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#### AMERICAN ACADEMY OF FORENSIC SCIENCES

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## **PROCEEDINGS** of the American Academy of Forensic Sciences 63rd 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 pathology, toxicology, physical anthropology, psychiatry, immunology, odontology, jurisprudence, criminalistics, questioned documents, digital evidence, and engineering. Similar submissions dealing with forensic oriented aspects of the social sciences are also included.

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

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## **PROCEEDINGS**

## of the American Academy of Forensic Sciences

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## **SPECIAL SESSIONS**





### S1 Eleven Sections: One Academy — Current Perspectives on the State of Relevant, Reliable, Valid Forensic Science in a Multidisciplinary Context

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After attending this presentation, attendees will have an understanding of the role of each section of the Academy and how they integrate to produce relevant, reliable, and valid forensic science.

This presentation will impact the forensic science community by highlighting the positive research and education being produced by the membership of the Academy and by providing a roadmap to the future.

The American Academy of Forensic Sciences is internationally renowned as a leader in the advancement of knowledge, continuing education, and cutting edge research in a multitude of disciplines. Currently, there are eleven sections in the Academy that represent a diverse, capable, and highly respected membership. The Interdisciplinary Symposium is designed to highlight each of these sections and to demonstrate how they interact across the membership in multidisciplinary collaboration. The goal of this important session is to celebrate the current achievements of the Academy membership and to set a course for the future of forensic science.

The theme of the 2011 AAFS meeting is "Relevant, Reliable and Valid Forensic Science: Eleven Sections–One Academy." The Interdisciplinary Symposium will dovetail into that theme by asking each section to detail how their membership actively participates in research and education and how they participate cooperatively with the membership(s) from other sections to achieve reliable, valid science. One member of each section was selected to provide a thirty minute glimpse into the current state of the discipline as well as to furnish future goals. The General section, the most diverse and wide-ranging of the eleven, acts as the "gatekeeper" for the Academy. Their membership includes 22 sub-disciplines and provides standards across the spectrum

of forensic science. As warriors on the battlefield of forensic science by providing standards and certification for a diverse membership. The newest addition to the Academy is the Digital and Multimedia Sciences discipline and as such, their focus will be on establishing definitions, codifying their discipline, and providing a roadmap for the future. They are involved in many Scientific Working Groups (SWG) in an effort to make the science more reliable and valid. Odontology, Pathology/Biology, and Physical Anthropology share many areas of common and overlapping interest. The research and development in each section is often augmented by the others. Odontology is currently involved in bringing human identification into the digital age, both for individuals and in mass fatality events. The membership is engaged in human rights and maintains an active role in both the understanding and analysis ("best practice") of human bite marks. The membership of the Physical Anthropology section is currently participating in a widereaching Scientific Working Group to determine best practices across the gamut of activities in which they engage. Pathology/Biology plays an active role in research, education, and frontline forensic science. Each of these disciplines provides cutting edge-research and technology to the overall forensic science community. Data collection and interpretation are critical for Criminalistics, Toxicology, Psychology, and Engineering. Each of these disciplines produces internal and external standards that enhance any forensic science investigation. By necessity, the membership in each of these sections is actively engaged in advanced research techniques, laboratory accreditation procedures, and systematic investigations. The Jurisprudence section of the Academy provides a framework for the rest of the disciplines. Jurisprudence also defines the legal world in which the Academy membership operates. One of the current question being addressed by this section is the way in which judicial officers and attorneys are being kept abreast of rapidly changing forensic science. "Eleven Sections-One Academy" truly defines the medico-legal community. This session will demonstrate the current capabilities of each of those sections and how they integrate in multidisciplinary collaborations that will have far reaching implications for forensic science.

Interdisciplinary Symposium, Current Perspectives, Forensic Science Community

## S2 Interdisciplinary Approaches to Solving Crimes in Forensic Science

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After attending this presentation, attendees will have a better understanding of the scope of the different fields of forensic science. Attendees will learn how the different fields of forensic science work together to each play a significant role in case work. Both casework and research will be presented by the speakers. This will show how casework and research are intertwined and how they both contribute to advancing forensic science. In addition, participants will learn about each section represented by the AAFS and about the benefits of membership in the Academy. Attendees will learn about various cases and research being done by their peers at the posters and slides sessions, and they will learn valuable skills needed to secure a job within the forensic science field at the breakfast session.

This presentation will impact the forensic science community by providing the encouragement, tools, resources, and support needed to give new and future professionals the ability to positively contribute to the forensic science field.

For the past fifteen years, the Young Forensic Scientists Forum has provided a program for a group of Academy members ranging from students to professionals new to their career in forensic science. Young Forensic Scientists Forum has even had some nonmembers attend. The program has grown and changed drastically since its establishment in order to provide students and scientists with five years' experience or less, with the most quality information possible. The continuing goal is to provide this audience with topics relevant to their education, training, and skill levels. The event also seeks to provide a comfortable means for students and professionals new to their respective fields to contact and communicate with experienced members and fellows of the AAFS. The session planned for the 63rd AFS meeting in Chicago, Illinois will be mirroring President Joseph Bono's message, of unity, eleven sections, one academy - with the theme: "Interdisciplinary Approaches to Solving Crimes in Forensic Science." The goal of the 2010 Young Forensic Scientist Forum is to show how collaborative forensic science can be. The special session will highlight how some disciplines require the analysis of others in order to release reports. The session will show how important it is for different sections to process evidence in a particular order so that the processing by one section will not damage the evidence that another needs. A panel of speakers will present the same case from the different perspectives of each discipline, showing how each contributes to the final conclusions of identity and cause of death. Finally, we will show how multiple analyses fit together to present a cohesive theory of a crime in court. All of these presentations will demonstrate that forensic analysis does not occur in a vacuum, but rather requires the work of multiple analysts to form a complete picture.

The annual YFSF Bring Your Own Slides Session, with

presentations from students and emerging forensic scientists, is scheduled for Wednesday evening. The program will continue Thursday morning with the annual YFSF Breakfast Meeting with a CV/resume review.

It is the goal of the YFSF to foster relationships between the participants of the session with peers as well as established members of AAFS and to provide for a smooth transition from student, to emerging scientist, to established member. With the forum group setting provided and the variety of programs offered throughout the week, the YFSF will not only provide academic and relevant technical information to attendees, but will also cultivate relationships that will last a career.

The Breakfast meeting will focus on professionalism, "Professionalism 101: What everyone should know!" The presenters at the Breakfast Meeting will focus on a variety of topics relating to the importance of professionalism when emerging into the forensic science field and will share their knowledge with participants through an open question and answer forum discussion. The session will conclude with a CV/resume review.

**YFSF, Special Session, Interdisciplinary** 

#### ES1 Relevant, Reliable, and Valid Forensic Science — Application and Utilization in Pre-Trial Case Analysis and Trail Testimony

Cyril H. Wecht, JD\*, 1119 Penn Avenue, #404, Pittsburgh, PA 15222-4205; Michael Welner, MD\*, 224 West 30th Street, Suite 806, New York, NY 10001; and Henry C. Lee, PhD\*, University of New Haven, 300 Boston Post Road, West Haven, CT 06516

After attending this presentation, attendees will learn how to deal with overly aggressive, abusive, and biased attorneys, and various governmental officials.

This presentation will impact the forensic science community by demonstrating how to objectively review a forensic case; how to deal with biased or unethical attorneys and law enforcement personnel; how to avoid being abused or misrepresented as a forensic expert.

The overall field of forensic science is inherently fraught with intellectual challenges and controversy. Quite disturbingly and rather surprisingly to many forensic scientists, the National Academy of Sciences identified numerous kinds on analysis that have been frequently misrepresented and overstated regarding their degree of specificity and exclusion of alternative conclusions. Further compounding the obvious evidentiary significance of this dramatic review of long standing forensic misconceptions are three additional areas of criticism; namely, inadequate training, lack of experience, and absence of proven competence by national certification entities; and the likelihood of bias (conscious or unconscious) engendered by virtue of the all too frequently encountered, governmentally sponsored, integrated relationship between forensic scientific facilities (medical examiners, "crime" labs, toxicology labs, etc.) and prosecutorial offices (local, regional, state, or federal).

When all these unrecognized, unappreciated defects are brought into play in any given case, the potential for eliciting and crafting biased, non-objective, and even occasional, deliberately false and grossly misrepresented test analyses, autopsy findings, and ultimate courtroom testimony, is understandably and quite predictably a very real occurrence.

Any kind of biased test analysis and trial testimony is unacceptable, whether it be the result of deliberate malevolence, professional negligence, or incompetence. Unfortunately, it is sometimes impossible or very difficult to demonstrate and prove such serious misconduct. Deceitful cover-ups, loss of physical evidence, inexperienced and scientifically ignorant attorneys, and indifferent judges are all obstacles that must be overcome in order to ferret out, identify, and corroborate suspicions of invalid, distorted, or contrived test conclusions and expert opinions.

It is not the least bit surprising that many of the most egregious examples of these kinds of biased forensic scientific endeavors have occurred in cases that acquire much prominence and notoriety, either because of the celebrity of the people involved or because the case for whatever reason captures that attention of the national news media and the general public. In these instances, the stakes are high for everyone directly involved or intimately concerned. Hence, the greater need and likelihood of all kinds of pressures being brought to bear upon the forensic scientists on both sides.

In the adversarial system of both civil and criminal justice that exists in the United States, with public pressures and clandestine manipulative factors exercised by news media, special interest groups, and political forces, it is absolutely essential that forensic scientists remain as professionally independent, intellectually objective, and free from undue influences as possible. Only then can there be an appropriate milieu in which on can function as a reliable expert and present evidence that is relevant and valid in every case, no matter what the particular professional role and job capacity may be.

What constitutes relevant, reliable, and valid forensic scientific evidence, testing, and expert testimony? Who should be the ultimate arbiter of such evaluations? Can there be effective, reasonable, fairly uniform standards applied to ensure the application of such standards?

This presentation will address these critical questions based upon their own personal experiences in a large number and variety of both civil and criminal litigation. Several highly controversial, well-known cases will be utilized as appropriate background sources from which the audience can develop a better understanding and appreciation of this extremely important subject.

Aggressive, Abusive, Biased

# ES2 Forensic Analysis and the Re-Investigation of the Death of Emmett Till

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After attending this presentation, attendees will learn about the history and background surrounding the 1955 death of Emmett Till, the re-investigation of the death initiated as part of the Civil Rights- Cold Case Initiative, and about the interdisciplinary forensic analyses conducted upon the exhumation of his body in 2005.

This presentation will impact the forensic science community by serving as a case study for how the various fields of forensic sciences can effectively work together and with investigative authorities. Additionally, this presentation will highlight the roles of the investigator, jurisprudence, and forensic sciences in civil rights cases and cold cases, and the impact forensic science has on victim's families and the nation as a whole.

In 1955, 14-year-old Chicago native Emmett Till was killed after allegedly whistling at a white woman during a trip to visit family in Mississippi. The circumstances of Till's death received national attention and became a flash point for the civil rights movement when his remains were returned to Chicago and placed on public display in a glass topped casket. Two suspects in his death were acquitted of all charges by an all-white jury in Mississippi in the 1950's. In 2004, the Federal Bureau of Investigation (FBI) and Mississippi authorities initiated a re-investigation into the death of Emmett Till as part of the FBI Civil Rights - Cold Case Initiative. The purpose of the reinvestigation was to determine if there were any outstanding criminal charges that could be brought to court and to deal with issues that had previously undermined the case in the 1950's. To assist in the investigation, the casket linked to Emmett Till was exhumed from the Burr Oak Cemetery in Alsip, IL and transported to the Cook County Medical Examiner's Office. The purpose of the exhumation was to confirm the identity of the body as Emmett Till, which was a fact that was argued during the original trial and to document any trauma through a full autopsy or anthropological exam, as the remains permitted. The remarkable preservation of the body allowed a wide range of forensic analyses to be conducted, answering several questions remaining from the original investigation and trial.

This multidisciplinary symposium will present an overview of the investigation of Till's death, the exhumation, the autopsy, and the subsequent forensic analyses to identify Till and to document the trauma. Speakers will include: members of Emmett Till's family to discuss the impact of Till's death and the results of the investigation 50 years later; investigative agents and law enforcement officials from Mississippi and the FBI to discuss the background of the case, the reinvestigation, and the exhumation; and forensic scientists who performed a variety of analyses on the remains, including members of the pathology/biology, physical anthropology, and criminalistics sections of the American Academy of Forensic Sciences. Scientific topics will include autopsy, radiography, anthropology, odontology, and DNA.

**Civil Rights, Exhumation, Pathology** 



## **BREAKFAST SEMINARS**





### B1 Psychology of Homicide: What Every Forensic Scientist Should Know About Homicidal Behavior

#### Emanuel Ta'nay, MD\*, 2980 Provincial Drive, Ann Arbor, MI 48104

After attending this presentation, attendees will familiarized with the classification of homicidal behavior based on the anatomy of personality.

This presentation will impact the forensic science community by providing the psycho-social context of homicidal behavior relevant to all forensic involvements with homicide investigation.

All behavior is psychiatrically categorized as egosyntonic, egodystonic, or psychotic. These terms can be translated as selfharmonious, self-disharmonious, and psychotic behavior. For example, a parent instructing a child is acting in an egosyntonic manner, the selfimage of a parent involves assisting and teaching the child. However, when the parent loses self-control and beats the child and inflicts injury, this is egodystonic behavior, since it is inconsistent with parents' selfimage as caregivers and protectors from harm.

Egosyntonic homicide is a killing committed without disruption of personality. The egosyntonic homicide is a rational, goal-directed act, committed in order to fulfill a consciously acceptable wish. Killings by police, soldiers in combat, and those acting in self-defense are egosyntonic. Killings committed by criminals are also in the same category, even though they are the result of the antisocial motivation.

Egodystonic homicide is often the result of a sadomasochistic relationship between two individuals whose personalities and life situations determine the deadly outcome. The passive participant in the sadomasochistic relationship has failed the developmental task of transforming infantile rage into the adaptive anger of adulthood. He or she fluctuates between passivity and breakthrough rage, during which egodystonic violence takes place.

Psychotic variety of homicide is based upon delusional ideation of the perpetrator. For example, Andrea Yates drowned her five children based upon delusions of saving them from Satan. John Hinckley's attempt to kill President Reagan is an example of a psychotic homicide attempt. In his delusional state, the schizophrenic John Hinckley believed that killing President Reagan would secure for him the love of Jodie Foster. Hinckley's behavior was neither impulsive nor rational, yet it was consistent with his delusional thinking.

Modern neuro-science makes it is possible to show that there is a difference between different types of homicide. Some impulses bypass the neo-cortex and the behavior of the actor is the result of activation of subcortical parts of the brain. Thus, first-degree murder and manslaughter differ not only legally but neuroscientifically. In the egosyntonic homicide, different areas of the brain are activated in order to bring about the physical actions designed to cause the victim's death; these are areas that deal with intentionality.

Case examples, specifically the cases of Jack Ruby, John Hinckley, Ted Bundy, and Sam Sheppard will be discussed. Number of less known cases will provide illustration of the basic concepts. The Jack Ruby case is a classic example of impulsive homicide. A special variety of impulsive homicide (egodystonic) is spousal homicide.

Husbands do kill wives and wives do kill husbands, but rarely in the premeditated way we see depicted in the media. Spousal homicide is usually impulsive and happens after years of aggressive tension within the marriage. A sudden increase in hostility may result in an altered state of consciousness, popularly known as "rage," that leads to a physical assault. If a knife or gun is readily available, a homicide may result.

Ted Bundy is a classic example of a sadistic serial psychopath. Excerpts of the author's interview with Bundy and testimony will be included in the presentation.

The presentation will stress the significance of expert testimony in the adjudication of the legal varieties of homicidal behavior. Egosyntonic, Egodystonic, Psychotic Homicide

#### B2 Coping With the CSI Effect: From the Perspective of a Career CSI The Horrific, The Outrageous, and the Amusing

Thomas L. Martin, BS\*, Crime Scene Forensics, LLC, New York State Police, PO Box 515, Red Hook, NY 12571

After attending this presentation, attendees will understand the legal and investigative issues created by the Hollywood portrayals of forensic science and crime scene investigations. Actual case examples outlining the realistic and sometimes amusing aspects of CSI work, will put forensic crime scene investigations back in perspective, and will refocus attendees to the practical aspects of conducting criminal investigations, and presenting evidence in court.

This presentation will impact the forensic science community by explaining the objectives of collecting physical evidence to support or refute information as it develops during the course of an investigation. Every crime scene tells a story and every person having information or knowledge about that crime scene also tells a story. At times that story can be quite detailed and complex; at other times, the story can be quite simple. The job of the crime scene investigator is to collect and document physical evidence in an effort to determine whether or not the physical evidence corroborates the story behind the case.

As the field of forensic science continues to progress, we see science taking center stage in more and more criminal cases. Science and technology have their appropriate place in criminal investigations and subsequent court proceedings, but should not replace the basic common sense and logic that has solved cases for many years. The inception of the CSI fad has notably caused a change in the expectations of jurors who constantly watch forensics related programming. This realization is somewhat understandable, given the fact that most people know about forensic science by what they've learned from their favorite television show. The cause for greater concern is the fact that the CSI fad is having an effect on the criminal justice community. Most criminal cases are solved with hard work and perseverance; compiling information, documenting and collecting physical evidence, tracking persons of interest, and interviewing anyone and everyone with viable information. Investigations should not be limited to forensic science, but should rather be supported by forensic science. The basic observations made at a given crime scene and the subsequent documentation of those observations will corroborate or refute the "story," or the information being gathered. In analyzing the physical and informational evidence together, a just and reasonable conclusion can be drawn.

This presentation will detail the pertinent observations and methodology that should be utilized and documented by the crime scene investigator; as well as some of the technology currently available to display that information. Several case examples will be utilized for illustrative and entertainment purposes.

Forensic Evidence, CSI, Technology

#### B3 Lighting Strikes Twice: The Case of a Femme Fatale

Robert J. Morton, MS\*, CIRG/NCAVC, FBI Academy, Quantico, VA 22135

After attending this presentation, attendees will understand the need for forensic sciences in circumstantial murder cases, including GSR, as well as, understand the concept of staging in crime scenes, and the importance of timelines, financial records, and inconsistent statements.

This presentation will impact the forensic science community by highlighting the intricacies of circumstantial murder cases and the significant role forensic sciences plays in assisting the prosecution of such cases.

This presentation is designed to highlight two unique murders committed by a female offender and the great efforts she undertook to stage the murders to look like other crimes. The historical basis for the term "Femme Fatale" will be explored, as well as the famous case of Lizzie Borden.

The FBI's National Center for the Analysis of Violent Crime (NCAVC) is routinely consulted by federal, state, and local authorities in a variety of cases of bizarre and repetitive violent crimes, especially homicides. NCAVC assistance was requested by local authorities in regards to a case involving the murder of a local man who was found murdered in his residence, apparently during the course of a burglary.

The victim had been scheduled to meet his wife for lunch and when she could not reach him by telephone, she returned home. The victim had been shot in the back of the head and was found lying in his home office. Several drawers in the office and in the bedroom had been rifled through, although nothing was reported missing. The investigation quickly focused on the wife as a possible suspect, because of an earlier incident in which the victim was in his vehicle when someone attempted to set the gas tank on fire.

During the investigation, it was discovered that the wife had forged several checks on a bank account that the victim had sole access. Also, gunshot residue was discovered on the bottom of her shoes and on the carpet in her vehicle. The suspect received a threatening card in the mail shortly after the murder that contained the message "you are next." An investigation of the background of the suspect revealed that she was present when her first husband committed suicide by shooting himself in the head while lying in bed. That case was ruled a suicide, even though the suspect was lying on the bed with her husband when he supposedly shot himself.

The suspect was indicted and tried for the murder of her husband. The prosecutor's office presented a complex, circumstantial case that highlighted the forensic evidence, the suspect's inconsistent statements, and testimony on the staging of crime scenes. The suspect took the stand in her own defense. She was convicted and sentenced to life in prison plus 108 years.

This case highlights the need to understand the role of forensic evidence in circumstantial cases and the factors relating to the staging of crime scenes, as well as careful crime scene assessment.

Forensic Sciences, Staging, GSR

#### B4 Internationalization of Forensic Science Disciplines: Why Certification is Necessary in Forensic Anthropology

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After attending this presentation, attendees will understand the complexity of practicing forensic anthropology and related disciplines globally.

This presentation will impact the forensic science community by proposing a certification program that intends to validate a minimum set of criterion to practice forensic anthropology globally.

During the last decade, there has been an exponential increase in the popularity of forensic anthropology due in part to the popular media and to the internationalization and globalization of the field with the advent of numerous wars, natural and manmade disasters. Current forensic anthropology practitioners come from various professional backgrounds ranging from archaeology to medicine, which is the result of the different academic requirements of various countries.

Since forensic casework has significant legal ramifications such as the documentation of human rights violations and identification and disposition of remains, preparation in traditional physical anthropology (e.g., archaeology, primatology, etc.) or pathologists with expertise in soft tissues, no longer suffice to qualify one as an expert. To further complicate this problem, legal precedence may be used across international courts, which means that national borders are irrelevant and international standards and protocols must be universally relevant.

Thus, with the legal implications there is an urgent need for the certification and of properly vetting of individuals to practice forensic anthropology and universally applied methods in a global setting. Otherwise we will find ourselves addressing miss-identifications and other legal errors. A recent example which illustrates the importance of properly vetting practitioners, is the situation that has recently occurred in Chile, where the disappeared were being erroneously identified, and as such an international committee and team had to be brought in by the Chilean courts to undo and bring resolution to the grieving families who suffered the assault of losing their family members for a second time when the remains had to be exhumed and returned to the legal system for these gross errors. It is not likely that this is unique to Chile and with the continued globalization of forensic anthropology and with multinational teams assisting in the identification of victims of human rights violations and mass disasters.

Forensic anthropology is at crossroads. An international certification program is critical to address the different educational, training, and professional experience for practitioners; as well as the methods used from field recovery to human identification. The certification program would improve the quality of the work performed by the various agencies (e.g., NGO's) who contract anthropologists from various countries and would help to ensure that practitioners have the necessary minimum qualifications for forensic casework. Importantly, the purpose of certification is not to exclude individuals nor is it to keep anyone from practicing anthropological sciences, but again it is to ensure they meet the minimum standards to practice. Even here in the United States, field recovery methods, report writing, acceptable methods,

standards for research, training, and practice are highly variable and inconsistently applied. For individual certification, it is proposed that in order to meet these minimum standards, individuals would have to submit their professional Curriculum Vitae, submit examples of their cases (cases reports), and pass a standardized written examination. Unlike other forensic professions such as forensic pathologists, forensic anthropologists are not licensed and are not required to be board certified in order to practice-the courts solely determine whether a forensic anthropologist is accepted as an expert witness, which will also vary by jurisdiction. In the United States, the American Board of Forensic Anthropologists (ABFA) certification program provides one type of credential for osteological analysis but is exclusive for U.S. residents and does not address the growing global challenges, issues for courtroom testimony, field recovery, or forensic facial imaging. Therefore, pragmatically, North American, Latin American, and European experiences should be brought together in order to address the globalization and internationalization of forensic anthropology as it is applied in all of its many contexts. Specific examples and cases from a variety of countries and international courts will be presented.

Forensic Anthropology, Certification, Globalization

#### B5 Quincy vs. Ducky: Scalpels at Dawn — An American Forensic Pathologist and a British Home Office Pathologist Square Off

Wendy M. Gunther, MD\*, Office of Chief Medical Examiner, Tidewater District, 830 Southampton Avenue, Suite 100, Norfolk, VA 23510-1046; and Stuart J. Hamilton, MB ChB\*, 9 Troon Close, Consett, DH8 5XF, UNITED KINGDOM

After attending this presentation, attendees will gain an "inside view" of the differences between two major forensic pathology systems and the working lives of two similar yet very different professionals – the British Home Office pathologist and the American forensic pathologist. Utilizing the medium of their favorite cases and demonstrating a lighthearted sense of competition with each other, these two forensic physicians will demonstrate how relevant, reliable, and valid evidence is obtained from autopsy pathology in each of their systems.

This presentation will impact the forensic science community by expanding their understanding of what the British and American systems of forensic pathology together find relevant, how far they go to establish reliability, and how they attempt to ensure a valid outcome despite their sometimes very different approaches to the same problem, the body in a suspicious death.

This presentation will take a competitive approach to a comparison of the American and British systems of forensic pathology, using case presentations to highlight the differences. There are few American medical examiners who would know what to do if they were suddenly transplanted to the British system – or vice versa! But both systems must establish relevant evidence, perform reliable autopsies, and develop valid observations and opinions from the dead bodies of victims.

Although the current British and American systems grew from the same historical medicolegal roots, a Home Office pathologist and an American forensic pathologist, if switched suddenly, would find each other's milieu quite alien. Medical school is different. Residency is different. Conflict with colleagues in surgical pathology is handled differently. Reporting cases is different. Testimony is different. Even the word for "autopsy" is different (Britons say "p.m.ing"). The American forensic pathologist may do many more suspicious cases per year than her British counterpart, and death by multiple gunshot wound is far more frequent in the United States; but death by stomping, stabbing, and baseball bat (yes, *baseball* bat) is more common in the

United Kingdom. Americans deal with the differences between coroner systems and medical examiner systems, state by state; Britons use a centralized system in which the only conflict is between Scotland and England. Some American forensic pathologists do hospital autopsies, but few find malpractice cases making up a large part of their daily load like their English counterparts. Each system leads to internal contradictions and each specialist has to deal with the contradictions without making the situation worse.

Through a light-hearted sense of competition, with a less than modest sense of rivalry, these two physicians will utilize some of their most challenging and interesting cases to illustrate the fascinating differences between the British and the Americans as they practice forensic pathology.

British Home Office Pathologist, American Forensic Pathologist, Comparison of Systems

#### B6 Criminal Profiling - With a Little Help From My Friends

Dayle L. Hinman, BS\*, 3830 South Highway A-1-A, Suite 4 #200, Melbourne Beach, FL 32951

After attending this presentation, attendees will gain a greater understanding of criminal profiling and better appreciate the efficacy of collaborative working relationships between the various professional disciplines involved in criminal investigation.

This presentation will impact the forensic science community by providing a unique perspective on profiling and the team approach to investigation.

Criminal profiling has long been the subject of countless movies, television programs and novels. Profilers are frequently cast as individuals with special psychic abilities coupled with personal pathologies that are somehow linked with their creativity in solving complex crimes. In reality, profilers draw upon their own law enforcement experience, specialized training in forensic and behavioral science, and empirically developed information about the characteristics of known offenders. Essentially they are the historians of crime information.

Far from a magical event, profiling is an investigative technique that was developed and refined by the Federal Bureau of Investigation. It is a process of systematically reviewing and analyzing crime scene information. The major personality, behavioral, and demographic characteristics of the offender are suggested based upon an analysis of the crimes. People are not profiled; rather, the offender's interactions with the victim(s) within the context of the crime scene(s) are examined in great detail. Using specific case examples, the attendees will be given the opportunity to understand how these complicated cases were resolved.

Criminal Profiling, Criminal Investigation, Odontology



## **LUNCHEON SEMINARS**





### L1 The Psychological Autopsy: Its History, Applications, and Legal Ramifications

James P. Cho\*, 238 County Down Lane, Loveland, OH 45140; Scott Bresler, PhD\*, University of Cincinnati Medical Center, Department of Psychiatry, 260 Stetson, Box 670559, Cincinnati, OH 45267-0559; and Carl N. Edwards, JD, PhD\*, 4113 Sunflower Lane, Temple, TX 76502

After attending this presentation, attendees will understand the implications of the psychological autopsy across multiple settings. Analysis of the circumstances leading to death will be analyzed though actual case presentations. The uses of psychological autopsies across legal contexts will be presented by an attorney knowledgeable in this field.

This presentation will impact the forensic science community by comprehensively covering a topic specifically relevant to forensic mental health experts, police investigators, and attorneys to equivocal deaths in the community at large and in the military.

Psychological autopsies have been used by professionals to review the circumstances leading up to and including equivocal deaths. That is, deaths in which it cannot be quickly or cleanly determined whether or not the decedent acted purposefully to kill him or herself. In such cases, there may be extremely important questions that must be answered and are relevant to subsequent investigative and/or legal questions. For example, coroners and law enforcement must determine if someone killed himself or was murdered.

Families may have a vested interest in the determination of a psychological autopsy. For example, the decedent may have dependents that stand to benefit from his or her death through insurance, workman's compensation, or potentially a tort.

There are different methods for carrying out the psychological autopsy. Some of these methods include finding out several key elements in the decedent's history such as the presence of a mental illness, substance use, previous suicide attempts, or ownership of a gun. Another method involves recreating the recent and relevant events of the decedent's life up until the time of death. Other methods include incorporating the coroner's determination as to the cause of death, presence of a suicide note, and recent communications from the decedent to his or her loved ones that could have been interpreted as a farewell.

A case study will be presented and will give an overview of the research in progress in the area of the psychological autopsy. The tools and methods used in the process will also be presented and how these tools have been considered by the courts both in terms evidentiary issues and case law. The topic is one where it is believed medicolegal investigators and the legal community must be familiar. **Psychological Autopsy, Equivocal Death, Suicide** 

#### L2 Forensic Jeopardy

#### Carl Wigren, MD\*, 1008 West Galer Street, Seattle, WA 98119

The goal of this presentation is to present an interactive and fun parody of the Jeopardy game giving a forensic twist to the television hit.

This presentation will impact the forensic science community by providing a fun, thrilling and knowledge gaining presentation of forensic facts. This is Jeopardy just as you remember it but with a forensic twist. Categories include Mind Your Manners (manner of death), Annie Get Your Gun (gun trivia for the gun nuts), Rotten Stuff (postmortem changes), Tinker Toys (anatomy with forensic significance), You Make Me Sick (disease entities encountered during postmortem exams), Hollywood Hits (forensics as depicted in the popular culture), Arsenic and Old Lace (postmortem toxicology), and many others. Participants will be treated to a Jeopardy round, Double Jeopardy, and Final Jeopardy. See if you can answer basic forensic knowledge questions and maybe the occasional extremely difficult question. This fun session is sure to elicit cheers as well as collective "boos" from the crowd. Many of the questions (answers in the form of a question) have been submitted by eminent forensic pathologists from around the world.

All disciplines and levels of expertise are encouraged to attend including surly forensic pathologists and forensic newbie's. If you don't know the answer, root on your luncheon table teammates.

Luncheon tables are equipped with state of the art lockout buzzer systems and will be assigned a Hollywood celebrity moniker.

Participants are politely requested to not answer questions while their mouths are full of food.

Forensic, Jeopardy, Forensic Facts



## WORKSHOPS



#### W1 Tips and Tricks to Improve the Interpretative Value of Postmortem Toxicology

Jayne E. Thatcher, BS\*, H272 Health Science Center, Box 357610, Seattle, WA 98195; Michele Merves, PhD\*, Pinellas County Forensic Lab, 10900 Ulmerton Road, Largo, FL 33778; Dennis J. Wickham, MD\*, Office of the Medical Examiner, PO Box 5000, Vancouver, WA 98666-5000; Dan T. Anderson, MS\*, Los Angeles County Department of Coroner, 1104 North Mission Road, Los Angeles, CA 90033; William H. Anderson, PhD\*, Washoe County Sheriff's Office, Forensic Science Division, 911 Parr Boulevard, Reno, NV 89512; Robert A. Middleberg, PhD\*, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Ruth E. Winecker, PhD\*, Office of the Chief Medical Examiner, Campus Box 7580, Chapel Hill, NC 27599-7580; Graham R. Jones, PhD\*, Medical Examiner's Office, 7007-116 Street, Northwest, Edmonton, AB T6H 5R8, CANADA; and Carl J. Schmidt, MD\*, Wayne County Medical Examiner's Office, 1300 Warren, Detroit, MI 48207

After attending this workshop, attendees will be able to: (1) Understand how specimen collection and quality can influence toxicological findings; (2) have an improved understanding of how alternative matrices should be processed and interpreted; (3) identify the key elements that should be evaluated when deciding if the toxicology findings are significant to the cause and manner of death; and, (4) identify resources that can be referred to and referenced when determining the role of toxicology in death investigation cases.

This presentation will impact the forensic science community by providing foundational knowledge on factors that affect the interpretation of postmortem toxicology results. Experienced toxicologists and pathologists will address key elements that affect the way specimens are collected, processed, and interpreted. The role of scene and autopsy findings, specimen collection and testing, tolerance, drug interactions, and postmortem redistribution in determining the cause and manner of death will be evaluated and interpretative value discussed. Case examples will be used to illustrate concepts presented.

This workshop will provide knowledge and direct attendees to additional resources needed to improve the interpretative value of postmortem toxicology. Experienced toxicologists and pathologists will address key factors that influence how toxicology can effectively be used in death investigations.

The objective of this workshop is to provide foundational knowledge on factors that affect the interpretation of postmortem toxicology results. Open communication between toxicologists, pathologists, and other community partners is essential when toxicology is considered to have contributed to death.

Postmortem, Toxicology, Interpretation

#### W2 Identifying and Managing Errors in Case Analysis: Introduction to Human Error Analysis

Susan M. Ballou, MS\*, National Institute on Standards and Technology, Law Enforcement Standards, 100 Bureau Drive, MS 8102, Gaithersburg, MD 20899-8102; Scott Shappel, PhD\*, Clemson University, Department of Industrial Engineering, Clemson, SC 29634; Melissa K. Taylor, BA\*, National Institute of Standards and Technology Office of Law Enforcement Standards, 100 Bureau Drive, Gaithersburg, MD 20899; Melissa Gische, MFS\*, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135; Deborah Boehm-Davis, PhD, George Mason University, College of Humanities and Social Sciences, 4400 University Drive MS3F5, Fairfax, VA 22030-4444; Linda Connell, MA, NASA, PO Box 189, Moffett Field, CA 94035-0189; Karen S. Runyon, BA\*, 400 South 4th Street, Suite 505, Minneapolis, MN 55415; and Douglas A. Wiegmann, PhD\*, University of Wisconsin -Madison, Department of Systems and Industrial Engineering, 3214 Mechanical Engineering, 1513 University Avenue, Madison, WI 53706

After attending this presentation, participants will learn the fundamentals of human error analysis, the relationship between human error and human factor influences, common human error prevention techniques, how process mapping can identify error-prone tasks, and innovative methods for identifying and managing human factors associated with forensic investigation.

This workshop will impact the forensic science community by providing an overview of the field of human error, illuminate the benefits of process mapping as a valuable technique to identify potential "failure" points in the various forensic disciplines, and explore strategies for how errors can be reasonably controlled or prevented.

Attendees will be guided through sources of error in forensic pattern recognition analysis, educated on methods used in other professions to eliminate or mitigate sources of error, and evaluate various approaches to numerically quantifying error within forensic pattern recognition disciplines. The information learned can be refined and applied to other forensic disciplines such as but not limited to drug chemistry, trace, and fire debris analysis.

