specificity of this test is 100% and 100%, respectively. This panel includes 25 genes and 196 variants based on the Food and Drug Administration's (FDA's) work group guidance and the recommendations of the Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG). Further investigation revealed the patient was prescribed and taking 80mg of fluoxetine per day, 6mg of risperidone per day, and 50mg of topiramate per day.

Results: The capsules were assayed and found to contain 20mg of fluoxetine per capsule. Toxicological findings revealed initial blood screen, fluoxetine 6.4 micrograms/mL. Quantitation of chest blood revealed fluoxetine 4.0 micrograms/mL. Quantitation of liver yielded greater than 160 micrograms/gram. Pharmacogenomic testing revealed the genotype of CYP2D6 *1/*4, phenotype of an intermediate metabolizer. The variant found was an intermediate metabolizer of fluoxetine.

Conclusions: Review of all three variables suggested that they had all contributed to the fluoxetine intoxication. The deceased intermediate metabolizer phenotype along with concomitant administration of another substrate, risperidone, combined with a high dose of fluoxetine created the perfect storm, resulting in the demise of the individual from fatal concentrations of fluoxetine. The patient had CYP2D6 intermediate metabolizer phenotype, which slowed down the metabolism of fluoxetine, thus resulting in a high concentration of fluoxetine in the blood. This high concentration, combined with risperidone, a known inhibitor of CYP2D6, further compromised the metabolism of fluoxetine. All of these variables converged into the perfect storm of toxicity. The medical examiner ruled the cause of death fluoxetine intoxication and the manner of death undetermined.

Pharmacogenomics, Drug Interactions, Fluoxetine

K59 The First Case of Loperamide Toxicity in Jefferson County, Alabama

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After attending this presentation, attendees will be aware of the potential for loperamide toxicity and of loperamide's emerging presence and significance in communities in which opioids are commonly abused.

This presentation will impact the forensic science community by emphasizing the importance of considering loperamide toxicity in deaths of individuals with histories of opioid abuse.

Shortly after being treated and released from a drug rehabilitation center, a 23-year-old male with a long history of heroin abuse was found dead, sitting in a chair. No drug paraphernalia was discovered at the scene. Upon examination, the only evidence of recent injury to the body was a punctate needle mark on the right antecubital fossa. Autopsy did not reveal any underlying disease.

Toxicological analysis of blood from the iliac veins detected therapeutic and subtherapeutic concentrations of trazodone (385ng/mL), citalopram (105ng/mL), diazepam (9ng/mL), and nordiazepam (43ng/mL). Loperamide was detected at a concentration of 374ng/mL (therapeutic range is up to 10ng/mL).[1] All analytes were quantified via Gas Chromatography/Mass Spectrometry (GC/MS) following n-butyl chloride liquid-liquid extraction. Death was attributed to accidental loperamide toxicity. Review of medical examiner cases in Alabama from 2013 to 2016 revealed six additional cases in which loperamide was detected at toxic concentrations (130ng/mL to 1,400ng/mL).

Loperamide is an over-the-counter diphenoxylate analogue that is commonly used for its anti-diarrheal effects. Loperamide is an opioid receptor agonist and, at therapeutic concentrations, acts on μ-opioid receptors along the gastrointestinal tract, causing suppression of normal bowel motility that ultimately results in increased water absorption from the small and large intestines; however, at particularly high concentrations or in cases in which another drug reduces the integrity of the blood-brain-barrier, loperamide exhibits a more centralized opioid action.

Drug discussion forums on the internet suggest that loperamide is thus being used recreationally to simulate the effects of other opioids like heroin and morphine or even to mitigate withdrawal symptoms associated with heroin and prescription opioid disuse.[2] Naloxone can even be given to reverse loperamide's effects in cases of suspected toxicity. Recently, loperamide has also been shown to produce both QRS and QT prolongation at high concentrations, and deaths have been reported directly related to its cardiotoxic effects.[3,4] Some toxicologists even advocate putting restrictions on the sale of loperamide, likening its potential for abuse in a manner similar to pseudoephedrine's when commercial sales limits were implemented in 2006 due to its non-intended use in the manufacturing of methamphetamine.

It is certainly possible that an increasing number of loperamide-related deaths are related to the current opioid epidemic, and it is important that investigators, forensic toxicologists, and medical examiners consider the possibility of loperamide toxicity in decedents with histories of drug abuse.

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Loperamide Toxicity, Withdrawal, Opioids

K60 Postmortem Tissue Distribution of U-47700 Following Lethal Intoxication and Novel Scheduling in the State of Ohio

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After attending this presentation, attendees will better understand the synthetic opioid U-47700, a Novel Psychoactive Substance (NPS), and its concentration in postmortem cases involving single and multiple drug intoxications.

This presentation will impact the forensic science community by informing forensic professionals on abuse trends for opioid NPS designer drugs. This presentation adds to the small amount of published data concerning the potential toxicity of these opioid NPS designer drugs and provides an approach for the analysis of these substances.

3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methyl-benzamide, also known as U-47700, is structurally categorized as an opioid and is an isomer of AH-7921. U-47700 was produced by the Upjohn Compnay in 1978 as a synthetic alternative to morphine. U-47700 exerts its pharmacologic effects as a μ-opioid receptor agonist and has approximately 7.5 times the potency of morphine. Opioid NPS drugs are popular recreational substitutes for heroin, fentanyl, or morphine. NPS drugs can be taken orally, intravenously, or rectally or can be smoked or snorted. They are sold on the internet as "legal highs" and are often mixed with heroin or other psychoactive substances. There have been more than 60 deaths in the United States and European countries involving U-47700.

The effects of U-47700 are similar to other opioids and include analgesia, sedation, and mild euphoria. U-47700 toxicities should be similar to the opiate toxidrome and include respiratory depression, altered mental states, pulmonary edema, coma, bradycardia, hypotension, hypothermia, nausea, and vomiting.

Due to drug scheduling research initiated by the Ohio Attorney General's Bureau of Criminal Investigation (BCI), a swift and unanimous vote by the State of Ohio Board of Pharmacy classified U-47700 as a Schedule I opium derivative under rule 4729-11-02 of the Ohio Administrative Code. The next day, Ohio Governor John Kasich signed an executive order authorizing the Board to take emergency action and subjected U-47700 to criminal drug penalties as of May 4, 2016. U-47700 is also scheduled in the states of Wyoming and Georgia and the countries of Finland and Sweden.

Case 1: A 35-year-old White male with a history of heroin abuse was found unresponsive, seated on a couch. Autopsy findings were unremarkable, except for moderate pulmonary vascular congestion and pulmonary edema (right lung 950g, left lung 710g).

Case 2: A 49-year-old White male was found at home, prone on the bathroom floor. The decedent's past medical history included back and neck pain, sleep apnea, arthritis, and migraines. Autopsy findings were remarkable for moderate coronary artery disease, a 540g heart, fatty liver/cirrhosis, moderate pulmonary vascular congestion, and pulmonary edema (right lung 840g, left lung 1,060g).

Case 3: A 29-year-old White male was found on the bedroom floor. The case was reported to the Lake County Sheriff's Office as an accidental overdose. This was the first confirmed U-47700 death in Ohio. No autopsy was performed.

No apparent foul play or trauma was noted in any of these cases. The decedents in allof the cases purchased U-47700 from the internet.

Standard comprehensive toxicology and drug chemistry analyses were performed on multiple specimens/drug exhibits using Gas Chromatography/Mass Spectrometry (GC/MS). U-47700 detection in tissues was accomplished using GC/MS, Ultra High-Performance Liquid Chromatography/Tandem Mass Spectrometry (UHPLC-MS/MS) after Solid-Phase Extraction (SPE) and liquid-liquid extraction and in hair using HPLC-MS/MS after SPE.

U-47700 concentrations:

Specimen (ng/mL)	Femoral Blood	Heart Blood	Urine	Gastric	Vitreous	Bile	Liver (ng/g)	Kidney (ng/g)	Brain (ng/g)	Hair
Case 1	456	137	Present	Present	55.2	360	605	NTDN	NTDN	Present*
Case 2	90.1	116	Present	Present	117	1070	561	253	123	Present**
Case 3	242	NTDN	NTDN	NTDN	NTDN	NTDN	NTDN	NTDN	NTDN	NTDN

NTDN=No testing performed.

Case 1: No other drugs, including synthetic cannabinoids, fentanyl analogs, novel opioids, or other NPS drugs, were found to be present in the femoral blood, except cotinine. Consecutive 30-day hair segments from the root end contained U-47700 at 10,619, 12,391, and 13,185pg/mg*.

Case 2: Femoral blood contained 0.016mg/L alprazolam, 3.2mg/L topiramate, 0.091mg/L diphenhydramine, and 0.155mg/L bupropion. Cotinine was present. Fentanyl analogs, novel opioids and other NPS drugs were negative in the femoral blood. The unsegmented 0-90-day hair segment contained U-47700 at 12,536pg/mg.**

Case 3: Femoral blood contained 5.3ng/mL delta-9-carboxy THC with presumptive positive benzodiazepines and cannabinoids in the urine. U-50488 and furanyl fentanyl were negative.

Drug chemistry exhibits found near the decedents contained U-47700 in all three cases.

The cause of death in Case 1 was ruled a drug intoxication by U-47700. The cause of death in Case 2 was ruled an intoxication by the combined effects of U-47700, alprazolam, topiramate, diphenhydramine, and bupropion. The cause of death in Case 3 was ruled an acute intoxication by U-47700. The manner of death for all three postmortem cases was accidental. Tissue and fluids associated with detoxification had higher concentrations of U-47700.

Based on the U-47700 concentrations in Cases 1 and 2, there appears to be postmortem redistribution.

U-47700, Postmortem Drug Distribution, Scheduling of NPS Drug

K61 On the Statistical Distribution of V_{max} for Ethanol Pharmacokinetics

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After attending this presentation, attendees will better understand how to model a sample population distribution that is applied to the pharmacokinetics of ethanol and the calculation of appropriate prediction intervals.

This presentation will impact the forensic science community by explaining the difference between confidence, prediction, and tolerance intervals and how they can be calculated and applied to ethanol pharmacokinetics.

Pharmacokinetic parameters such as volume of distribution, maximum blood concentration, and rate constants have been found to be lognormally distributed.[1-3] Although this has been taken by pharmacokineticists to apply to all parameters in pharmacokinetic models, data for V_{max} in ethanol has been lacking. Jones, in his review, gives a frequency histogram for V_{max} values (~ elimination rates) with a normal distribution curve superimposed and stated that the distribution fit well to a normal curve but did not give the results of any goodness of fit tests.[4] Jones also stated that the tails of the distribution are unreliable. This would be the case if they follow a lognormal distribution.

This study extracted a sample of V_{max} values from the literature and used additional data to fit 121 values to both a normal and lognormal distribution.[5-9] Studies were selected if at least a regression was conducted on the terminal values. Studies with only two levels were excluded since ethanol follows a two-compartment open pharmacokinetics model with Michaelis-Menten elimination. The best estimates of V_{max} will be obtained by regression or non-linear regression of the appropriate pharmacokinetic model as outlined by Wagner.[10] A fit of the values to a normal distribution was tested by the Anderson-Darling test, the Cramer-von Mises test, the Kolmogorov-Smirnov (Lilliefors) test, the Pearson chi-square test, and the Shapiro-Wilk test. Only the Kolmogorov-Smirnov test did not reject at the 0.05 level. All tests were conducted in the statistical program R using the package "nortest."

The data was log transformed to obtain the mean ($\bar{x}$=-4.152) and standard deviation (s=0.322) and repeated the tests. None of the tests rejected the null hypothesis. That is, the lognormal distribution seems to be an excellent model for the sample of ethanol elimination rates, V_{max}.

This then brings us to the use of values for the simulation, either forward or backward, of ethanol blood levels. The use of a single value is scientifically invalid and the appropriate way to make a prediction that is used repeatedly is to calculate a tolerance interval. That is, one would like values to predict a range of ethanol blood levels that would cover some specified percentage of the population with 100(1 - α)% tolerance or a lower (or upper) confidence bound to be exceeded by (or to exceed) at least 100*p*% of the population.[11] The concept of prediction and tolerance intervals allows one to calculate the required interval.

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K62 The Benefits and Challenges of Implementing Blind Proficiency Testing in Blood Alcohol Analysis

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After attending this presentation, attendees will better understand the advantages and challenges of implementing a blind proficiency program in blood alcohol analysis.

This presentation will impact the forensic science community by illustrating the benefits of blind testing, subsequently raising the bar for proficiency test requirements. Attendees will learn the value of evaluating an entire quality management system and discover targets that can be assessed through blind testing.

Accrediting bodies require laboratories to participate in a minimum of one proficiency test annually in each area listed on the scope of accreditation. In the forensic community, annual proficiency testing is a tool laboratories use to evaluate the performance and competency of staff, test their quality systems, including evidence handling, instrumentation, standard operating procedures, and to review their case review process. The most common type of proficiency test is one purchased from a commercial vendor. In this type of "open" test, the test taker is aware he/she is participating in a proficiency test and the vendor knows how the test was made.

A limitation of such commercially made tests is they do not mimic actual casework and are often predictable. Furthermore, when an analyst knows they are taking a test, they tend to be more cautious, which can create bias. While the external proficiency exams are a valuable tool in ensuring the quality of test results through inter-laboratory comparisons, the Houston Forensic Science Center (HFSC) sought to use blind samples to objectively test its overall quality system.

As a measure of continuous quality improvement, HFSC implemented a blind proficiency testing program in blood alcohol analysis in September 2015. HFSC purchased blood alcohol material prepared to its requested specifications. The material consisted of human blood in gray-top blood vials filled by puncture and spiked with ethanol in a range of concentrations. The case submission forms used fabricated subject information and the samples were packaged in the same blood alcohol collection kit used by HFSC's clients. The cases were then tagged at the client's property room and submitted to the laboratory to mimic a typical chain of custody and true casework. The proficiency test samples then flowed through the process unrecognized by the participating analyst and back to the client. The greatest challenge in this project was keeping the cases blind. HFSC discovered that even the smallest deviations from normal casework would raise red flags for the analysts and create suspicion. Still, as the cases flowed through the system incognito, analysts did not know if they were analyzing a real case or participating in a blind test.

The completed tests were compared to the expected concentration and control charts plotted standard deviation. These control charts allowed the data to be searched for deficiencies. Additional targets evaluated included training gap assessment, precision of instrumentation, and general stressors on the system.

The blind proficiency program has been highly successful and has proven to be a true test of the entire quality management system. Feedback from participating analysts and clients has been overwhelmingly positive. Data compiled from the blind proficiency program has shown that the methodologies used by the section and the quality system are capable of obtaining accurate and reliable results. Furthermore, satisfactory results obtained by participating analysts provides them with added confidence in court as they are able to discuss an additional quality control measure that attests to the reliability of the results.