Human error is an inevitable part of everyday life. In most instances the results of human error are harmless and correctable, but in circumstances, such as forensic analysis, where errors may lead to the loss of life or liberty, error prevention is imperative. The forensic science community has traditionally addressed human error in two ways: (1) by demanding examiners to adhere to strict protocols and perform perfectly; and, (2) by instilling the fear of punishment for performance errors. Human factors research in a variety of industries, including aviation and medicine, has proven that these two approaches are highly ineffective at controlling error. Some key tenets of human factors theory are that:

- 1. Humans are incapable of sustained perfect performance;
- 2. Task analysis is critical in understanding activities that pose the greatest risk for errors;
- 3. Errors can be prevented by designing processes that minimize dependency on weak cognitive functions; and
- 4. Punitive measures inhibit error reporting.

This multidisciplinary panel will provide perspectives from various disciplines within the forensic sciences and other industries such as aviation and medicine. Case studies in forensic sciences and other industries will be presented.

Human Errors, Error Reporting Systems, Process Map

Joshua A Perper, MD\*, Broward County Medical Examiner's Office, 5301 Southwest 31st Avenue, Fort Lauderdale, FL 33312; Ruth E. Kohlmeier, MD\*, El Paso County Coroner's Office, 2743 East Las Vegas Street, Colorado Springs, CO 80906; Michael M. Baden, MD\*, 15 West 53rd Street, #18B-C, New York, NY 10019; Bruce P. Levy, MD\*, 108 Bancroft Court, Nashville, TN 37215; and Tom C. Hall, JD\*, Hall & Bates, LLP, 115 East Travis Street, Suite 700, San Antonio, TX 78205

After attending this presentation, attendees will have a greater understanding of the role and responsibility the forensic scientist has in accurately and clearly communicating with the public. The participant will be exposed to various experts in forensic medicine and the law who will offer examples of techniques that may be helpful when communicating with the public. There will be opportunity for the attendee to participate in a mock press conference.

This presentation will impact the forensic science community by providing information and offering techniques that could be utilized to improve communication with the public.

Although professionals in the forensic community are required to communicate with the public on a routine basis, little is offered in training and at professional meetings to show how to accomplish this. This workshop will provide exposure to internationally known experts in forensic medicine and the law who have years of experience dealing with the public who will offer their "pearls of wisdom" when it comes to talking with family members, interacting with the media and appearing in court. The goal of this workshop is to help improve communications with family members, law enforcement, and the media, and to enhance performance in court. The "Communication in Forensics" workshop offers an interactive educational experience with an opportunity to meet and talk with a variety of entertaining speakers who have diverse backgrounds. Among the speakers is Dr. Michael Baden who is a well known figure in forensic medicine for a myriad of reasons, to include his consultation on a host of famous cases, such as the deaths of John F. Kennedy and Nicole Simpson Brown. Another speaker is forensic pathologist Dr. Bruce Levy, who is an expert media consultant and a frequently invited speaker on a variety of cable network programs. Dr. Levy will share useful techniques that may assist you in dealing with the media. Dr. Joshua Perper is the Chief Medical Examiner for Broward County, Florida and is a respected forensic pathologist with a tremendous amount of talent and experience who was most recently seen by the public when he participated in the press release following the passing of celebrity, Anna Nicole Smith. Dr. Perper will lecture on various real life examples of forensic professional's successes and mishaps when communicating with the public. Dr. Tom Hall who has been practicing law for over 30 years, will share techniques the participant may find useful to apply when presenting in court as an expert witness in either criminal or civil proceedings. There will be a mock press conference and attendees will be encouraged to participate. Attendees may volunteer to be a "presenter" at the mock press conference and he or she will then receive questions from their fellow attendees who will be acting as the media. The mock press conference promises to be a fun educational experience that will allow the attendee to practice what they have just learned at the workshop and to do so in a supportive and encouraging environment. Throughout the "Communication in Forensics" workshop, there will be opportunities for the attendees to interact with the guest speakers to ask questions, to share ideas and get constructive feedback about their personal experiences in dealing with the public. Attendees of all experience levels are welcome to attend and participate and include those who are just starting out in the field and need an introduction-type course in communication as well as those who have tremendous experience and desire a refresher course in the subject.

Communication, Forensic Science, Public Speaking

### W4 Staged Crime Scenes: Crime Scene Clues to Suspect Misdirection of the Investigation

Arthur S. Chancellor, MA\*, 1910 Stroud Road, McDonough, GA 30252-7841; Grant D. Graham, MFS\*, Office of the Armed Forces Medical Examiner, 1413 Research Boulevard, Building 102, Rockville, MD 20850; and Richard D. Walter, MA\*, RR 3, Box 364, Montrose, PA 18801

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 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 thrust 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 will 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

#### W5 Bones and Children: An Interdisciplinary Approach to Forensic Issues

Andrew M. Baker, MD\*, Hennepin County Medical Examiner's Office, 530 Chicago Avenue, Minneapolis, MN 55415; Laura C. Fulginiti, PhD\*, Forensic Science Center, 701 West Jefferson, Phoenix, AZ 85007; B.G. Brogdon, MD\*, University of South Alabama Medical Center, Department of Radiology, 2451 Fillingim Street, Mobile, AL 36617; Steven A. Symes, PhD\*, Mercyhurst Archaeological Institute, Mercyhurst College, 501 East 38th, Erie, PA 16546-0001; Susan M.T. Myster, PhD\*, Hamline University, MB 196, 1536 Hewitt Avenue, Saint Paul, MN 55104; and Rich Kaplan, MD\*, University of Minnesota Medical School, 717 Delaware Street Southeast, Minneapolis, MN 55414

After attending this workshop, attendees will: (1) understand the strengths and weaknesses of the various disciplines (radiology, anthropology, pediatrics, and radiology) that can be brought to bear on the evaluation of bone fractures in children; (2) describe those medical conditions that can mimic fractures or predispose to fractures in the pediatric population, and understand typical medical conditions that are regularly offered in court as arguments against non-accidental child injuries; (3) understand the medical workup and differential diagnosis of fractures in living children; (4) recognize that in most instances, the collaboration of various medical and forensic specialists is the most productive way to find and evaluate fractures: (5) understand the strengths and limitations of various techniques for chronological age estimation in the living and the dead, and how various factors impact these determinations; and, (6) understand the techniques used in recovering and evaluating pediatric remains that may be burnt, unidentified, and/or skeletonized, and how these analyses compare to the fresh autopsy and the interpretation of traumatized bone.

This workshop will impact the forensic community in its evaluation and interpretation of fractures in children.

This presentation will inform attendees of something they do not know, something they do not do, and something they do not do correctly. This presentation will address the lack of knowledge regarding appropriate use of postmortem skeletal surveys in deceased infants; identifying fractures radiographically and correlating the fracture findings with the histology; and properly engaging outside consultants (anthropology, radiology, pediatrics) to aid in the recognition and interpretation of the significance of bony injury in infants.

The finding of fractures in children, particularly the very young, can have profound medicolegal implications if the fractures are suggestive of inflicted injury. The radiological, histological, and anthropological aspects of the fracture may provide useful information as to the mechanism and/or age of the injury. Correlation with published studies and the experience of the pediatric and radiological communities may prove vital in assessing whether a fracture might be accidental, or whether the child may have a medical condition predisposing to, or mimicking fractures. By bringing a variety of medical and forensic specialists together to examine all aspects of fractures, including accidental fractures, conditions that might mimic fractures, and conditions that can predispose to fractures in the pediatric population. This workshop will help attendees gain significant knowledge of fracture evaluation within their own disciplines, and develop a deeper appreciation for the understanding of fractures that other medical and forensic specialists can bring to the table. The workshop will emphasize the benefits of a multidisciplinary approach to one of the most difficult and emotional forensic questions that an examiner encounters.

This workshop will also impact the forensic community by going beyond fractures to examine skeletal features of children that may prove useful in estimating or establishing the child's chronological age, verifying identity, estimating time since death, and confirming the presence of antemortem trauma. The workshop will further impact the forensic community by exploring and explaining anthropological and other forensic techniques—and the unique challenges—in recovering and identifying pediatric remains.

The complex intersection of skeletal trauma analysis, bone pathology, clinical pediatrics, and radiology is centered on children, and necessitates a multidisciplinary approach. The presence of unexplained fractures in living and deceased children-particularly infants and the very young-may have profound implications for how injuries are assessed and deaths investigated. Pediatricians, radiologists, pathologists, and anthropologists all have expertise to offer in the assessment of the pediatric skeleton, but may be unaware of other disciplines' skills that can be brought to bear on questions of considerable legal and forensic significance. Furthermore, some diseases may mimic fractures, or predispose to fractures, and these conditions must be recognized to ensure proper treatment and/or avoid miscarriages of justice. Conversely, some medical conditions are regularly and systematically offered in court as arguments against nonaccidental child injuries, or as explanations for the child's death.

Just as the finding and interpreting of fractures in children is different than in adults, so too is the recovery and identification of pediatric remains. Thermal injury, decomposition, and a variety of other factors present significant challenges in recovery and identification. In the living or the deceased child, even the simple question of chronological age may be difficult to answer.

This workshop will utilize multiple medical and forensic disciplines (anthropology, pathology, radiology, and pediatrics) to examine features of children's bones that have forensic and legal implications: the recognition and interpretation of fractures, age estimation in children, and aspects of recovering, identifying, and evaluating fresh, skeletonized, burnt, or decomposed pediatric remains. A significant portion of the workshop will be dedicated to explaining and demonstrating how various disciplines, working together, can potentially provide enhanced information that stimulates more precise conclusions than any one discipline alone. Specific examples of multidisciplinary approaches to the pediatric skeleton will include the complementary expertise of the pathologist and anthropologist in finding, removing, and interpreting fractures; the radiological and pathological correlation of fractures; clinical and radiological consultation with regard to pediatric development and likely fracture mechanisms; clinical and radiological features of diseases that mimic or predispose to fractures; and the multidisciplinary approach to the recovery and interpretation of pediatric skeletal remains. Historical and technical aspects of the various disciplines-anthropology (fracture identification, tissue maceration, fracture photography, assessment of gross healing, skeletal recovery, invasive versus standard autopsy procedure, techniques for aging), pathology (fracture identification, specimen handling, microscopic bone development, normal and abnormal bone histology), radiology (the historical development of the recognition of abuse, techniques in radiological evaluation), pediatrics (examination of the patient, differential diagnoses of skeletal findings)-will be addressed.

Ultimately, the goal of this workshop is to combine the impacts and benefits of a multidisciplinary approach to one of the most difficult and emotional questions encountered by forensic examiners: "Is the child a product of physical abuse?" **Bones, Children, Fractures** 

#### W6 Fracture Match of Papers, Tapes, and Miscellaneous Materials for Document Examiners

Stephen McKasson, BS\*, Document Consulting Service, 899 Rowan Road, Makanda, IL; and Robin K. Hunton, BA, and Judy A. Gustafson, BS, Internal Revenue Service, National Forensic Laboratory, 29 North Wacker Drive, 3rd Floor, Chicago, IL 60606

After attending this presentation, attendees will be able to make fracture comparisons and identifications with confidence in the underlying theory and practice.

This presentation will impact the forensic science community by providing training in fracture matching theory and technique. This training should help to answer *Daubert*-type challenges.

Fracture matching is considered by many to be the "easiest" of all identification fields requiring only minimal experience to refit torn or broken items back together. This is a misconception; however, as it requires the same depth of understanding and has the same danger of misidentification or missed identification as any other field. Eventually, no matter what the discipline, an examiner will receive a case that contains evidence for possible fracture match. There are concepts and methods which can be applied to such a case to ensure a thorough examination and a sound scientific conclusion. A theoretical basis for identification will be discussed before sample materials are distributed.

This workshop will provide the background theory for making fracture identifications followed by a number of unknowns to compare and a short summary of report wordings and court testimony options.

In Questioned Documents, it is expected to see torn paper and tapes, so this workshop will focus on those materials, although others will be addressed as well. Attendees should bring a hand lens or portable microscope and an auxiliary light source.

Fracture, Identification, Paper Tears

#### W7 Psychiatry and Behavioral Sciences for Lawyers and the Courts: A Crash Course

Vivian Shnaidman, MD\*, Jersey Forensic Consulting, LLC, 181 Cherry Valley Road, Princeton, NJ 08540; Karen B. Rosenbaum, MD\*, 262 Central Park West, Suite 1A, New York, NY 10024; and Clarence Watson\*, JD, MD; and Robert G. Thompson, PsyD\*, Delaware Department of Health and Human Service, Delaware Psychiatric Center, 1901 North DuPont Highway, New Castle DE 19720

After attending this presentation, attendees will be able to understand what their psychiatric and psychological experts are saying and why. Attendees will also understand how to really provide the appropriate services for their clients and the legal system.

This presentation will impact the forensic science community by providing a new and much-needed bridge between what happens in court and what the experts are actually saying. Forensic scientists can be the most intelligent, capable, and amazing experts in their fields, but if the courts do not understand how to use their testimony, or what questions to ask, the brilliance of the expert becomes irrelevant. Those who practice forensic psychiatry or psychology want the courts to understand what we are talking about. More than half of all inmates in U.S. prisons are reported to be mentally ill. In fact, prisons are currently the primary providers of psychiatric treatment in this country. How did those prisoners get locked up? The courts put them there! And the courts are made up of lawyers.

Over a million lawyers are currently active in the United States and a good portion of their cases and clients include some reference to psychiatry or mental illness. When substance abuse, psychopathy, sexual offenses, learning disorders, birth defects, and other behavioral issues are included, there is not a lawyer practicing in this country today who does not encounter some sort of psychiatric problem, information, or testimony in his or her work.

Law students study mental health law in law school; they might learn about insanity defenses and how to examine and cross-examine experts—but what about understanding what the expert is actually saying?

This presentation is designed to teach lawyers a bit about psychiatry and behavioral sciences - not just what color suit their expert should wear, but how to really understand:

- When a case needs a psychiatric evaluation;
- At which stage of the process the evaluation should take place;
- What its legal purpose would be; and,
- How to understand the reports and testimony.

Forensic Psychiatry, Psychiatric Expert Testimony, Mental Health Law

#### W8 Method Validation and Estimating the Uncertainty of Measurements in the Modern Forensic Laboratory for Compliance With ISO/IEC 17025:2005

Terry Mills, MS\*, Mills Forensic Services, 5360 Galberry Lane, Gulf Breeze, FL 32563; Robert J. Ollis, BS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; and Sudhir K. Sinha, PhD\*, Forensic Quality Services, Inc., 5300 West Cypress Street, Suite 180, Tampa, FL 33607

After attending this workshop, participants will have a better understanding of what is expected in the ISO 17025 standards concerning method validations and the requirements of Uncertainty of Measurements (UM). Validation and UM are cited more than a dozen times in the standards. Attendees will understand what ISO 17025 standards require regarding validation and UM, will understand how to measure UM, when to measure UM, and what to do about reporting and recording UM. Since activities needed for method verification are a subset for validation, a table of required performance characteristics for validation will be introduced to the participants. For uncertainty of measurements, details examples using various techniques will be presented.

This presentation will impact the forensic science community by familiarizing forensic scientists and quality managers to the requirements of the ISO 17025 standards regarding method validation and measurement uncertainty and by providing tools to satisfy the ISO standards.

As ISO/IEC 17025 accreditation gains more significance to forensic science, many laboratories are faced with the seemingly daunting task of validating procedures and determining uncertainty of measurement (UM). This workshop consists of two sections.

The first section deals with validation of analytical methods and procedures. The objective of validation of an analytical procedure is to determine that the method is suitable for its intended purpose. In the ISO 17025 standards, method validation is one of the measures recognized as a necessary part of a comprehensive quality system. In method validation, the key questions to be answered are:

• What is the test objective?

• What performance characteristics must we look for?

- Is the performance acceptable for the applications?
- Can we have confidence in the results?
- How can we demonstrate assay validity?

USP, FDA, IUPAC, AOAC, and other groups have published specific guidelines for method validation. The defined steps in most of these guidance documents include accuracy, precision, specificity, limit of detection, limit of quantitation, linearity and range, ruggedness, robustness, trueness, recovery, fitness of purpose, matrix variation, and measurement of uncertainty. Using the example of categories of chemical test methods, this workshop will demonstrate schemes on which of these performance characteristics are to be included in a validation study. Based upon these discussions, the laboratory should be able to develop a validation protocol, an operating procedure, or a validation master plan for the desired validation.

The second part of this workshop involves one of the most daunting tasks in ISO accreditation - determination of measurement uncertainty. As a critical part of method validation, determination of measurement uncertainty is one of the least understood concepts. This part of the workshop will provide a common sense approach to measurement of uncertainty. First, the workshop will focus on how to evaluate measurement uncertainty and its affect on the customer. Each step of a quantitative process is evaluated to determine the contribution to the overall uncertainty. Concepts such as uncertainty budget, and compounded uncertainty are investigated in detail. Common pitfalls and misunderstandings during this evaluation are introduced to the attendee.

Second, methods of measuring the uncertainty of a process are discussed. Determining uncertainty through the use of control charts, and statistical concepts such as mean, standard deviation, confidence intervals, and the proper, legal, and responsible application of these statistical principles to forensic work are described. Ideas such as Type A and Type B uncertainty are presented. Some forensic applications do not require a rigid, metrologically robust method of determination of measurement uncertainty. In those cases, compliance with the ISO standard is still a requirement. This workshop will instruct the scientist in the skills necessary to determine when statistics should be involved, and how to treat determine and report measurement uncertainty when it is not.

Lastly, the workshop will focus on reporting measurement uncertainty. A common myth is that the ISO standard requires measurement uncertainty to be reported to be reported to the customer always. This is not the requirement. The workshop will instruct the attendee on how to determine when measurement uncertainty needs to be reported to the customer, and how to report it responsibly, when needed. Time will also be spent instructing the attendee on the proper presentation of the concept of measurement uncertainty in a courtroom setting.

This hands-on, no-nonsense approach to the application of the ISO standard will help the attendee realize that going ISO is easier than previously thought.

Method Validation, Uncertainty of Measurement, ISO 17025

#### W9 Identification of Animal Hairs

Skip Palenik, BS\*, and Christopher S. Palenik, PhD\*, Microtrace, 790 Fletcher Drive, Suite 106, Elgin, IL 60123-4755

After attending this workshop and actively participating, the attendee will become familiar with the theoretical foundation and practical techniques for identifying animal hairs received in evidence with forensic investigations.

This presentation will impact the forensic science community by presenting and demonstrating the state-of-the-art techniques and presenting the theoretical basis for the accurate identification of nonhuman animal hairs in the forensic science laboratory. Animal hairs are frequently encountered as microscopic trace evidence. Their identification by microscopical methods is possible with a high degree of certainty when such examinations are undertaken in a rigorous scientific manner. This workshop is intended to present the fundamental knowledge and techniques to forensic scientists that will enable attendees to begin identify animal hairs that are received as evidence.

The course will begin with a discussion of the basic microscopic anatomy of mammalian hairs and how they are exploited for identification. A discussion of mammalian taxonomy, the scientific literature and the collection and curating of a hair collection follow.

The session will begin with lecture/demonstrations followed by the laboratory exercises in which attendees will have the opportunity to practice the techniques and examine their results. These will begin with the macroscopic characteristics of hair including color, length, diameter, shape, banding, etc. Microscopical characteristics and the techniques for their study will follow. These will include the examination of the cuticle by light and electron microscopy and the technique for preparing and examining scale replicas. Next cross-sections will be prepared and examined. Finally, the medulla and cortex will be cleared and examined. Hairs from a variety of commonly encountered animals will be available for study and students may bring any hairs they wish to examine as well.

At the conclusion of this workshop, the attendees should have acquired the knowledge and techniques required to begin an animal hair identification program in their own laboratory. This will, of course, require the accumulation of a reference collection and supplies, the perfection of technique and experience the observation of these characteristics and their correlation with specific species.

Hair, Microscopy, Identification

#### W10 Quality Assurance in Human Identification

Vincent J. Sava, MA\*, John E. Byrd, PhD\*, and Thomas D. Holland, PhD\*, Joint POW/MIA Accounting Command, Central Identification Laboratory, 310 Worchester Avenue, Building 45, Hickam AFB, HI 96853

After attending this presentation, attendees should be able to understand the basic quality assurance principles and measures applicable to human identification. Participants will learn the unique challenges faced by professionals involved in human identification when striving to have their facilities, procedures, and casework meet the standards demanded by the criminal justice and medical-legal systems. Attendees should be able to utilize the material presented to formulate a quality assurance program for their organization.

This presentation will impact the forensic community by demonstrating how quality assurance in forensic laboratories and forensic programs has led to objective and measurable standards of performance that ultimately strengthen and elevate the forensic profession as a whole.

Quality assurance programs in forensic laboratories and activities have been a growing trend over the past decade. The publication of the National Academy of Sciences report "*Strengthening Forensic Science in the United States: A Path Forward*" and its recommendations have made quality assurance programs and accreditation an increasing priority for forensic human identification laboratories. Since 1999 the Joint POW/MIA Accounting Command, Central Identification Laboratory (JPAC-CIL) has implemented a stringent quality assurance program to ensure the scientific integrity of its casework. The CIL's quality assurance program ultimately led to its accreditation by the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB) in 2003, the first forensic skeletal identification laboratory to be so credentialed. In 2008, the CIL was reaccredited under the ASCLD-LAB International Program using ISO 17025 Criteria. The goal of this workshop is to introduce the attendee to the CIL's Quality Assurance Program and to convey the lessons learned resulting from its implementation and growth. A video overview of the JPAC-CIL is presented followed by an overview of its quality assurance program. In the latter, the concept of the scientific integrity of the laboratory is discussed followed by a summary of the "Surety" model of quality assurance.

The participants will become familiar with each measure that comprises the surety model of quality assurance. The importance of integrating and synchronizing all of the surety measures discussed during the workshop will be continually stressed. In Part I, infrastructure and support considerations necessary for a successful quality assurance program are also presented. Surety measures addressed include, but are not limited to:

- Desired qualities of a laboratory manual and other vital documentation and their control
- Adequacy and safety of laboratory facilities
- Policies and procedures conducive to a positive work environment
- Evidence management and security
- Training and professional development

Gathering and interpreting evidence is the focus of Part II where quality assurance in field operations and trace evidence analysis is discussed. The surety measures directly related to casework, the peer review process, validation of technical procedures, case file management, analytical notes, and documentation are presented for consideration.

Quality assurance procedures and programs are ineffective in the absence of monitoring, enforcement, and corrective action. These are accomplished through a myriad of surety measures including proficiency testing, review of court testimony, audits, annual reports, and corrective action policies which are presented in Part III.

In closing, the attendees will become acquainted with the problems that hindered, and the processes that led to the accreditation and reaccreditation of the JPAC CIL. Surety assistance programs offered by the CIL will be discussed in the event an attendee's organization desires assistance with their surety programs or accreditation efforts. Additionally, the contributions, to date, of the Scientific Working Group in Forensic Anthropology (SWGANTH) to the human identification profession will be briefly discussed.

Quality Assurance, Human Identification, Forensic Anthropology

#### W11 Microscopy Workshop for Trace Evidence Examiners and Forensic Serologists

Richard E. Bisbing, BS\*, William L. Chapin, BA, Kristen D. Wiley, BS\*, Ruben J. Nieblas, BS\*, Heidi M. Ullberg, BS\*, Kirsten T. Kelley-Primozic, BS\*, and David A. Wiley, BS\*, McCrone Associates. Inc., 850 Pasquinelli Drive, Westmont, IL 60559

After attending this workshop, attendees will understand and have practiced techniques for handling, isolating, and preparing for analysis microscopic-sized forensic evidence including trace evidence and DNA evidence.

This presentation will impact the forensic science community by giving hands-on instruction and practice in the handling of microscopic evidence, and will result in more relevant, reliable, and valid forensic science.

The workshop is specifically designed for the trace evidence examiner, the forensic serologist responsible for isolating small particles of biological evidence for DNA analysis, and other forensic scientists who must recover and analyze microscopic evidence.

There are two parts to this workshop. Part A is a lecture session that will be offered only once at the beginning of the workshop. Part B is a

half-day hands-on session that consists of a rotation through five workstations. All workshop participants will register for the first morning lecture session (Part A) and one of the three identical half-day hands-on sessions (Part B).

During the first morning session, microscopists will explain and illustrate various particle handling, sample isolation, sample preparation, and imaging techniques along with some on-line resources for the forensic trace evidence examiner and forensic serologist.

Following the introductory morning session, three identical halfday hands-on sessions will follow, allowing workshop attendees to practice the techniques in small groups. The hands-on session will utilize five separate workstations through which the attendees will rotate.

The first workstation will focus on the tools used for isolating, mounting, and handling microscopic-sized particles. Making tools and supplies for isolating particles of interest from a variety of samples and for preparing samples for further analysis by light microscopy, infrared microspectroscopy and scanning electron microscopy will be demonstrated and practiced. Some of the sample-handling tools and supplies made in the workshop can be retained by the participants. The workstation will have a stereomicroscope and polarizing microscope available for use.

The second workstation will be for digital imaging. Many forensic laboratories are routinely documenting their evidence and data with digital imaging. This hands-on workshop station will deal with the challenges associated with archiving and managing digital images and files. Participants will use digital cameras on a stereomicroscope and polarizing microscope to document microscopical observations and to save images to a database. The participants will learn some fundamental aspects of digital photomicrography.

The third workstation will demonstrate specialized techniques for isolating and mounting small particles and microtraces from examples of trace evidence. Workshop participants will use a stereomicroscope and specialized micro-tools. Upon completion of the session, attendees will have learned techniques for isolating trace evidence for analysis, proper handling of micro-tools, and basic stereomicroscopy. Attendees are encouraged to bring samples with them to the workshop to learn how to isolate, mount, and manipulate them.

At the fourth workstation, participants will learn the techniques used for isolating microscopic-size individual blood crusts and skin particles for identification and individual spermatozoa from smear slides. These techniques are faster and more economical than laser capture micro-dissection. Participants will use stereo and polarizing microscopes and special micro-tools. Upon completion of this workshop, participants will be able to use manual techniques for isolating microscopic blood crusts, skin cells and spermatozoa from forensic evidence, proper handling of micro-tools, basic stereomicroscopy and polarized light microscopy.

The fifth workstation, in a central chat zone, will allow participants to access a number of on-line microscopy websites selected for their value to forensic microscopists, trace evidence examiners and forensic serologists.

Instruction and practice in the handling of microscopic evidence will result in more relevant, reliable and valid forensic science.

Trace Evidence, Forensic Serology, Microscopy

#### W12 Grief – Forensic Practice and Family Interaction

Gregory G. Davis, MD\*, Jefferson County Medical Examiner's Office, 1515 6th Avenue, South, Room 220, Birmingham, AL 35233-1601; Daniel W. Dye, MD\*, Arkansas State Crime Lab, PO Box 8500, #3 Natural Resources Drive, Little Rock, AR 72215; and Jonathan C. Davis, PhD\*, Samford University, Department of Family Studies, 800 Lakeshore Drive, Birmingham, AL 35229-2239

After attending this workshop, attendees will be able to: (1) list the stages of grief; (2) discuss the principles of elementary grief counseling; (3) recognize situations that call for counseling from trained counselors; and, (4) recognize statements and situations that herald the development of an antagonistic relationship with relatives of the decedent.

Forensic pathologists regularly talk with family members who are still mourning the loss of a loved one. Such interactions require empathy and, often, a measure of counseling. Training in handling grieving family members and situations that develop when those family members become angry with the death investigation process tends to occur only in response to a situation that has already developed. This workshop will impact the forensic science community by providing basic education in how to recognize and handle grief, both appropriate and inappropriate, so that forensic pathologists will better be able to serve their community and protect themselves.

The practice of forensic pathology is a special form of primary care medicine. Forensic pathologists regularly talk with relatives of decedents in order to explain the autopsy findings and simultaneously gather information. All such encounters are delicate, and despite the best efforts family can turn on the death investigation team as the family experiences anger during the normal process of grieving. This workshop will provide participants information on the process of grieving and on fundamental aspects and principles of grief counseling. Participants will work in small groups, discussing various scenarios in which families have directed their frustration toward the forensic pathologist. The groups and speakers will then discuss the scenarios and various approaches to handling situations and conflict. Training will include specific statements and actions that warn of approaching conflict. Each speaker will provide a handout covering his topic, and there will be time allotted for participant questions and discussion.

Forensic pathologists regularly talk with family members who are still mourning the loss of a loved one. Such interactions require empathy and, often, a measure of counseling. Training in handling grieving family members and situations that develop when those family members become angry with the death investigation process tends to occur only in response to a situation that has already developed. This workshop is intended to provide basic education in how to recognize and handle grief, both appropriate and inappropriate, so that forensic pathologists will better be able to serve their community and protect themselves. **Grief, Family, Counseling** 

#### W13 Indentation Sequencing Workshop

Brian S. Lindblom, BA\*, 389 Roosevelt Avenue, Ottawa, ON K2A 1Y9, CANADA; Robert W. Radley, MSc, The Forensic Document Laboratory, Macartneys-Kings Road, Silchester, Reading, RG7 2NS, UNITED KINGDOM; and Larry A. Olson, MFS\*, IRS National Forensic Lab, 29 North Wacker Drive, Chicago, IL 60606

After completing this workshop, attendees will understand the underlying theory of indentation sequencing, its application to an array of cases, and will appreciate the complexities and limitations of employing this technique. This workshop will impact the forensic science community by increasing understanding of the importance of the sequencing technique as another potential means of determining if documents have been backdated or altered.

Research into the technique of indentations sequencing was begun by one of the authors almost twenty years ago.<sup>1</sup> A correlation was observed between: (1) the appearance on the ESDA "lift" of the intersection of writing on a document and the indentations developed thereon; and, (2) the sequence in which they were placed on the document. Blind testing showed that the sequence could be determined with a high degree of confidence in one of the instances with no false positives, but more care had to be taken in the evaluation of the other instance, to avoid false negatives.

The original research was performed using specimens of writing from ballpoint pens. Since that time, additional work has verified that the method is also successful in determining: (1) the sequence of indentations and writings made with fluid-type inks (from porous tip, fiber tip, and rollerball pens); as well as, (2) the sequence of writing

found on the front and reverse sides of a single sheet of paper.<sup>2</sup> This workshop explores the indentation sequencing technique. Presented are the theory and practice of determining the execution order of visible ink lines and intersecting indented impressions. The critical factors to consider when conducting such examinations are addressed in detail. This technique has application to a variety of casework situations, particularly where issues arise with respect to back-dating, and insertions in documents such as diaries, notebooks, agendas, medical charts and police incident books. Numerous examples involving various writing instruments (both ballpoint and non-ballpoint) will be shown. Attendees will be provided with practical materials to analyze during the workshop.

#### **References:**

- <sup>1.</sup> Radley, R.W. Determination of sequence of writing impressions and ball pen strokes using the ESDA technique. Journal of the Forensic Science Society 1993; 33(2): 69-72.
- <sup>2.</sup> Radley, R.W. Determination of sequence of intersecting ESDA impressions and porous tip, fibre tip and rollerball pen inks. Science & Justice 1995; 35(4): 267-272.

Indentations, Questioned Documents, Sequencing Written Entries

#### W14 Images in the Court Room: The Impact of SWGIT Guidelines in Court

Carl R. Kriigel, MA\*, 568 Kings Walk, Douglasville, GA 30134; William R. Oliver, MD\*, Brody School of Medicine at East Carolina University, Department of Pathology and Laboratory Medicine, Division of Forensic Pathology, 7S-10 Brody Medical Sciences Building, Greenville, NC 27834; and Richard W. Vorder Bruegge, PhD\*, Federal Bureau of Investigation, OTD-DES, Building 27958A, Pod E, Quantico, VA 22135

After attending this workshop, attendees will gain: (1) familiarity with forensic imaging guidelines; (2) knowledge of their use in court; and, (3) strategies for dealing with guidelines-based challenges in court.

This workshop will impact the forensic science community by providing a better understanding on the use of imaging guidelines in expert testimony and on dealing with guidelines-based challenges to their testimony.

Expert witnesses should be aware that practice guidelines in forensic imaging are being used by the courts and should be prepared to address them.

Image acquisition, processing, and analysis have become integral parts of forensic investigation. In the late 1990s, the FBI convened a Scientific Working Group on Imaging Technology (SWGIT) to address practice issues related to possible court challenges to the admission of digital image evidence in court. In the ensuing decade, SWGIT has proposed a number of best practices in the area of imaging technologies. Other organizations, such as Law Enforcement and Emergency Services Video Association (LEVA) and the United Kingdom Home Office Police Scientific Development Branch, have also promulgated guidelines. These and other guidelines have, in turn, been referenced in numerous court cases when deciding on admissibility. The SWGIT guidelines have undergone revision, expansion, and updating, and are in a continual improvement cycle. There are now sections that deal with most standard tasks in forensic imaging.

As these guidelines have become better known, they have been adopted by an increasing number of agencies. In addition, they have on occasion been used as a consideration for admission of evidence in *Daubert* and other evidentiary hearings. The speakers have collected a number of instances of this, have reviewed the transcripts of hearing and trial testimony, and have testified themselves in such hearings.

Criticisms of forensic imaging have included questions of artifact, image integrity, image provenance, resolution, algorithm validation, and demands for probabilistic rather than cognitive evaluation. In the area of forensic pathology, what began as attacks on digital imaging techniques have moved to attacks on the visual evaluation of wounds in general. For instance, in a recent appeal, it was claimed that it was not possible to recognize a patterned injury unless an image was acquired and displayed at an arbitrary resolution, regardless of the field of view or size of the lesion. Emphasis will be placed on issues of documentation, image integrity, validation, and reasoning in the application of imaging to forensic problems, as well as the differing requirements for differing uses of forensic images. In addition, as more formal structure has been imposed on forensic imaging, proposals for certification, proficiency testing, and external evaluation have arisen. These requirements have, in part, been adopted by accreditation organizations such as The American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB).

While the recent National Academy of Sciences Report, "Strengthening Forensic Science in the United States: A Path Forward," did not address imaging issues specifically, there has been, and will continue to be, an increasing and healthy critical analysis of conclusions based on imaging. The imaging expert should expect these challenges and be prepared to address them on the stand. The guidelines will be discussed, including the history of their development, how to access them, how to comment on them, and how they compare to guidelines in other countries. A brief comparison of the guidelines from selected different organizations will be presented. Past attacks on admissibility based on guidelines will be reviewed, as well as responses to them, using transcripts and pleadings from trials in which the guidelines have been discussed. Future possible developments from the perspective of both experts and lawyers will be presented.

Forensic Imaging, SWGIT, Admissibility

# W15 Veterinary Forensic Sciences: Animals as Evidence

Jason H. Byrd, PhD\*, University of Florida, Maples Center for Forensic Medicine, University of Florida, 4800 Southwest 35th Drive, Gainesville, FL 32608; Elizabeth Wictum, BS\*, University of California - Davis, Vet Genetics Lab, Forensic Unit, One Sheilds Avenue, Davis, CA 95616; Kenneth F. Cohrn, DDS\*, 422 Teague Trail, Lady Lake, FL 32159; Randall Lockwood, PhD\*, 2214 Tulip Drive, Falls Church, VA 22046; Kathryn Destreza, C-ACI\*, ASPCA, 520 8th Avenue, 7th Floor, New York, NY 10036; Melinda D. Merck, DVM\*, ASPCA, PO Box 100126, Gainesville, FL 32610; and Michael W. Warren, PhD\*, CA Pound Human ID Laboratory, 2033 Mowry Road, Room G17, PO Box 103615, Gainesville, FL 32610

After attending this presentation, participants will: (1) become aware of the national database for canine DNA; (2) understand the reasons to contact and enlist the specialized skills of a veterinary forensic pathologist; (3) develop an understanding of animals as indicator crimes; and, (4) will become aware of additional educational opportunities in veterinary forensic sciences.

This presentation will impact the forensic science community by encouraging greater networking and professional use of forensic scientists in legal cases involving animal crime, abuse, neglect, and death.

This workshop is intended to provide the forensic practitioners, law enforcement investigations, crime scene analysts, attorneys, and animal control officers with information on how techniques in the forensic sciences have been successfully applied to legal investigations involving animal and wildlife crime.

This program will provide detailed information on how various areas of forensic science are being applied to cases of animal crime. Techniques once common only in the investigation of crimes against humans are now being adapted to the rapidly expanding field of veterinary forensic sciences. Creation of a nationwide DNA database "canine CODIS," an international professional organization dedicated to the application of the forensic sciences to animal crime, and formal educational programs at the graduate level are all working in conjunction to bring the level of scientific integrity to the investigation animal crimes that is regularly noted in human death investigations. This program will highlight forensic biology, anthropology, entomology, odontology, psychology, pathology, and investigative techniques.

Veterinary Forensic Sciences, Animal Crime Scenes, Animal Forensics

### W16 Sex-Related Homicide and Death Investigation: Significance of Pornography, Sexual Deviance, Autoerotic Fatalities, Investigative and Behavioral Analysis, Signature, and MO

Vernon J. Geberth, MS\*, PHI Investigative Consultants, Inc., PO Box 197, Garnerville, NY 10923; Peter I. Collins, MD\*, Law & Mental Health Program, CAMH - Unit 3-4, 1001 Queen Street, West, Toronto, ON M6J 1H4, CANADA; and Anny Sauvageau, MD\*, Office of the Chief Medical Examiner, 7007 - 116 street, Edmonton, AB T6H 5R8, CANADA

After attending this workshop, attendees will: (1) understand the role of fantasy in sex-related death; (2) collect and preserve physical and psychological evidence in sex-related incidents; (3) determine the M.O. and Signature characteristics in crime scenes; and, (4) understand investigative and behavioral analysis in criminal profiling.

This presentation will impact the forensic science community by better informing attendees of the dynamics and proper procedures in the investigation of sex-related homicides and death investigations.

Sex-Related homicides and deaths occurring during sexual events 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 represent 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 a year in the United States and how that may influence the increase in sex-related events.

Attendees of this presentation will obtain a better understanding of the significance of sexual deviancy, fantasy and pornography in sexrelated events as well as the investigative and behavioral analysis applied to these types of incidents. Attendees will understand the importance of the collection and preservation of evidence in sex-related events and the attendees will appreciate the impact of signature characteristics and modus operandi as applied to sex-related homicides.

In the first segment, examples of sexual deviance will be provided as well as the paraphiliac considerations in these type death investigations. There are over 35 paraphilias described in the literature and a number of paraphilias are cross-associated with sexual homicides. These will be explored at length in the presentation. The connection between pedophilia and the sexual homicide of children will be explored with appropriate case studies provided for illustration. The attendees will then be apprised of the current knowledge regarding autoerotic fatalities

Attendees will be apprised of the current knowledge regarding autoerotic fatalities including definitions, incidence, crime scene characteristics, typical and atypical methods, and victims. It will be demonstrated that the widely-cited incidence of 500 to 1,000 autoerotic deaths per year in the United States is not accurate anymore. It will be explained that an incidence of 0.2 to 0.5 cases per million inhabitants per year is a better estimate of the incidence of autoerotic deaths. New epidemiological data have demonstrated that this incidence is higher in big cities compared to rural areas. There is no clear evidence of a preferential time of day for these deaths, but there appear to be slightly more autoerotic deaths during summer. The typical and atypical methods of autoerotic deaths have also been revisited recently, based on the new standardized classification of asphyxia. New data on the crime scene characteristics will also be presented, along with a discussion of four types of atypical victims: non-white, female, children, adolescent, and the elderly. This presentation will impact the forensic science community by providing a comprehensive and practical illustration of specific examples and cases with a discussion of the investigative and clinical considerations, which will include pathology, pathophysiology, as well as the investigative and behavioral and forensic aspects of these events.

The second segment of the workshop will focus on the dynamics of sex-related homicides, which will include rape, lust murder and serial killing. Using a case history format, the importance of "Signature" and "modus operand" will be demonstrated to illustrate the application of investigative and behavioral analysis to the crime scene examination. This will include current research regarding the frequency of sexual posing in homicide crime scenes.

Each of the presenters bring years of practical experience and research into various aspects of sex-related events and will share their expertise with the attendees. The overall goal will be to provide comprehensive and practical information which will serve an investigative guide in sex-related homicide and death inquiries.

Sex-Related Homicide, Sexual Deviance, Autoerotic Deaths

### W17 DNA Mixture Analysis: Principles and Practice of Mixture Interpretation and Statistical Analysis Using the SWGDAM STR Interpretation Guidelines

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After attending this workshop, attendees will learn the basic principles and practice behind DNA mixture interpretation and statistical analysis utilizing the SWGDAM STR Interpretation Guidelines published in April 2010 (http://www.fbi.gov/hq/lab/html/codis\_swgdam.htm). Attendees will examine the elements of mixture interpretation using the STR guidelines and work through specific examples using appropriate statistical approaches.

This presentation will impact the forensic science community by providing attendees with a better understanding of applying the principles within the SWGDAM STR Interpretation Guidelines to validating mixture protocols, resolving DNA mixtures, developing strategies for statistical analysis, and reporting the results.

DNA mixture interpretation of evidence from sexual assaults or volume crimes such as burglaries - especially when the stain is degraded or compromised - can be challenging for the forensic scientist to decipher. The workshop is divided into two sections. The "Principles of Mixture Interpretation" will provide the attendee an overview of the new SWGDAM guidelines and a review of the forensic literature on the development of mixture interpretation guidelines from laboratories in the global DNA community. An overview of the elements and principles of DNA mixture interpretation will be discussed with a goal to include ideas on developing protocols and validation experiments to implement the concepts within the guidelines, such as stochastic thresholds. Finally, an overview of the statistical approaches to analyze mixtures will be presented. In the second half of the workshop, "Practical Applications of Mixture Interpretation," examples of challenging mixtures will be discussed starting with a two person mixture and concluding with discussions of how to approach mixtures with three or more individuals. An example of how one laboratory changed their interpretational guidelines, and the lessons learned from this change, will be presented. A survey of currently available mixture interpretation software to assist in mixture interpretation will be discussed. Finally, a presentation on how to train multiple individuals to consistently interpret mixtures will be discussed.

DNA Mixture Interpretation, Statistical Analysis, Forensic STR Typing

#### W18 K2 and Beyond: A Synthetic W19 Cannabinoid Primer

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After attending this workshop, attendees will be able to: (1) explain cannabinoid pharmacology; (2) understand the history and chemistry of synthetic cannabinoids (SCS) and their role in therapeutics; (3) develop and implement analytical methods for the identification of SCS in biological and non-biological matrices; (4) describe the current knowledge on the metabolism of SCS and the results of controlled drug administration studies; and, (5) explain the current legal status of SCS.

This workshop will impact the forensic science community by providing valuable and up-to-date information on a class of compounds which have garnered a large amount of media attention for their use and abuse.

First developed to study the cannabinoid receptor system, synthetic cannabinoids are now being used as an alternative to cannabis. Marketed as "herbal incense" under a wide variety of names including Spice, Yucatan Fire, Smoke, Sence, K2, Skunk, Space, K2 Citron, and K2 Blonde these compounds such as HU-210; JWH-018; CP 47,497; JWH-073; and JWH-200 are mixed with plant material and smoked. These synthetic analytes have a varying degree of selectivity and affinity for cannabinoids (CB<sub>1</sub> and CB<sub>2</sub>) receptors and thus have different therapeutic and abuse potentials. In addition to analytes that have been identified in herbal incense products, there are many other known CB<sub>1</sub> and CB<sub>2</sub> receptor agonists that could potentially be used in a similar manner.

As the popularity of these drugs increases, there is a developing need for analytical methods to identify and quantify the parent compounds in the herbal incense products as well as in biological matrices. On-going research will help identify metabolites of these compounds which can be used as markers of use in humans and develop methods for identifying these metabolites in a wide range of human biological specimens. Controlled administration studies will enable researchers to further study the pharmacokinetics, pharmacodynamics, and toxicity of this class of compounds.

Currently only HU-210 is controlled on the federal level; it is a schedule I drug. It may be possible that other synthetic cannabinoids could be controlled under the Federal Analogue Act. However, the law only applies to substances sold for human consumption. Since the herbal incense is labeled "not for human consumption," it is more difficult to prosecute users of these analytes under this act. Several states have been successful in passing legislation to control specific synthetic cannabinoids and many more are in different stages of developing and implementing laws to control their use.

This workshop is designed to provide an overview of the current issues related to synthetic cannabinoids including cannabinoid pharmacology and chemistry; history, development and evaluation of new synthetic cannabinoids for therapeutic use; diversion of synthetic cannabinoids to illicit use; current methodology for the identification of synthetic cannabinoids in herbal incense and biological matrices; metabolism of synthetic cannabinoids; what is known about their pharmacokinetics, pharmacodynamics and potential toxicity, and the current legal status of these compounds across the united states and around the world.

#### Synthetic Cannabinoids, K2, Spice

#### W19 Monitoring Conditions of Detention and Documenting Torture: An International Perspective

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After attending this workshop, attendees will become familiar with the main normative and practical considerations for international factfinding investigations into conditions of detention, including the forensic documentation of torture and ill-treatment.

This workshop will impact the forensic community by providing an updated review of current knowledge and practice in investigations into conditions of detention and the documentation of torture and illtreatment.

A multidisciplinary panel of international experts will share their experiences, lessons learned, and recommendations regarding practical considerations for international fact-finding investigations into conditions of detention, including the documentation of torture and illtreatment. Topics for discussion will range from the international normative and ethical frameworks and procedures which guide and are involved in such investigations; the role and duties of forensic experts; the preparation and implementation of international fact-finding investigations of conditions of detention, torture and ill-treatment; logistical issues and practical considerations for practitioners; to the ethical dilemmas faced during such investigations. In addition, the development and use of international standards for the medical documentation of torture will be addressed.

**Torture, Ill-Treatment, Places of Detention** 

#### W20 Forensic Multimedia Analysis

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After attending this workshop, the participant will be aware of the different methods that are available within multimedia analysis, as well as the possibilities and limitations within this field.

This workshop will impact the forensic science community by showing the possibilities within multimedia analysis and giving special

attention to validation and uncertainties in measurements and how to report them.

During this workshop information will be provided on new developments of forensic investigation of (digital) images and video streams and the use of 3-dimensional computer modeling in forensic investigations. The workshop will be interactive and many examples of case material will be shown.

Traditional sources of images as evidence concern crime scene photography, and more specifically, photographs of fingerprints, tool marks, shoe prints, and other impressions. A short overview of image processing techniques is given. Special attention is given to the introduction of artifacts by image processing (e.g., FFT on fingerprints), quality assurance aspects. During the last decades the use of CCTVcamera systems and digital cameras on phones has become widespread. Typical questions have arisen regarding the quality and the selection of images from a specific camera in a multi-camera-recording, and combining the information. Digital processing of video streams for presentation and storage purposes, and the compression techniques that are applied in digital CCTV-systems, lead to questions about the integrity and authenticity of recordings. Also questions about image interpretation of facial recognition, body height, or car speed, often in low resolution, time lapse, or compressed images have increased. A special focus will be given on image analysis.

Since more images are being processed for forensic investigation, new methods have been developed for answering questions about the interpretation of images: Is it possible to read a license plate number? Is a suspect, or his car, the one depicted in the image? What is the body length of the robber or the speed of a car?, and, Is it possible to do a reconstruction of an accident or a shooting incident from the information in these images? Methods for image comparison, facial comparison with non standardized images, image reconstruction, and photogrammetry are presented and discussed. Special attention is given to measurement uncertainties of the results and the impact on the conclusions from these investigations.

Common sources of video streams and images are video recordings from handy cams, digital photo camera's, internet, and cellular phones.

Typical questions about these recordings concern the integrity and authenticity of the recordings, the data compression techniques used, the synchronicity of sound and images, compensation for camera movement, and the conversion of a video stream to a higher resolution image. This session will focus on methods for state of the art image enhancement techniques as contrast stretching and deblurring, as well as methods as super resolution, stabilizing, and automatic tracking. It will also cover the issue of erased videofiles, and how to recover these when they are partly erased.

The state-of-the-art methods for camera comparison will also be presented, examples of comparing Photo Response Non-Uniformity with software. With this method a camera can be linked to a camera. Also, the cautions of identification and limitations of the technique are discussed, and solved within the Bayesian framework of likelihood ratios.

Finally, an overview is given to the use of 3-dimensional computer modeling in forensic investigations, since these techniques have an impact on traditional crime scene photography.

Computer models and animations have been recently used for analyzing video by superimposition of computer generated views of the model on the video images, for the visualization of complex scenarios in animations and for testing scenarios against video footage and evidence in crime scene photographs. Examples: the reconstruction of car accidents from photographs, analysis of blood spatter patterns from photographs using a computer model of the crime scene, the visualization of wound channels in computer models of human bodies, the reconstruction of bullet trajectories, the reconstruction of a burglary using the limited information in dark images from a multi-camera video recording, and the analysis of firework explosions from video recordings, photographs, and geographical data. Special attention is given to modeling techniques, the accuracy of the models, methods for visualizing uncertainties and possibly erroneous suggestions coming from these visualizations.

Multimedia Analysis, Image Analysis, 3D Visualizations

#### W21 Blood Alcohol Concentration (BAC) Evidence: Extrapolation, Interpretation, and Testimony in the Post-NAS Era

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After attending this workshop, attendees will understand the salient legal and practical considerations for expressing the scientific principles and methods available for interpreting and extrapolating BAC results from chemical analyses to a particular time of interest. Emphasis on preparing this evidence for use in court will provide hands on working knowledge of the predominant methods of testifying about extrapolating BAC results, and understanding the limitations imposed by law concerning expert interpretation and testimony when reporting and testifying to extrapolated BAC results.

This presentation will impact the forensic science community by providing the participants with the knowledge necessary to promote the just and scientifically valid practices necessary for the successful expression of alcohol test results and calculations and interpretations relating to those results according to substantive and procedural legal rules. It will thus improve the ability of our justice system and the witnesses, lawyers and judges to whom this evidence is vitally important to deal with forensic BAC results in a scientifically valid and rational manner.

Alcohol is one of the most widely tested chemicals in forensic laboratories, and test results carry probative weight as evidence. Alcohol concentration (BAC) evidence is usually reported as a singular numerical value without accounting for the range of values that express uncertainty and limitations affecting analysis, which should be accounted for in interpretation and testimony about the results. This practice is no longer acceptable as noted in the recently published report by the National Academy of Sciences Report entitled, "Strengthening Forensic Science in the United States: A Path Forward." Specifically, the report recognizes that "methods for measuring the level of blood alcohol in an individual...can do so only within a confidence interval of possible values." In this context, retrograde calculations are also often presented in court as singular values without any estimation of uncertainty or an uncertainty budget identifying the sources of uncertainty associated with the calculations. Accurate retrograde estimations of BAC must be expressed in a manner that reflects the uncertainty associated with the calculations, that is as the range of values that can actually and reasonably be attributed to the result. Failure to do so precludes objective interpretation of, and testimony concerning, the results which interferes with the quest for truth in the courtroom. Multiple methods are available for estimating BAC under a variety of circumstances, but they can be complex and time consuming often rendering them impractical and leading to human error when done by hand

The workshop focus is on the forensic use of blood and breath alcohol concentration test results. It is directed toward forensic toxicology practitioners, managers, attorneys, and jurists who must appreciate the necessity for accounting for uncertainty in chemical testing for alcohol and calculations utilizing those chemical test results. Those attending the workshop will be better equipped to promote the just and scientifically valid practices necessary for the expression of alcohol test results and calculations and interpretations relating to those results. It will thus improve the ability of our justice system and the witnesses, lawyers, and judges to whom this evidence is vitally important to deal with forensic BAC results in a scientifically valid and rational manner.

Easily obtained software can address many of these concerns, rendering the estimation of uncertainty both simple and relatively error free. Methods utilizing such software to improve the quality of forensic reports, as well as limitations of a software-based approach in these types of cases will be discussed. This workshop will, therefore, assist forensic science practitioners, advocates, and jurists to understand, interpret, and recognize the limitations of such calculations, and the methods by which they are conducted. By doing so it will facilitate the quest for truth in the courtroom leading to producing more just outcomes consistent with scientific reality.

Alcohol, Uncertainty, Interpretation

#### W22 Introduction to Expert Witness Testimony

Thomas J. David, DDS\*, 1000 Johnson Ferry Road, Building H, Marietta, GA 30068; Haskell M. Pitluck, JD\*, 573 Lake Avenue, Crystal Lake, IL 60014; and Paul G. Stimson, DDS\*, 902 Lakespur Drive, Sugar Land, TX 77479-5909

After attending this presentation, participants will have the basic skills to develop into an accomplished expert witness.

This presentation will impact the forensic community by producing more skilled expert witnesses who are members of the AAFS.

The skills required to become an accomplished forensic scientist are entirely different from those necessary to become an accomplished expert witness, even though both are important components of a successful career in the forensic disciplines. Expertise in the forensic sciences requires intense study of scientific principles as well as knowledge of technical detail and the ability to apply what has been learned. On the other hand, expertise as an expert witness requires mastery of an entirely different skill set. The basis for this difference revolves around where the two skills are applied. Forensic science skills are applied in the laboratory where scientific principles and technical knowledge are paramount. In this setting, the forensic scientist is in familiar territory with respect to rules that affect testing and results. On the other hand, when a forensic scientist steps out of the laboratory and into the world of attorneys and courtrooms, a completely different set of rules apply and you are no longer in your comfort zone. You are now playing in someone else's "sandbox" and the rules have been set by attorneys, not forensic scientists. If you fail to recognize this important distinction, you will quickly become aware that you are engaged in an adversarial process without any knowledge of the rules that govern it. This scenario would be akin to playing a baseball game without knowing the rules of baseball.

The purpose of this workshop is to familiarize forensic scientists with the necessary skills that are crucial to becoming an accomplished expert witness. This workshop will present a number of important concepts that are essential to presenting evidence and opinions in a forensic context. In order to present evidence and opinions in the proper forensic context, you must consider the perspectives of the people that take part in the process. Therefore, you need to consider the perspective of the following individuals: (1) the judge; (2) the attorneys; (3) the jury; (4) other experts; and, (5) spectators.

These perspectives will be discussed by the panel of presenters, including a Judge/ attorney, and forensic scientists/expert witnesses. In addition to consideration of perspectives, other topics to be presented include: (1) curriculum vitae guidelines; (2) report writing; (3) subpoenas; (4) depositions; (5) trial testimony; (6) expert witness guidelines; (7) do's and don'ts of court testimony; and, (8) cross examination skills. These topics will be discussed in detail with ample opportunity for questions.

At the conclusion of the presentations, there will be a panel discussion, so that participants can clarify questions that they may have

about the topics that were presented. The objective of the workshop is that all individuals who complete the instruction process will have an awareness of the necessary skills as well as the methods necessary to implement those skills in order to become an accomplished expert witness.

Expert Witness, Forensic Evidence, Court Testimony





## A1 Validation of a Qualitative TLC Analysis of A2 Seized Ecstasy Tablets Using Easy Ambient Sonic-Spray Ionization Mass Spectrometry (EASI-MS)

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After attending this presentation, attendees will be able to use TLC as an analytical tool in forensic ecstasy analisys. Attendees will also discover the application of EASI-MS technique in forensic analysis.

This presentation will impact the forensic science community by the insertion of a new application of mass spectrometry in forensic sciences. This presentation will show a rapid and conclusive technique to be used in forensic chemistry.

Ecstasy is the popular name of an illicit drug sold as tablets constituted by the compound 3,4-methylenedioxymethamphetamine (MDMA). Thin Layer Chromatography (TLC) is the most widely used analytical technique in Brazil as it is a low-cost analysis. The process of validation of drug TLC analysis is crucial to generate trustworthy, credible, and quality results in forensic chemistry.

Among the elution systems studied,  $CH_3OH / NH_4OH$  (98:2 ml); and  $CH_3CH(CH_3)OH / CH_3OH$  (95;5 ml) showed the best resolution results by TLC analysis. All mobile phases developed show similar Rf values between main active substance present in ecstasy tablets and metamphetamine (MDMA), a good resolution of MDMA in relation to other standards studied (MDA, MDEA, amphetamine, ketamine, and caffeine) were obtained from the systems described above.

TLC results were confirmed using a new technique of ambient mass spectrometry, easy ambient sonic spray ionization (EASI-MS). EASI was developed by Eberlin *et al.* and has already been successfully applied to different analytes in different matrixes such as ecstasy and m-CPP tablets, perfumes, surfactants, and biodiesel. The combination of TLC and EASI-MS techniques provides a valuable tool in forensic analysis, generating conclusive results in reduced time.

Several standards in this study (MDMA, MDA, MDME, caffeine, ketamine, methamphetamine, and amphetamine), and ecstasy tablets (street samples) are analyzed with TLC plates. A TLC/EASI-MS system for a rapid analysis of ecstasy tablets was developed to validate TLC analysis using EASI-MS technique, identifying compounds present in the eluted spots with great sensitivity.

The TLC/EASI-MS system can be used as a valuable tool in forensic analysis, confirming the presence of MDMA in the sample. Among 25 tablets analyzed, three show negative results for MDMA, where the presence of lidocaine and caffeine were identified. TLC/EASI-MS was not found to be appropriate to confirm caffeine due to its high plate affinity and typically low concentration in the ecstasy tablets. GC-MS analysis is a better analysis technique for the confirmation of caffeine.

Ecstasy-Like Tablets, MDMA, TLC/EASI-MS System

### Analysis and Characterization of Several Varieties of Herbal Blends Containing Synthetic Cannabinoids

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After attending this presentation, attendees will be familiar with the various methods in which synthetic cannabinoids may be analyzed and how cannabinoid content within marketed products may vary.

This presentation will impact the forensic science community by demonstrating various methods in which products containing synthetic cannabinoids may be analyzed. As a number of states have controlled these substances within the past year, with more expected to follow, it is important to have an understanding of which successful analysis methods are available.

Forensic identification of synthetic cannabinoids involves the detection of an increasing number of synthetic compounds. The specific compound(s) included within the product varies greatly among brands and, in some instances, batches. Sample content, in some cases, has been reported to rapidly change in response to local regulations; presumably to maintain a presence within the "legal high" market. An increasing number of states in the United States, including Kentucky, and local jurisdictions, have introduced legislation making the sale, purchase, possession, and/or use of these substances illegal. However, many states have no such regulation and the majority of these substances are not federally regulated.

The rapid evolution of the presence of these compounds presents a challenge for forensic investigators in instances where synthetic cannabinoids are banned, as opposed to those instances in which specific cannabinoid compounds are regulated. Some of the most frequently reported synthetic cannabinoids include HU-210, CP 47,497(and its homologs), JWH-018, JWH-073, JWH-398, and JWH-250. These compounds are added to a mixture of vegetative material in order to produce cannabis-like effects when smoked. They are commonly marketed as herbal incense or potpourri with the disclaimer that these substances are "not for human consumption." Reported effects are similar or greater in intensity than the effects of actual marijuana. The number of brands and types of these products are continuously growing and a current list of brand names rapidly becomes obsolete.

Standard marijuana color tests do not produce positive results as no delta-9-tetrahydrocannabinol (THC) is present within these products. Toxicological identification of the presence of synthetic compounds within blood samples is commonly available. However, it is limited in the ability to detect usage after a short period of time from consumption. Urine analysis allows for detection of these compounds over a longer period of time, currently, methods of detection are for the identification of a few synthetic cannabinoid compounds.

A simple methanol extraction followed by GC/MS analysis is sufficient for the identification of many of these compounds utilizing a small amount of sample of the unburned product. An analysis of the burnt residue and ashes remaining after burning a portion of synthetic cannabinoid sample indicates a diminished yet identifiable and sustained presence of several of these compounds. Derivitization of these substances was also studied for optimization of the analysis and detection of the synthetic compounds. Analysis of several different brand name samples revealed the presence of various concentration and types of known synthetic compounds, including JWH-073, JWH-018, and CP 47,497-C8. Samples included in this study were obtained on the local market, from the internet, or as evidence submitted to the laboratory for analysis and identification.

Marijuana Mimics, Cannabinoids, Drug Analysis

#### A3 Trifluoroacetyl Derivatization of Amphetamine, Methamphetamine, 3,4-Methylenedioxymethamphetamine, and Other Controlled Substances With Similar Mass Spectra

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The goal of this presentation is to provide attendees a look into a possible new way to analyze and identify controlled substances in a GC-MS with the use of chemical derivatization.

This presentation will impact the forensic science community by explaining how the use of TFA derivatives will enable drug analysts to utilize GC-MS to uniquely identify a number of amphetamine class compounds who have very similar mass spectra for the underivatized forms.

For the identification of a controlled substance, gas chromatography/mass spectrometry is the most commonly used method. However, there are some cases where the controlled substance shares a similar mass spectrum with a drug of a lower scheduling or a compound that is not even considered to be a controlled substance, such as methamphetamine and phentermine. Drug analysts then need to use chromatographic retention times to make a positive identification of the suspected compound. Here, it is proposed that the derivatization of these compounds will create mass spectra that are sufficiently different enough to make a positive identification without relying solely on retention time.

The major ion observed for amphetamine (AMP) was 44 m/z, sharing a similar mass spectra with 3,4-methylenedioxyamphetamine (MDA), 2,5-dimethoxy-4-bromoamphetamine (DOB), 2,5-dimethoxy-4chloroamphetamine (DOC), and 2,5-dimethoxy-4-methylamphetamine (DOM). Methamphetamine (MA), with a major ion at 58 m/z, had a similar mass spectra to phentermine and 3,4methylenedioxymethamphetamine (MDMA, ecstasy). 3,4-Methylenedioxyethylamphetamine (MDEA) and fenfluramine both had a major ion peak at 72 m/z.

Controlled substance standards, such as MA and AMP, were dissolved in chloroform and derivatized with trifluoroacetic anhydride (TFA), with pyridine acting as a catalyst and sodium hydroxide as a neutralizer, and analyzed by a GC-MS, resulting in unique, identifiable spectra for each standard. For example, after TFA derivatization it was observed that MA had major ions at 154, 110, and 118 m/z, while phentermine had major ions at 154, 91, and 59 m/z and MDMA at 154, 162, 135, and 110 m/z.

In most cases, a suspected controlled substance could be mixed with other drugs. Ecstasy tablets, for example, can be found containing MDA, MDEA, AMP, MA, and ketamine. When combining in a single GC vial equal amounts of these drug standards after TFA derivatization, the derivatized substances were shown to have adequate separation and could be uniquely identified by both their mass spectra and relative retention times through the gas chromatographic column. Future studies should be made using different controlled substances, derivatizing agents, and GC and MS conditions. This will help to determine which derivatizing reagents would produce the most unique and identifiable mass spectra for controlled substances, and also which chromatographic conditions would give the most ideal separation of drug mixtures.

Amphetamines, TFA Derivatization, GC-MS

### A4 Investigation of the Stability of Cannabinoids in Cannabis, Decarboxylation Reaction, and the Validation of HPLC Method for the Quantitative Determination of Cannabinoids

Stephanie H.J. Lim, BSc\*, Angeline T.W. Yap, PhD, and Chiu Guay Phua, BSc, Health Sciences Authority, 11 Outram Road Singapore, 169078, SINGAPORE

After attending this presentation, attendees will gain a better understanding into stability of cannabinoids in cannabis and the quantitative determination of cannabinoids by HPLC.

This presentation will impact the forensic science community by enriching attendee knowledge in the chemistry of cannabinoids.

Cannabis refers to any part of a plant of the genus Cannabis. It is used for fiber (hemp), for medicinal purposes, and as a recreational drug. The three main forms of cannabis products are the herb (marijuana), resin (hashish), and oil (hash oil). Tetrahydrocannabinol (THC) is the main psychoactive ingredient in cannabis and is known to degrade into cannabinol through oxidation over time. The extent of degradation could be co-related to the age of the seized exhibits. The stability of cannabinoids in cannabis stored under different conditions over a period of time was investigated. The influence of temperature and exposure to light, on the stability of the exhibits was investigated. The rate of degradation of THC in cannabis was evaluated by Gas Chromatography/Flame Ionization Detection (GC/FID). The results indicated that the degree of degradation is highest in exhibits exposed to sunlight under ambient temperature while those kept in the freezer showed the highest stability. In addition, the stability study of a THC standard solution was conducted in parallel to evaluate the influence of matrix on the degradation process.

Total THC content often represents the maximum potency of the smoked cannabis and is of great importance to legal systems. Measurement of total THC content comprises the sum of free  $\Delta^9$ -Tetrahydrocannabinol  $(\Delta^9$ -THC) precursor  $\Delta^9$ and its Tetrahydrocannabinolic acid A ( $\Delta^9$ -THCA-A). The influence of extraction solvent on the ratio of  $\Delta^9$ -THC and  $\Delta^9$ -THCA-A extracted was studied. Since cannabinoids are easily soluble in most organic solvents, such as methanol, petroleum ether, n-hexane, toluene, chloroform and methanol:chloroform combinations, these solvents are suitable for extraction. However, non-polar solvents such as n-hexane and petroleum ether will only extract the free cannabinoids quantitatively, while other solvents extract both cannabinoids and its acids. Therefore, for total THC determinations, the choice of extraction solvent plays an important role in determining the amount of the different types of cannabinoids extracted.

GC/FID is can be used for the quantitative analysis of cannabis samples.  $\Delta^9$ -THCA-A converts to the psychoactive  $\Delta^9$ -THC when heated. During the GC analysis, thermal conversion occurs as the acidic forms of the cannabinoids are decarboxylated into the neutral counterparts. Therefore, the sum of free  $\Delta^9$ -THC and  $\Delta^9$ -THC generated from the decarboxylation of  $\Delta^9$ -THCA will be measured in the GC. Previous studies have shown that thermal conversion of  $\Delta^9$ -THCA-A to  $\Delta^9$ -THC in GC is only partial, yielding about 70% at the maximum. Decarboxylation reaction can be conducted prior to GC analysis. The temperature and duration of heating parameters influencing the extent of decarboxylation and establish the optimum condition for the reaction were investigated.

When the free  $\Delta^9$ -THC and  $\Delta^9$ -THCA-A needs to be determined independently, derivatisation (trimethylsilyl derivatives) has to be performed prior to GC analysis. Alternatively, the High Performance Liquid Chromatography (HPLC) method has to be applied. Analysis of  $\Delta^9$ -THCA-A by GC involves derivatizing the carboxyl and phenol functions of the molecule, while HPLC does not cause any decomposition as the method does not involve any input of heat.

The application of a HPLC method for the direct quantitative determination of the cannabinoids will be discussed. The major advantage that HPLC has over GC is in the direct analysis of the thermally labile carboxylic acid derivatives of the cannabinoids. HPLC method will be validated and the ratio of  $\Delta^9$ -THCA-A and  $\Delta^9$ -THC in local cannabis exhibits will be investigated. Reversed-phase columns and solvent programmed gradient systems are required for the separation of cannabinoids and their acids.

Tetrahydrocannbinol, Degradation, Cannabinoids

#### A5 Identification of Alkaloids and Cutting Agents in Illicit Brazilian Cocaine Using Liquid Chromatography-Mass Spectrometry

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After attending this presentation, attendees will be briefed on a new methodology to analyze cocaine alkaloids and adulterants using liquid chromatography-mass spectrometry (LC/MS). Also, attendees will understand what compounds can be found in refined illicit cocaine seized in Brazil and discuss the experimental results with the authors.

This presentation will impact the forensic science community by serving as an appealing alternative to the traditional methods used in the analysis of cocaine alkaloids by GC/MS or GC/FID both requiring prior derivatization with MSTFA.

Drug impurity profiling can generate important information for drug law enforcement authorities. In fact, chemical correlation between samples can be established, and material from different seizures can be classified into groups of related samples and determined if different seizures were derived from the same source. Consequently, specific links between different suppliers and users can be structured, drug distribution routes and networks can be built up, and the geographic origin of drug samples may be identified.

Since 2007 Brazilian Federal Police has been working on its own cocaine impurity profiling program ("Perfil Químico de Drogas" also known as the "PeQui" Project). An alternative methodology to analyze cocaine alkaloids that can be reliable, faster and free of derivatization agents, which are expensive and toxic, were suggested in the establishment of the Brazilian Signature Program.

A LC/MS method is proposed for simultaneous identification and quantification of some cocaine alkaloids: ecgonine, methylecgonine, tropacocaine, benzoylecgonine, norcocaine, N-formylcocaine, trimethoxycocaine; and typical cutting compounds: benzocaine, phenacetin, caffeine, lidocaine, levamisole, hidroxyzine diltiazem. Samples of illicit hydrochloride cocaine and cocaine base from Brazilian Federal Police apprehensions, in the period between 2009 and 2010, were analyzed by this methodology.

LC/MS analysis was performed on a time-of-flight (TOF) mass spectrometer with electrospray ionization (ESI) in positive ion mode, using full scan data. Compound identification is based on accurate mass, isotope pattern, and retention time information. Protonated molecules  $(M+H)^+$  were the ions selected in the quantification of all analytes. The HPLC conditions tested were two different reversed-phase C18 columns. The method was optimized using a gradient of formic acid/ammonium formate in water and formic acid in acetonitrile.

The analysis of the same compounds were also tested on a LC/MS/MS system based on UHPLC and triple quadrupole (QQQ) mass spectrometer with electrospray ionization (ESI) in positive ion mode, using MRM (multiple reaction monitoring) mode, monitoring two transitions to each compound. The preliminary results obtained on ESI-QQQ experiments were comparable with ESI-TOF results and demonstrate that MRM experiments are a promising analytical scheme for identification and quantification of cocaine alkaloids and adulterants.

Tandem mass spectrometry (MS/MS) screening techniques using MRM monitoring are able to detect only the target compounds previously defined in the method, while TOF can look for untargeted compounds not originally sought, from accurate mass full scan data without rerunning the samples. For instance, it is possible to detect new adulterants in cocaine samples and to reprocess the original data with screening software/database commercially available in modern mass spectrometry equipments.

The methodology based on LC/MS analysis of refined illicit cocaine samples is a novel and reliable approach and it can complement existing gas chromatographic methods for cocaine impurity signature programs. **Cocaine, LC/MS, Profiling** 

#### A6 Detection of Altered Bloodstains with BlueStar®

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The goal of this presentation is to discuss how the ability to prevent detection of blood stains deposited on common building materials as substrates by the use of common household cleaning products was investigated with the use of the relatively new Bluestar® blood detection reagent.