Blind Proficiency Test, Quality Control, Alcohol Analysis

K63 The Simultaneous Determination of Gamma-Hydroxybutyric Acid (GHB) and GHB-Glucuronide (GHB-Gluc) in Urine Using Hydrophilic Interaction Liquid Chromatography-Tandem Mass Spectrometry (HILIC-MS/MS)

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After attending this presentation, attendees will better understand a new method using HILIC-MS/MS to determine GHB and GHB-Gluc in urine. A new separation method is necessary to detect GHB and its glucuronide metabolite in urine to better understand the role of the glucuronide in the metabolism of GHB and the potential usefulness of the metabolite in the analysis of GHB in urine samples.

This presentation impacts the forensic science community by introducing a novel separation procedure using HILIC-MS/MS to simultaneously determine GHB and its glucuronide metabolite in urine.

GHB is an endogenous compound in mammalian tissue. It is classified as a Schedule I controlled substance that is highly addictive with low medicinal properties and has been abused in health clubs, raves, and in Drug-Facilitated Sexual Assault (DFSA) cases.[1] GHB is rapidly eliminated from the body after its absorption, making it difficult to detect.[2] GHB-Gluc is a recently discovered metabolite of GHB whose role in the metabolism of GHB still requires investigation and is not well understood.[3] There is currently no method to detect GHB and its metabolite, GHB-Gluc, simultaneously in biological fluids. Difficulty in the analysis of GHB and its glucuronide metabolite can arise due to the polarity of the compounds. Because they are small and polar molecules, HILIC can be utilized to achieve optimum separation.[4]

A Macherey-Nagel NUCLEODUR® HILIC column (100mm x 2mm, 3µm) connected to an MS/MS with an Electrospray Ionization (ESI) source operated in the negative ion mode was used for all analyses. Mass spectrometric analysis was performed in the Multiple Reactions Monitoring (MRM) mode using appropriate collision energy for each selected precursor ion. MRM transitions monitored for GHB included m/z of 103 to 85, 103 to 101, and 103 to 59 for quantitation. The MRM transitions monitored for quantitation of GHB-Gluc were m/z of 279 to 103, 279 to 113, and 279 to 59. Chromatography was performed at 50°C using a binary flow method with mobile phases of 0.1% (v/v) formic acid in water (pH=7) as the strong phase and 0.1% (volume/volume (v/v)) formic acid in acetonitrile for the weak phase. The weak phase was held at 90% for two minutes, then decreased to 60% for five minutes, and held for three minutes. The weak phase was increased back to 90% for five minutes to allow the column to re-equilibrate for the next sample. The total acquisition time was 18 minutes. GHB and GHB-Gluc eluted at approximately two and nine minutes, respectively. The spiked urine samples were diluted 1:4 with deionized water, filtered, then 5µL was injected into the HILIC column. The method displayed good linearity in the concentration range of 1µg/mL to 100µg/mL for GHB and GHB-Gluc with a R^2=0.99. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for GHB and GHB-Gluc were approximately 13µg/mL and 40µg/mL. This method was calculated to be repeatable through Analysis of Variance (ANOVA) testing.

The method was validated in urine samples with regard to linearity, sensitivity, selectivity, precision, accuracy, and recovery. This method could be used in forensic toxicology laboratories for victims of DFSA, Driving Under the Influence (DUI) suspects, and postmortem investigations.

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GHB, Glucuronide, HILIC

K64 Forensic Toxicology Findings in 101 Cases of Drug-Facilitated Sexual Assault (DFSA) in the United States

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After attending this presentation, attendees will better understand the number and variety of substances encountered in DFSA cases in 17 different states and one territory in the United States.

This presentation will impact the forensic science community by providing data on sociological and toxicological statistical information, such as gender, age, concentrations of drugs, prevalence, and the amount of biological specimens collected.

DFSA involves the act of slipping a drug into a beverage to incapacitate without consent and the subsequent practice of sexual assaults. In 2015, 10,035 rapes were reported to law enforcement, according to the Federal Bureau of Investigation (FBI) Uniform Crime Reports.

The most common substances used in DFSA cases include: ethanol, GHB, benzodiazepines, opioids and opiates, Z-drugs, antihistamines, barbiturates, and traditional drugs of abuse, such as cocaine, cannabinoids, and amphetamines.

The data from 101 DFSA cases submitted to NMS Labs in 2016 were reviewed. All positive results from screening (Enzyme Immunoassay (EIA)) were verified by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS), headspace Gas Chromatography (GC), or Gas Chromatography/Mass Spectrometry (GC/MS). In cases in which gender was available, 84 were female with an average age of 25.6 years (median 22, range 14-53 years) and two were male with an average age of 17.5 years (median 17.5, range 13-22 years). Blood and urine were collected from the alleged victims. Urine was submitted in 77.2% of the cases, and blood was submitted in 63.3% of the cases. Both matrices were collected in 40.5% of the cases.

Overall, 40 substances were found positive in blood and 67 substances were found positive in urine. There were no differences in the drugs detected in blood and urine for subjects for whom both samples were submitted. The most prevalent substances identified overall in blood and urine samples from the cases reviewed are listed in Table 1 below. Sixty-seven other substances were detected in less than 10% of the samples. The mean concentration of the most common drugs in blood were ethanol (116.77mg/dL); Δ^9 carboxy THC (19.55ng/mL); Δ^9 THC (2.07ng/mL); amphetamine (43.20ng/mL); and methamphetamine (367.50ng/mL). The drugs most frequently found in combination with alcohol were: ethanol + THC/metabolites (19.8%); ethanol + amphetamine/methamphetamine (13.86%); ethanol + antidepressants (12.87%); ethanol + benzodiazepines (11.88%); ethanol + antihistamines (8.91%); and ethanol + cocaine/metabolites (4.95%). Benzodiazepines and GHB are frequently reported as being associated with DFSA. In this series, benzodiazepines were found in a total of 15 cases: lorazepam (five cases); clonazepam/7-amino clonazepam (four cases); alprazolam/α-hydroxyalprazolam (four cases); midazolam/1-hydroxymidazolam (one case); and oxazepam/temazepam (one case). GHB was found in three urine samples at concentrations of 4.3mg/L, 4.5mg/L, and 6.0mg/L. Cut-off concentrations of 5mg/L or 10mg/L have been proposed to distinguish endogenous from exogenous GHB. This study demonstrates the variety of substances that are commonly encountered in alleged DFSA victims in the United States. The absence of alcohol and drugs in some cases may represent a time delay in collecting samples. The purpose of this research is to contribute to a better understanding regarding the factors involved in DFSA.

Table 1. Most common substances in DFSA cases.

Overall		Blood		Urine	
	%		%		%
Ethanol	36.6	Ethanol	35.9	Ethanol	33.3
Δ^9 Carboxy THC	33.6	Δ^9 Carboxy THC	23.4	Δ^9Carboxy THC	30.7
Amphetamine	14.8	Δ^9 THC	15.6	Amphetamine	8.9
Methamphetamine	10.8	Amphetamine	15.6	ß-phenethylamine	7.6
Δ^9 THC	9.9	Methamphetamine	12.5	Methamphetamine	6.4

K65 Buprenorphine and Metabolites in Paired Breast Milk and Maternal and Infant Plasma Specimens

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After attending this presentation, attendees will be able to describe the transfer of Buprenorphine (BUP) from maternal sublingual buprenorphine therapy to infants via breastfeeding.

This presentation will impact the forensic science community by strengthening evidence that BUP should be an approved therapy for medication-assisted, opioid-dependent women who breastfeed and abstain from illicit drug use.

Opioid abuse during pregnancy is a growing public health concern and is associated with fetal growth restriction, placental abruption, fetal death, and Neonatal Abstinence Syndrome (NAS). Lactation is known to reduce NAS severity, and women receiving methadone therapy are encouraged to breastfeed if they do not use illicit drugs. Recently, the Academy of Breastfeeding Medicine revised their protocol to also recommend BUP-treated mothers to breastfeed, despite a lack of conclusive infant safety data. This study sought to quantify BUP and its active phase I and phase II metabolites in breast milk and maternal and infant plasma of BUP-maintained women and their infants.

Ten opioid-dependent, BUP-maintained women (2mg-22mg/day, sublingually) provided paired breast milk and plasma specimens (2h-2.5h after daily sublingual dose) on days 2, 3, 4, 14, and 30 post-delivery, and nine infants provided plasma specimens on day 14, as part of this Johns Hopkins University School of Medicine Institutional Review board-approved study. Plasma and breastmilk samples (100µL) were quantified for BUP, Norbuprenorphine (NBUP), BUP-Glucuronide (BUP-Gluc), and NBUP-Gluc by a previously validated liquid chromatography/tandem mass spectrometry method. Briefly, samples were fortified with deuterated internal standards and proteins precipitated with acetonitrile. Supernatants were diluted with phosphoric acid and extracted on solid phase polymeric, strong cation exchange cartridges. Linear ranges were 0.1-20 (BUP, BUP-Gluc), 0.25-50 (NBUP-Gluc), and 2-100 (NBUP) µg/L. Bias and imprecision were <±16%. Non-parametric correlation coefficients (Spearman) assessed correlations between BUP dose and concentrations with a $p < 0.05$ significance threshold.

Women were 26.1 ± 4.7 years old. Ninety percent of women were cigarette smokers and only three exclusively breastfed their infants. Infants were born at term with appropriate birth weights; only one infant required treatment for mild NAS for 12 days. BUP (median (range)) was detected in all breast milk (2.4 (0.2-20.8) µg/L) and plasma (1.9 (0.4-7.0) µg/L) samples on all days from all mothers. BUP-Gluc, NBUP, and NBUP-Gluc were detected > Limit Of Quantification (LOQ) in 16/44 (0.1-0.3µg/L), 18/44 (1.0-4.1µg/L), and 36/44 (0.3-5.1µg/L) breast milk samples, respectively. BUP-Gluc, NBUP, and NBUP-Gluc were detected > LOQ in 44/46 (0.1-9.3µg/L), 27/46 (2.1-12.4µg/L), and 46/46 (1.2-42.2µg/L) maternal plasma samples, respectively. Ratios of median BUP in breast milk to median BUP in maternal plasma were 0.7 (day 4)-2.0 (day 14). Statistically significant correlations (rho) between maternal dose and maternal plasma BUP concentrations were 0.67 (day 14)-0.85 (day 2). Statistically significant correlations between maternal dose and breast milk BUP concentrations were 0.64 (day 3)-0.88 (day 4). BUP was detected in 4/9 infant plasma samples at 0.2µg/L, 0.7µg/L, 1.0µg/L, and 2.9µg/L. Only one exclusively breastfed infant had detectable BUP (0.7µg/L). No metabolites were detected > LOQ in any infant plasma sample. Using a 600mL/day breast milk approximation for an exclusively breastfeeding infant at two weeks of age and a 4.8µg/L median BUP breast milk concentration, a maximum relative infant dose of 2.9µg/day of BUP is possible and well below BUP doses tested to treat NAS (15.9µg/kg/day).

BUP was detected in less than half of the infants and at low concentrations. Metabolites associated with sedation were not detected in infant plasma. The significant correlations between maternal BUP dose and maternal plasma and breast milk concentrations in this study were not observed in previous methadone studies and could be due to BUP's higher lipophilicity. Although increasing BUP concentrations in breast milk warrant further study, data from this small cohort contribute to the recommendation for breastfeeding in BUP-maintained women.

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Buprenorphine, Breast Milk, Neonatal Abstinence Syndrome

K66 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will have gained an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 18th Annual Special Session within the Toxicology Section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information exists involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmaco-toxicokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths one must go to in some cases. Karen Ross, MD, Erik Christensen, MD, Greg Schmunk, MD, and Laura Labay, PhD will be reviewing cases from their years of experience as forensic pathologists and toxicologist, respectively, that highlight the issues and confounders in the pediatric population.

Dr. Ross will discuss the difficulties associated with interpreting drug findings in perinatal cases when exposure took place *in utero*. Multiple variables in the context of *in utero* exposure present unique challenges in assessing any contribution to death. Included in the complexities are considerations of pharmacokinetics and pharmacodynamics of both the mother and the fetus. These, and other issues, will be discussed.

Dr. Christensen will examine the potential role of olanzapine in the death of a 2-week-old infant and whether breastfeeding represents a competent cause of exposure, in addition to other considerations. Breastfeeding is often attributed to causing adverse effects in nursing neonates/infants without careful consideration of the complexities of such conclusions.

Dr. Schmunk will review a case involving the death of a 12-year-old after administration of the neuromuscular junction blocker succinylcholine. He will speak regarding cautions with the use of this commonly used agent in the pediatric population and what the cutoff for "pediatric" should be for this compound.

Dr. Labay will present a case that highlights the lengths to which one must go in the toxicological investigation related to the death of a young child. Analyses of a pillow case, mattress pad, and hair from the exhumed child were used to bring light to the role of diphenhydramine, dextro/levo methorphan, and/or doxylamine in the cause and manner of death of this 10-month-old child. This case reinforces the fact that routine toxicological examinations may not suffice in every case.

Pediatric, Postmortem, Toxicology

LW1 Were Moses and Aaron the First Bioterrorists?

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After attending this presentation, attendees will better understand the history of the Ten Plagues of Egypt described in the Bible, specifically, the causes of plagues five and six, and their interrelationship.

This presentation will impact the forensic science community by demonstrating the importance of critical thinking, the use of primary source materials, and the application of contemporary scientific knowledge to explain remote historical events.

The exodus of the Israelite slaves from Egypt was momentous in religious history. The Pharaoh had refused to free them from bondage until a series of ten plagues occurred. Biblical and scientific scholars have been fascinated by these events and numerous theories have been proposed.[1-5] Theologians believe the cause of the plagues was the supernatural power of God, while Sigmund Freud called the Passover story "a pious myth."[6] Scientists offer more naturalistic mechanisms. In *Miracles: A Preliminary Study*, C.S. Lewis wrote that the cause of a miracle is the activity of God, but its results follow according to natural law. During the fifth plague, death of livestock occurred from an unnamed infectious disease and during the sixth, boils appeared on humans and animals. A variety of infections have been suggested as the causes of plagues five and six, including, but not necessarily limited to: malaria, cholera, glanders, African horse sickness, bluetongue disease, West Nile fever, Rift Valley fever, and anthrax. Exodus 9:3 states, "Behold, the hand of the Lord is upon thy cattle which *is* in the field, upon the horses, upon the asses, upon the camels, upon the oxen, and upon the sheep: *there shall be* a very grievous murrain (plague)."[7] Most warm-blooded animals, especially hoofed herbivores, are susceptible to anthrax. Exodus 9:6 reads, "And the Lord did that thing on the morrow, and all the cattle (livestock) of Egypt died….."[7] Presumably, the dead animals were either buried or burned.