This presentation will impact the forensic science community by highlighting the fact that bloodstains that have been "washed" or potentially altered at a crime scene, can still be revealed days and months after attempts at removal.

After attending this presentation, attendees will understand that latent blood that has been attempted to be removed, destroyed, or altered can still be detected days and months after deposition and "clean-up." Using the following list of common building materials as substrates, and household cleaning products to eliminate blood evidence, the blood could still be detected with Bluestar®.

Substrates	Household Cleaning Products
Linoleum	Water
Tile	Dawn Ultra Concentrated Liquid Soap-Original Scent
Drywall	Bleach- Clorox Liquid Bleach: Clean Linen Scent
Carpet	Resolve Triple Action Spot Carpet Cleaner
	Hoover Premium Pet Formula Detergent
	Lysol Disinfectant All Purpose Cleaner 4 in1

Substrate materials (1ft x 1ft) were prepared in groups of four sets and kept for each designated time interval after deposition of blood (1,7,30 and 60 days). The total number of squares used for the experimental study was sixty-four. 15 ml Blood was deposited on each substrate and allowed to dry indoors for two hours. The linoleum, tile, and drywall were cleaned using water, soap and water, Clorox Bleach, and Lysol. The carpet was cleaned using water, soap and water, Resolve Carpet Cleaner, and a Hoover Premium Pet Formula detergent in combination with a Hoover Steam Cleaner.

Latent blood was found to be detectable, regardless of cleaning method, throughout the two month period that the substrates were being evaluated. This study showed that certain types of cleaning methods will alter detection of blood not only immediately, but also over extended periods of time. Results showed that overall those substrates cleaned using a chemical method (i.e., Clorox Bleach, Lysol) presented a significant decrease in detection of the latent blood. This detection was demonstrated by decreased luminescence using the Bluestar® reagent. The effects of time on latent blood detection with Bluestar® appeared to be in direct correlation to the attempted clean-up method used. Clean-up of blood using chemical methods presented decreased detection, presumably because of altered peroxidase activity of hemoglobin in the blood trace evidence.

Detection was rated on a visual scale defined as negative or positive (+ to +++++) when viewed in dim light. The results were recorded photographically and showed that Bluestar® is capable of detecting latent blood on a wide variety of substrate types often encountered at crime scenes after attempts to "clean-up" the evidence by the use of a variety of common household cleaning products.

For optimal effectiveness, Bluestar® should be used as soon as possible after opening. The elapsed time from which the Bluestar® was opened and when it was used to process the substrates appears to have some effect on its chemiluminescent quality. Overall, Bluestar® was effective in detecting the latent blood on all substrates regardless of the cleaning method to remove the blood evidence.

The detection of latent blood during criminal investigations can provide clues about the reconstruction of events at a crime scene. This research demonstrated the resilience of human blood to total eradication using common household cleaning products and after delay until detection (up to two months). The ability to detected latent blood using Bluestar® was after attempts at removal and long periods of delay until detection.

Bluestar®, Bloodstain, Chemiluminescent

#### A7 Massacre of AWA Indians in Colombia: Genetic Identification

Martha L. Camargo, BSc\*, Instituto de Medicina Legal, Calle 4B N° 36-01, Cali, COLOMBIA

After attending this presentation attendees will learn about the problems posed by the parties to the armed conflict in Colombia, including law enforcement officers who fight against illegal groups, namely FARC, ELN, rightist paramilitary groups, such as the so-called self-defense groups of Nariño, the Black Eagles, and the "Rastrojos" which are at the service of drug traffickers. The AWA Indian community is caught in the middle of this conflict and it has been the victim of massacres, displacement, and forced disappearance. At least ten Indians, whose bodies were not found at the time, were stabbed to death during a massacre that occurred in February 2009. In March and May 2009, five bodies were found and referred to the Legal Medicine in Tumaco for the corresponding autopsy and sample collection for purposes of genetic identification by the Genetics Lab at the Southwestern Regional Office in Cali. The technical problems of collecting samples from family members will be described, as well the difficulties of analyzing the genetic results

obtained, due to the high rate of endogamy among victims and family members.

This presentation will impact the forensic science community by describing the key aspects of these cases in terms of the identification of victims that belong to protected populations covered by the International Humanitarian Law, as well as the evaluation of genetic information provided by family members, considering the high level of endogamy existing in geographically isolated Indian communities. This presentation will describe the methods of DNA extraction from skeletal remains and PCR amplification of DNA via autosomal STRs and Y chromosome used by the Genetics Lab of the Legal Medicine. The contribution of the genetic study that supports criminal investigations involving populations protected by the International Humanitarian Law will be highlighted. These vulnerable communities require humanitarian assistance to protect their rights, their beliefs, their children, and their land. The contribution of the Genetics Experts of the Southwestern Regional Office of the Institute of Legal Medicine in court is noteworthy. Expert reports supported the positive identification of five AWA Indians who were recovered.

Four massacres of Indian communities were reported in 2009. Forty victims belonged to the AWA community, which is made up of approximately 30,000 individuals who live in small villages, spread out in twenty-six Indian reservations in the areas of Roberto Payan and Barbacoas in Nariño. During the massacre of February 2009, the victims' bodies disappeared. Five bodies were subsequently found in May of the same year. The bodies recovered had signs of torture and stabbing. The victims were positively identified through genetic analyses of the samples provided by family members who were located in forest and mountain areas. The genetics lab was able to determine kinship among the five victims and eleven family members. Three family groups were targeted in this massacre. Some of the profiles obtained from family members were entered in the national genetic profiles database and will be compared with unidentified bodies that may be found and recovered as part of on-going investigations conducted in the area. The efforts of government agencies, NGO's, and international organizations in terms of body recovery, location of family members, and positive identification of the victims must be highlighted. The discovery of the events and its causes show the AWA community is a highly vulnerable population in the Colombian domestic conflict.

AWA Indians, Human Rights, Massacre

#### A8 Unique Evidence and Examination in Methadone Suspected Death

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After attending this presentation, attendees will have an idea of the types of evidence examined and analytical approaches employed in solving cases at the United States FDA's Forensic Chemistry Center (FCC).

This presentation will impact the forensic science community due to the evidence received for evaluation and the approaches used to obtain results, in particular, direct analysis in real time with mass spectrometric detection (DART-MS).

The FDA's Forensic Chemistry Center (FCC) encounters many different types of cases involving foods, drugs, and medical devices. Many articles discuss methadone fatalities involving children and associated toxicology results. This case review is unique due to the evidence received for evaluation and the approaches used to obtain results, in particular, direct analysis in real time with mass spectrometric detection (DART-MS).

The FCC received a case involving the death of a 17-month-old child. It was suspected the child had received a fatal dose of methadone. The evidence sent to the laboratory included the t-shirt the child was wearing at the time of death, various liquids possibly ingested by the child, two bottle nipples with attached bottle rings, a bottle of over-thecounter children's oral suspension pharmaceutical product containing a semi-solid, and an oral syringe.

The child's t-shirt had visible areas of discoloration, and was analyzed for areas of fluorescence using a multiple wavelength light source. The fluorescent spots of interest as well as other areas on the tshirt, were subsequently analyzed for controlled substances and pharmaceuticals. Fourier Transform infrared spectroscopy (FT-IR), DART-MS, gas chromatography with mass spectrometric detection (GC-MS), and liquid chromatography with mass spectrometric detection (LC-MS) were employed in the analyses. Methadone and cocaine were identified on the front collar of the t-shirt. The lower front of the t-shirt, as well as the back collar, shirt tail, and middle back of the t-shirt were also consistent with the presence of cocaine.

The liquids and semi-solid were extracted with solvents, and the resulting solutions screened for the presence of methadone, other pharmaceuticals and poisons using GC-MS. The inside and outside surfaces of the ring-nipple units were rinsed with methanol, and the resulting solution screened for the above substances using GC-MS. The tip and inside of the oral syringe barrel were also rinsed with methanol and analyzed using GC-MS. These same items were analyzed by LC-MS to obtain improved sensitivity. No methadone, drugs, poisons, or other pharmaceuticals were detected in the extractions of the liquids. Methadone was identified in the semi-solid from the bottle of over-thecounter children's oral suspension, and quantitated at 4 µg/g of semisolid. Acetaminophen, chlorpheniramine, and methorphan were also identified in the semi-solid. These three components were listed as ingredients on the bottle. Methadone was also identified in the rinse solutions of the ring-nipple units and oral syringe. Methadone was quantitated at 0.2 µg in one ring-nipple unit extraction, 18 µg in the extraction of the second ring-nipple unit, and at 600 µg in the syringe extraction. Acetaminophen, chlorpheniramine, and methorphan were also identified in the extraction from the syringe.

The investigators, with results in hand, encouraged the mother of the child to tell the story of what led to her child's death. Her son had been born with a drug dependency and was treated for withdrawals by a physician. However, the mother suspected the child was still having withdrawals. So on the advice of a "friend," the mother continued to give the child methadone for a year without a doctor's supervision. She found her toddler unresponsive in his crib. The mother pled guilty to a Class A felony neglect of a dependent. She was sentenced to 20 years in prison to be followed by ten years of probation.

Methadone, Death, Toddler

#### A9 Some Error Rates Associated With Angle of Impact Calculations

Kaitlin Leslie\*, and Taylor Lowis, University of Nebraska-Lincoln, Department of Entomology, 202 Entomology Hall, Lincoln, NE 68583-0816; Larry Barksdale, MA, Lincoln Police Department, 575 South 10th Street, Lincoln, NE 68508; and David O. Carter, PhD, University of Nebraska, Lincoln, Department of Entomology, 616 Hardin Hall, Lincoln, NE 68583-0996

After attending this presentation, attendees will have a better understanding of the effect of impact angle and surface texture on error rates associated with angle of impact (AOI) calculations.

This presentation will impact the forensic science community by increasing the understanding of the accuracy, precision, and reliability of

AOI calculations. This will enhance bloodstain pattern analysis and crime scene reconstruction.

Bloodstain pattern analysis is useful in reconstructing the bloodletting events at a crime scene. The presence of transfer stains, projected stains, passive stains, miscellaneous stains, or impact stains all can act as informative physical evidence. Accurately calculating angle of impact (AOI) of an impact stain can be used to estimate the three-dimensional point in space from which blood originated (the area of origin). However, little experimental data is available concerning the error rates associated with AOI calculations. Further, the effect of surface texture and impact angle on the error rates associated with AOI calculations has received little experimental investigation. Controlled laboratory experiments were conducted to investigate the effect of surface texture and impact angle on the error rates associated with AOI calculations.

Experiments were conducted by intravenously drawing human blood (blood density equaled 1.07 grams per milliliter  $\pm$  0.003 grams per milliliter) into vials containing no anticoagulants or preservatives. Blood (30 microliters  $\pm$  1 microliter) was dropped from a height of 35 cm onto three different surfaces: ceramic, porcelain, mirror; at three different angles: 10°, 50°, 90° within three minutes (to avoid clotting). Ceramic tile had the greatest amount of texture while mirror tile had the least amount of texture. AOI was calculated using standard protocol and error rates were calculated as standard error of the mean. Four analysts, ranging in expertise from novice to expert, measured the bloodstains for angle of impact calculations. Data did not meet the assumptions set forth for normality and homogeneity of variance. Thus, a comparison of the calculated AOI means was conducted using the Kruskall-Wallis H and Mann-Whitney U statistics.

Surface texture had a significant (P < 0.05) effect on AOI calculation at 10° and 50° but not at 90°. Estimates of 10° on mirror were significantly (P < 0.05) greater than on porcelain. Estimates of 50° on ceramic were significantly (P < 0.001) greater than on mirror and porcelain. Error rates increased as angle of impact increased. Yet, error rates were relatively small, as they all were less than or equal to 1.2°. Yet, despite this precision, only one AOI calculation was accurate (50° on mirror). That is, only one calculation overlapped the actual angle of impact.

Impact stain analysts typically impose an error rate of  $\pm 5^{\circ}$  to  $\pm 7^{\circ}$ . To achieve 100% accuracy in the current study, an error rate of 3.5° for AOIs of 50° and 90° and an error rate of 1.9° for AOIs of 10° would need to be imposed. Applying reliable, smaller error rates to angle of impact analysis should increase accuracy and precision in determining area of origin, which could be the difference between a self-defense plea and a homicide conviction. It was also determined that a one millimeter division ruler is not sufficiently precise to measure angle of impact for bloodstains with a volume of approximately 30 microliters.

Bloodstain Pattern Analysis, Impact Stain, Reliability

#### A10 Determining Buried Human Decomposition Odor Profiles in Various Biotopes

Patricia T. Caldwell, PhD\*, and Douglas Beussman, PhD, Oak Ridge Institute for Science and Education, FBI Laboratory, CFSRU, Quantico, VA 22135; Christopher Tipple, PhD, FBI Laboratory, CFSRU, Quantico, VA 22135; Mark Sabo, PhD, Catawba College, 2300 West Innes Street, SCI 207, Salisbury, NC 28144; Nishan Dulgerian, MS, Rex Stockham, MS, and Wynn Warren, BS, FBI - ERTU, FBI Laboratory, Quantico, VA 22135; and Brian A. Eckenrode, PhD, FBI Laboratory, CFSRU, Building 12, Quantico, VA 22135

After attending this presentation, attendees will learn that buried human remains emit a unique odor profile of volatile organic compounds that analytical instrumentation can detect and identify. With these data, canine training aids can be created for use in locating clandestine burials. This presentation will impact the forensic science community by providing canine handlers with a consistent and testable mixture of the components that best simulate the odor of a buried human. A mixture that can produce a decomposition odor profile for presentation to canines should improve the likelihood of locating clandestine burials and reduce false positives due to other sources such as decomposing animals or waste products. This research will also help confirm canine alert responses in law enforcement cases.

Current canine training aids for victim recovery investigations vary widely by the handler. The development of scientific methods for determining the exact chemical composition required when creating training aid mimics is difficult. Other complicating factors include both endogenous and exogenous influences. The unique scent associated with human remains changes during the decomposition process as the body's building blocks such as proteins, nucleic acids, carbohydrates, and lipids are broken down into smaller components. A buried human odor profile also changes due to variations in temperature, rainfall, bacteria/microbes, and humidity. Delineating the chemical constituents of the odor over time and in various biotopes is a major goal of this research. Developing the appropriate mixture levels that correspond to the stages of buried (anaerobic/aerobic putrefaction) human decomposition is another objective.

Verifying a canine alert response with on-site field instrumentation would further support canine evidence in law enforcement cases. Human decomposition odor is complex but should not differ significantly between individuals since the internal physiological structural are similar. Thus, identifying a generalized odor profile, even with the variations and quantities of compounds emanating from the source, may be possible. Solid-phase microextraction (SPME), whole air sampling and an Agilent 6890N GC system coupled with an Agilent 5973 mass selective detector were used to analyze the odor of human decomposition. Previous research on human burial subjects identified seven volatile organic compound (VOC) groups produced by decomposition, including: acids/acid esters, alcohols, aldehydes, halogens, aromatic hydrocarbons, ketones, and sulfides.

Soil and soil gas VOC data from two different land sites and from two forensically relevant cases will be presented, utilizing SPME and thermal desorption methods. Preliminary data comparisons between soil and air samples collected at each of the sites indicate there are different VOC profile variances emitted with the various phases of human burial decomposition. If so, development of more specific training aids for human burial investigations might be beneficial for casework. Animal decomposition will also be compared to the human decomposition to show the differences in odor profiles.

Development of more precise training aids should improve the accuracy of canines used in the field for clandestine burial investigations. Thus, training exercises utilizing specific training aids that mimic buried human odor profiles are critical. If a human has a unique odor profile equivalent to that of a fingerprint, this research will be extremely useful in differentiating humans from other sources of decomposition, such as animal remains, and to reduce false positives and increase confidence in canine alerts.

Human Decomposition, Detection Canines, Clandestine Burials

#### A11 Evaluation of the Scent Transfer Unit (STU-100) for the Collection of Human Odor from Porous Objects

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After attending this presentation, attendees will have been presented data from both male and female subjects demonstrating the potential that the Scent Transfer Unit-100TM (STU-100) has in collecting human odor from porous objects.

This presentation will impact the forensic science community by exhibiting the capabilities of the Scent Transfer Unit-100<sup>TM</sup> (STU-100), as well as demonstrating an appropriate technique with which law enforcement personnel can collect human scent evidence from a porous object, and in turn utilize it as corroborating evidence for the apprehension and prosecution of a suspect.

Human scent collection, although considered fairly new within the United States, has been successfully used in European countries as a form of evidence during criminal proceedings for many years. It has been previously reported that human odor is characteristic to an individual, thus making it ideal for use as a form of evidence in court. Within the United States, human scent collection is recognized as a form of corroborative evidence that can be collected, non-invasively, from other physical evidence and used to connect a suspect to a particular crime scene. The manner in which human scent can be collected without disturbance is through the implementation of the Scent Transfer Unit-100<sup>™</sup>. The STU-100 is a portable vacuum source that utilizes airflow to transfer human odor from the surface of an object to a gauze pad. The gauze pad is then presented to scent-discriminating canines that are used to assist law enforcement officials in their investigations. These canines are able to locate a suspect by following the suspect's odor from the scene of the crime and in some countries, identify a suspect through the use of a scent line-up.

Though the abilities of these canines have been demonstrated, there is still limited analytical data that assesses the collection of human odor from various objects. Thus, it is the objective of this study to evaluate the STU-100 for the collection of human scent evidence from porous objects. Utilizing Headspace Solid-Phase Microextraction coupled to Gas Chromatography-Mass Spectrometry (HS-SPME-GC/MS) the hand odor released from a porous object and collected with the STU-100 was evaluated from both male and female subjects. The process of the human scent collection consisted of washing the hands and forearms for 30 seconds with fragrance free soap, rinsing for two minutes, air drying for four minutes, rubbing hands and forearms for five minutes and then the subject was given a porous object to clasp in his/her dominant hand for five minutes. The STU-100, containing a four inch x four inch gauze pad, was then utilized for one minute to collect the human odor from the porous object. The gauze pad was then retrieved with sterilized tweezers, placed into a vial and allowed to equilibrate for 24 hours. Solid-Phase Microextraction was then used to extract the volatile organic compounds from the headspace of the samples for 21 hours and then analyzed using Gas Chromatography-Mass Spectrometry.

Within the law enforcement field, the STU-100 has shown to be an invaluable tool when conducting criminal investigations due to its ability to collect human odor from evidence commonly left by suspects at a crime scene, such as weapons or clothing. This presentation will validate this practice through the use of instrumental analysis.

Scent Transfer Unit-100<sup>TM</sup>, Human Scent, Corroborative Evidence

#### A12 Genetic Detection of Serial Rapists in Sexual A13 Assault Cases in Colombia

#### Andrea Pinzon, MSc\*, Medicina Legal, Calle 4B Nº 36-01, Cali, COLOMBIA

After attending this presentation, attendees will learn that the number of sex crimes reported has escalated, according to the National Reference Center on Violence in Colombia (CRNV). These cases require the involvement of interdisciplinary teams capable of interdisciplinary investigations. The genetics lab of the Southwestern Regional Office of the Institute in Cali has been providing investigators with genetic profiles of autosomal STRs and Y Chromosome of unknown offenders who are currently committing or have committed sex crimes. Attendees will learn how this has contributed to the association of various investigations and has helped orient the search for these offenders.

This presentation will impact the forensic science community by discussing the performance and significance of the role of the genetics lab in terms of keeping the authorities informed on the matches found in the genetic profiles database. These profiles are obtained from sexual assault cases that help orient the investigations and establish associations in order to prioritize the pursuit of the offenders. The procedure used by the lab to obtain post-coital DNA samples will be highlighted. This process obtains differential and clear profiles that minimize mixture reports. It enhances clean or major profile reports on suspects, which enhances the likelihood of finding matches between the genetic profiles of the reference population.

Sex crimes account for eighty percent of the casework of the forensic biology lab. These cases are accepted for preliminary microscopic examination utilizing Christmas Tree staining and PSA analyses, regardless of whether the offender is known. The preliminary analysis of the evidence is a priority in sexual assault cases when the perpetrator is unknown. A major drawback is the lack of a team of investigators who interact and share their findings in order to "correlate" cases and expand the information required to investigate serial rapist cases.

The need for a centralized team that understands the facts of the case and is capable of linking various sexual assault cases was recognized. Therefore, the Elite Sex Crimes Team (GEDES) was created in Colombia. GEDES is currently operating in Bogotá and has carried out routine operations where the investigators have analyzed cases for similarities, common areas, and "modus operandi." Simultaneously, the lab is required to obtain a genetic profile from the evidence and conduct a search in CODIS.

The Southwestern Regional Office is currently working on the creation of a GEDES team. This effort is required to detect serial rapists, based on the medical history obtained as a result of forensic sexual assault examinations conducted by the medical examiners of the Institute of Legal Medicine. This will guide law enforcement in terms of prioritizing their investigations and arresting the offenders. Based on the genetic findings from the evidence, the regional genetics lab has made contributions to the investigation of eight sexual assault cases. The matches of the genetic profiles obtained from the evidence have confirmed the involvement of two unknown perpetrators.

Additionally, the genetics lab of the Institute recognizes the importance of obtaining genetic profiles for purposes of comparing them to the national CODIS database. The analysis of sexual assault cases is the lab's priority. Consequently, the implementation of standard procedures to obtain genetic profiles from both perpetrators and the victims is essential.

Sexual Assault, Genetic Profile, CODIS

#### 13 Determination of Cyanide as an Indicator of Bitter Almonds in a Shipment of Organic Almonds

Valerie M. Toomey, BS\*, Elisa A. Nickum, BS, and Cheryl L. Flurer, PhD, United States Food and Drug Administration, 6751 Steger Drive, Cincinnati, OH 45237

After attending this presentation, attendees will see how the detection of cyanide indicated the presence of bitter almonds that had been commingled with sweet almonds.

This presentation will impact the forensic science community by demonstrating how the detection of cyanide can be used to remove potential health hazards from the market, in order to ensure the safety of the food supply.

Several complaints were received from consumers in Washington, who had purchased organic almonds at local stores around August 2010. Although some of the almonds tasted "normal," the consumers indicated that some "tasted very bitter." The original shipment of almonds was declared as a product of Uzbekistan, a region of the world in which bitter almonds grow. This raised the possibility that wild, bitter almonds had been commingled with the sweet almonds that are typically consumed in the United States.

Bitter almonds contain the cyanogenic glycoside amygdalin, which undergoes acid hydrolysis to produce glucose, benzaldehyde, and cyanide. According to the literature, cyanide levels in bitter almonds can range from 4 mg to 9 mg per almond.<sup>1</sup> A minimum lethal dose of cyanide is reported as 0.5mg per kg, or 50mg for a 100kg (220lb) adult. Due to the potential health hazard associated with the ingestion of cyanide through consumption of bitter almonds, samples of the organic almonds were collected and submitted to the Forensic Chemistry Center for analysis. One sample was a bulk bag of almonds, obtained from the store where one of the consumers made his purchase. The second sample consisted of portions taken from pallets of almonds that were placed on hold in the dealer's warehouse. Although bitter almonds are described as being "shorter and rounder" than the sweet almonds with which most U.S. consumers are familiar, the variation in size, shape and color of the organic almonds received made physical separation unreliable.

The results presented will include the detection of cyanide in both ground composites and individual almonds, the optimization of sample extraction protocols for the removal of amygdalin from the product, and the use of amygdalin and cyanide as indicators of the presence of bitter almonds in a bulk shipment of raw organic almonds. **Reference:** 

<sup>1.</sup> Shragg TA, Albertson TE, Fisher, Jr, CJ. Cyanide Poisoning After Bitter Almond Ingestion. West J Med 1982; 136: 65-9.

Cyanide, Amygdalin, Bitter Almonds

#### A14 Morphological Variation in Hair From Mammals of the Order Carnivora

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The goal of this presentation is to illustrate the variability and subtleties of hair structure (and its relationship to function) in mammals of the order Carnivora. HAIRbase<sup>TM</sup> is a valuable online reference tool that can be used by trace evidence examiners to discriminate between the hairs of many carnivores and common furbearers. Potential users will learn a fast and reliable method of accessing morphological information regarding the microscopic and macroscopic characteristics of the hair of many species across the order Carnivora.

This presentation will impact the forensic science community by introducing attendees to HAIRbase<sup>TM</sup>, which offers a wealth of information regarding the structural characteristics of mammalian hair of the order Carnivora and goes beyond traditional references in its coverage of both species and variability on individuals.

Carnivora contains approximately 279 species, separated into 129 genera, and 12 families that collectively display an incredible array of morphological variation in hair characteristics. Owing to this, the microscopic examination of guard hairs is paramount to the forensic identification wildlife. Although primary guard hairs are often used, secondary guard hairs are more variable and, therefore, have the potential to be more diagnostic. Current references and atlases of hair morphology fail to illustrate the range of variation, including hair grades, and regions on the body, of the order Carnivora. A digital database of both primary and secondary guard hairs from three different body regions–dorsal, ventral, and tip of tail–using bright field and scanning electron microscopy images to adequately display the variation inherent in the hair of common carnivores has been constructed.

Animal specimens were obtained from the collections at the U.S. Fish and Wildlife National Forensic Laboratory in Ashland, Oregon and the Biology Department at California State University, Fresno. Hair was collected from each specimen by either plucking or cutting as close to its base as possible with a sterile razor blade. Hair was collected from three body regions: (1) the dorsal region, between the shoulder blades; (2) the ventral region, on the midline between the forelimb and the hind limb; and, (3) at the tip of the tail. Approximately 20-25 hairs were collected from each body region of each individual, for a total collection of approximately 60-75 hairs from each animal. After the hair from each body region was collected, it was placed in separate sterile sealable bags.

Approximately three to five primary and secondary guard hairs were selected from each collection bag. Several hairs of each type were then plated onto individual glass microscope slides using a commercial mounting medium with a refractive index close to that of hair. Each hair on each slide was examined and photographed in a manner that documented microscopic fields containing the most representative hair characteristics for the particular hair type and section under view. A transmitted light microscope coupled with a camera was used to acquire digital images of the basal, sub-shield and shield portions of the hair of each specimen at 200-400 X magnification. Macroscopic and microscopic evaluations of each specimen were conducted. The macroscopic characteristics recorded included hair color, form, and banding pattern. Microscopic observations, such as medullar, cuticle, and cortex characteristics were then recorded. A user interface was created that allows the publishing of website content quickly and easily.

Separating the hair of carnivores requires an aggregation of characteristics in order to make a taxanomic determination. The digital database proposed here will aid investigators by giving them a reliable reference that contains diagnostic information regarding the structure of the hair of the order Carnivora, such as traits of the hair shield, medullary configurations, and cuticle scale patterns that can be used for identification.

This digital database is readily available on the Internet, allowing the addition of specimens and the accommodation of the needs of the forensic trace evidence community in real time. Currently, the database contains over 60 species, 42 genera from 12 families of the order Carnivora. Through the addition of relevant specimens and the ability to adapt to the changing needs of its user groups this digital database will remain relevant and remain a valuable resource to investigators and researchers across multiple scientific disciplines.

Hair, Morphology, Trace Evidence

### A15 Morphological Variation in Hair from Mammals of the Order Artiodactyla

Elsbeth S. Murata\*, and Michael V. Gonzalez, BS, California State University, Fresno, 2555 East San Ramon Avenue, MS SB70, Fresno, CA 93740; and Kevin W.P. Miller, PhD, California State University, Fresno, Chemistry & Criminology, 2555 East San Ramon Avenue, Fresno, CA 93740-8034

The goal of this presentation is to illustrate the variability and subtleties of hair structure (and its relationship to function) in mammals of the order Artiodactyla. HAIRbase<sup>TM</sup> is a valuable online reference tool that can be used by trace evidence examiners to discriminate between the hairs of many artiodactyls, including domesticated and wild game animals that are endangered and highly trafficked. Potential users will learn a fast and reliable method of accessing morphological information regarding the microscopic and macroscopic characteristics of the hair of many species across the order Artiodactyla.

This presentation will impact the forensic science community by introducing attendees to HAIRbase<sup>™</sup>, which offers a wealth of information regarding the structural characteristics of mammalian hair of the order Carnivora, and goes beyond traditional references in its coverage of both species and variability on individuals.

Artiodactyla is an extremely large and diverse order that contains approximately 220 species separated into ten families that collectively display an incredible array of morphological variation in hair characteristics. Owing to this, the microscopic examination of guard hairs is paramount to the forensic identification wildlife. Although primary guard hairs are often used, secondary guard hairs are more variable and, therefore, have the potential to be more diagnostic. Current references and atlases of hair morphology fail to illustrate the range of variation, including hair grades, and regions on the body, of the order Artiodactyla. They also tend to omit the endangered and highly trafficked, which are of the greatest forensic importance. A digital database of both primary and secondary guard hairs from three different body regions-dorsal, ventral, and tip of tail-using bright field and scanning electron microscopy images to adequately display the variation inherent in the hair of select Artiodactyls including many endangered species and African big game animals has benn constructed.

Animal specimens were obtained from the collections at the U.S Fish and Wildlife National Forensic Laboratory in Ashland, Oregon and the Barry Gilcrease collection at California State University, Fresno. Hair was collected from each specimen by either plucking or cutting as close to its base as possible with a sterile razor blade. Hair was collected from three body regions: (1) the dorsal region, between the shoulder blades; (2) the ventral region, on the midline between the forelimb and the hind limb; and, (3) at the tip of the tail. Approximately 20-25 hairs were collected from each body region of each individual, for a total collection of approximately 60-75 hairs from each animal. After the hair from each body region was collected, it was placed in separate sterile sealable bags. Approximately three to five primary and secondary guard hairs were selected from each collection bag. Several hairs of each type were then plated onto individual glass microscope slides using a commercial mounting medium with a refractive index close to that of hair. Each hair on each slide was examined and photographed in a manner that documented microscopic fields containing the most representative hair characteristics for the particular hair type and section under view. A transmitted light microscope coupled with a camera was used to acquire digital images of the basal, sub-shield and shield portions of the hair of each specimen at 200-400 X magnification. Macroscopic and microscopic evaluations of each specimen were conducted. The macroscopic characteristics recorded included hair color, form, and banding pattern. Microscopic observations, such as medullar, cuticle, and cortex characteristics were then recorded. A user interface was created that allows the publishing of website content quickly and easily.

Artiodactyls have become increasingly important to humans both as wild game and domesticated animals. With the increasing importance of artiodactyls in and around society at large, the ability to differentiate hair from these animals has increased significance to forensic examiners. Separating the hair of artiodactyls requires an aggregation of characteristics in order to make a taxonomic determination. The digital database proposed here will aid investigators by giving them a reliable reference that contains diagnostic information regarding the structure of the hair of the order Artiodactyla, such as traits of the hair shield, medullary configurations, and cuticle scale patterns that can be used for identification.

This digital database is readily available on the Internet, allowing the addition of specimens and the accommodation of the needs of the forensic trace evidence community in real time. Currently, the database contains over 73 species, 53 genera from eight families of the order Artiodactyla. Through the addition of relevant specimens and the ability to adapt to the changing needs of its user groups this digital database will remain relevant and remain a valuable resource to investigators and researchers across multiple scientific disciplines.

Hair, Morphology, Trace Evidence

#### A16 A High Throughput Protocol for Using Soil Molecular Biology as Trace Evidence

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After attending this presentation, attendees will understand how soil sample handling and storage can alter soil microbial community fingerprints using capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) and fatty acid methyl ester (FAME) analysis.

This presentation will impact the forensic science community by presenting a method of using soil molecular biology for comparative analysis, which will ultimately lead to more robust crime scene reconstruction.

Trace evidence, although often found in small quantities, can be vital in a forensic investigation. The primary contribution of this form of physical evidence is typically to trace the movement of an object or a person. Soil as trace evidence is the main focus of this experiment. Soil has complex mineralogical, physical, chemical, hydrological, and biological properties that can be specific to its location. These properties can be an accurate way to determine whether a person or an object has been at a certain location.

In order to test whether the storage and/or treatment before storage has an effect on microbial communities within the soil, samples were taken from a depth of zero to five cm at four different locations in Nebraska representing a variety of soil types of varying texture and organic matter content. The samples from each location were divided into seven subsamples. Microbial DNA and fatty acids from the first subsample were extracted immediately, while the other subsamples went through a selected storage treatment under varying conditions (-80 °C, -20 °C, 4 °C, freeze dried, air dried, oven dried). Soil fatty acids and microbial DNA from the stored samples were analyzed at a later date. This process was done for each of the four selected soil types. Fresh samples were collected and processed in the same manner within two weeks of initial collection at each location to determine whether soil microbial communities fluctuate significantly over short periods of time (similar to the time between the commission of a crime and the collection/analysis of evidence). Soil samples will also be collected seasonally to identify the effects of seasonal impacts on microbial communities in the soil.

The polymerase chain reaction was used to amplify and fluorescently tag amplicons of the V3 region of 16S rDNA. This region is highly conserved throughout prokaryotes, yet it has enough variability to allow for different conformations during capillary electrophoresis single-strand conformation polymorphism (CE-SSCP). The CE-SSCP peaks and their relative heights represent the microbial community and its diversity for an individual soil sample. These sets of peaks were compared to determine if soil samples can be matched, thereby locating a crime scene. Fatty acid methyl ester (FAME) analysis was used as a second method to fingerprint soil samples. Results will be presented to show the effectiveness of these two methods to detect small variations in the soil microbial community.

Capillary Electrophoresis, Fatty Acid Methyl Ester, 16S rDNA

#### A17 Reliability in Forensic Science: Pedagogical Implications

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After attending this presentation, attendees will better understand the relationship among the components of reliability analysis.

This presentation will impact the forensic science community by improving the ability of practitioners and educators to assess their knowledge of the components of reliability analyses.

The goal of reliability in forensic science can only be achieved when the concepts relating to uncertainty are understood and implemented by the practitioners. Accomplishing this goal; however, is a real challenge. The uncertainty involved with the different types of examinations varies dramatically and therefore so does the approach to measuring the probability. Learning to quantify probability in a classroom is daunting but even more complex is applying it to the different decision-making styles within the scientific method.

In order to achieve reliability in their scientific examinations, forensic scientists obviously need to know how uncertainty and probability impact each phase of the scientific method. These impacts are: (1) the validity of the theoretical basis requires knowing the probability distribution of the variables being measured; (2) the reproducibility of the empirical protocol requires knowing the expected error rate for the measurement technique; and, (3) the objectivity of decision criteria requires knowing how the observed results compare to the expected probability distribution. Since the nature of the uncertainty is different for each phase of the scientific method, the probability techniques applicable to each phase must also be different.

Reliability analysis; however, is not only a complex subject but is applied in a complex human system. Therein lies the real problem. The typical forensic scientist applies the principles of science within constraints imposed by attorneys, managers, consultants, and reformers who not only are ignorant of the principles but who unabashedly dictate their ignorance via obfuscation and whimsy. The crux of the problem for the profession of forensic science is that forensic scientists cannot protect themselves against the obfuscations posed by those who wish to control them without first eliminating their own ignorance of reliability analysis.