During WWII, Winston Churchill ordered the development of tens of thousands of anthrax bombs, to be dropped over every city and town in Germany in the event of an invasion of Great Britain. A small anthrax bomb was tested on Gruinard Island, in the Hebrides, off the northwest coast of Scotland. Thirty sheep were taken to the island and tethered. A 25-pound bomb was dropped and all the sheep died within a week. The project was stopped after an anthrax outbreak in cattle and sheep occurred on the Scottish coast that directly faced Gruinard Island. This small island was contaminated with anthrax until 1986, when tons of topsoil were removed and incinerated. Because the highly heat-resistant spores persisted, formaldehyde mixed with sea water was then used to complete the cleanup. Thus, anthrax spores are not eliminated with burning.

Exodus 9:8 declares, "And the Lord said unto Moses and unto Aaron, Take to you handfuls of ashes of the furnace, and let Moses sprinkle it toward the heaven in the sight of Pharaoh."[7] The carcasses of the animals that died during the fifth plague were probably incinerated in a "furnace" with resultant "ashes." The blood of the animals would have contained as many as one billion bacilli/ml, which sporulate on exposure to air. Thus, the "ashes" would have contained countless anthrax spores. Exodus 9:9 states, "And it (the anthrax-contaminated ashes) shall become small dust in all the land of Egypt, and shall be a boil breaking forth *with* blains (sores) upon man, and upon beast, throughout all the land of Egypt."[7] Cutaneous anthrax is described as a "boil-like" lesion that ruptures (i.e., breaks forth) into an ulcer (i.e., sore), affecting humans and animals.

In conclusion, this study agrees that anthrax was the most probable cause of the fifth and sixth plagues of Egypt and suggests a novel interrelationship and transmission mechanism between the two plagues.

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Moses, Bioterrorism, Anthrax

LW2 Joyeux Anniversaire, Commissaire! An (Im)Possible Interview With Jules Maigret — The "Mender of Destinies"

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The goal of this presentation is to familiarize attendees with the world-famous, fictional detective Jules Maigret on the 130th anniversary of his birth (1887-2017) by discussing the importance of Maigret's method of investigational rediscovery in a world dominated by a blind faith in technology.

This presentation will impact the forensic science community by presenting a multidisciplinary framework, based on the analyses of both the crime stories and television series inspired by Maigret's investigations, showing how the fat, grumpy commissioner created by the French novelist George Simenon has been wrongly accused of not having a scientific methodology. The goal of this study is to recognize the central features of Maigret's method in ethnology and the ability to reconstruct psychological and social profiles.

In recent decades, the hundreds of novels and short stories featuring Commissar Maigret have been unfairly neglected. Maigret lacks the mental acuteness of Hercule Poirot and Nero Wolfe as well as the charm of Philo Vance and Ellery Queen; however, Maigret has something more: his extraordinary humanity. Maigret's humanity is not just a drink at Brasserie Dauphine or a walk in the Parisian neighborhoods he patrols. It is the core of his investigational model, a model that rests on a single question: why?

Maigret's personal concept of justice lies indeed in the moral maxim *comprendere sans juger* (understanding without judging) that is profoundly connected to the final object of his work: understanding the criminal mind. The principal determinant of an investigation is the detective's ability to arrive at a complete physical and psychological identification with the circumstances he must investigate in order to understand all the people involved in the case from their own points of view. In ethnology, individuals must be understood before the rules of society, and Maigret does likewise. Using an anthropological language, Maigret conducts his investigations according to the principles of fieldwork and participant observation.

This presentation will illustrate how, for Maigret, crime always arises from individual meanings and not as a consequence of social facts. The novel *La tête d'un homme* (published in English as *A Battle of Nerves*) will be analyzed. Maigret organizes the "escape" from death row of the prisoner Joseph Heurtin, who was convicted of killing Mrs. Henderson, an American heiress, and her French maid, Élise Chatrier. Heurtin had been seen in the neighborhood when the murder was committed and his finger- and footprints were found everywhere in the victim's house. Nonetheless, Maigret casts doubt on Heurtin's guilt because of a lack of motive. Physical evidence can direct the detective's attention, which pertains to "what" happened and "how" it happened. Although Maigret is very passionate about Dr. Moers's criminal analysis (i.e., from his handwriting and graphological expertise), we learn that the probable offender is left-handed and extremely smart, with science being just one aspect of a more complex situation. His investigation model indeed shows many similarities with clinical criminology. Thus, it is not a coincidence that Maigret had studied medicine for three years, is very interested in positivist criminology, and almost all of his inquiries deal with deviance and criminal responsibility. In this regard, the novel *Maigret Hesitates* analyzed Article 64 of the French Penal Code, which states that an insane person who commits a crime is not responsible. Even if this is the only article concerning human responsibility, Maigret wonders who is the right professional to decide whether a defendant is insane. His moral question is now at the center of the contemporary neuroethical debate.

This presentation will conclude by presenting attendees with an interview with Commissaire Maigret regarding some famous Italian criminal trials in which physical evidence, investigation methods, and criminal profiling seem to contradict each other.

As Hegel wrote, "Minerva's owl spreads its wings with the falling of the dusk," meaning wisdom takes flight after the day's main events have taken place.[1] This is why it is important to rediscover Maigret's investigational methodology on the 130th anniversary of his birth, because the shadow of the past helps us step into the light of the future.

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Investigation Methodology, Crime Story, Trial

LW3 O.J.: If the Case Were Tried Today, What the Science Would Say

Adam Itzkowitz, JD, 736 Vallance Way, NE, Saint Petersburg, FL 33716*

After attending this presentation, attendees will better understand how today's advances in forensic science could affect the O.J. Simpson trial if the case were tried today.

This presentation will impact the forensic science community by illustrating: (1) the challenges that were presented in the collection of evidence; (2) the methods that were available to accurately test the evidence; as well as, (3) how the evidence was presented in the courtroom. Attendees will be shown that 20 years later, the science has changed and prosecutors and defense attorneys are better prepared to present scientific evidence to a jury.

Despite all the evidence in the case, most people think that the most damaging evidence was the scientific evidence. A time of death estimated by the coroner fit perfectly with O.J. Simpson's lack of an alibi. Hairs and fibers found at the scene, in a knit cap possibly dropped by the assailant, and on the shirt of Ronald Goldman were matched to those from O.J. Simpson. Bloody size-12 shoeprints were found at the crime scene, which were the same size as shoes Simpson wore. Those shoeprints were determined to match the prints of a rare Bruno Magli shoe. Rare, yes; however, in his subsequent civil trial, a picture surfaced of Simpson, who wears a size 12 shoe, wearing a pair of Bruno Magli shoes. But most damaging of all the scientific evidence was the DNA.

Most experts claim there has never been a trial in which there was more DNA evidence. If prosecutors could dream up a scenario where there was scientific evidence linking someone to a crime, this was it. They described it as a trail of evidence, from the crime scene, into O.J. Simpson's white Ford Bronco, then ending back at his house.

While the defense's explanation of this evidence included well-documented racial animus, the defense team also argued that the results of testing were tainted due to sloppy evidence handling by the police department.

While nobody knows what affect, if any, the defense argument had on the jury, one thing is certain: despite all the evidence pointing toward him, O.J. Simpson was acquitted of the double homicide of Nicole Brown Simpson and Ronald Goldman.

In conclusion, this presentation will evaluate a variety of scientific evidence presented in the case. The first segment of this presentation will examine the blood evidence in the case and address how the science, storage, and handling affected the evidence. This presentation will further examine how the science has evolved since the trial and how those advances would cause the evidence to be viewed in a different light today.

The second portion of this presentation will examine how a scientific evidentiary issue, such as the admissibility of luminol testing, affected the trial and how, 20 years later, the courts would rule on such an issue in light of the advances in forensic science since this trial.

DNA, O.J. Simpson, Admissibility of Evidence

LW4 Investigating Death or Raising the Dead? The Saga of William Wynn Westcott: A 19th-Century London Coroner

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After attending this presentation, attendees will gain an appreciation of the role of 19th-century coroners in the development of an effective public health system in England and will gain insight into the narrow path those in public life tread between fame and infamy.

This presentation will impact the forensic science community by reminding them, in particular, of the need to keep confidential papers confidential.

Martindale: The Complete Drug Reference, now in its 38th edition, was first published in 1883; William Wynn Westcott collaborated in its production with the principal author, William Martindale. Then, as now, Westcott's name can be found on its frontispiece. This, apart from several books on esoteric topics such as the Rosicrucians and numerology, is his legacy.

Born in 1848, Westcott graduated in medicine from London University in 1871. He spent ten years as a general practitioner in rural Somerset and became a Freemason in 1875. He progressed rapidly up the Masonic ranks, becoming interested in the more esoteric aspects of ceremonial Masonry.

Westcott moved to London in 1878, working first as a deputy coroner, then from 1894, as Coroner for North East London. He published extensively on death investigation in the medical literature and his occult interests were hinted at in an 1890 *British Medical Journal* paper on mandrake root.

Westcott's parallel life in occult studies developed apace. He was a member of Societas Rosicruciana in Anglia, a society with membership restricted to Master Masons, rising to become its general secretary, and was also involved with the Theosophical Society. Both societies were interested in the theoretical study of "high magic" ritual, but not in its practical application. To further an interest in practical magic, Westcott, with others, founded the Order of the Golden Dawn in 1888. Its structure was derived from a likely forged, ciphered manuscript, translated by Westcott. Alleged to have originated with the original Rosicrucians, this document was used as the basis of a pseudo-Masonic structure for the new organization.

Because membership did not require a Trinitarian Christian belief and was not restricted to males, it attracted an eclectic membership from the glitterati and intelligentsia, including artists, actresses, scientists, and doctors. Prominent members included W.B. Yeats, Algernon Blackwood, Constance Wilde (Oscar Wilde's wife), and Alistair Crowley; however, all did not go smoothly, particularly after Westcott had to resign from the Order in 1897.

Westcott gave this reason for his resignation: "It somehow became known to the State officers that I was a prominent official in a society in which I had been foolishly posturing as one possessed of magical powers – and if this became more public it would not do for a coroner of the crown to be made shame of in such a mad way." Allegedly, this was due to him leaving papers relating to the order in a taxi, which were then forwarded to the Home Office, the ministry with oversight of coroners. Crowley's take on this was that Westcott had been told by the Home Office, "That he was paid to sit on corpses not to raise them; and that he must choose between his coronership and his adeptship." Another possible reason is that at around this time, allegations surfaced that Westcott knew that some of the documents used to develop the constitution of the Order were forgeries. There may also have been family pressures on Westcott. The Order schismed, notably with Crowley splitting to develop the thelemic system of magic.

In 1901, two American adherents were tried and convicted at the Old Bailey of rape. By this time, Westcott was no longer associated with the order and was less prominent in public life.

In 1919, Westcott traveled to Natal and remained there, resigning his coronership in March 1920. His wife may not have joined him there, as she died after a fall from a house in Tunbridge Wells in 1921.

Westcott died in South Africa in 1928. While he had a fulsome, if sanitized, obituary in the *Lancet*, his death was only mentioned in passing by the *British Medical Journal*, to which he had contributed greatly, in a review of the 19th edition of *Martindale*.

LW5 Double-Induced Suicide of a Father and Adult Daughter: A Case Report

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After attending this presentation, attendees will better understand a rare case of double-induced suicide of a father and a daughter, in which the daughter was the perpetrator.

This presentation will impact the forensic science community by illustrating that, even though very rare, these cases do exist and by providing a detailed insight into how the forensic investigation was conducted in this particular case.

Double-induced suicide is a known forensic entity that most often occurs in emotional relationships or can be caused by social issues. It is a prior agreement by the partners to end their lives. In heterosexual relationships, almost without exception, the perpetrator is a man who kills his partner first, then takes his own life.

A case of double-induced suicide of a father (53 years old) and a daughter (30 years old), both intellectuals with a university degree, will be presented.

Forensic expertise primarily determined that there had been an agreement between these two people to commit suicide, and that surprisingly the perpetrator was the daughter. A farewell letter that was found at the scene was analyzed by a handwriting expert, who determined that she wrote certain parts of the letter, but there were also parts of the letter that had been written by the father. Two guns were used as tools and Gunshot Residues (GSR) were found on the hands of both persons.

At autopsy, it was discovered that the father had four gunshot wounds: one distant-range gunshot wound to the right hip, one tangential gunshot scratch to the frontal region, with gunpowder tattooing, and two separate close-range gunshot wounds to the temporal region. The daughter had only one close-range gunshot wound to her chest, with the injury to the heart. Therefore, it was determined that the only possible scenario was one in which the daughter was the perpetrator who killed her father first, then took her own life.

This case is particularly interesting because the daughter was a young doctor, who obviously tried to present the murder of her father as his own suicide (close-range gunshot wound in the right temporal region). The analysis of her medical records discovered that she was diagnosed with acute depression, for which she was treated with antidepressant medications only 15 days prior to this tragic event. Furthermore, a few months before the event, the daughter published a book of poetry (as the author), the detailed analysis of which can also shed light on the motive of suicide — unattainable, forbidden love.

Double-Induced Suicide, Father and Daughter, Female Perpetrator

LW6 The Mysterious Case of Lori Ruff — Solved!

Colleen M. Fitzpatrick, PhD, 18198 Aztec Court, Fountain Valley, CA 92708; and Reg Brown, BS, Identifinders International, 18198 Aztec, Fountain Valley, CA 92708*

After attending this presentation, attendees will learn how high density autosomal Single Nucleotide Polymorphism (SNP) testing methods borrowed from the genetic genealogy community were used to identify a Jane Doe in a case in which forensic identification methods could not be applied. Although the identification methods used here have been very successful for the genetic genealogy community for identifying adoptees and long-lost family members, they are not presently available for use by law enforcement. Therefore, attendees will learn about identification techniques they may not yet be aware of, but that have great potential for future use by the forensic community.

This presentation will impact the forensic science community by demonstrating the successful resolution of a unique case of human identification using techniques borrowed from genetic genealogy. This presentation will broaden the understanding of various types of SNP metadata that can be extracted to reduce the complexity of analyses, leading to a better appreciation of high-density autosomal SNP testing as an alternate means of DNA identification.