Education is the traditional solution to ignorance. However, in the case of reliability analysis in forensic science, there are some novel challenges due to its comprehensive nature. The three phases of the scientific method involve logic, technology, and decision-making, three topics seldom discussed together. The theoretical basis requires applying inductive, abductive, and deductive logic. The forensic scientist also employs one or more of three types of examination–classification, individualization, and reconstruction–each of which approaches

probability differently. Analyzing uncertainty requires combining the theoretical, empirical, and subjective probability approaches. And, all of these aspects convolute.

Both those who have not performed the particular examinations, and those who have performed them without knowledge of probability, are challenged when trying to teach them. The pedagogical questions thus boil down to three succinct matters: (1) what to teach; (2) how to teach it; and, (3) equally importantly, who to teach it. This presentation will focus on the easiest of the three, what to teach.

This presentation presents a concept map of the key interactions of reliability considerations, providing a topical outline of the key areas. This format summarizes the topics in a way that allows practitioners to review their knowledge of reliability components and assess whether they have the knowledge background to make an informed, reliable decision, not only on the results of the examination but also on the formulation of the questions, and the evaluation of the results. This assessment is an appropriate first step whether one is a practitioner educating oneself, or an educator attempting to educate a practitioner.

an educator attempting to educate a practitioner.

Reliability, Forensic Science Education, Probability

# A18 The Implementation of the Brazilian DNA Database

Guilherme S. Jacques, MSc\*, Hélio B. Lima, PhD, and Paulo R. Fagundes, BS, SAIS 07, Lote 23, Brasília, 70610-200, BRAZIL

After attending this presentation, attendees will learn how the Brazilian DNA Database was planned and implemented as well as understand the perspectives for the evolution of this database. Issues to be addressed include the crime and violence scenario, the evolution of the DNA laboratories, the implementation of the FBI's CODIS, and the architecture of the system and the difficulties of such an effort in a developing country where the Constitution strongly emphasizes the individual rights.

This presentation will impact the forensic science community describing the national DNA database implementation in a developing country and providing elements to better understand the evolution and application of this technology outside the United States and Europe.

In a dedicated effort to fight against the impunity through forensic science, during the last six years Brazil has developed an organized network of DNA laboratories and has finally implemented a national DNA database. In 2004 there were only five DNA crime labs and this number reached 17 in 2010. DNA technology has a great potential to change the high crime rates but the expansion of the program and the change of the legislation is a major challenge.

The creation of a federal DNA program in 2004 was the catalyst to spread the DNA technology in Brazil. This program permitted the reinforcement of the existing DNA labs, the creation of new labs, the education and training of analysts and the formation of a group of specialists who established the main guidelines of the program and the technical procedures of the analysis. The program included a high degree of cooperation between labs. Brazil has 27 states and analysts from states without a DNA lab could perform tests in the most experienced labs under supervision.

In 2009, Brazil signed the letter of agreement to use the FBI's Combined DNA Index System (CODIS) software and in the same year CODIS 6.0 was used to identify the victims of the Air France flight disaster. In 2010, the largest installation of CODIS software outside the United States was made, including in 15 state labs, one federal lab, plus the national databases of both CODIS 5.7.4 and CODIS 6.1.

A recent study showed that Brazil has the sixth highest rate of homicides across the globe (25.8 homicides in 100,000 inhabitants/year) and some studies show similar findings in sexual crimes (official statistics is about nine sexual crimes in 100,000 inhabitants/year but the actual number might be much higher due to the lack of reporting). The crime

solving rate is extremely low, with less than ten percent of the murderers being identified and convicted, and the absence of physical evidence is one of the main causes of this situation. It is clear that the expansion of the DNA database program would benefit Brazilians but the current understanding of the Constitution established in 1988, after a dictatorial military regime, is a major constraint. Suspects and convicted offenders have the right to refuse providing reference samples. The strategies to overcome this difficulty will be discussed.

Brazil, CODIS, DNA Database

#### A19 Internal Validation of Yfiler<sup>®</sup> for Casework at the Saint Louis Metropolitan Police Department

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After attending this presentation, attendees will be able to perform an internal validation of Yfiler<sup>®</sup>. Attendees will have an idea of what tests should be conducted for a Yfiler<sup>®</sup> internal validation and the results that could be expected. They will also gain an understanding of why Yfiler<sup>®</sup> is important technology for DNA casework and its limitations.

This presentation will impact the forensic science community by informing attendees about the Yfiler<sup>®</sup> benefits and limitations.

AmpFlSTR® Yfiler® amplification kit is a Short Tandem Repeat (STR) kit specific to the sex determining region of the Y-chromosome. Yfiler<sup>®</sup> kit can be used to detect male DNA from samples where a male profile cannot be generated from an autosomal kit. Yfiler® kits are valuable when a large presence of female DNA is in a sample as compared to the male DNA, when absence of sperm in an azoospermic male prevents a differential extraction, and in determining the number of individuals in a multiple assailant rape. Most of these instances prevent a full male profile from being generated. Single source Yfiler® profiles typically consist of haplotypes containing one allele at each locus. A single source Autosomal kit profile consists of diplotypes containing one or two alleles at each locus. Multiple alleles can be expected in situations of multiple assailant rape. Thus the Yfiler® kit may be useful in determining the number of assailants in a multiple assailant rape. Haplotypes only show one allele at most loci for each person involved. The manufacture conducts developmental validations that aids in the laboratory's internal validation when implementing the Yfiler® STR kit. The St. Louis Metropolitan Police Department hypothesized, based on SWGDAM guidelines, the developmental validation preformed by Applied Biosystems and the work of Gross et al. (Journal of Forensic Science, 2008, Vol. 53, No. 1), the internal validation of Yfiler® kit for casework could be performed by completing sensitivity, precision, mixture, probative/non-probative, stutter, and specificity studies. DNA was extracted from male and female lab members and from probative and non-probative sources for use in the internal validation studies. The nonprobative samples were collected in various common places in and around the lab. Examples include door handles and other commonly touched items including a parking pass button from a nearby parking lot. Probative sources were collected from cases previously adjudicated. The concentration of the DNA was determined using Quant Duo and diluted to appropriate concentrations depending on the study being conducted. DNA was amplified on an AB 9700 using the Yfiler® kit and electrophoresis was conducted using a 3130 genetic analyzer. Results of the experiments were analyzed using the AB software Genemapper v3.2. The results of the sensitivity study showed the target amount of DNA for an amplification using Yfiler<sup>®</sup> is 0.8 ng, which gave average RFU levels of 1012.25. A full profile was generated with 0.1 ng of DNA. Allele drop

out was seen with 0.08 ng template DNA in most of the loci. The peaks were still present below Genemapper's threshold for allele calls of 50 RFU. The results of the precision study suggested amplifications using Yfiler<sup>®</sup> were precise across multiple injections of samples. The mixture study showed Yfiler<sup>®</sup> to amplify only male DNA in the presence of a large amount of female DNA. Male mixtures could still generate male profiles, which could be deconvoluted in some cases or at least suggest how many people were in the mixture. The probative/non-probative study suggested the Yfiler® kit could work with casework consistent with the samples encountered by the lab. The stutter study generated an anomaly in the male B NIST standard that was reproducible in multiple extractions and in multiple injections. The specificity study showed the primers used in the Yfiler® kit were specific to human male DNA and did not cross react with dog, chicken, cow, orangutan, baboon, deer, or mouse DNA. The Yfiler® kit has limitations of not being as discriminative, since the loci are physically linked, as autosomal kits with 16 loci. Additionally the Ychromosome is shared in paternal lineages making the resulting profile less unique within families. The completion of these internal validation studies will allow the lab to start using the Yfiler<sup>®</sup> kit in casework. The laboratory can increase the efficiency of determining the male(s) associated wit'h a crime.

Yfiler<sup>®</sup>, Validation, Male

#### A20 An Interesting Mutation at Locus DYS385 in an Uncle-Nephew Pair in a Fatherless Paternity Case

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After attending this presentation, attendees will understand how the Y-chromosomal short tandem repeat polymorphisms (Y-STRs) included in the AmpFISTR® Yfiler® amplification kit are currently used for forensic and evolutionary applications, therefore a consistent knowledge on mutation properties is necessary for correct data interpretation.

This presentation will impact the forensic science community by presenting the necessity to know mutation rate of Y-STRs according to ISFG guidelines.

The Y-chromosomal short tandem repeat polymorphisms (Y-STRs) included in the AmpFISTR® Yfiler® amplification kit are currently used for forensic and evolutionary applications. Therefore knowledge on mutation properties is necessary for correct data interpretation.

Recently, in a fatherless paternity case, an interesting mutation at locus DYS385 in an uncle/nephew pair was observed. The alleged father was unavailable due to his death several years ago in South America; where he is currently buried. Consequently the paternity test was conducted without the father's DNA profile.

DNA typing was conducted on the reference samples from the son, his mother, and his alleged uncle (father's brother) using AmpFlSTR<sup>®</sup> Identifiler<sup>®</sup> amplification kit. The obtained data showed that in this particular fatherless case the analysis of 15 STRs was not sufficient to establish the paternity. The paternity probability value was improved by conducting additional DNA typing using three miniSTRs markers (NC01 systems: D10S1248, D14S1434, D22S1045) and AmpFlSTR<sup>®</sup> Yfiler<sup>®</sup>. The combined DNA profiles obtained were statistically analyzed with Probabilistic Expert Systems (PES) FINEX and Familias; a high paternity probability value was of P=0.9993 obtained. The relationship between the son and his uncle was confirmed by conducting Y STR typing using the AmpFISTR® Yfiler® amplification kit. However, an incompatibility at locus DYS385 was observed; the uncle's genotype was 15-16 opposing the nephew's genotype of 14-16 at that locus. A second amplification of the son and the alleged uncle samples was conducted using the PowerPlex Y amplification kit (Promega) and the mutation was confirmed.

PCR was carried out using AmpFISTR<sup>®</sup> Identifiler<sup>®</sup>, AmpFISTR<sup>®</sup> Yfiler<sup>®</sup> (Applied Biosystems), PowerPlex Y (Promega) amplification kits, and three miniSTRs markers with a homemade multiplex. All PCR products were detected by capillary electrophoresis in the ABI Prism310 Genetic Analyzer and alleles were typed using Genemapper software. Experiments were performed according to the ISFG guidelines.

Sequencing of the DYS385 locus was performed. DNA was extracted from two blood samples using DNA IQ<sup>TM</sup> System (Promega) according to the manufacturer's protocol. DYS385 alleles were amplified using available primers on Gene Bank (Accession code: AC022486). The separated alleles were removed by means of surgical scalpel and placed in a spin tube with Chelex (20%) incubated overnight at 56°C. Three cycles of freezing – thawing were performed. The amplification products were recovered, re-amplified using the same conditions, purified with Exosap, sequenced with BigDye Terminator v 1.1 kit (Applied Biosystems), re-purified with Centrisep Columns and finally detected by capillary electrophoresis in the ABI Prism 310 Genetic Analyzer. The sequenced alleles showed a regular repeat structure [GAAA] with 15 repeats in one case and 14 repeats in the other.

Mutation Rate, Y-STR, Probabilistic Expert Systems

#### A21 STR Data for Three Closely Linked X-Chromosomal Markers in an Argentinean Population

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After attending this presentation, attendees will be become familiar with X-chromosome STR loci data from an Argentinean population.

This presentation will impact the forensic science community by providing statistical data of forensic interest for three closely linked STR markers located on the X-chromosome in an Argentinean population.

Autosomal (AS) and Y-Chromosome (ChrY) STRs play a pertinent role within forensic science, but can be limited in their applications. Therefore the application of X-Chromosome (ChrX) markers may be necessary in cases where a biological sample from the putative father is unavailable and a sample for analysis is from a paternal relative. ChrX are also useful within trace analysis, complex kinship and incest cases. Furthermore, the use of ChrX makers may be beneficial for anthropological purposes. Despite the numerous applications, ChrX is rarely used within forensic practice.

Forensic application of ChrX markers can be difficult due to the lack of data. Research continues to find suitable markers. Rather than performing analysis with a single STR locus, it is beneficial to utilize a cluster of closely linked ChrX STR markers. These markers could potentially produce stable haplotypes for forensic use.

The study consisted of a metropolitan population database containing ChrX STR markers DXS10079, DXS10074, and DXS10075 that are within a 280-kb region at Xq12. Blood and/or buccal swabs were obtained from 100 males and 100 females from the Argentinean population. The samples were extracted by the chelex method or by using a blood mini kit, quantified by one step real time PCR, amplified and genotyped. Upon determination of the sample's profiles, statistical

analyses were performed. Population analyses tested for deviation from Hardy-Weinberg equilibrium and linkage disequilibrium. Parameters of forensic interest were also determined, including polymorphic information content (PIC) and power of discrimination (PD). The statistics obtained determined the use of the three ChrX markers within an Argentinean population appropriate for forensic usage.

X-Chromosome, Population Database, STR

#### A22 Entire mtGenome Sequencing: A Strategy for High-Quality Samples

Elizabeth A. Lyons, MFS\*, Kimberly Sturk-Andreaggi, MFS, Jodi A. Irwin, PhD, and Rebecca S. Just, MFS, 1413 Research Boulevard, Rockville, MD 20850

After attending this presentation, attendees will gain an understanding of considerations particular to entire mtGenome sequencing and insight into the development and implementation of an automated mitochondrial genome (mtGenome) protocol for population and reference samples.

This presentation will impact the forensic science community by providing an amplification and sequencing strategy designed to produce complete forward and reverse sequence coverage over the entire mtGenome with minimal manual reprocessing.

There has been a steady rise in the number of entire mitochondrial genome (mtGenome) haplotypes generated for medical genetic, phylogenetic and population studies, as well as for forensic applications in recent years. Although the number of complete human mitochondrial DNA (mtDNA) sequences in GenBank now exceeds 6500, many of these sequences contain errors and few meet the criteria for use as forensic reference data. Given this, along with new techniques that will simplify access to mtDNA coding region data in forensic specimens, the creation of mtGenome reference databases appropriate for forensic use is needed.<sup>1</sup> However, the development of complete mtGenome haplotypes is labor intensive, expensive and fraught with opportunities for human error. An optimized, automated process is thus essential for high volume generation of mtGenome reference data that meet forensic standards.

The Armed Forces DNA Identification Laboratory (AFDIL) has generated more than 500 complete mtDNA sequences using a 12amplicon, 108-reaction Sanger sequencing strategy over the past ten years.<sup>2,3</sup> Though the process results in error-free data, the protocol, originally developed more than a decade ago,<sup>4</sup> frequently requires extensive manual reprocessing to produce complete haplotypes. Therefore the goal of this work was to develop an automated amplification and sequencing strategy for high-quality (non-degraded) samples that would routinely produce data sufficient to cover the entire mtGenome.

Suitable placement for amplification and sequencing primers was assessed using published mtGenome substitution rate data and haplogroup-specific polymorphism information.<sup>5,6</sup> A web-based tool was then used for amplification and sequencing primer design. Candidate amplification primer pairs were subjected to varying PCR conditions to determine optimal thermal cycling parameters, and a capillary-based detection instrument was used to gauge amplification efficiency. Enzymatic, column, and bead-based purification protocols were evaluated to balance efficacy, cost, and automation. Sequencing primers were subsequently tested to assess sequence data quality, defined by the degree of "background" sequence and the range of high-resolution data.

An eight-amplicon mtGenome strategy that allows eleven samples to be processed on each 96-well amplification plate was designed. Amplification set-up was performed on a robotic instrument to ensure correct sample placement, and an optimal PCR extension time has been implemented. Amplification success was assessed using an automated capillary electrophoresis system that requires no manual pipetting. Amplicons, which range in size from 1,197 to 2,544 base pairs, were sequenced in 16 reactions each for a total of 128 sequencing reactions per sample. Alternating forward and reverse sequence primer placement ensured redundant sequence coverage across the entire mtGenome. All post-PCR pipetting steps (post-amplification purification, sequencing setup, post-sequencing purification and sequence detection set-up) were performed robotically, and sequence detection was performed on a 48capillary electrophoresis instrument.

This optimized, highly automated protocol reduced cost, hands-on laboratory time, and opportunities for human error by substantially decreasing the number of manual production steps and the extent of sample reprocessing necessary to construct complete mtGenome haplotypes. The high-throughput strategy will facilitate regular generation of high-quality entire mtDNA profiles for forensic reference databases and other applications which would benefit from error-free mtDNA data.

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Mitochondrial DNA, Sequencing, Protocol

#### A23 Determination of an Effective Housekeeping Gene for the Quantification of mRNA for Forensic Applications

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After attending this presentation, attendees will understand the underlying concepts of current serological methods, the need to establish techniques that confidently and precisely identify biological fluids other than those commonly used in blood and semen identification, the importance of mRNA quantification, and the impact that this procedure might have on serological applications.

This presentation will impact the forensic science community by providing insight into a method that might improve the already existing technologies for the identification of body fluids.

The potential application of mRNA for the identification of biological fluids using molecular techniques is a recent development in

forensic serology. However, the rate of degradation and instability of mRNA is relatively high compared to DNA. Since the amount of biological material is often limited in forensic cases, it is critical to determine the appropriate quantification method to gather the most amount of information from a given sample. Constitutively expressed housekeeping genes can be used to assess the amount of mRNA recovered from a sample and establishes its suitability for downstream applications using real-time polymerase chain reaction (RT-PCR).

After an in-silico query to determine commercial availability, amplicon size, level and consistency of expression among a variety of tissues, six housekeeping genes were selected for this study. Relative quantification was utilized to compare the degree of expression of the selected genes from forensic-like body fluid stains obtained from semen, saliva, blood, menstrual blood, and vaginal secretions, in order to establish which would be the most appropriate housekeeping gene for the assessment of human mRNA quantity prior to profiling. Five consenting donors provided the samples of the various fluids. The housekeeping genes glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta actin (ACTB), beta-2-microglobulin (B2M), cyclophilin A (PPIA), phosphoglycerate kinase 1 (PGK1), and ribosomal protein large P0 (RPLP0), were compared to determine which was the most consistently expressed between the different donors for any one of the examined body fluids. The number of cycles necessary for the sample fluorescence to exceed that of the background fluorescence, called the cycle threshold (Ct), were compared using GAPDH as a reference for normalization across samples.

The results indicated that overall,  $\beta$ -2-microglobulin and  $\beta$ -actin exhibited the highest expression level across all body fluids examined. The same results were observed when an across donor comparison was made. In addition, both genes were highly expressed after exposure to environmental conditions (June, no precipitation) for up to 24 hours, indicating that mRNA is suitable for analysis if recovered during this period of time.

Consequently, ACTB or B2M appear to be the best candidates from the set of housekeeping genes analyzed for human-specific mRNA quantification prior to mRNA profiling for forensic applications. Serology, Messenger RNA, Real-Time PCR

#### A24 ABO Blood Group Antigens and Mucin-Like Proteins in Human Buccal Cells

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After attending this presentation, attendees will gain an understanding of previously undescribed polymorphisms in buccal cell proteins, their expression of ABO blood group antigens and their comparison to soluble salivary fluid proteins. Knowledge of salivary and buccal cell proteins may translate into applications for forensic testing in pathology and drug abuse.

This presentation will impact the forensic science community by showing how salivary components such as soluble proteins and buccal cells can be analyzed by fluorescence microscopy, microtiter plate assays, and lectin staining of electrophoresis gel transfers, and by informing attendants that saliva represents an easily accessible body fluid and is often present at crime scenes.

Buccal cells are a major component of saliva and are a major source of DNA for forensic and biomedical analyses. Little is known; however, about the protein components of these cells but such information could be used to identify biomarkers for forensic testing and for pathological conditions such as exposure to toxic chemicals and chronic drug abuse.

The characterization of buccal cell and cell-free salivary fluid proteins, their variations among individuals, and their reactivity with Ulex europaeus-I lectin was studied by different techniques. Buccal cells were isolated from whole saliva and washed twice by centrifugation and resuspension in fresh buffer. The identification of blood group O-bearing glycoproteins was conducted by separating, buccal cell proteins by SDS gel electrophoresis and electrotransferred to nitrocellulose. These gel replicas were incubated with biotinylated UEA-I lectin which recognizes blood group O. The proteins bound by UEA-I were revealed by binding of streptavidin conjugated to alkaline phosphatase and a colorimetric substrate. The same approach was used to identify proteins bound by other lectins such as those from peanut agglutinin, Artocarpuis integrifolia and Vicia Villosa which recognize galactos and N-acetyl galactosamine terminated saccharides on glycoproteins.

In order to test for the presence of blood group O structures on intact buccal cells, buccal cells were examined by fluorescence microscopy after incubation with fluorescent UEA-I (UEA-I FITC) or biotinylated UEA-I followed by Alexa conjugated streptavidin.

The relative amounts of blood group O antigens on buccal cells and in cell free saliva from different donors were measured by binding of UEA-I to samples immobilized on microtiter plates.

After biotin-UEA-1 staining of nitrocellulose transfers of Laemmli SDS electrophoresis gels of 48 salivary fluid samples from different individuals, 42 had staining of the stacking gel. A 150 kDA band was present in 20 samples, a 75-100 kDA was found in 22 samples and six samples had UEA-1 staining in the region 40-50 kDa. Six out of nine buccal cell samples had UEA-I binding in the stacking gel of nitrocellulose transfers and differed in staining of 130kDa to 200 kDa proteins.

Five of eight salivary fluids and three of four buccal cell samples coated onto microtiter plates bound biotin-UEA-1 and the amount of binding varied among individuals for the same number of cells. By fluorescence microscopy, five out of seven buccal cell preparations from donors with different ABO blood groups bound UEA-1 and the staining intensity varied among individuals.

A number of novel findings were made in this study: (1) a high molecular mass glycoprotein with blood group O antigensthat bound UEA-I lectin was identified in human buccal cells; (2) buccal cells from different individuals varied in the amount of UEA-I lectin they could bind and in the molecular masses of UEA-I binding proteins; (3) the high molecular mass UEA-I binding glycoprotein only poorly bound to lectins such as PNA, JCA and VVL that characteristically recognize O-linked oligosaccharides; and (4) A lower molecular mass 120kDa-200kDA buccal cell protein did not bind UEA-I lectin but did bind to PNA, JCA and VVL lectins.

Saliva, Buccal Cells, Glycoproteins

#### A25 Interesting Case - XO Male in Forensic Casework

Robin Freeman, MS, MBA\*, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will become familiar with an unusual STR genotype for a male individual and the ways in which male quantification and Y-STR typing can be used to aid in the confirmation of male DNA.

This presentation will impact the forensic community by providing education and a case study on a forensic casework sample that appeared genotypically female but was actually male. Attendees will observe how male quantification and amplification using Y-STRs can help resolve apparent discrepancies such as these.

A homicide case was submitted to the Harris County Institute of Forensic Sciences-Forensic Biology section for analysis. The case involved two male defendants charged with capital murder for the beating and shooting death of a victim during a convenience store robbery. Swabbings taken from a bat used during the commission of the crime and swabs from the counter area where the crime occurred were
presumptively positive for blood. DNA analysis was performed on these samples and the DNA profiles obtained were compared to the known reference samples from the defendants and the victim. Mixtures of DNA were obtained from the bat and two of the counter area samples which were consistent with the reference from the victim (major contributor). The suspects were excluded from these mixtures. Single source DNA profiles were obtained from two of the counter area samples that were also consistent with the reference sample from the victim.

The profiles from the crime scene, which were consistent with the victim, yielded an "X" at the Amelogenin locus. Since the decedent in this case was male, a genotype of "XY" was expected at the Amelogenin locus. Three explanations for this discrepancy were explored: (1) the absence of a "Y" chromosome was due to the fact the victim was female; (2) deletion on the Y-chromosome; and, (3) mutation in the primer binding site of the AMEL locus on the Y-chromosome of this individual. The autopsy of the victim also had been performed; therefore the medical examiner on the case was contacted for more information. The medical examiner confirmed that the victim was physically male.

The Forensic Biology section had validated and implemented combined human/male DNA quantification and Y-STR analysis during the tenure of this case. It was decided to use these DNA analysis tools to resolve the issue of the discrepancy of the sex of the victim. The commercial human/male quantification kit used determines the amount of human DNA in a sample and also utilizes a male probe that targets the sex determining region of the Y chromosome (SRY) to determine the amount of male DNA present in a sample. Male DNA was detected at quantification. To determine whether a region of the Y-chromosome had been deleted or a point mutation in the primer binding site of AMEL was the culprit, Y-STR testing was performed on the victim's standard. A full Y-STR profile was obtained from the victim's reference sample with the exception of DYS458 which is the closest locus to the AMEL locus of the Y-STR loci tested.

Literature research documented that Amel Y and DYS458 are adjacent to each other on the Y chromosome (6.79 Mb and 7.92 Mb, respectively). Therefore, a deletion occurring within this area could affect the detection of both loci. Publications have also documented a 3.2-3.6% sex test failure rate in individuals of Indian descent; the victim in this case was of Indian descent. This case study demonstrated the advantages of a quantitation system that determines both human and male DNA and the utility of Y-STR analysis to provide a comprehensive analysis of genetic information in casework samples.

Amelogenin, Y-STR, STR

## A26 Protein Based Identification of Epithelial Cell Types in Forensic Samples

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After attending this presentation, attendees will be aware of the proteomic studies being undertaken to identify a biomarker that will specifically identify vaginal cells from buccal cells.

This presentation will impact the forensic science community by advising on epithelial cell identification.

The use of DNA technology to identify people associated with a crime scene has been one of the most important weapons in the arsenal of the forensic scientist. However, research in forensic biology is now moving towards questions that cannot be answered using DNA analysis alone. Although the value of DNA profiles is indisputable, there is increasing importance in also identifying the cellular source of the DNA, as evidence regarding the cellular source from which the DNA profile originates increases the evidential value of the sample. One example where the cellular origin of a DNA profile is important is in sexual assault investigations where it is alleged that an object, such as a bottle, is used

in the assault. Neither side disputes that the complainant's DNA is on the bottle, but the defence may suggest she had simply handled, or drank, from the bottle, while the prosecution claim she was sexually assaulted with it.

Epithelial cells are of particular interest, as samples such as oral or vaginal cells are indistinguishable by other methods. Previous work has resulted in the development of a histological staining method that has application in the identification of vaginal epithelial cells. However, during validation studies it was shown that this method was best applied to samples that contained a high concentration of a single type of cell, and was less effective for identifying the components of cell mixtures. A method that is based on the presence, or absence, of a particular marker that is specific to an epithelial cell type would greatly assist in the identification of, and subsequent selection of, cells from cell mixtures. After the identification of protein specific markers for vaginal and buccal epithelial cells, we propose to use an immunohistochemical method to label cells containing the cell specific protein marker from mixed cell samples commonly encountered in forensic casework. One of the benefits of a cellular identification test based on immunohistochemistry is that it is compatible with laser microdissection analysis. Laser microdissection allows removal of selected cells from a slide sample of mixed cells, from whence a DNA profile can then be isolated.

Several candidate protein markers, known to be present in various types of epithelial cells, and have investigated whether or not any of these proteins have potential for use as cell specific markers for buccal or vaginal epithelial cells were selected. Western analysis was used to initially test the specificity of these putative biomarkers. Any proteins that show differential expression after western analysis are then progressed to the next phase of specificity testing by undertaking immunohistochemical analysis of intact cells on microscope slides.

Cell Identification, Vaginal, Buccal

## A27 A Comparison of Chemical Enhancements for the Detection of Latent Blood

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After attending this presentation, attendees will have reviewed the components of blood, the basic principles and biochemical reactions behind the major serological tests for latent bloodstains, and understand the effects that dilution and substrate play in observed sensitivity of the tests. Limitations of the tests will be discussed. Participants will also be able to better interpret future research in serological blood testing as it can be applied to forensic casework, as impacted by the type of blood or artificial blood used in testing.

This presentation will impact the forensic science community by presenting a careful and balanced evaluation of the modern detection methods for latent bloodstains, by exploring differences in observed sensitivities, and making appropriate conclusions for applying the research to forensic casework, as dependent upon the type of blood or blood substitute used in the research.

In forensic investigations, the presence of latent bloodstains can be critical information to the case. Often, the blood is not detectable for a variety of reasons, to include time, weather, and attempts by the perpetrator to clean the crime scene. In these types of cases, the use of forensic chemical enhancements for the detection of blood is important for location of the latent stains, so that subsequent confirmation, followed by forensic DNA analysis, can be performed at the laboratory.

Luminol and Fluorescein are chemicals commonly used in the detection of latent bloodstains. Both classes of reagents, including commercial preparations, share a common chemical pathway in that they

result in a visible chemical reaction based on the peroxidase-like activity of blood, specifically the Heme group of the Hemoglobin protein in red blood cells. Both tests present the Heme group with a substrate to act upon, releasing free oxygen radicals. These free radicals act upon the chemical indicators to yield a visible response. In the case of luminol, 3aminophthalhydrazide is oxidized to 3-Aminophthalate, releasing energy in the form of visible light. In regards to the fluorescein reaction, fluorescin is oxidized to fluorescein, which fluoresces when under a 450nm alternate light source. Both methods require relative darkness for visualization. While the fluorescein method requires an additional light source and filter to visualize, it is nontoxic. In contrast, the luminol reaction requires no additional equipment, but dries to leave a residue that is slightly toxic.

This study compared and contrasted the relative sensitivities and specificities of laboratory and commercial preparations of the luminol and fluorescein detection tests. This comprehensive study also examined the relative sensitivity of each reagent in regards to differing substrates, ranging from smooth fabrics to carpet, tile and hardwood, porous and nonporous surfaces, and the effect of colors and dyes on the substrate in altering observed sensitivity of the tests. As a further exploration, each preparation was tested against fresh human blood with no additives, as well as synthetic, pig blood and human blood collected in EDTA-vacuum tubes, all commonly used in blood detection forensic research. Results indicated that observed sensitivity varies depending on commercially prepared versus homemade preparations, and, as seen in other studies, that sensitivity is drastically impacted by the substrate that the latent bloodstain is present upon.

**Blood Detection, Latent Blood, Enhancement** 

### A28 Optimization and Application of DNA Repair Enzymes to Damaged DNA for Short Tandem Repeat DNA Analysis

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After attending this presentation, attendees will be familiar with environmental and chemical agents causing DNA damage, the need for methods to repair such damage, and the importance and impact of a successful repair method for Short Tandem Repeat (STR) DNA analysis of damaged samples.

This presentation will impact the forensic science community as it discusses a method that may augment traditional STR analysis by restoring previously unobtainable and incomplete STR profiles.

In forensic investigations, DNA analysis plays a major role in human identification. However, DNA evidence collected from a crime scene may have been damaged from exposure to environmental and/or chemical stresses. UV radiation, heat, humidity, and oxidation have been shown to damage DNA, generating double-strand breaks, single-strand nicks, and/or modified bases. Such damage may prevent procession of DNA polymerase during Polymerase Chain Reaction (PCR), inhibiting STR amplification, and potentially resulting in full or partial loss of the DNA profile.

In living cells, excision repair pathways can correct lesions in DNA caused by either endogenous processes or exogenous agents. These repair mechanisms include enzymes such as glycosylase to excise modified or mismatched bases, endonuclease to remove abasic sites, DNA polymerase to fill in gaps, and DNA ligase to seal nicks in the DNA.

DNA evidence recovered from a crime scene, however, does not have the protection and benefit of these cellular processes. Thus, previous studies have investigated using and applying mixtures of these enzymes to repair damaged DNA in vitro.

In this study, a commercially available DNA repair kit was evaluated for the repair of damaged DNA samples showing partial STR profiles. HL-60 cell line DNA was damaged artificially with UVC radiation, heat/acid, and oxidation treatments. Damage was assessed initially using a human DNA real-time PCR quantification assay. An increase in cycle threshold value for treated DNA compared to untreated DNA indicated DNA damage. STRs from the artificially damaged DNA were amplified, separated using capillary electrophoresis, and analyzed. Damaged DNA samples that produced partial STR profiles were chosen to test and optimize the DNA repair kit.

The initial results indicated that using the repair kit following a protocol developed by the Bureau of Alcohol, Tobacco, Firearms and Explosives and Armed Forces DNA Identification Laboratories for forensic-like samples was more effective in repairing damaged DNA samples than the manufacturer's standard protocol. This modified protocol was optimized for DNA repair with respect to repair reaction time, temperature, and repair mix volume. The optimized repair protocol was then applied to DNA samples showing partial STR profiles that had been extracted from UVC- and environmentally-exposed bloodstains.

The results indicated that when using the modified and optimized protocol, treatment of damaged DNA with the repair enzyme mixture led to an overall increase in average peak heights for STR analysis. Particularly for the larger STR loci, repair successfully recovered alleles that had previously shown peak heights below the detection (50 rfu) and/or stochastic thresholds (200 rfu).

The modified repair method may provide a means to obtain a full STR profile from environmentally and/or chemically damaged DNA that would otherwise be refractory without multiple, time-consuming treatments. In addition, the modified method only requires a few additional steps that could easily be incorporated into the current STR analysis procedure.

Damaged Samples, DNA Repair, STR Analysis

#### A29 Voluntary Interruption of Pregnancy (VIP): STR Profile From Chorionic Villus as Evidence of Sexual Violence

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After attending this presentation, attendees will understand the main issues associated with the isolation of chorionic villus from maternal decidua and subsequent DNA extraction to obtain a genetic profile of the product of the conception. Moreover, the possibility of comparing this profile with that of the suspect represents important evidence used in cases of sexual violence.

This presentation will impact the forensic science community by discussing how the suggested methodology proves useful for the purpose of identifying the profile of a fetus, as well as in cases of sexual violence where the identity of the suspect is known and there are no other elements that may be used in order to ascertain the truth.

Italian law regulates the voluntary interruption of pregnancy (VIP) under the Law no. 194 of March 22, 1978. VIP is not to be considered as a tool for birth control. However, where the woman reports circumstances by which the continuation of the pregnancy, the delivery, or the maternity would imply severe risks to her physical or mental wellbeing, within the first ninety days she can address a family guidance center, a social healthcare facility, or a trusted physician. In the presence of conditions signaling the need for urgent intervention, the latter will immediately issue the woman with a certificate stating the emergency. With this certificate the woman can go to one of the centers authorized to carry out the interruption of pregnancies. The institution is bound to send the provincial physician a notice declaring the intervention has taken place as well as sending the relevant documentation on the basis of which the intervention was made. No reference to the identity of the woman is made.

The originality of the methodology described here is underlined by the fact that in literature, unlike the case of prenatal diagnosis, not many descriptions are available regarding cases of investigation for obtaining an individual profile on the product of an abortion within the third month of gestation for justice purposes.

In the case under study, the pregnancy of the woman who had suffered sexual violence was not beyond the 90<sup>th</sup> day of gestation when she lodged the complaint and there was evidence of serious risks for her mental integrity. The product of the abortion was seized by the Investigating Authority immediately after the abortion and was frozen at a temperature of -20 °C until the enactment of genetic investigations. The profile of the fetus was obtained from the chorionic villus isolated from the residuals of maternal decidua. The methodology applied was not particularly complex. Several tissue fragments were collected which, at a first macroscopic observation, and at the following histological observation, certified their derivation from the trophoblastic syncytium. Particularly noteworthy was the presence of edges of normally structured pregnancy decidua, with epithelialized chorionic villus and tissue fragments belonging to the foetus and having a regular morphologic appearance. The sample fragments were subsequently rinsed with PBS at pH 7.2 and finally washed in SDS 2% (P/V), and subjected to centrifugation in order to separate the supernatant from the material at the bottom. The operation was carried out several times.