The true identity of Lori Kennedy Ruff baffled authorities since her death on Christmas Eve of 2010. Although Lori was married and the mother of a young daughter, her family discovered that she had created a new identity nearly two decades earlier. A collection of personal items found in her strongbox after she died included a letter of reference written on stationery from a hotel in Thailand, a certificate from a parachute school, and a sheet of scribbled phone numbers of individuals who claimed they had never met her. There were also documents that revealed that in 1988, she had assumed the identity of Becky Sue Turner, a 2-year-old who had died in a fire in Fife, WA, in 1971, after which she immediately had her name changed legally to Lori Erica Kennedy.

Lori's identity change had been well planned. Despite investigations by the federal authorities, her life prior to 1988 remained an enigma. Various theories were advanced regarding who she was, but they were based on speculation. When no further leads were produced, the case was closed.

Yet the Ruff family still wanted to identify Lori for the sake of her young daughter. Because Lori had been cremated and her DNA was not available, the family had her daughter and Lori's husband tested by 23andMe, a commercial DNA testing company. Software tools provided by the company enabled this research to back out Lori's autosomal DNA results through phasing, which revealed a few close matches that could potentially provide clues to her identity; however, there were challenges in exploiting these matches because they were either anonymous, adopted, or unresponsive.

Using an innovative DNA mapping technique, the Geographical Relationship ID System (GRIDS), applied to the genealogies of more distant matches, this study was able to visualize geographical overlap among their families. Coupled with what little was known about her activities prior to 1988, this study was able to focus on areas where her family probably resided and identify possible family surnames.

Through autosomal phasing analysis and GRIDS, a first cousin was successfully identified, which led to Lori's family. None of the theories about Lori Ruff were true. She was simply a runaway teen from Philadelphia who could not get along with her stepfather.

Lori Ruff, Autosomal DNA, Genetic Genealogy

LW7 Do You Do Voodoo? Zombies, Ritual Crime, and Forensic Investigations

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The goal of this presentation is to illustrate the complex challenge of ritual crime investigation in modern multicultural Western society.

This presentation will impact the forensic science community by proposing an effective approach for the investigation of suspected ritual crime.

In the last few decades, the mass movements of people from different parts of the world have transformed several countries into multicultural societies. Different populations, beliefs, and traditional religious systems have left their places of origin to merge with other cultures.

Despite the fact that Western society is inclusive, some ritual aspects of ethnic religions are perceived as particularly mysterious and looked at with suspicion and fear. Among them, voodoo is frequently associated with horror plots and dark crimes in movies.

The scientific community has been attracted by some of the darkest ritual phenomena, including zombification, and has attempted to explain them. Resuscitating a dead body has been viewed with fascination since Mary Shelly's *Frankenstein*; similarly, the possibility of transforming someone into the living dead by a voodoo ritual has attracted public interest. A dispute has begun about the alleged chemical origin of zombification, initially attributed to the use of tetrodotoxin. More recently, the possibility of a zombie invasion has been considered from an epidemiological and legal point of view, transfiguring the walking dead into a biological phenomenon, separate from the religious world.

However, even if scientists have put moderate effort into the investigation of these mysterious figures, the majority of ethnic cultures and their symbolisms are still poorly explored. The combination of mystery, fear, and lack of in-depth knowledge raises misunderstandings and misinterpretations. If crime scene elements seem to be out of the ordinary, it is not uncommon to lend them a ritual significance. The victim's bruises could appear to be arranged in a geometric symbol or some objects are scattered around as alleged indicators of an obscure ceremony. Media in particular, more than investigators, have emphasized and inflated these aspects since the Jack the Ripper murders, perhaps the first case of a modern serial killer being accorded media coverage.

In addition to the risk of "recognizing" symbols even when they are not, the ritual phenomenology is simplified into abstract categories because of a poor knowledge of ethnic beliefs. When the ritual shows pagan symbols of a possible ancient Western origin, it is classified as Satanic, while if the evidence exhibits a more exotic nature, it is considered voodoo related. Clearly this simplistic approach can lead to misinterpretation, driving the investigation and public opinion in the wrong direction, sometimes with terrible consequences (e.g., racist witch hunts).

During forensic investigations, it is central to identify the actual presence of symbols at the scene. Only then it is possible to recognize their origin and ascription to a particular cultural group. The same symbol may have very different and sometimes opposite meanings, depending on the culture using it. Symbols and rituals are languages of a specific group that must be identified in order to interpret their significance. Thus, it is important that the investigative agencies are able to identify whether there are discordant elements in a crime scene and whether these elements have a potentially symbolic meaning. After the first screening, the alleged symbolic elements must be carefully interpreted and classified according to the group to which they belong, without making simple generalizations. When the symbol is recognized and classified, the investigation needs to clarify the forensic relevance and whether a correlation between the ritual and the crime exists. Moreover, the symbolic elements may provide a cultural/ethnic profile of the persons involved at the scene.

Modern Western society is a multi-ethnic organism that includes a variety of beliefs and religious systems, representing a new challenge for investigators who require effective support (e.g., training and specialized units) in order to recognize and interpret symbolic elements present at a crime scene.

Ritual Crimes, Voodoo, Satanism

LW8 The Tamam Shud Mystery — Old Case, Modern Forensics

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After attending this presentation, attendees will better understand the physical, chronological, and historical information that can be provided about a John Doe by using a wide range of modern investigative technologies not available in the past. This presentation will emphasize how such information can be exploited to create a more accurate and less speculative theory of the John Doe's identity.

This presentation will impact the forensic science community by demonstrating how new technologies can generate investigative leads even in very old cold cases. This presentation will broaden understanding of how human identification has advanced over recent decades from relatively primitive private investigation techniques on a local level to the advanced research and analysis methods used today on a global basis.

The identity of a deceased male discovered in the early morning of December 1, 1948, on Somerton Beach near Adelaide, Australia, has become one of the most baffling cold cases in that country's history. The autopsy of the estimated 40-to-45-year-old "Somerton Man" (SM) indicated his death could not have been natural; poisoning by barbiturate or a soluble hypnotic was suspected, although no poison was detected. The case took a puzzling turn when a small scrap of paper was found in the man's watch pocket with the words "Tamam Shud" (Finished) printed on it. These are the last words of *The Rubaiyat of Omar Khayyam*.

A thorough police investigation produced no identifying information. SM's fingerprints, the wide publication of his autopsy photos, and a review of United Kingdom and United States missing person's reports produced nothing. His suitcase, recovered from the nearby Adelaide train station, also revealed no identifying information, except for several items of probable American origin, including a double-breasted jacket with featherstitching that at the time was produced on machinery present only in the United States.

SM's identity has remained a mystery for more than 60 years.

This study reports the first major advances in the identification of SM since the 1940s and 1950s, using DNA and isotope analysis not available during the original investigation. Because permission to exhume SM has not yet been granted by the South Australian government, only strands of his hair have been available for analysis; however, SM may have fathered a son named Robin. Robin's mother was associated with SM because her phone number was penciled on the back page of a copy of *The Rubaiyat*. This book was also found to be the source of the scrap of paper in the man's pocket. Robin and SM share two rare genetic conditions that add significant weight to the hypothesis of a father/son relationship. Unfortunately, Robin passed away in 2009 and his DNA is unavailable; however, advanced analysis has been possible using his family's DNA.

Major developments presented in the analysis of the Tamam Shud mystery are: (1) a new and more comprehensive analysis of the items found in SM's suitcase; (2) isotope and mitochondrial (mtDNA) analysis of strands of SM's hair taken from the plaster cast made of his upper torso; (3) an analysis of Robin's DNA based on autosomal DNA testing results of his daughter and her mother (SM's assumed granddaughter and daughter-in-law); (4) use of a novel DNA mapping technique that has revealed SM may have had American ancestry; and, (5) application of chromosome matching to discover a possible genealogical connection to Thomas Jefferson.