The genetic profile of the rape victim was obtained from a blood sample taken during the abortion, while the profile of the suspect was obtained from a saliva specimen left on a coffee glass and on the spoonlike stick used to sweeten it.

The samples were then treated with a lysis solution containing protein kinase K and SDS. The extracted DNA was purified via selective adsorption on silica gel columns and subsequent elution in a TAE buffer. The DNA amount in the extracts was assessed with a REAL-TIME PCR. Individual profiles were obtained with multiplex amplification and following separation in capillary electrophoresis.

The comparison of the genetic profile obtained from chorionic villus with those of the mother and the suspect showed, with the exclusion of the allele deriving from the mother, that for any genetic area under study, the suspect always had a common allele with the genetic profile obtained from the chorionic villus.

From a statistical elaboration of the results it could be inferred that this sharing was not random.

The suggested methodology proves useful for the purpose of identifying the profile of a fetus, as well as in cases of sexual violence where the identity of the suspect is known and there are no other elements that may be used in order to ascertain the truth.

VIP, STR Profile, Sexual Violence

## A30 Accuracy Matters When Quantitative, Manually-Pipetted PCR Assays Transfer to Automation: A Story in Diagnosing and Troubleshooting

Keith J. Albert, PhD\*, Artel, 25 Bradley Drive, Westbrook, ME 04092; and Lisa Knapp, MS, Agilent Automation Solutions, 5301 Stevens Creek Boulevard, Santa Clara, CA 95051

After attending this presentation, attendees will learn how to successfully transfer a manually-pipetted assay to automation by paying attention to liquid handling variables and processes.

This presentation will impact the forensic science community by presenting a case in which the automation was not to blame. Many assays are transferred from the bench (handheld pipetting) to liquid handlers with success; when they are not successfully transferred, one has to look at all variables.

It is often the case that assays are initially performed on the benchtop using handheld pipettes before they are transferred to an automated liquid handler. Automating a manual method may take time and patience, but automation will help lower costs, increase throughput, and potentially avoid errors associated with a manual method. During the transfer process, however, the manual assay should be directly compared to the automated assay for consistencies in pipetting performance. An undetected variability in accuracy will impact the integrity of the assay as the automation process continues. Liquid handling accuracy and precision information, for both the manual and automated method, are critical to determine any deviation of dispensed volumes between the two processes. Therefore, as it is shown in this presentation, that validating the liquid delivery steps for each assay will help uncover discrepancies in pipetting performance. This presentation discusses the importance of knowing both accuracy and precision information when a manual method is transferred to a robotic liquid handler. It was determined that the ratelimiting reagent in the RT-PCR assay was not being accurately pipetted between the manual and automated methods per the protocol, and the automation was not to blame.

Method Transfer, Automating PCR assaysA, Pipetting and Liquid Handling

#### A31 Geographic Origins of Illegally Harvested Hawksbill Sea Turtle Products

Elizabeth F. Shattuck, BS\*, and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will learn about the extraction of mitochondrial DNA (mtDNA) from illegal tortoiseshell items made from hawksbill sea turtles shells. The origin (natal beach) of the poached sea turtle can be estimated using mtDNA haplotype analysis, which can be used to focus conservation and anti-illegal poaching efforts.

This presentation will impact the forensic science community by identifying a methodology to forensically process tortoiseshell and offer insight on how mtDNA haplotypes can be used to inform wildlife and government officials about where illegal poaching is occurring. For example, the Dominican Republic government recently cracked down on tortoiseshell sales, confiscating the illegal items and drastically reducing the number of items openly available. Yet, hawksbill objects are still found in markets both in the Dominican Republic and around the world. This research will directly aid law enforcement capabilities by determining from where these endangered animals are being illegally harvested. The hawksbill sea turtle (*Eretrmochelys imbricata*) is one of seven sea turtle species, all of which are protected by international law. One of the distinguishing characteristics of the hawksbill sea turtle is its carapace (shell), which is covered with keratinous overlapping plates called scutes. These scutes are the source of tortoiseshell products, which are widely though illegally available in countries around the world. Scutes are molded and pasted together with water and heat into products such as glasses, jewelry, and small boxes. The tortoiseshell trade is one of the main causes of the hawksbill sea turtle endangered status. Unfortunately, given the global range of hawksbills, it has been impossible to discern the origin of illegally sold tortoiseshell items; such items could originate locally or be shipped from distant locations. This makes conservation efforts difficult or impossible, as illegal harvesting and trafficking routes are not defined.

In the research presented, DNA was first extracted from the shell of hawksbill museum specimens to test the feasibility of DNA recovery. Species specific PCR primers were designed to amplify approximately 440 base pairs of the mitochondrial control region. The amplicons were then sequenced using the same primer set, after which mtDNA sequences were analyzed and haplotypes determined. Next, tortoiseshell items were obtained from the U.S. Fish and Wildlife Service from stockpiles confiscated from individuals entering the United States. mtDNA was again purified, amplified, sequenced, and haplotypes determined. Importantly, haplotypes corresponding to the geographic origins of hawksbills are available as a result of population structure studies of this species, which show nesting site uniqueness and fidelity. Such uniqueness allows for insight into the geographic origin of poached turtles, which was then determined for all illegal hawksbill items.

The extraction of mtDNA from tortoiseshell items has never before been accomplished, thus it represents a unique and powerful new tool for law enforcement and endangered species conservation. Methods developed and data collected during this research, combined with the existing database of sea turtle haplotypes, for the first time allows precise targeting of efforts to stem the illegal taking of these endangered and internationally protected turtles.

Mitochondrial DNA, Wildlife forensics, Tortoiseshell

### A32 Probative Value of Male DNA on Underwear Collected From Female Children With Adult Male Caretakers

Sarah Geering, BS\*, Stephanie King, PhD, Shelly Steadman, MS, Steven Hoofer, PhD, and Robert C. Hansen, MS, Sedgwick County Regional Forensic Science Center, 1109 North Minneapolis, Wichita, KS 67214

After attending this presentation, attendees will appreciate the results of autosomal and Y-STR analysis of the interior and exterior aspects of female children's underwear under normal caretaking situations.

This presentation will impact the forensic science community by examining the probative value of the presence of male DNA from a primary caregiver on children's underwear in the absence of semen.

Y-STR technology in public forensic biology laboratories has allowed for male-specific testing on an increased number of evidentiary items across the United States. Cases submitted for biological examination to the Sedgwick County Regional Forensic Science Center (Wichita, Kansas) by the Exploited and Missing Children's Unit often are candidates for Y-STR typing primarily because the nature of the assault involves very low quantities of male DNA. As child crimes typically are not reported immediately to authorities, secondary items such as clothing and bedding often are submitted for examination in lieu of sexual assault examination kits.

In these situations, when the alleged male suspect cohabitates with the complainant, the probative value of secondary items arguably diminishes because it can be reasonable to expect DNA from the male suspect to be present on those items. This can be true for secondary items that are shared (e.g., bed linens) or unshared (e.g., clothing) between the victim and male suspect. Even when examining underwear from the victim – a typical unshared item with high probative value – it can be reasonable to expect DNA from the male suspect to be present due to DNA transfer during caretaking activities. These activities include folding laundry, commingling of garments prior to or after washing, batched washing and drying of clothing, and general assistance with lavatory use or routine dressing.

The purpose of the present study was to investigate the probative value of male DNA on children's underwear in the absence of semen by examining the quantity and quality of male DNA present on underwear from female children that were exposed to normal caretaking activities or commingled laundry situations, or both. Samples were collected from exterior (tapings) and interior (swabbings) aspects of ten pairs of worn underwear from three female children (ages two to six).

Human DNA was detected on the exterior and interior aspects of all ten pairs of underwear, and autosomal STR testing yielded major contributor profiles that were consistent with each underwear donor. Minor contributions to the autosomal STR profiles were detected in five exterior tapings. Foreign contributions in three of these five samples were minimal, whereas the other two produced foreign contributions that were substantial. Notably, Amelogenin Y was observed as a minor allele in just one exterior sample. For the interior swabbings, only one autosomal allele foreign to the underwear donor was observed in one sample.

Male DNA was detected on the exterior aspects of the underwear in all ten cases, whereas it was detected on the interior crotch linings in just four of the ten cases. Y-STR profiles were obtained from all exterior samples, three of which were single source profiles, and seven were mixtures of two or more individuals. For nine of the ten exterior samples, the father could not be excluded as a contributor to the profile. Y-STR profiles were obtained from just eight of the interior samples, four of which were single source profiles, and four were mixtures of two or more individuals. For seven of the interior samples, the father could not be excluded as a contributor to the profile.

Results of this preliminary study confirm that it can be reasonable to expect male DNA to be present on unshared clothing (underwear) from female children due to DNA transfer during caretaking activities. This can be true even for the interior crotch aspect of the underwear and/or DNA samples with undetectable quantities of male DNA. In conclusion, in the absence of confirmed seminal components, the probative value and/or relevance of male contributors on secondary items should be considered carefully prior to subjecting such items to additional timely and costly Y-STR testing.

Y-STR, Clothing, Trace DNA

## A33 Casework ICPMS/IRMS Examples in the Netherlands

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After attending this presentation, attendees will understand the advantages of the transparant and interactive nature of reporting of forensic ICPMS and IRMS results to both police as well as court authorities in the Netherlands.

The presentation will impact the forensic science community by providing insight into the use of forensic (LA-)ICPMS/IRMS results

throughout the complete judicial process, including the police investigation phase and the court phase.

In this presentation, a number of forensic IRMS/(LA-)ICPMS applications are discussed demonstrating the strong discriminating power of this technique combination. These techniques are used for a wide variety of forensic material casework investigations (various tape types, glass, XTC, drug precursors, paper, sawdust, ink, bullets, brass, and other metals, rope materials, cables, polymeric jerrycan remains in arson residues, human materials for tracing geographic origin unidentified human victims). For a selected number of general casework investigations experiences are shared below and general trends and aspects discussed.

LA-ICPMS and IRMS casework investigations mostly center on material comparisons, e.g., does this piece of material as found at the crime scene and a similar material as found with the suspect originate from one source? As one hypothesis the materials are therefore considered to originate from one source (e.g., roll of tape). Most of the investigated materials are industrially produced in production batches. As alternative hypotheses we will typically consider that materials are from the same production batch; from another production batch but the same producer or from other random producers.

Weighing of the evidence is based on scientific literature results and Netherlands Forensic Institute (NFI) investigations on limited numbers of samples to test literature information applicability for the Dutch situation. An interactive process is used in reporting. Mostly (fast, softer) forensic intelligence is generated for the police investigation phase.

For the court evidence phase in first instance we will report the findings as of that moment and mention possible follow-up studies. Dependant on the court response some aspects of the first investigation may be further substantiated in a follow-up study.

In one example a series of police cars were torched near police stations throughout the Netherlands. The modus operandi (MO) often consisted of placing a jerrycan filled with petrol on top of a police car and torching it. At two crime scenes (A+B) almost completely burnt jerrycan remnants were recovered and offered for a comparison to see if there was a link. Burnt jerrycan remnant samples were cut to gain access to visually appearently unchanged core material. Visual, FTIR and  $\mu$ -XRF investigations could not discriminate materials from both crime scenes A and B. Both jerry can remnants contained poly ethylene.

Samples were therefore investigated with IRMS and LA-ICPMS and from the results could be discriminated.

In a second example explosives and other materials were found in various Dutch cities and believed to be in preparation for a terrorist attack. Forensic investigations were made on possible links between materials from different sites. Compared were a device consisting of two packages of pentrite (PETN) explosives next to a metal frame with two magnets and grey duct tape (figure 3) with a roll of grey duct tape from another location. The focus of the comparisons with visual, FT-IR, LA-ICPMS and IRMS was on these tapes and results were also combined with results from physical fit investigations. Interesting was also the unexplained presence of small orange foil particles in the glue layer of both these tape materials.

In a third example two murdered males were found walled in an empty building. In one forensic investigation red polypropylene ropes as bound around a crime scene carpet and as found at a location controlled by a suspect is compared using IRMS. The crime scene rope samples were heavily contaminated with human decomposition products and were cleaned ultrasonically before analysis. FTIR was applied but results were insufficient for discrimination. IRMS results for the three rope samples vary more than expected from repeatability experiments but still offer potential for discrimination. One of the reference ropes was easily discriminated but another rope was closer to the crime scene samples. Interestingly the heavy contamination does not appear to influence variability for the crime scene samples.

In this presentation the high discrimination power of the IRMS/LA-ICPMS technique combination is demonstrated. If samples are also not discriminated using IRMS and LA-ICPMS, for the police investigation phase this is normally already important information in view of the high discrimination power of this combination. Some examples are presented where samples were potentially too contaminated (rope) or transformed (jerrycan) to be used for forensic purposes. Still useful information could be extracted for these samples.

**IRMS, ICPMS, Rope** 

### A34 The Questionable Thought Processes in Requiring Error Rates in Forensic Science

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After attending this presentation, attendees will better understand the questionable requirement some have demanded of forensic scientists to answer the question: What is your error rate?

This presentation will impact the forensic science community by discussing a number of concepts, which have arisen over the past few years, some in light of the National Research Council report, and others, which have been discussed in detail, especially since 1993. Some of these concepts without substantive definitions have taken on a life of their own. The number of purported experts who advocate a point of view does not determine the legitimacy of an argument. Legitimacy should be determined by the relationship of an argument to the real world. One of these arguments for the "requirement of a science," and more specifically forensic science, is the demand for specifying an "error rate" in a forensic discipline. Some claim that the validity of any forensic discipline cannot be determined without an "error rate." The requirement for any definition must go beyond providing examples. Without a definition, the term has no substantive meaning; and without a substantive meaning of the term, there is little credibility in the arguments of those who insist on a requirement for this concept, which cannot be defined.

In researching the literature there are a number of articles (mostly from those who do not have a degree in a natural or physical science) which advocate an inherent requirement to specify the "error rate" in order to determine whether a scientific method is valid. Some have said publicly that without an error rate, a method is not scientific. There are a number of inconsistencies in these "requirements" which lead the uninformed to ask an apparently basic question: What is your error rate? This is a question without an answer.

One can calculate the number of unacceptable results in a specific proficiency test in a forensic science discipline administered to a defined set of test takers. However, this number cannot be used to extrapolate a conclusion regarding the number of unacceptable results that would occur in actual case work in the same forensic science discipline across the spectrum of those who perform a similar forensic science analysis. Giving examples without a definition does not provide an answer to the basic question: What are you talking about?

Most can remember a definition from fourth grade math: the term "rate" meant and is still defined by "numerator/denominator." Until those who continue to pontificate this purported requirement for an error rate define the numerator, and then the denominator, expressing any term which includes the word "rate" is mathematically illogical. There is a difference between claiming that an error can occur in a forensic science method, and claiming that an error has occurred in a forensic science analysis. The first argument is based on a philosophical/statistical discussion; the second is based on an evaluation of data or images in a specific case. The decisions in the judicial system are, or should be based on what has transpired in the individual case which is being tried. Arguments in trial should be evaluated by the presentation of facts in the form of data and justification for the methodology rather than by a discussion of whether an error can occur. The question should be: Has an error occurred? There is no arguing that there is a nonzero probability of an error having occurred with a resulting erroneous conclusion. The question should be: Did an error occur in this case?

Mistakes/errors do occur in forensic science laboratories; however, no method exists for quantifying the "rate" at which these mistakes/errors occur in actual casework. The other side of this issue which is even more interesting is that these arguments related to forensic science are usually attributed to philosophers, psychologists, statisticians, and lawyers. This presentation will also deal with shortcomings in the arguments of lawyers, based on fundamental science and resulting opinions in the Supreme Court cases cited in *Daubert vs. Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579 (1993) and Kumho Tire Co. vs. Carmichael (97-1709) 526 U.S. 137 (1999) 131 F.3d 1433, Reversed.* 

This presentation will evaluate both "quasi scientific" and legal arguments erroneously arguing for the requirement of "error rates." The presentation will also look at some examples used to describe "error rate" which have little if any application to forensic science.

Error Rate, Daubert vs. Merrell Dow Pharmaceuticals, Kumho Tire Co. vs. Carmichael

## A35 How to Cope With Monopoly Forensic Science

Roger G. Koppl, PhD\*, Fairleigh Dickinson University, Institute for Forensic Science Administration, M-MS2-02, Madison, NJ 07940

After attending this presentation, attendees will know how the organization of forensic science may influence the testing and interpretation of forensic evidence. Attendees will learn that forensic science in the United States is often characterized by a two-fold monopoly in the testing and interpretation of data.

This presentation will impact the forensic science community by helping lawyers improve their performance when engaging forensic science and the forensic scientists. This presentation will help lawyers to better understand monopoly forensic science. It will also help lawyers understand what is deemed appropriate behavior when dealing with monopoly forensic science. This presentation will improve the interface between law and science by improving the participants' understanding of the incentives created by the current organization of forensic science in the United States and elsewhere. This improved understanding will help close the gap in understanding between law and science.

Participants will learn that the monopoly in testing increases the risk of one-sided "evidence filtering" whereby some evidence and some tests are filtered out of the case file. Filtering creates a one-sided picture if the elements passing the filter (the evidence that is tested and the tests that are performed) all tend to support only one theory of the crime, while the elements blocked by the filter (evidence that is not tested and the tests that are not performed) tend to support alternative theories of the crime. The dangers of evidence filtering are compounded if the crime lab does not report the results of all tests performed.

Participants will learn that the monopoly in interpretation increases the risk of one-sided interpretations of the scientific evidence. They will learn that when evidence is ambiguous, forensic scientists must interpret the evidence in the light of their prior expectations and the relative importance they place on different risks of error. These effects arise even when forensic scientists are conscientious and free of cognitive bias.

Participants will be encouraged to draw practical implications for trial practice. They will learn how monopoly forensic science influences each step in the forensic science process from the initial event being investigated to expert testimony in the courtroom. Lawyers will be encouraged to adapt their practices to the contingencies created by forensic-science monopoly.

Monopoly Forensic Science, Evidence Filtering, Prior Expectations

#### A36 Errors in Forensic Science: What Does It All Mean?

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After attending this presentation, attendees will be able to: (1) to define "error" in the forensic science context; (2) recognize and classify the types of errors made in forensic work; (3) understand and describe the roles of bias in forensic science; and, (4) describe how forensic scientists deal with the concept of individualization in the analysis of forensic evidence.

This presentation will impact the forensic science community by shedding light on the types of errors that are made in forensic analysis, how they can be mitigated, and what role bias plays in the propagation of errors.

In *Daubert v. Merrill Dow*, The United States Supreme Court listed several factors that a judge could consider in determining if proffered scientific evidence was scientifically valid. Among these is the "known or potential error rate." The *Daubert* decision didn't contain a definition of "error rate" or even "error." In the past two decades, The Innocence Project(s) have exonerated more than 200 persons who were falsely convicted of serious crimes. Attorneys for the Innocence Project have asserted that many of these false convictions occurred because of errors made by forensic scientists. They describe some of these types of errors and their definitions raise some issues about the nature of such alleged errors. The National Academy of Sciences released a report in 2009 about the state of forensic science in the U.S. The report contains references to errors being made in crime laboratories and how they may be minimized in the future.

These developments raise a number of questions concerning error:

- 1. First and foremost, what is error in forensic science? Is there more than one type of error?
- 2. What is "error rate"? How is this measured? Is there an error rate for each individual test? For a whole scheme? Once error rate has been measured, how should it be used in framing conclusions and in court testimony?
- 3. How does bias affect error in forensic analysis?
- 4. If conclusions of individuality in the association of evidence lack scientific validity and are in need of scientific data, what do forensic scientists and courts do until the needed data is developed?

In the absence of probabilistic conclusions of associations of evidence, forensic scientists use terms such as "similar to," "consistent with," "match," and "could not eliminate as the source of." The most contentious association is "came from" which denotes "individualization." It is not known what these terms mean, how often they are erroneous, or how to get at the answers.

This panel discussion will explore: (1) the nature of errors in forensic science; (2) how to properly define, describe and measure them; and, (3) how this might lead to the reduction of errors in forensic analysis. A panel of experts in errors and error measurement will be invited to discuss these issues. It is expected that this panel will attract an audience from across the spectrum of AAFS sections.

Errors, Bias, Comparison of Evidence

#### A37 The Path Forward: Two Years Later

Gerald M. LaPorte, MSFS\*, National Institute of Justice, Investigations & Forensic Sciences Division, 810 Seventh Street, Northwest, Washington, DC 20531

After attending this presentation, attendees will be knowledgeable about what the forensic community has accomplished since the National Research Council (NRC) report was issued in February 2009.

This presentation will impact the forensic science community by providing a detailed synopsis of what the forensic science community did in response to the NRC report and what may be on the horizon.

On February 18, 2009, the National Research Council (NRC), an arm of the National Academy of Sciences (NAS), published "Strengthening Forensic Science in the United States: A Path Forward." This report had been commissioned by Congress in 2005 and concluded that forensic science, as a whole, produces valuable evidence contributing to the successful prosecution and conviction of criminals, as well as to the exoneration of the innocent. However, the report also identified what the committee considered to be systemic weaknesses in the use of forensic evidence that can and have led to wrongful convictions. The report contains 13 recommendations designed, in the committee's opinion, to remove or ameliorate these systemic weaknesses.

The purpose of this presentation is to discuss what has been done since the NRC report. The National Institute of Justice (NIJ) is the research, development, and evaluation agency of the U.S. Department of Justice and is dedicated to researching crime control and justice issues which impact our Nation's ability to fight and prevent crime. NIJ has been a strong funding source to the forensic science community for research, training, laboratory improvement, and increasing forensic technology capacity. Since the NRC report, NIJ has awarded grants in fundamental research to improve understanding of the accuracy, reliability, and measurement validity of forensic science disciplines. As well, NIJ provides financial support to numerous scientific working groups and has collaborated with other agencies in creating new working groups to examine human factors in latent print analysis and questioned document examination, preservation of biological evidence after it leaves the forensic laboratory, AFIS interoperability, and the use of methods for the reporting of probabilistic statements on forensic evidence in court.

There has been a significant effort to address the recommendations put forth in the NRC report, but has it been enough?

Forensic Science, National Institute of Justice, NAS Report

#### A38 There is an Integrity Problem at the Lab -What Do You Do?

Kristine Hamann, JD\*, Office of the Special Narcotics Prosecutor, 80 Centre Street, 6th Floor, New York, NY 10013

After attending this presentation, attendees will learn how to deal with an integrity problem at a forensic laboratory from the perspective of the laboratory director, the police, the district attorney, the defense bar, and the independent Coverdell investigator.

The presentation will impact the forensic science community by discussing the variety of issues raised when an integrity problem is uncovered in a forensic laboratory, including how to investigate the problem, what to disclose to various stakeholders, what corrective actions should be taken by the laboratory, and the legal consequences to criminal prosecutions.

There are over 400,000 arrests in New York City every year. Approximately 100,000 of them are drug related. The New York City Police Laboratory tests about 40,000 controlled substances every year, more than any other laboratory in the nation. In 2007, a dry-labbing scandal hit the NYPD lab. It was alleged that chemists in the laboratory were cutting corners and not properly testing the drugs they were assigned.

**Coverdell Investigator:** Pursuant to federal guidelines, the New York State Forensic Commission had to designate an independent Coverdell Investigator. In the Spring of 2007, the NYS Inspector General's Office was given that designation. Shortly thereafter the drylabbing allegations arose. The allegations were investigated by the New York State Inspector General and a report citing serious irregularities in the controlled substance section of the NYPD laboratory was issued. The problems uncovered included sloppy testing, cutting corners, failure to properly report to regulatory authorities, and the lack of communication with customers. Though re-testing demonstrated that the items tested were in fact controlled substances, so that no defendants were wrongfully accused, the irregularities did call into question the propriety of the laboratory's procedures.

**Responding to the Investigation**: In 2008, the author joined the Office of the Special Narcotics Prosecutor and had the responsibility of coordinating the response to the Inspector General's report for the office and the five District Attorneys for the City of New York. The response to the Inspector General's report was coordinated by the Office of the Special Narcotics Prosecutor. The multi-faceted issues faced were: (1) the creation of a citywide strategy; (2) the practicalities of identifying the affected cases; (3) working with the NYPD on a remediation strategy;(4) appearing before the NYS Forensic Commission; and, (5) providing disclosure to defense counsel.

It Happens Again - New Investigation into the NYPD Laboratory – 2010: In 2010, another integrity issue was discovered in the controlled substance section of the NYPD laboratory. It was alleged that a chemist was cutting corners in lab work and possibly switching vials of evidence.

Learning From Experience – Improved Approach to Integrity Issues at the Lab: All parties learned from their experience with the 2007 matter. In this instance there have been significant improvements in how the information was reported, how the District Attorneys are working with the laboratory, and the corrective action to prevent similar problems in the future. Though the matter is still under review and investigation, the procedures to address the problem will be discussed.

Dry-Labbing, Investigation, Corrective Action

#### A39 Privatization of Forensic Science Laboratory Functions — Caveat Emptor

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The goal of this presentation is to stimulate thinking and discussion about the nature and role of the criminalistics laboratory.

The presentation will impact the forensic science community by making decision makers aware of the negative consequences of criminalistic laboratory privatization.

Recently publicized crises in government operated forensic science laboratories in the United States and the issuance of the National Academy of Sciences Report, "*Strengthening Forensic Science in the United States – A Path* Forward," have resulted in increased discussion concerning the perceived benefits of the outsourcing of sample testing or the outright privatization of forensic science laboratory services. If the perception of the forensic science laboratory as merely a testing facility were accurate, the arguments in favor of outsourcing and privatization would have some validity. They are not, and to proceed in this direction would have a severely adverse impact on the potential of science to serve justice. It would exacerbate an already artificially compromised ability of forensic science to contribute its full potential to an understanding of the physical evidence record from crime scenes. The physical evidence record can provide the ground truth in criminal investigations. No other source of information in criminal investigations enjoys anything approaching this potential. There is no close second. Unfortunately, this potential is not widely appreciated, and as a result, many forensic science laboratory systems are prevented from contributing what they could due to structural and budgetary constraints. This problem needs to be recognized and addressed. The traditional methods of seeking truth in criminal investigations – e.g., eyewitnesses, interrogation, confessions, etc. are far less reliable than is a scientifically derived understanding of the physical evidence record.

Forensic Science Laboratory, Criminalistics Laboratory, Laboratory Privatization

#### A40 Evaluation of MCLAB Buffer and NanoPOP-4<sup>TM</sup> as Alternative Consumables for Applied Biosystems Capillary Electrophoresis

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After attending this presentation, attendees will have a better understanding of how capillary electrophoresis (CE) buffer and polymer affect resolution and precision of CE instruments. Further, attendees will appreciate the differences between MCLAB consumable CE products and Applied Biosystems consumable CE products.

This presentation will impact the forensic science community when evaluating MCLAB consumable CE products, as a cheaper alternative, for use on Applied Biosystems CE instruments.

The enhancements in forensic DNA analysis that have occurred over the past 15 years can be partially attributed to the capabilities of CE. The successes of such systems rely on accuracy, reproducibility, and precision, all of which allow for the ability of associated software to assign discrete allele values to DNA fragments. Resolution and precision are two means of evaluating the overall health of a CE system. Resolution is a measurement that predicts the ability of an instrument to effectively separate two components, such as two DNA fragments of similar size. On the other hand, precision is the degree to which repeated measurements under unchanged conditions show the same result upon replicate testing. Factors such as resolution and precision can be affected by variations to the reagent components used in a CE instrument. Both Applied Biosystems (ABI) and MCLAB manufacture buffer and polymer that are compatible with ABI CE instruments. The evaluation of resolution and precision are necessary measurements for the validation of any component used on a CE instrument. MCLAB's consumable CE products could be a cheaper alternative to ABI products; however, information on the performance and quality of MCLAB products has not been made available to the forensic community. Therefore, this study was designed to evaluate the performance of MCLAB consumable CE products using ABI AmpFlSTR® Yfiler™ PCR Amplification Kit with analysis on a ABI 3100Avant Genetic Analyzer. In this study, Y-STR data quality, resolution, precision, and cost were evaluated to determine if MCLAB consumable CE products perform comparably to ABI consumable CE products. As described by Heller et al., resolution was calculated for the two allele peaks at the DYS385 locus using RSL=W<sub>h</sub>/( $\Delta X/\Delta M$ ). In this study, W<sub>h</sub> and  $\Delta X$  were measured in CE scan time units (ms) and  $\Delta M$  was measured in bases. Precision was calculated

using the 250 bp DNA fragment of GS500 LIZ ILS for both sample groups. Regardless of the consumable CE products used, all expected Y-STR alleles were detected for each sample analyzed in this study. For samples analyzed with ABI consumables, 36.1% of the loci produced artifacts above threshold, whereas 55.5% of the loci had artifacts when MCLAB consumables were used. Consistent artifacts included pull-up and baseline for both products. However, the average number of loci with artifacts above threshold was not significantly different for ABI and MCLAB products. ABI consumables produced significantly higher peak heights, on average, than MCLAB consumables. Peak heights for ABI analyzed products were slightly over 500 RFU higher than MCLAB, on average. However, both manufacturers' products had peak heights well above typical stochastic thresholds. Further, both MCLAB and ABI consumable CE products were comparable in all other measures of STR data quality, resolution, and precision. Both manufacturer products produced size ranges for the 250 bp fragment that varied less than 0.2 bp. Therefore, if laboratories decide to evaluate a different manufacturer consumable to implement, other factors, such as cost, may be considered. While formamide cost differences are negligible, ABI buffer and POP-4<sup>TM</sup> are approximately 4.5 and 2 times more expensive than their analogous MCLAB products, respectively. This study suggests that MCLAB products are appropriate for DNA analysis on ABI CE instruments and may provide a cheaper alternative for DNA separation. **Applied Biosystems, MCLAB, Polymer** 

A41 Comparison of Automated DNA Extraction Instruments for DNA Extraction From Various Forensic Samples

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After attending this presentation, attendees will understand that AutoMate Express, developed lately by Applied Biosystems, gives sufficient quantity of DNA for STR typing or mitochondrial DNA analysis from various forensic samples, and understand its performance in comparison to EZ1 Advanced XL, Maxwell 16 and QIAcube.

This presentation will impact the forensic science community by providing basic data about automated DNA extraction using commercially available instruments.

Automated DNA extraction instruments were compared for forensic purpose. AutoMate Express was used with "PrepFiler Express Forensic DNA Extraction kit" (Applied Biosystems), EZ1 Advanced XL with "EZ-1 DNA Investigator kit" (QIAgen), Maxwell 16 with "DNA IQ Casework Sample kit for Maxwell 16," and "Tissue and Hair Extraction kit" (Promega), and QIAcube with "QIAamp DNA Investigator kit" (QIAgen). DNA was extracted from fresh bloodstains (3 µl of whole blood and 3 µl of 10-time diluted blood) on cotton and denim, three 3-mm punches of FTA cards containing buccal cells collected by EasiCollect (GE Healthcare), aged bloodstains, hair roots and hair shafts. For each category of samples except for the aged bloodstains, five samples were prepared from three persons to give 15 samples in total. DNA was eluted in 50 µl of water by EZ1 Advanced XL and QIAcube, in 50 µl of TE by AutoMate Express, and in 50 µl of Elution buffer by Maxwell 16. The genomic and/or mitochondrial DNA was quantified by real-time PCR assay using D17Z1 locus and/or hyper variable region 1 (HV1), respectively. The extracted DNA was used to amplify 15 STR loci of Identifiler kit (Applied Biosystems) and/or the HV1.

The highest DNA concentration was obtained by AutoMate Express from the bloodstains and the diluted bloodstains on cotton and denim, and the hair shafts. Concerning the FTA cards and the hair roots, similar concentration was obtained by AutoMate Express, EZ1 Advanced XL and QIAcube, but the DNA concentration obtained by Maxwell 16 was lower. Full STR profiles were obtained by all the instruments from the bloodstains on cotton and denim, the FTA cards and the hair roots. Out of the 15 diluted bloodstains, full STR profiles were obtained from 14, 11, 0, and 8 samples on cotton, and from 15, 14, 6, and 14 samples on denim for AutoMate Express, EZ1 Advanced XL, Maxwell 16, and QIAcube, respectively. The denim extract obtained by AutoMate Express and Maxwell 16 slightly inhibited the PCR amplification of Identifiler kit, when 20  $\mu$ l of the extract was concentrated and amplified with 1ng of 9947A DNA. However, nine  $\mu$ l of the denim extract obtained by both the instruments did no inhibit the PCR.

When the hair root DNA was amplified in the A region (443bp) and the C region (231bp) in the HV1, the similar thickness of bands on agarose gel were obtained in both the regions by all the instruments. On the other hand, when the hair shaft DNA was amplified, the bands of the A region were weaker than those of the C region in the use of all the instruments especially AutoMate Express. This result raises a possibility that AutoMate Express tends to recover small fragment of DNA preferentially from hair shafts compared to the other instruments.

In conclusion, there is a possibility that AutoMate Express can extract DNA from various forensic samples as other conventional instruments do. Further study is needed to validate this instrument to know its performance, feature, and usefulness.

Automated DNA Extraction Instrument, STR, Mitochondrial DNA

#### A42 Rapid Pentameric STR Screening Using Short Microchip Capillary Electrophoresis

Maurice J. Aboud, MS\*, Florida International University, Chemistry & Biochemistry CP304, 11200 Southwest 8th Street, Miami, FL 33199

After attending this presentation, attendees will understand the development of a fast and portable DNA screening method that uses microchip electrophoresis for the detection of a set of three newly designed pentameric STRs. Attendees will also gain an understanding for how this system works, the limitations of the system and how these limitations were surmounted to achieve the desired resolution on the microfluidic chip.

This presentation will impact the forensic science community by addressing the problems and limitations encountered with the current commercial microfluidic systems such as poor resolution, and the ability to only detect double stranded DNA.

Forensic DNA analysis involves the amplification and separation of length polymorphisms in the human genome for the purposes of identification. The power of this technique for assisting law enforcement in solving crimes has resulted in a rising backlog of untested samples needing to be screened and analyzed. As a result of this problem and a similar need to develop procedures to screen evidence in remote locations, there is need for the development of rapid and portable genotyping systems. While short tandem repeat (STR) DNA analysis by capillary array electrophoresis is capable of high resolution and has a large power of discrimination in forensic identification, these instruments are not portable and require a relatively long sample run time. It is because of this problem that the project to develop a portable DNA screening method using a commercially available microchip system that utilizes short fluidic channels was started.