Tamam Shud, Autosomal SNPs, Isotope Analysis

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National Institute of Standards and Technology (Employee). - B202
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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National Institute of Justice (Grant Support). - A124
Discloses no financial relationships with commercial entities. - S2

C

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Desert Research Institute (Employee).
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Kevley Technologies, PIKE Technologies (Discussion of Commercial Products or Services).
National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (Grant Support).
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Applied Biosystems, Craftsman®, Onset Computer Corporation, QIAGEN, Inc, Thermo Fisher Scientific Inc (Discussion of Commercial Products or Services). - B131
Applied Biosystems, Beckman Coulter, Inc, Craftsman®, Illumina, Inc, Onset Computer Corporation, Promega Corporation, Thermo Fisher Scientific Inc (Discussion of Commercial Products or Services). - H10
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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University of North Texas Health Science Center (Employee).
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ESRABI (Discussion of Commercial Products or Services). - B185
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Microsoft Corporation (Discussion of Commercial Products or Services).
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Promega Corporation, QIAGEN, Inc, Thermo Fisher Scientific Inc (Discussion of Commercial Products or Services).
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Apple Inc, Raspberry Pi Foundation, Samsung (Discussion of Commercial Products or Services and Unlabeled/Investigational Use of Product/Device).
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National Institute of Justice Sam Houston State University (Grant Support).
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Discloses no financial relationships with commercial entities.
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F

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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Ashleigh M. Faris, MA - H91
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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National Institute of Justice, Slice and Algee-Hewitt (Grant Support).
The University of Tennessee - Thomas Fellowship (Other Financial/Material Support). - A46
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CAPES (Grant Support). - K16
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G

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Netherlands Forensic Institute (Employee). - C14, C21, W18
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Figure 1, Instagram, Twitter, Inc (Discussion of Commercial Products or Services).
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National Institute of Justice (Grant Support). - K44
SCIEX (Discussion of Commercial Products or Services). - K23
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Discloses no financial relationships with commercial entities.
Kenji Kurosawa - C12
Discloses no financial relationships with commercial entities.
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Japan Society for the Promotion of Science - KAKENHI (Grant Support).

L

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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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National Institute of Justice (Grant Support). - A32
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UVA (Employee).
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Federal Bureau of Investigation (Employee).
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Agilent Technologies (Discussion of Commercial Products or Services).
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Chrysler (Discussion of Commercial Products or Services). - D16
General Motors, Takata Corporation (Discussion of Commercial Products or Services). - D18
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Promega Corporation (Discussion of Commercial Products or Services). - B212
National Institute of Standards and Technology (Grant Support). - B212
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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3D Systems, Cad Cam Technologies (Discussion of Commercial Products or Services).
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Katie Thompson, MD - H106
Discloses no financial relationships with commercial entities.
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CBI (Employee).
Ronald R. Thrasher, PhD - I42
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GEDNAP German DNA profiling, Leica Microsystems, Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services).
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National Institute of Justice (Grant Support).
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Agilent Technologies (Discussion of Commercial Products or Services).
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Nikon Camera Company (Discussion of Commercial Products or Services and Unlabeled/Investigational Use of Product/Device).
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U

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IonSense, Inc, Thermo Fisher Scientific Inc (Discussion of Commercial Products or Services).
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V

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Netherlands Forensic Institute (Employee).
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Sigella Vargas, MD - I10
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Michigan State University (Grant Support).
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PerkinElmer Inc, VUV Analytics, Inc (Discussion of Commercial Products or Services).
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Discloses no financial relationships with commercial entities.
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JEOL, Ltd. (Discussion of Commercial Products or Services).
National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. (Grant Support).
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Discloses no financial relationships with commercial entities.
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W

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Forensic Analytical & Clinical Toxicology (Paid Consultant).
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Armed Forces Medical Examiner System (Employee).
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National Institute of Justice (Grant Support).
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Magle Life Sciences (Discussion of Commercial Products or Services).
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IU McKinney School of Law (Employee). - F35
Cybergenetics (Discussion of Commercial Products or Services). - F11
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Big Heart Pet Brands, Bumble Bee Seafoods, Nestlé Purina Pet Care Company (Discussion of Commercial Products or Services).
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Discloses no financial relationships with commercial entities.
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Pennsylvania State University (Other Financial/Material Support).

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Strava, Inc (Discussion of Commercial Products or Services). - D34
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Battelle Memorial Institute, Illumina, Inc, Life Technologies Corporation, Promega Corporation (Discussion of Commercial Products or Services).
Harris County Institute of Forensic Sciences (Employee).
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Discloses no financial relationships with commercial entities.
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Cellebrite Company, Niantic, Inc (Discussion of Commercial Products or Services). - C16
AccessData, Cellebrite Company, Griffeye, Guidance Software, Inc, Magnet Forensics, (Discussion of Commercial Products or Services). - C6
Teresa A. White, MA - W15
Discloses no financial relationships with commercial entities.
Jonas Widness - B29
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Illumina, Inc, MO BIO Laboratories, Inc (Discussion of Commercial Products or Services).
Defense Forensic Science Center (Employee).
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National Institute of Justice (Grant Support).
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National Institute of Standards and Technology (Employee).
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Discloses no financial relationships with commercial entities.
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Sigma-Aldrich Co. LLC (Discussion of Commercial Products or Services).
Cedar Crest College Forensic Science Program (Other Financial/Material Support).
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Defense Threat Reduction Agency (Grant Support).
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Discloses no financial relationships with commercial entities.
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Stroz Friedberg, LLC (Employee).
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Discloses no financial relationships with commercial entities.
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VCU Quest for Innovation Commercialization Fund (Grant Support).
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.

X

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Y

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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Kim Younsu, PhD - C1
Discloses no financial relationships with commercial entities.

Z

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National Institute of Standards and Technology (Grant Support).
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Discloses no financial relationships with commercial entities.
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Colt's Manufacturing Company LLC, Glock, Inc, Sig Sauer, Smith & Wesson, Sturm, Ruger & Co., Inc (Discussion of Commercial Products or Services).
National Institute of Standards and Technology (Employee).
Brita Zilg, PhD - H83
Swedish National Board of Forensic Medicine (Employee).
Joel A. Zlotnick, MSFS - J5
Adobe Systems Incorporated, Articulate Global, Inc, Foster + Freeman, Ltd, Lego (Discussion of Commercial Products or Services).
U.S. Department of State (Employee).
Peter D. Zoon, PhD - B178
Discloses no financial relationships with commercial entities.
Silvia Zoppis, MD - B52
Applied Biosystems, Bio-Rad Laboratories, Inc, MP Biomedicals, LLC, Promega Corporation, Streck, Inc, TaKaRa Bio, Inc, Whatman plc (Discussion of Commercial Products or Services).
University of Rome "Sapienza" (Other Financial/Material Support).

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PRESENTING AUTHOR INDEX

The presenting author index can provide a quick reference to find when and in what section presenting authors are scheduled to present at the 2017 Annual Scientific Meeting. The reference table below assists you in finding the section in which the presentation will be given. Letters correspond to the scientific discipline/section in which the presentation is being made while the number corresponds to the numerical sequence of the presentation within the section.

A	Anthropology	J	Questioned Documents
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