Generally speaking microfluidic systems require fairly long channels and complex detection systems for proper resolution and accurate identification using multiplex STR loci for forensic DNA samples. However, there remains a need for portable systems with a small footprint for use in evidence screening. The Agilent 2100 Bioanalyzer uses small two cm microfluidic chips, which are approximately the size of a postage stamp. Due to the short path length of these chips and the fact that they were designed to analyze double stranded DNA (dsDNA), most four base repeats will not properly

separate. As a proposed solution, a set of primers for known pentameric STRs that permits the use of smaller, easier to separate polymorphic amplicons was designed. Pentameric nucleotide repeat units have been shown to reduce the amount of stutter in the amplified sample, a useful characteristic when dealing with mixtures. In addition, pentameric STRs are highly polymorphic and have relatively few microvariants, which make them ideal for forensic analysis. Secondly, a novel mixture of polyvinyl pyrrolidone (PVP) and hydroxyethyl cellulose (HEC) that permits facile DNA separations in the shorter fluidic channels was developed. Lastly a modified chip platform that permits single stranded DNA analysis was utilized. The result is a dramatically improved separation. The data demonstrates that baseline separation is possible for pentameric STR markers using very short fluidic channels. In addition because this new technique is based on a beta modification of an existing commercial system, chip loading is relatively quick and easy. As a result this system should provide a useful tool for quick and portable screening in forensic DNA analysis.

The net result of this research will be to address the problems and limitations encountered with the current commercial microfluidic systems such as poor resolution, and the ability to only detect double stranded DNA.

Pentameric STR, Forensic DNA, Microfluidics

#### A43 Recovery of DNA From Black Powder Enhanced Latent Fingerprint Lifts Archived Against Matte Acetate

Shelly Steadman, MS\*, Steven R. Hoofer, PhD, Sarah C. Geering, BS, and Stephanie King, PhD, Sedgwick County Regional Forensic Science Center, 1109 North Minneapolis Street, Wichita, KS 67214

After attending this presentation, attendees will gain an understanding of the inherent problems with recovering DNA from black powder enhanced prints. Attendees will be introduced to performance verifications conducted by the laboratory after receiving a court order to conduct post-conviction testing on matte acetate cards collected over 30years ago. Attendees will also be introduced to relevant judicial issues, especially those regarding hearings before a trial court concerning whether this testing would be conducted on a post-conviction case.

This presentation will impact the forensic science community by highlighting the laboratory's performance verification which was heard by the judge at a Bruner hearing and was instrumental in his decision whether there is DNA to be tested, and if so, whether that DNA could possibly be exculpatory. This decision is important because it sets precedence for future post-conviction test requests in the state of Kansas.

The purpose of this study was to examine methods for removal, extraction, and profiling of DNA entrapped between latent tape and matte acetate. The study was driven by court order to conduct DNA analysis on latent lifts collected from a crime scene in 1977. The tape lifts were archived against matte acetate cards and varied in size; however, all were of substantial area (comprising no less than 25 cm<sup>2</sup>) and extraction in full was not feasible. Since the laboratory does not routinely type such latent lifts, a performance verification was conducted that tested swabbing and scraping recovery techniques from fingermarks placed on adhesive, latent lifts collected from glass, and latent lifts collected from glass following routine black powder enhancement. Preliminary results indicate that while the adhesive on common lift tape and hexane do not inhibit one's ability to obtain full fingermark donor profile, recovery of appreciable quantities of DNA was more difficult once the adhesive was fixed to the matte acetate card and lessened even further when enhancement by black powder was used during processing. Samples selected for STR amplification following the dusting/lifting procedure did not result in profiles suitable for comparison purposes and detection of extraneous peaks not expressed by print donors occurred for some extracts. A Bruner hearing was set to argue whether there was DNA remaining to be tested, and if so, whether that DNA could possibly be exculpatory in this postconviction matter. Laboratory testimony concluded that this historical evidence should not be tested further since these processes demonstrated little potential for generating accurate profiles from laboratory simulated samples; these studies weighed heavily in the judge's decision to not require the testing.

Fingerprint, DNA, Latent Lift

#### A44 Collection of Touch DNA by a Handheld Vacuum Device

Shahida K. Flores, BS\*, 1319 Smither Drive, Apartment 7301A, Huntsville, TX 77340; and David A. Gangitano, PhD, 14723 TC Jester Boulevard, Apartment 1118, Houston, TX 77068

After attending this presentation, attendees will become familiar with a new collection technique for touch DNA evidence.

This presentation will impact the forensic science community by demonstrating that a handheld vacuum device can be used for enhanced collection and recovery of touch DNA. This may prove to be a useful tool during evidence collection at the crime scene or in the laboratory.

Touch DNA itself has revolutionized the forensic community in the last few years by increasing the number of items of evidence that can be processed for DNA analysis. This has not been more evident than with property crimes where it is common for an object or surface to come in contact with a perpetrator's skin. During the contact of skin to surface, epithelial cells are sloughed off and deposited along with oil, sweat and other cellular components present on the skin. A hand grasping a doorknob, a face pressed against a window, a finger on the trigger of a gun, are all examples of skin to surface contact that can leave behind valuable touch DNA evidence. However, this kind of contact typically leaves behind only a small number of cells so an efficient method of recovering and collecting such evidence would greatly increase the likelihood of generating a full DNA profile.

Currently, the most common method for recovering and collecting touch DNA from a hard surface is a swabbing method. A cotton swab moistened with either water or detergent is rubbed over the entire surface area where touch DNA is suspected to be present. Other methods such as cutting, scraping, and tape lifting aren't as applicable to hard surfaces as swabbing. The use of a handheld vacuum device for recovering and collecting cells would not only provide another method for collecting touch DNA from hard surfaces but would also provide a comparative method in order to determine the percentage recovery of DNA from swabbing.

The concept of the handheld vacuum device and the way it operates is fairly simple. Liquid buffer containing a surfactant is applied to the surface where the touch DNA is present. The device is turned on after attachment of a collection nib and vial. The negative pressure created by the vacuum allows the liquid buffer containing the suspended cells to be propelled into the collection area. The nib, acting as a filter, collects the epithelial cells while the vial, acting as the liquid storage unit, collects the buffer solution.

In the first part of the study, optimization of the device was carried out by testing various liquid buffers and surfactants in addition to testing nibs of various pore sizes and surface areas. Quantification results and generated profiles relating to each variable were analyzed. The optimal buffer and nib were chosen for the second part of the study. A comparison study between the handheld vacuum device and a moistened cotton swab was performed to determine if there was a significant difference in the quantity and quality of touch DNA collected. This was done by comparing quantification results and generated profiles. A standard organic extraction was performed on all samples in combination with a concentration and purification step. The samples were treated as low yield and the final elution volume was 40 ul. Samples were amplified with PowerPlex 16 and run on an ABI 310 Genetic Analyzer. **Touch DNA, Collection System, Handheld Device** 

#### A45 Optimization of Touch DNA Collection

Jordan L. Williams, BS\*, and Laura L. Perrella, BS, Sam Houston State University, 1003 Bowers Boulevard, Huntsville, TX 77341; and Andrew P. McWhorter, and MS, and Jennifer N. Watson, MS, Department Of Public Safety, 12230 West Road, Houston, TX 77065; and, 12230 West Road, Houston, TX 77065

After attending this presentation, attendees will understand the importance of testing for trace DNA in a crime laboratory by observing the number of successful touch DNA cases for the Texas Department of Public Safety Houston Crime Laboratory. Attendees will also learn the best liquid for recovering touch DNA using the double swab technique.

This presentation will impact the forensic science community by introducing the number of Combined DNA Index System (CODIS) hits for touch DNA at the Texas Department of Public Safety Houston Crime Laboratory since 2007 and by presenting an optimal method of recovering touch DNA.

The Texas Department of Public Safety Houston Crime Laboratory considers touch DNA, also known as trace DNA, to be any DNA deposited through touch. This does not include DNA from blood, saliva, or tissue. Approximately 19% of the crime laboratory's property crimes including robbery, burglary, theft, and auto theft cases have resulted in CODIS hits from touch DNA evidence alone; this study spanned from 2007 to present cases. Most laboratories, including some in the Department of Public Safety system, do not collect touch DNA from evidence. This percentage indicates an importance for the collection of touch DNA and how often it succeeds. To improve this number, several touch DNA collection methods were analyzed and the best one selected. The Texas Department of Public Safety Houston Crime Laboratory currently uses deionized (DI) water and a single swab method to obtain trace DNA from evidence. Pang and Cheung (2007) have suggested the use of a double swab method, which utilizes one wet swab followed by a dry swab to collect the DNA. The wet swab collects a portion of the DNA but also leaves a residue behind for the dry swab to collect. The two swabs are then extracted together. The method has been found to collect more DNA than the single swab method. Therefore, this technique was utilized; however, the wet swab was composed of varying liquids other than just DI water.

Three different liquids were selected for testing, and these included DI water, sodium dodecyl sulfate (SDS), and SDS:isopropanol:deionized water. DI water acted as the control since the laboratory already uses this method. In a previous study, SDS was determined to be an optimal detergent for DNA collection; based on its hydrophobic properties and DNA properties such a detergent attracts DNA. In addition to the DI water and SDS, a combination of SDS, isopropanol, and DI water was also utilized. According to a previous study, isopropanol yields more DNA due to its lack of surface tension. With the lack of surface tension from the isopropanol, the liquid will disperse more evenly covering more surface area, and with the high attraction from SDS, the liquid will also collect more DNA. Additionally, isopropanol dries quickly, creating an almost instant collection time. Isopropanol has also been found to preserve DNA from bacterial degradation. For the experiment, DNA was deposited on four different surfaces: a steering wheel, a laboratory coat collar, a revolver, and a hard hat. Using the double swab technique and the three different liquids, the DNA was collected.

The swabs were extracted according to the Qiagen Standard Operating Procedure (the most popular method at Department of Public Safety Houston, TX). Quantitation, amplification, and analysis were also performed according to the Department of Public Safety Houston, TX Standard Operating Procedures. The profiles were compared to known profiles for a better analysis. The interpretation of results was based on the quantity of DNA yielded as well as the profile produced. Although some samples did not result in quantification, DI water yielded the most DNA for the other samples. SDS produced a substantial profile with little to no peak height imbalance. Further testing should be performed in order to better the conclusions.

Touch DNA, Trace DNA, DNA Collection

#### A46 Direct Amplification of Low Copy Number DNA on Two Substrates

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After attending this presentation, attendees will have a better understanding of the potential of direct amplification when applied to low copy number DNA situations. Attendees will also be informed on the effect that porous and non-porous substrates have on direct amplification of LCN DNA.

This presentation will impact the forensic science community by exploring inexpensive methods of LCN DNA profile recovery through direct amplification. This presentation will also test whether direct amplification of LCN DNA can be done on both porous and non-porous substrates.

The successful production of short tandem repeat (STR) profiles from evidence samples is the goal of analysis in a forensic biology laboratory. In order to provide the best chance for successful amplification of STRs, investigators must maximize the amount of DNA that is recovered from evidence samples and is available for amplification. Although modifications can be made to the amplification and analysis parameters, these modifications do not always eliminate the problems associated with low copy number (LCN) DNA analysis. With all of the inherent problems in LCN DNA recovery and analysis, increasing the amount of DNA in the amplification reaction would be most beneficial. Since some DNA is lost in the process of extracting DNA from a substrate, eliminating the extraction step and adding the substrate directly to the amplification reaction might be more effective in producing full STR profiles.

In the past few years, direct PCR methods, which eliminate the extraction step, have been developed by several organizations. In direct PCR methods, the solid substrate containing DNA is added directly to the amplification reaction. The conventional extraction preparation is bypassed, thus shortening the time between collection and analysis, and minimizing sample loss and contamination.

In this research, DNA was amplified directly from a porous piece of regular white cotton cloth, a non-porous plastic credit card, and a swab of a non-porous plastic credit card. Small areas containing DNA were cut out and directly inserted into the amplification tube. The amount of DNA on all samples was controlled by using a quanititated solution of extracted DNA. For comparison, a second set of credit card and cloth samples were organically extracted. Additional sets of mock evidence samples were processed containing DNA from volunteers that had either touched the credit card or worn the cloth. All extractions were quantitated using Applied Biosystems Quantifiler Human DNA Quantification Kit. All samples were amplified using Applied Biosystems AmpFISTR Identifiler PCR Amplification Kit.

LCN DNA, Direct Amplification, Cloth

#### A47 Analysis of N-4 STR Repeat Slippage with Amplification Enhancer on Low-Quantity DNA Samples

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After attending this presentation, attendees will better understand how the amplification enhancer affects allele and stutter peak height of low-quantity DNA samples. Attendees will also learn that the enhancer causes the stutter peaks to increase proportionately the peak heights. This is evident in some mixture DNA profiles.

This presentation will impact the forensic science community by introducing a possible new enhancer that could be used to enhance lowquantity DNA samples in criminal cases.

Forensic DNA profiles are based on small repetitive nucleotide sequences called short tandem repeats (STR) that vary by size in the population. Due to the stochastic effects, low copy number (LCN) DNA samples (~<100pg) produce more random fluctuations in allelic peak heights and more reproducible anomalies or artifacts than larger quantities of DNA. Artifacts in the electropherograms can interfere with the interpretation of the true DNA peaks. One artifact, called repeat slippage or stutter, is due to the strand slippage during the PCR process. Based on sequence analysis of tetranucleotide STRs, stutter products are generally one repeat unit or n-4bp smaller than the true allele. Stutter percentages, or the ratio of the stutter height to the corresponding allele height, have been found to be ~<15% for tetranucleotide STR's. Stutter can complicate interpretation of stutter products is important when analyzing low-quantity DNA samples.

A new PCR enhancer, STRboost (SB), has been used to enhance amplification from low-quantity samples. SB has been reported to enhance alleles by 5-fold and is based on the natural biological mechanism called androhydrobiosis or life without water. The purpose of this study is to evaluate the percentage of stutter formation with various volumes of SB. The hypothesis is that amplification of low-quantity DNA samples will result in increased sensitivity (and higher peak heights) with no significant change in stutter percentage. Controlled human male DNA was diluted to 0.5ng/µl, 0.25ng/µl, and 0.125ng/µl. Extracted male and female DNA samples were quantified using real time quantitative PCR or QPCR and diluted to a 0.5ng/µl mixture of a 9:1 female to male ratio. The DNA samples were amplified in triplicates using the AmpFISTR Identifiler STR Multiplexing kit (Applied BioSystems) using SB at three volumes: 2.5µl, 5.0µl, and 9.0µl. The 0.5ng/ul mixed sample was tested at only 9.0ul of SB. The amplicons were separated by capillary electrophoresis using the ABI 310 Genetic Analyzer. A genetic profile containing sixteen core loci and amelogenin was generated using the software GeneMapper ID. From the profiles, the analyses of allele and stutter peaks were performed. The stutter percentages in the triplicate runs at each concentration were compared using the Single-Factor ANOVA and the Independent Two-Sample t-test with alpha being 0.05. Results at the single-source DNA samples and the 0.5ng/µl mixture show that the allele and stutter heights increased with no significant change in stutter percentage in most of the loci. Stochastic effects greatly increased at lower concentrations of DNA causing the pvalues to approach alpha. The Two-Sample t-test was used for the mixture sample and for loci that showed reproducible stutter peaks at two volumes of SB. Highest peak heights and sensitivity were obtained using between 5-9µl of the enhancer in most of the loci. Preliminary results of the 0.5ng/µl mixture show higher stutter percentages than in the 0.5ng/µl single-source sample. The proportional enhancement of stutter and the corresponding true allele supports the hypothesis. This may be explained

by the fact that the stutter sequence only differs from the true alleles by one repeat (4bp).

Stutter, STR Boost, Low-Quantity DNA

#### A48 Expedited Enzyme-Based Generation of PCR Ready DNA From Forensic Biological Samples on Glass or PMMA Microdevices

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After attending this presentation, attendees will have gained an understanding of a microfluidic enzyme-based method for generating PCR ready DNA.

This presentation will impact the forensic science community by demonstrating that microchip liquid extraction can greatly reduce the amount of time necessary for DNA extraction, leading to the possibility of an increase in sample throughput.

Solid phase extraction (SPE) is a widely-used method for the extraction and purification of DNA from biological samples. Typically, a silica-based solid phase is used to reversibly bind the DNA under high salt conditions, while impurities are rinsed away using an organic solvent. DNA is released from the solid phase upon addition of a low salt buffer. This method generally has a number of sample handling steps which may provide the opportunity for the introduction of a contaminant or a reduction in DNA yield. The use of a closed, single-tube, liquid extraction eliminates these issues and may be more amenable to automation. An enzyme-based extraction method has recently been developed that uses a thermophilic neutral proteinase from *Bacillus* sp. EA1 to lyse cells and degrade proteins and nucleases, leaving only DNA in a PCR-ready buffer in 20 minutes.<sup>1</sup>

Microfluidic devices provide a unique alternative to conventional methods that is rapid and cost-effective. Reduction of sample and reagent volume, analysis time and incidence of contamination make microfluidic devices ideal for forensic applications. Additionally, a microfluidic platform allows for integration of multiple techniques on a single device,<sup>2</sup> potentially allowing for a portable DNA analysis system. SPE has been successfully adapted to a microdevice;<sup>3</sup> however, it may be hindered by uneven packing of the solid phase or high back pressure. Adapting the enzyme-based extraction method described above to a microdevice would not only reduce the extraction time, but eliminate any issues that occur with SPE.

Traditionally, microdevices are fabricated in glass for a number of reasons, including a high understanding of the surface chemistry and reusability. However, fabrication of glass devices is time-consuming, involves the use of hazardous chemicals and is expensive. Recently, there has been a shift towards the use of polymers, such as polymethylmethacrylate (PMMA), for microdevices due to the ease of fabrication and low cost.<sup>4</sup> Liquid DNA extraction can be easily adapted to a microdevice, since a packed bed and centrifugation are not required, providing an ideal platform for integration with downstream analyses.

The current work focuses on the development of an expedited enzyme-based DNA extraction method on a microdevice and a comparison of glass versus PMMA substrates. A fragment of a dried buccal swab was added to the liquid extraction solution, which contains buffer and enzyme. A small portion of this mixture was loaded onto a microdevice and incubated using an infrared (IR)-mediated heating method<sup>5</sup> for a short period of time. The sample was removed from the microdevice and added to a conventional PCR master mix for STR amplification. Results show that both glass and PMMA are adequate substrates for the liquid extraction method. Additionally, the incubation time on a microdevice can be reduced to as little as one minute, without a loss of STR peak height. This represents a 20- and 60-fold reduction in extraction time compared to conventional liquid and solid phase extraction methods, respectively.

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PMMA, Liquid Extraction, Microdevices

## A49 Refining Laboratory SOPs for Enhanced Consistency Through Incorporation of SWGDAM's Guidelines for Autosomal STR Typing

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After attending this presentation, attendees will learn how the incorporation of SWGDAM's Interpretation Guidelines for Autosomal STR Typing into existing laboratory Standard Operating Procedures (SOPs) can standardize interpretation among analysts.

This presentation will impact the forensic science community by illustrating the complexity of DNA mixture interpretation and how it can be structured to yield reproducible conclusions.

Advancements in technology and instrumentation have expanded the variety and complexity of samples typically encountered in a forensic DNA laboratory. As a result of these improvements, analysts are increasingly confronted with challenging profiles arising from poor DNA quality/quantity and complex mixtures. Standard Operating Procedures (SOPs) for DNA mixture interpretation may harbor vague language to accommodate the widest variety of casework scenarios, relying heavily on the individual analyst's experience and training. Although affording flexibility, these generalized SOPs can lead to interpretation differences among analysts.

In January 2010, The Scientific Working Group on DNA Analysis Methods (SWGDAM) approved guidelines for the interpretation of autosomal STR typing to provide direction to the forensic community. Built upon a solid foundation of validation studies, these guidelines serve as a decision making framework within a laboratory's well-defined SOP. It is impractical to establish a guideline for every scenario; however, a well-defined SOP containing scenario-specific structure will consistently direct interpretation toward reproducible conclusions. As a result, any individuals adhering to the same SOPs should ultimately arrive at the same conclusion.

Three mock casework scenarios were prepared with non-probative DNA samples to specifically challenge mixture interpretation consistency among analysts. Using these scenarios, potential discrepancies are more likely to occur due to ambiguously written SOPs was illustrated. Using SWGDAM's Interpretation Guidelines for Autosomal STR Typing to strengthen SOPs, analysts will interpret data more consistently within

Sperm, DNA, Differential Extraction

#### A50 Application of a Handheld Vacuum Filter Device for Differential Sperm Separation

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After attending this presentation, attendees will understand the practical application of a handheld vacuum filter device, as well as, its use for specific separation of the male fraction in mixed sample evidence.

This presentation will impact the forensic science community by providing a methodology to obtain a cleaner suspect DNA profile. This profile is obtained with reduced contamination and increased speed and specificity. Data interpretation is more accurate upon the cleaner DNA profile obtained using the handheld vacuum filter device.

A critical aspect of the crime laboratory is the processing and analysis of evidence related to sexual assaults. Usually this evidence is a mixture of sperm and female epithelial cells. When mixtures of different sources of DNA are present, the analyst must separate the contributors into fractions. This separation, when different cell types prevent clean fractions, can produce significant problems upon DNA profile presentation in court. To improve this separation of sperm and female epithelial cells, a handheld vacuum-filter device was used during collection, sampling, and fraction separation. The resultant, male fraction produced a cleaner DNA profile for the perpetrator. Upon presentation in court, the information and correlation with the suspect's DNA could be made with more clarity and a decreased possibility of re-trial and retesting.

The handheld vacuum-filter device can be adjusted to various degrees of porosity, depending on the type of evidence to be evaluated. The adjustment can be made at either initial collection or during sample analysis. Selecting a smaller pore size filter during differential mixture analysis more specifically and accurately targeted the male fraction of the body fluid mixtures, producing a cleaner male DNA profile. The use of the handheld vacuum-filter device during evidence collection would permit for minimal contamination, further improving the quality of the fractions obtained during DNA analysis.

The mixtures evaluated included liquid and dried samples, mimicking the type of evidence collected at crime scenes. This was done to determine scope and limitations of the handheld vacuum-filter device. Performance was also compared when using different filter porosities with the different sample types to identify the limitations of the instrument during collection, sampling, and DNA extraction. DNA was extracted using the organic extraction method coupled with concentration by filtration. The DNA recovered was verified by qPCR prior to amplification and genotyping. The use of the handheld vacuum-filter device was also applied to samples with various male to female ratios. The same procedures were followed to determine the applicability to degraded or limited quantity/quality samples. The filters permitted concentration of the DNA, resulting in the quantification of DNA with these challenging samples. The results of quantification and STR profiling were compared to traditional differential extraction procedures on both sample types.

The resultant cleaner suspect DNA profile, obtained with reduced opportunity for contamination and increased speed and specificity, will significantly impact the forensic science community. Data interpretation would be more accurate upon the cleaner DNA profile obtained using the handheld vacuum-filter device. However, of even greater significance will be the impact upon the DNA case backlog and efficiency of analysis. Cleaner samples at collection and more efficient and specific separation Bianca N. Vigil, MS\*, and Kirsten T. Kelley-Primozic, BS\*, McCrone Microscopes and Accessories, 850 Pasquinelli Drive, Westmont, IL 60559

The goals of this presentation are to discuss micro-isolation of epithelial cells, evaluation of different adhesive tapes, and histological staining.

This presentation will impact the forensic science community by introducing a more reliable method for getting a profile from epithelial cells, it will eliminate the extraneous particulate and adhesive that is usually present during extractions by isolating the cells.

Adhesives are currently used widely for the collection of impression evidence and microscopic trace evidence; and, more recently recommended for biological evidence. However, the choice of adhesive can affect the success with which the evidence is obtained and subsequently analyzed. If the adhesive tape of choice contains too much adhesive, it will tend to remove more particulate than desired from the substrate, requiring the analyst to sort through more irrelevant particles and sometimes confusing the evidence with complicated mixtures. Not enough adhesive will lead to inadequate material for analysis. Through a critical evaluation of various adhesives, the collection and subsequent analysis can be optimized.

Three adhesives were evaluated: (1) SPI Supplies carbon tape tabs; (2) Neschen Filmolux<sup>1,2</sup> S23; and, (3) Gel-Pak "0." The tapes were chosen because of their extreme differences in adhesiveness, thickness, and composition. The SPI supplies carbon tape tabs are currently used for gunshot residue analysis because of their suitability for scanning electron microscopy. Neschen Filmolux has been used by German forensic scientists for epithelial cell identification, and removal of trace evidence from cadavers<sup>3</sup>. Gel-Pak "0" has many materials science applications, but is not currently being used in biological or forensic analysis.

These adhesives were evaluated by seeding relevant forensic materials (i.e., pillowcase, shirt, direct skin contact) with epithelial cells and then removing the cells with each tape type. The tape was gently pressed on each substrate to remove the cells to ensure only the top layer of particulate was removed. Each tape was then viewed using a stereomicroscope (Olympus MVX10) to search for epithelial cells and estimate the number recovered on the lift. When possible epithelial cells were suspected on the Neschen Filmolux S23 and Gel-Pak "0," an amido black staining procedure was carried out to verify the presence or absence of epithelial cells. Due to the opaque nature of the SPI carbon tape, the amido black staining procedure could not be performed. Once the presence of epithelials was confirmed, the tapes were evaluated for ease of cell removal during micro-isolation.

Micro-extraction was accomplished by hand using sharpened tungsten needles while observing the suspected epithelial cells with a stereomicroscope. The recovered cells are then easily transferred to a micro-tube for PCR.

The SPI supplies carbon tape tab, with its thick layer of gooey adhesive, greatly complicates the micro-isolation and manipulation techniques. This tape also removes more than just the surface particulate and makes it difficult to determine which particles were loosely associated with the material or which were inherent. It also is an opaque tape, so it cannot transmit light, and therefore does not allow for any histo-chemical staining of suspected cells. The Neschen Filmolux S23 adhesive is slightly less tacky than the carbon tape. It also removes more than just surface particulate, but not as much in comparison to the SPI carbon tape. The fact that it is colorless (transparent) is an advantage allowing the staining of suspect cells. The drawbacks are its thick adhesive layer which make isolation cumbersome, and its hydrophobic nature that may interfere with PCR analysis.

Gel-Pak "0" adhesive was so thin that it only removed the "newest" or "loosest" particulate that tends to be most relevant in forensic evidence. Typical results on the Gel-Pak "0" displayed mostly epithelial cells, and very little extraneous particulate. Like the Neschen Filmolux S23, the colorless and transparent adhesive allowed for easy staining and transmitted illumination. A further advantage over the Filmolux, however, is the adhesive backing that allows it to be mounted on many different collection surfaces (i.e., glass slides, plastic cartridges, etc.). Due to the fact that Gel-Pak is in reality a gel film instead of a true adhesive, removal of the cells is less inhibited than with the other two tapes. Also, because of the lack of adhesive, it does not interfere with PCR analysis.

Tape lift collection is commonly used for impressions and trace evidence; it can also be an efficient method for the collection and isolation of DNA evidence. When individual cells or a small aggregate of cells can be recovered and isolated for analysis, the DNA results can be more relevant and reliable.

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Amido Black, Adhesive Tape, Micro-Isolation

#### A52 Comparison of Room Temperature Forensic DNA Extract Sample Preservation Methods

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After attending this presentation, attendees will be informed of room temperature DNA sample preservation methods.

This presentation will impact the forensic science community by informing the community about the potential of room temperature sample preservation of DNA extracts.

In most cases, only a portion of the entire DNA extract volume is consumed during forensic analysis. Once extracted, the remaining DNA is typically stored in a refrigerator at 4°C, a freezer at -20°C or at -70°C for long-term storage to avoid sample degradation. While these are acceptable DNA storage methods, use of refrigerators and freezers may be viewed as costly when factoring in the individual cost to purchase and maintain as well as energy and space requirements. The potential loss or degradation of evidentiary samples when such systems fail must also be taken into consideration as well as when refrigeration and/or freezers are not readily available. For these reasons, alternative room temperature biological evidence storage systems and methods are of interest to most forensic DNA units.

Historically, forensic DNA has been stored dry and/or cold since these conditions reduce the rate of bacterial growth or degradation by DNases. This study evaluated three room temperature storage techniques which included; Whatman<sup>®</sup> Micro FTA cards, QIAsafe<sup>™</sup> DNA Tubes, and sterile swabs. Swab samples were dried using the SafeSwab<sup>™</sup> swab dryer, and a contamination study was conducted to ensure that the drying process would not cause cross contamination.

Sample types tested included liquid blood, dried trace blood, hair, buccal swabs, sweat/wearer, mock sexual assault, and touch DNA. All samples were extracted with Promega's DNA IQ<sup>TM</sup> system on the BIOMEK® 3000 Laboratory Automation Workstation, quantified with Applied Biosystems Quantifiler Duo® Quantification Kit on Applied Biosystems 7500 Sequence Detection System, and amplified using Promega's multiplex STR PowerPlex<sup>®</sup> 16 system and capillary electrophoresis run on ABI Prism® 3130*xl* Genetic Analyzer. Raw data from the 3130*xl* was analyzed using Genemapper® ID v3.2.1. To test the efficiency of each storage technique, samples were eluted in TE buffer and recovered at two weeks, six weeks, and finally six months. Each method was evaluated according to its ability to provide the highest recovery of DNA, as well as to provide a quality profile as compared to the initial quant value and profile obtained from the sample.

**Preservation, Storage, DNA** 

### A53 Optimizing DNA Storage at Room Temperature: Teflon vs. Polypropylene Tubes With and Without Polymers

Erica DiNaro, BS, Clarissa Trogdon, BS, and Steven B. Lee, PhD\*, San Jose State University, One Washington Square, Macquarrie Hall 521, San Jose, CA 95192

After attending this presentation, attendees will have a better understanding of technologies used for long term storage of low quantity, low quality DNA samples at room temperature as compared to -20 °C freezer storage. Attendees will also learn how teflon coated storage tubes may have an affect on the retention of DNA samples and also how newly designed polymers are able to aid in the recovery and stabilization of low quantity, low quality DNA samples over time.

This presentation will impact the forensic science community as results from this project may provide a more cost efficient way of storing low quantity, low quality DNA samples. A combination of commercially available polymers and teflon coated tubes appear to protect the samples at room temperature from damage and maximize recovery. Furthermore, replacement of cold with room temperature storage will result in reduced energy consumption.

Storage of DNA samples is of paramount importance in forensic DNA, epidemiological, clinical and virtually any genetic database laboratory. There is always the possibility that cases may be re-opened and any stored DNA sample may need to be re-tested. This is especially important when the amount of sample is limited.

Biological evidence may be consumed with the result that the DNA extracts may be the only remaining genomic resource to retest and test with new technologies for retrospective and prospective testing. Optimal storage of DNA is therefore critical to retrospective (retesting) or prospective (downstream analysis with additional or new genetic markers) testing. In addition to sample quantity, intrinsic differences in sample types resulting in differences in quality, extrinsic differences in the storage buffers especially ionic strength, tube surface type, exposure to UV and temperature of storage may lead to differences in the ability to recover and re-test the sample.

Previous DNA storage studies indicate that DNA samples stored in polypropylene tubes resulted in a lower DNA recovery than when stored in Teflon. One potential explanation is that DNA may be retained within the chemical lining of the polypropylene tubes. Additionally, DNA storage in a new polymer, SampleMatrix (SM), at room temperature has been found to result in higher DNA recovery than those frozen without SM.<sup>1</sup> Thus, the hypothesis states that Teflon tubes with SM will preserve samples more efficiently and increase the amount of DNA recovery versus those stored in polypropylene tubes.

Replicate dilutions of control DNA at 0.5, 0.25, and 0.05 ng/ $\mu$ L were prepared and quantified by qPCR to provide a starting baseline concentration of the amount of DNA. Enough replicates were prepared to be sampled at three days, seven days, three months, six months and one year in either Teflon or polypropylene tubes with and without SM at room temperature or as frozen liquid extracts at -20°C. Temperature and humidity were controlled in an environmental dry storage chamber with desiccant and electronically monitored over time with an electronic humidity and temperature logger.

Quantity and quality of the recovered DNA samples were evaluated using agarose gel electrophoresis; qPCR and fluorescent multiplex short tandem repeat amplification followed by capillary electrophoresis. Comparison of peak heights from replicate samples stored under different conditions was performed to evaluate differences in quality and quantity of recovered DNA. It is expected that recovery of DNA samples stored in Teflon with SM will outperform either treatment alone and will be better than those stored at -20°C.

Results from the first two time points were unexpected: Samples stored in polypropylene tubes at -20°C had a higher overall rate of DNA recovery than samples stored in Teflon with or without SM at room temperature and -20°C. Surface tension of DNA samples stored within the Teflon may have resulted in inaccurate recovery of the DNA. Results from the additional analyses of replicates stored for three months and six months will be reported.

This research was supported by NSF-REU grant.

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DNA Storage, Room Temperature, SampleMatrix, DNA Stable

#### A54 Fundamental Measurements for Trace Detection of Energetic Materials

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After attending this presentation, attendees will have an understanding of: (1) the importance of thermophysical and analytical properties in the detection of explosives; and, (2) the impact of reliable measurements and models of thermophysical properties of explosives.

This presentation will impact the forensic community by providing familiarity with thermophysical property and analytical measurements that are of importance in characterizing explosive vapors.

The continuous emergence of new and non-standard explosive compounds necessitates the need for reliable explosive detection devices. This presentation represents the work on fundamental measurements that enable the development of such devices. Vapor detection methods for sampling and detecting energetic compounds that may be components of improvised explosive devices (IEDs) are very attractive because they are sensitive, selective, and afford non-invasive, standoff detection. То develop reliable vapor detection devices for energetic materials, it is necessary to know: (1) what; and, (2) how much is in the vapor phase above the energetic material or IED. The method presented both identifies and quantifies components (even trace components of low volatility) above energetic materials with very low uncertainty. This method, a modified purge and trap approach, makes use of cryoadsorption on short alumina-coated porous layer open tubular (PLOT) columns. To illustrate this method, headspace measurements on practical military and industrial plastic bonded explosives (PBXs) including tagged C-4, Semtex-1A, Semtex-H, detonating cord (detcord), and sheet explosive

(Detaflex) are shown. Components of the headspace were identified and quantitated as a function of temperature and are presented in the form of van't Hoff equations. The linear relationship of the recovered mass as a function of inverse collection temperature reveals the predictive capabilities of the methodology. Thus, the necessary data for standardization and calibration of current and emerging in-the-field vapor detection devices is possible. This study also presents a novel apparatus and method for detecting and quantifying the permeation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) through polymer barriers (i.e., plastic bottle and containers). Measurements have been performed with 35% and 50% hydrogen peroxide by weight. Polymer barriers of several thicknesses made from polyethylene terephthalate (PET), both high and low density polyethylene (HDPE and LDPE), polylactic acid (PLA), and polypropylene (PP) were tested. Analytical methods were also used to measure two fundamental thermodynamic properties, vapor pressure  $(p_{sat})$  and enthalpy of adsorption ( $\Delta H_{ads}$ ), that are critical to the detection of energetic materials. p<sub>sat</sub> for three mononitrotoluene compounds was measured (which are used as detection taggants in plastic explosives) with a gas saturation apparatus. In this type of apparatus a carrier gas stream is saturated with the vapor of a condensed phase. The vapor is then stripped from a measured volume of the carrier gas, the amount of vapor is determined analytically, and p<sub>sat</sub> is calculated by assuming ideal gas behavior. Gas-solid chromatography was used to measure the  $\Delta H_{ads}$ for a variety of fuel-like compounds (acetone, benzene, n-alkanes, etc.) on concrete. This work is part of a larger project to study the surface energetics of chemicals on construction materials.

Adsorption, Explosives, Vapor Pressure

#### A55 Application of Pressure Cycling Technology (PCT) in Differential Extraction

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After attending this presentation, attendees will understand a new method for the differential extraction of DNA from sperm and epithelial cells in sexual assault casework.

This presentation will impact the forensic science community with a better understanding of how pressure cycling technology can be used to speed up and simplify the extraction process.

One of the stumbling blocks in obtaining a successful male genetic profile in sexual assault cases involves the separation of the evidence left behind by the perpetrator from that of the victim's. Conventional differential extraction method used for the separation of DNA from sperm and epithelial cells is time consuming and requires expertise. It is imperative to develop a method that addresses the issues of time, efficiency and ease of use.

Pressure cycling technology sample preparation system (PCT SPS) is a novel method that involves the use of pressure to disrupt tissues, cells and cellular structures enabling the recovery of their components. This research has utilized a commercially available instrument from Pressure Biosciences with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 35000 psi resulting in the lysis of cells Sample cells are placed in liquid suspension in microtubes and subjected to a range of on and off pressure pulses in an attempt to isolate and recover DNA. The microtubes are made from a fluoropolymer that renders them chemically resistant to improve sample recovery and limit adsorption.

The current study involves the application of pressure cycling technology in the extraction of nucleic acids from sperm cells and vaginal epithelial cells. The cells were suspended in 1X PBS buffer (pH 7.4) and subjected to 5,000 psi- 35,000 psi pressure in increments of 5,000 psi accompanied by varying number of cycles to determine the conditions at which one type of cell could be lysed differentially over the other.

Samples were placed in microtubes and introduced into the pressure chamber. This pressure treatment was followed by phenol chloroform isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with Alu-based real-time PCR method using SYBR green.

The initial studies indicate the potential of PCT application in analyzing samples from sexual assault cases in particular indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells. Overall these results provide new opportunities to explore the ability to generate male DNA profile by selectively lysing sperm cells from mixtures.

Differential Lysis, Pressure, Sexual Assault

#### A56 Evaluating the Applicability of Direct Polymerase Chain Reaction (PCR) Techniques to Samples from Human Skeletal Remains

Krista E. Latham, PhD\*, University of Indianapolis, Biology Department, 1400 East Hanna Avenue, Indianapolis, IN 46227; and Megan E. Madonna, BS, 4510 Marcy Lane, Apartment 40, Indianapolis, IN 46205

After attending this presentation, attendees will understand the possibility of using direct polymerase chain reaction (PCR) methods to perform genetic analysis on human skeletal remains. The ability to conduct direct PCR would potentially reduce the amount of biological sample destroyed for DNA isolation, as well as decrease the time and cost constraints of creating a genetic profile from skeletal DNA for positive identification purposes.

This presentation will impact the forensic science community by introducing a new tool that can potentially be employed in the positive identification of human skeletal remains via DNA analysis. It is important that the forensic community be aware of such advances that can decrease the amount of valuable biological evidence destroyed in attempts at producing a genetic profile.

Positive identification of skeletonized human remains using DNA analysis is becoming more and more frequent within the forensic community. While the forensic anthropologist plays an important role in estimating the descendant's biological characteristics such as age, sex and ancestry, as well as other essential tasks like ascertaining perimortem trauma, estimating the postmortem interval and identifying factors that have altered the remains since the time of death, the actual positive identification of human skeletonized remains is often the job of the forensic odontologist or DNA analyst. The process of obtaining analyzable DNA from bone has traditionally been a destructive, timely and costly process. While the starting amount of osseous material required for DNA analysis has decreased significantly over the past decade, many forensic DNA labs still require one or two whole bones from the decedent for analysis. As an expert in the human skeleton, the forensic anthropologist is often consulted regarding the appropriate bones or amount of bone to be submitted for DNA analysis.

The isolation of DNA from human cells entails the disruption of the structural components of the cells enclosing the nuclear and mitochondrial DNA. This process is simpler in a soft tissue cells in which the components are mostly fluid and membranous. The process becomes increasingly difficult in bone cells as the structural components also consist of a calcified extracellular matrix. Isolation of skeletal DNA has traditionally required the disruption of the inorganic hydroxyapatite structure of the osseous tissue, followed by a disruption of the organic collagen component of the osseous tissue, and finally the purification of the genetic material. Dependent upon the particular lab protocols, kits or chemical reagents used the process can require several grams of powderized bone, several weeks of preparation and hundreds of dollars

per sample. Recent advances in DNA analysis from soft tissue have led to the development of direct PCR techniques in which a microscopic piece of tissue, with no prior DNA purification, could be added directly to a tube prepared for PCR amplification. Such a scientific advancement saves copious amounts of time, expense and sample destruction.

This pilot experiment was designed to test the Finnzymes Phire® Animal Tissue Direct PCR Kit (New England BioLabs) using human bone as a source of template DNA. The study employed bone samples excised from two temporal periods: a modern bone from a recent death and an historic bone dating to a 19<sup>th</sup> century Euro-American cemetery. A small piece of bone (less than 0.2 grams) was powderized, and several grains of bone powder were directly added to the PCR tube. PCR amplifications targeted both a nuclear DNA locus and a mitochondrial DNA locus, as both are commonly employed in forensic DNA analyses. The direct PCR technique was found to amplify both loci from both temporal samples, although the results were not consistently repeatable. The preliminary results indicate that while it is possible to employ direct PCR techniques to human skeletal material it still requires significant optimization.

Skeletal DNA, Direct PCR, DNA Amplification

#### A57 Impact of Additional STR Loci on Random Match Probability Calculations and Kinship Analysis

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After attending this presentation, attendees will understand the value of additional autosomal STR loci in performing random match probability calculations, DNA database searches, and kinship analysis involving close relatives.

This presentation will impact the forensic science community by showing how DNA database searches and kinship analysis can be benefitted by additional STR loci.

Since their selection in November 1997, genetic information from a core set of 13 autosomal short tandem repeat (STR) loci have been required by the FBI for upload of DNA profiles to the national DNA database.<sup>1,2</sup> Unfortunately, only eight of the current 13 United States core loci overlap with data being gathered in the United Kingdom and most other European nations. Thus, international DNA comparisons can be hindered by lack of information overlap. As the United States considers expansion to additional core loci, recently adopted and previously used European STR markers should be considered to provide greater capabilities for international comparisons where needed. The European forensic DNA community has expanded the number of core loci for the same reason that the United States must do the same in the very near future–concern over potential adventitious matches between DNA profiles when trillions of comparisons are being performed with DNA database searches involving millions of profiles.

In November 2009, the European Union adopted five new autosomal short tandem repeat (STR) loci as part of their expanded European Standard Set (ESS). These new ESS STR loci, which include D12S391, D1S1656, D2S441, D10S1248, and D22S1045, were selected based on discussion over the past few years within the European Network of Forensic Science Institutes (ENFSI).<sup>3,4</sup> In the past year, Promega Corporation and Applied Biosystems have released new STR kits to enable coverage of these additional loci as well as the highly polymorphic locus SE33.

The probability of identity with different sets of loci will be illustrated in order to help assess the benefits of adding additional loci to the current 13 CODIS core loci. In addition, likelihood ratio calculations with parent-offspring, full siblings, and half-siblings will be shown.

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- <sup>3.</sup> Gill, P., et al. (2006) The evolution of DNA databases-Recommendations for new European STR loci. *Forensic Sci. Int.* 156: 242-244.
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Forensic DNA, New STR Loci, Kinship Analysis

#### A58 Indicting John Doe by His DNA – A DNA Analyst's Perspective

Noelle J. Umback, PhD\*, and Marie Samples, MS, Office of the Chief Medical Examiner, Department of Forensic Biology, 421 East 26th Street, New York, NY 10016

After attending this presentation, attendees will learn about Grand Jury presentations against DNA profiles in unsolved cases. These indictments have the potential to be amended to include the individuals' names if the cases are solved in the future.

This presentation will impact the forensic science community by demonstrating how John Doe indictments in New York City have created the potential for prosecution of heinous crimes which otherwise would move beyond the reach of the law.

For the last decade, the District Attorney's Offices (DAOs) in New York City have been making use of the John Doe Indictment as an instrument to stop the clock before the statute of limitations has run out. This method has been used extensively during the "DNA era" for sexual assault cases that occurred before DNA testing was available. These cases were tested years after the incidents occurred, but before their statutes of limitation had expired; on occasion, indictments were filed only days before the deadline. In 2006, the laws of the State of New York changed to allow sexual assaults to be prosecuted at any time after the incident when there is DNA evidence available. Previously to that, there was a five year statute of limitations for the prosecution of sexual assaults when the offender was known, with an additional five year extension when the offender was unknown. Other felonies (except homicides) have a five year statute within the State of New York.

Beginning in 2000, a joint project was undertaken by the New York City Police Departmen (NYPD), the five District Attorneys' Offices, and the Office of the Chief Medical Examiner (OCME) Department of Forensic Biology, to perform DNA testing on sexual assault kits collected during the years before it became standard for every NYC kit to be tested. This included approximately 17,000 kits, which were analyzed by three commercial labs and entered into CODIS by the Department of Forensic Biology. The "NYC Rape Kit Backlog Project" has been described previously at various forums.

Because these backlog cases dated back as far as 1989, many hundreds were already past the statute of limitations before testing even commenced. The most that could be done for them was to link up patterns and report any confirmed offender matches for the record. The majority, however, were cases where the statute of limitations had not yet expired. The inaugural John Doe indictment was filed in 2000 against the "East Side Rapist," a notorious Manhattan serial offender active from 1995-1997, who remains unidentified. As the Rape Kit Backlog Project progressed, the DAOs identified certain cases they desired to attempt to prosecute and the John Doe Indictment Project (as described in a 2003 press release by Mayor Bloomberg) went into high gear. Generally the cases were chosen because they were especially violent, had children as victims, were part of a rape pattern, or had other extenuating circumstances. Other characteristics of candidate cases were that the offender remained unknown, and therefore the case unsolved, despite due investigative diligence including CODIS; the victim being available and willing to testify; and that there was a single-source male DNA profile in the case (in order to cite statistics showing the profile was expected to be unique in many world populations' worth of people).

For a DNA analyst, a John Doe indictment in NYC is very similar to Grand Jury testimony where the perpetrator has been identified. There is a voir dire, background questions about what DNA is and how the testing works, and specific information about the case at hand. However, instead of proceeding to state that "the DNA in this case is the same as that of...," the statement is "the DNA in this case came from an individual with the following DNA profile" at which point the numeric profile must be read by the analyst into the record. If the Grand Jury votes a True Bill (meaning, votes to indict) "John Doe with known profile," the charges are filed and the statute of limitations clock is effectively stopped. Then it is simply a matter of waiting for a database match to occur, and thereafter amending the indictment to the name of now-identified perpetrator.

Dozens of John Doe indictments have been obtained in New York City in the last several years; however, only a small number have been later matched to a known individual via CODIS and converted to active indictments. Many of those which were converted have resulted in guilty pleas or verdicts.

Issues to be weighed when considering the use of John Doe indictments will be offered, as well as illustrative examples as time allows.

John Doe Indictment, Statute of Limitations, DNA Testimony

#### A59 Forensic Tissue Identification Based on DNA Methylation

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After attending this presentation, attendees will become acquainted with a new approach to forensic tissue identification based on methylation analysis.

This presentation will impact the forensic science community by demonstrating the potential of DNA methylation-based forensic tissue identification and the advantages of this approach over existing methods.

Identifying the source tissue of biological material found at crime scenes can be very informative in a number of cases. Current visual, catalytic, enzymatic, and immunologic tests for presumptive and confirmatory tissue identification are applicable only to a subset of samples, suffer limitations such as low specificity, can lack sensitivity of detection, and are substantially impacted by environmental insults. Moreover, these assays are incompatible and thus cannot be multiplexed and are less amenable to automation. In addition their results are operator-dependent. A better alternative approach is tissue identification based on messenger RNA (mRNA) and microRNA (miRNA); however, RNA is not as stable as DNA and requires the use of non-standard procedures by forensic laboratories.

A DNA-methylation based assay for forensic tissue identification can serve as an alternative and potentially overcome the limitations associated with extant methods. In mammalian DNA, methylation occurs at the C5 position of cytosine in some CpG dinucleotides. In the human genome 70 - 80% of all CpGs are methylated, while unmethylated CpGs are mainly grouped in "CpG islands" positioned at the 5' ends of many human genes. The exact biological function of DNA methylation remains poorly understood, however differential methylation patterns between different tissues have been demonstrated. Previous research found genomic loci that are consistently methylated and other loci that are consistently unmethylated in natural human DNA samples extracted from forensically relevant tissues, and showed that they could be used to differentiate between natural and artificially synthesized DNA. Herein genomic loci were utilized that were found to be differentially methylated between forensically relevant tissues forming the foundation of a DNAbased forensic tissue identification assay.

The presentation will demonstrate a DNA-based assay that performs tissue identification based on detection of tissue-specific methylation patterns. DNA samples are subject to digestion by a methylationsensitive restriction endonuclease followed by multiplex amplification of specific genomic targets with fluorescent-labeled primers, capillary electrophoresis of amplification products, and automatic signal analysis by dedicated software, yielding the source tissue of the sample. The single tube assay was designed for easy integration by forensic laboratories (as the assay utilizes the same platforms as current forensic STR profiling). Moreover, the system is fully automatable, provides operator-independent results, and allows combining tissue identification with profiling in a single procedure.

Results will be presented of the tissue identification assay performed in two modes: as a standalone assay and combined with DNA profiling. The assay was tested on 50 DNA samples from blood, saliva, semen, and skin epidermis, and source tissue was successfully identified in all cases. Detection of semen and DNA profiling were combined into one assay and the ability to detect mixtures of semen and saliva in various ratios was demonstrated. The assay correctly detected semen in all samples where it was present, and the calculated percentage of semen was comparable to the fraction of semen in the samples.

Tissue Identification, Differential Methylation, Forensic Science

#### A60 A Tale of Two Cities: Testing Your Rape Kit Backlog — Two Very Different Approaches

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After attending this presentation, attendees will be able to compare two different approaches to testing a jurisdiction's rape kit backlog. In one case, a forklift approach was taken and every kit of a 17,000 kit backlog was tested; in another, kits were triaged and carefully screened for maximum efficiency.

This presentation will impact the forensic science community by demonstrating results of two different approaches to backlog testing. Attendees can determine whether the most efficient approach is the best approach and compare outcomes in terms of cost, time, and whether the crimes were solved.

In 2000, when New York City's DNA lab joined CODIS, the New York Police Department (NYPD) and the Office of the Chief Medical Examiner (OCME) decided to tackle New York City's backlog of untested rape kits. At that point there were close to 17,000 sexual assault evidence kits in storage. After entering into contracts with three private laboratories, the NYPD began shipping close to 200 kits per month to each lab, for a total of 600 kits per month.

Because rape evidence kits are collected from victims at the very start of an investigation, they had been stored with only a police evidence voucher and no accompanying paperwork containing details of the crime or name of the suspect. This made it difficult, expensive, and time consuming to determine whether the case underlying each kit was prosecutable, whether an arrest had in fact been made, and if so, the disposition in court. In addition, the statute of limitations on thousands of cases was ticking away. For these reasons, a decision was made to test every single kit in the order they were removed from storage, with no regard to the status of the case.

In Los Angeles County, the decision to tackle the backlog of untested rape kits was based on policy changes. In 2008, increased media attention due to backlogs reported in Los Angeles City and pressure from rape treatment centers and victim advocate groups convinced the Sheriff and County Board of Supervisors to direct the crime lab to locate and examine all untested sexual assault kits.

After hand counting all 6,700 kits from the Sheriff's Department and its 87 client municipal law enforcement agencies, the crime lab devised a triage plan. The goal was to prioritize testing of kits most likely to get predators off the street in the fastest way possible. This required significant upfront administrative work to determine the status of the cases represented by each untested kit. Prior to testing, every agency was sent a detailed questionnaire to determine which category a kit fell into: Category 1, the highest priority, involved kits with unknown suspects, where DNA testing could potentially identify the perpetrator. Categories 2 through 6 involved known suspects, cases rejected by prosecutors, cases already adjudicated, cases where the police agency did not fill out the questionnaire, and cases where it could not be established whether a crime was actually committed. Ultimately, every kit was slated to be tested, regardless of investigative need.

Comparing statistics regarding crimes solved and costs involved will help jurisdictions facing backlog reductions to decide whether either approach meets their needs.

Rape Kit, Backlog Testing, Outsource Labs

#### A61 Team Effort: A Mid-Size DNA Lab's Approach to a Large Scale Validation Project

Kristy Kadash, PhD\*, Colorado Bureau of Investigation, Department of Public Safety, 690 Kipling Street, Suite 3000, Denver, CO 80215

After attending this presentation, attendees will understand the approach taken by the Colorado Bureau of Investigation laboratory to complete a large scale validation project involving a unique combination of commercial products through collaboration with the associated manufacturers and private laboratories.

This presentation will impact the forensic science community by providing an alternative to conventional models of laboratory validation. It will also provide some lessons learned by the CBI laboratory during the validation process.

In 2008, the Colorado Bureau of Investigation (CBI) laboratory Biological Sciences section launched a large scale validation project intended to improve efficiency. The National Institute of Justice DNA Backlog and Capacity Enhancement grants awarded to the CBI lab in the years 2006 through 2009 provided the funding for this long-term plan. This project involved new extraction chemistry, new STR amplification kits, and a liquid handling system to automate the extraction, quantitation, normalization, and amplification steps of the DNA analysis process.

Preliminary evaluations of extraction kits, STR kits, and robotic platforms were conducted initially in order to select the products that best fit the laboratory's need. A series of known samples and mock evidence samples were prepared to examine the DNA yield of three commercial DNA extraction kits and the profile quality of two single reaction STR amplification kits. Various robotic platforms were evaluated by conducting visits to four different forensic DNA laboratories that had already validated and were using robots either in casework, databasing, or research capacities. As per Colorado state fiscal rules, a competitive bid was opened for robotic platforms that met the requirements compiled during the various site visits. At this time, all of the components of the validation project were determined, including the commercial kits and robot manufacturer.

At the beginning of this project, the CBI Biological Sciences section was comprised of 15 analysts in three laboratories across the state. As with most forensic DNA facilities, the section was under tremendous pressure to improve turnaround time and decrease case backloads in the face of increasing case submissions and budgetary restraints. Analysts could not be spared the time necessary to complete a thorough validation of this magnitude. As a result, it was decided that the CBI Biological Sciences section would employ a private lab to assist in the project. Another competitive bid was opened to contract a laboratory to design an efficient plan to incorporate all aspects of validating an extraction kit, a CODIS STR kit, a mini-STR kit, and a robotic liquid handler. The analysts in the CBI laboratories would conduct the experiments to gain the important hands-on experience with the new techniques. The contract laboratory would then analyze the experimental data provided by CBI analysts and prepare and organize the validation summary according to the requirements listed in Standard eight of the most current version of the FBI Quality Assurance Standards document.

This team approach to validation, a collaboration of a caseworking DNA laboratory and a private contract laboratory, was intended to provide an efficient and effective means for a mid-size lab to successfully accomplish a large scale validation project. As the project evolved, the team encompassed not only the private contract lab, but also the manufacturers of the products used in the validation. The combination of extraction kit, STR kits, and robotic platform that the CBI lab was intending to validate had not been done before. As a result, the CBI was involved in bringing together application specialists and researchers from all of the manufacturers in order to troubleshoot the numerous issues encountered. Not only did this benefit the CBI laboratory, but it also provided each of the manufacturers with the opportunity to expand their product capabilities.

Validations, Robotics, STR

#### A62 The Optimized Use and Recovery of DNA From Tape Lifts

Shannon R. Peters, BS\*, and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will become familiar with the efficacy of multiple types of adhesive tape used to collect cells, as well as multiple methods for the retrieval of cells from the tape.

This presentation will impact the forensic science community by identifying efficient methods for using adhesive tape to collect cells. These methods can then be applied to various crime scene items from which a DNA profile is desired.

There has been minimal research into what type of tape is best suited for the collection of cells, or how to retrieve DNA from adhesive tape, despite the potential benefits. For instance, tape lifts may prove more effective on clothing than a moistened swab, given that the wetting agent may actually soak into the cloth. Likewise, there is the potential for a suspect to leave behind skin cells on tape used in a crime, such as holding together components of an improvised explosive device or sealing a container to prevent its opening.

The goal of this study was to identify the most effective tape(s) for collecting cells for subsequent DNA analysis by comparing the DNA yields from multiple types of tape (e.g., invisible tape, electrical tape, packing tape, surgical tape, fingerprint lifting tape, etc.). Different recovery techniques were also compared, including the use of foam or cotton swabs moistened with different adhesive removers, digestion buffer, and water. The isolated DNA was characterized throughout using commercial STR kits to ensure recovered DNA was of a quantity and quality adequate for analysis.

In preliminary experiments, known volumes of blood were spotted directly on the tape. Swabs moistened with one of the agents were used to wet the tape, loosen the cells, and collect as much of the stain as possible. DNA was then extracted organically and quantified using Real Time PCR to determine what percentage of the blood had been retrieved. Next, known volumes of blood were allowed to dry on Petri dishes and then lifted with tapes; any cells remaining on the surface of the dish were collected on a swab moistened with digestion buffer. Cells were again removed from the tape lifts using the solutions above, followed by extraction and quantification of the DNA.

Previous studies examining the isolation and purification of DNA from tape lifts have generally employed Chelex extractions; however, these are rarely conducted in crime labs today. For this reason, the more widely used organic extraction was compared to a commercially available kit using methods optimized in the tape lift portions of the study.

Taken together, the results show the utility of using tape to collect DNA from surfaces that may be associated with a crime scene, including clothing and firearms. Tape may likewise serve as a source of DNA from the perpetrator or someone involved in the crime. Clearly optimizing cellular retention on tape, along with its subsequent recovery and DNA analysis, is of prime importance if such adhesives are to be widely used by the forensic community.

**DNA**, Tape Lifts, Adhesive Remover

#### A63 DNA Analysis and Document Examination: Impact of Technologies on Respective Analyses

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After attending this presentation, attendees should appreciate how best to maximize evidence from documents that require DNA testing and document examination.

This presentation will impact the forensic science community by improving evidence obtainable from document exhibits.

With the enhanced sensitivity of specialized DNA profiling techniques there is now an increased demand to undertake DNA analysis on a range of samples that contain low amounts of template DNA (LtDNA). DNA can be deposited on a document by the writer of a ransom note, for example. Epithelial cells are likely to transfer from various parts of the hand and lower arm when these areas come in contact with the document during writing. Cellular material may also fall onto the document from the face and clothing. Therefore, DNA profiles from documents may provide useful probative evidence, particularly in conjunction with evidence obtained through document examination techniques. This research evaluated the ability to recover DNA from touched documents while maintaining the integrity of the document, so as to maximize the evidence from forensically examined documents. To ascertain which places on a document were most frequently touched when writing, a group of volunteers were asked to write a set paragraph and on completion, fold the document into an envelope, while being observed by the researcher. The writing observation study indicated that there were significant points of contact between the writer and the margins, central face area, and fold lines of the document. Non-invasive sampling methods were tested so that the integrity of the document could be maintained for subsequent examinations. Wet/dry swabbing and dry/dry swabbing were both found to be equally effective at recovering DNA from paper, however difficulties were experienced with a tapelifting method tested. The amounts of DNA recovered from the most commonly touched places were determined. These showed that the best sampling site for the recovery of touch DNA from documents were the fold lines of the document, as these samples consistently provided the highest DNA yields among the participants. This was not an unexpected

finding. When a document is folded, there is heavy pressure applied to flatten and crease the paper and this contact allows loosely adhering epithelial cells from the hands to transfer to the paper. DNA was also recovered from other sampling sites, including the face of the document and the top corners. DNA profiling results were successfully obtained after targeted sampling. DNA inhibitory effects were also evident in a number of the samples profiled, with DNA results being obtained after dilution of the extract.

This research also investigated the impact of document examination techniques on DNA recovery. Commonly employed document examination techniques; the electrostatic detection apparatus, UV light, and the video spectral comparator were investigated to determine their effect on DNA recovery. The results indicated that the ESDA examination had the most deleterious effect on DNA recovery. Furthermore, the impact of DNA recovery on subsequent document examination techniques were examined with results indicating that DNA sampling from fold lines, by swabbing, had the least impact on subsequent document examination. The findings from this research, and those of a larger investigation, assisted in recommending best practice guidelines for the examination of documents by forensic biologists and document examiners. The findings also indicated how the two disciplines could be coordinated in such a way so as to maximize the evidentiary value of document exhibits.

Document, DNA, Touch

#### A64 The Effect of Cyanoacrylate Fuming on DNA Recovery From Post-Deflagrated IEDs

Stephen K. Gicale, MS, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824; Timothy Ketvirtis, BS, Michigan State Police Bomb Squad, 42899 Merrill Road, Sterling Heights, MI 48314; and David R. Foran, PhD\*, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will learn about the pros and cons of cyanoacrylate fuming shrapnel from deflagrated pipe bombs as it affects subsequent DNA isolation and analysis.

This presentation will impact the forensic science community as it is not currently known if cyanoacrylate fuming is advantageous for later DNA isolation by helping to retain cells on the fumed item or disadvantageous due to interference with subsequent DNA analysis.

Previous research has shown that it is possible to generate handlers' DNA profiles from post-deflagrated improvised explosive devices (IED). This is beneficial as fingerprints rarely survive the heat and flame of the deflagration. However, it is still standard practice to send IED components to trace evidence and latent print units for examination. During this process, the components are often cyanoacrylate fumed in an effort to enhance any latent prints. If no prints or other individualizing evidence is found, the components may be sent to the DNA unit in an attempt to recover cells shed by the assembler of the device.

Earlier studies on producing DNA profiles from deflagrated IEDs and/or components associated with them (e.g., triggering devices, packaging) have been increasingly successful. However, those items were not fumed or otherwise preprocessed, as they typically would be in a crime laboratory. To examine what effect cyanoacrylate fuming might have on DNA analysis from IEDs, 24 pairs of pipe bomb components (steel end caps and one foot lengths of one inch steel pipe) were handled by volunteers. The bomb pairs were then assembled and filled with smokeless powder by members of the Michigan State Police bomb squad.

The bombs were deflagrated under controlled conditions and the fragments from one of the pair members was cyanoacrylate fumed on site, while the other was left unfumed.

The fragments were then placed in a brown paper bag, sealed, and returned to the Forensic Biology Laboratory at Michigan State. DNA was

isolated using the double swab technique, using six pairs of swabs (two per end cap and two per pipe). DNA was quantified using QuantiFiler and amplified using MiniFiler. Consensus profiles were generated based on results from all six pairs of swabs, the accuracy of which was then determined based on buccal swab profiles for each volunteer. Taken together, the results show what effects cyanoacrylate fuming has on DNA recovery and analysis from deflagrated IEDs and whether it should or should not be performed if DNA analysis may be undertaken.

DNA, Improvised Explosive Device, Cyanoacrylate Fuming

## A65 A Systematic Comparison of Methods for Recovery and Analysis of DNA From Handled Objects

Martha Petrovick, PhD, MIT Lincoln Laboratory, 244 Wood Street, Lexington, MA 02420; James D. Harper, PhD\*, Chemical and Biological Defense Systems Group, MIT Lincoln Laboratory, MIT/LL, Lexington, MA 02420; Johanna Bobrow, BS, and Jeffrey Palmer, PhD, MIT Lincoln Laboratory, 244 Wood Street, Lexington, MA 02420; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277

After attending this presentation, attendees will have learned about the relative performance of different combinations of collection, extraction, and amplification methods used to analyze samples of touch or contact DNA from materials and items relevant to the investigation of domestic and international crime scenes.

This presentation will impact the forensic science community by providing information to guide tactics, techniques, and procedures for the sampling and analysis of forensic evidence, and information on the relative probabilities of obtaining profiles from evidence in varying conditions.

DNA provides highly specific biometric identification and DNA profiles can be obtained from handled objects and even from individual fingerprints (van Oorschot 1997, Zamir 2000, Pesaresi 2003).<sup>1,2,3</sup> DNA profiles from handled objects can then be compared to profiles obtained from individuals to determine the likelihood that they were the source of the DNA recovered from the object. Increasing amounts of forensic DNA evidence are being analyzed in order to obtain biometric information about the criminals and their networks. As backlogs increase for the processing and analysis of forensic DNA evidence from domestic and international crime scenes, the need grows for more efficient analytical methods. A growing variety of new collection tools, extraction methodologies, and amplification kits have been developed with the goal of providing improved DNA analysis capabilities, even for DNA that is degraded or present in low quantities. It is challenging to weigh the success of one laboratory in obtaining profiles from handled objects against the failure of another laboratory to do so when different combinations of techniques are employed (van Oorschot 1997, Ladd 1999).1,4

In order to evaluate the relative performance of available methods for the collection and purification of DNA deposited on items, a systematic comparison was performed using seven different swab types with four wetting agents. In addition, organic extraction was compared to commercial extraction kits, including PrepFiler<sup>™</sup>, QIAmp DNA Investigator<sup>™</sup>, and DNA IQ<sup>™</sup>. Using the optimal swab/liquid/extraction combination, DNA was collected from a variety of objects that are relevant to typical crime scene investigations including items such as tape, wire, and cell phones. Prior to collection, subjects were asked to handle each type of object according to common instructions designed to minimize handling variability and simulate normal use of the object. Typical DNA collection and purification yields from these handled objects as determined by QuantiFiler<sup>™</sup> analysis and the ability to produce quality STR profiles using AmpFISTR IdentiFiler<sup>™</sup> PCR amplification kits will be presented.

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**Recovery, Extraction, Analysis** 

#### A66 Extraction Methods for Recovering Touch DNA

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Upon completion of this presentation, attendees will have an understanding of characteristics of touch DNA and current extraction methods. Attendees will be shown data comparing four common extraction methods for touch DNA. This evaluation of the different methods will be based on STR data quality, ease-of-use, time, and cost. Attendees will have a basis of which extraction method is best for recovering touch DNA and may be guided as to what method may be most appropriately implemented in their laboratory.

This presentation will impact the forensic science community by evaluating currently implemented methods when deciding which extraction method to validate for touch DNA analysis.

Crime scene investigators are collecting touch DNA samples more frequently to be submitted to forensic laboratories. With the development of new technologies in forensic laboratories, the number of casework samples submitted for touch DNA analysis has increased. This is due primarily to the investigation of property crimes, in which touch DNA is the predominant source of biological evidence. Touch DNA, defined as the transfer of shed DNA during physical contact between an individual and an object can be found in the form of shed skin cells, latent fingerprints, small quantities of saliva, or "wearer DNA" - composed of skin cells and sweat. Such samples are difficult to analyze due to the inability to see the shed skin cells or saliva which are left on objects during contact. Touch DNA samples typically have lower DNA yields than other body fluid samples. Due to the nature of these samples, an extraction method that maximizes recovery and minimizes further damage to the DNA would be most suitable. However, no consensus within the DNA community has been reached as to what extraction method is best for retrieving touch DNA/low-level transfer DNA. This study evaluated the performance of several common manual extraction methods for retrieving touch DNA from both porous (cigarette butts, white bond legal size paper, and worn cotton clothing; N=32) and nonporous substrates (plastic bags, aluminum cans, conical tubes, and desktops; N=44). These methods included Qiagen QIAamp<sup>a</sup>, Applied Biosystems Prepfiler<sup>TM</sup>, Promega DNA IQ<sup>TM</sup>, and traditional organic extraction. The goal of this study was to determine which of these extraction methods would provide the highest DNA yield, quality, and most successful STR profiles. Extraction methods were compared based on results obtained as well as overall costs, labor time, and ease-of-use. Results indicate that all methods are comparable to one another in total DNA yield and STR allele success. However, this study suggests that

DNA IQ<sup>TM</sup> may be a less suitable extraction method for touch DNA based on STR allele peak heights. QIAamp<sup>®</sup>, Prepfiler<sup>™</sup>, and traditional organic extraction had average STR allele peak heights that were two to three times higher that DNA IQ<sup>™</sup>, on average. Furthermore, QIAamp<sup>®</sup>, Prepfiler<sup>™</sup>, and traditional organic extraction methods perform comparably in all other measures of STR data quality, making them equally appropriate methods for touch DNA extractions. Although not statistically significant, QIAamp® produced more alleles on average than the other methods, when both porous and nonporous results were combined for STR allele success. In forensic casework, this could mean an additional one to two STR loci eligible for CODIS entry, which would also greatly improve the power of discrimination for statistical calculations. The data indicates that labs may need to consider other factors when selecting a DNA extraction method for touch DNA analysis. Evaluation of ease-of-use, time, and cost indicates that QIAamp® may be the best method for manual extraction of touch DNA samples. This information could be useful for forensic laboratories when evaluating currently implemented methods or when deciding which extraction method to validate for touch DNA analysis.

Touch DNA, Extraction Methods, Low Template DNA

#### A67 ALS Detection and Collection of Touch DNA From Porous and Nonporous Substrates

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After attending this presentation, attendees will clearly understand the advantages of using visual enhancement techniques for touch DNA on porous and nonporous substrates. Attendees will also learn about different DNA recovery techniques for touch DNA on porous and nonporous substrates.

This presentation will impact the forensic science community by providing forensic laboratories with the information to adopt or modify their current protocols for enhancement and DNA collection.

The analysis of touch DNA is now an extremely important tool for crime solving. However, there remains a lack of easily accessible screening tests that would allow for location and detection of inconspicuous (touch or contact) stains. Further, collection methods vary lab-to-lab and there is no clear consensus on what collection methods/devices work best with common touch or contact stain surfaces.

The development of new detection methods would improve the efficiency of touch DNA sample processing by offsetting the high costs and labor time frequently associated with repeated testing from these types of stains. Therefore, the first goal of this study was to determine if visual enhancement of potential touch or contact areas using an alternate light source (ALS) would be a viable method for improving DNA yield and subsequent STR analysis. If successful, ALS methods could be beneficial as they would mitigate the undesired effects of collecting "blind" swabs without generating the potential negative effects often associated with chemical enhancement. Equally important for a successful DNA analysis is the collection method used to retrieve the touch DNA from a substrate. The standard collection methods used in most laboratories remain either the double-swab technique (using deionized water) or cuttings taken directly from the substrate itself. In this study, several collection methods were investigated to determine which methods, if any, offer improved DNA yields and/or STR success.

In this study, three alternate light sources (UltraLite<sup>TM</sup> ALS combined with the Blue Merge Technology, KRIMESITE<sup>TM</sup> Imager, and Spectroline® short-wave UV lamp) were used in conjunction with four DNA collection methods (tape lift, gelatin lift, swab with ddH<sub>2</sub>O, swab with 0.01% SDS, and cutting) to detect and collect touch DNA from a

variety of forensic-type substrates (porous and non-porous). For all samples, DNA was extracted with Qiagen QIAamp<sup>™</sup> DNA Mini kit, quantified with Quantifiler<sup>™</sup> Human DNA Quantitation kit, and amplified with AmpFISTR® Identifiler® PCR Amplification kit. Results showed that the use of an alternate light source greatly improved the DNA yield and resulting STR profiles when compared to blind collections. Based on the DNA sources included in the study, a regular short-wave UV light (Spectroline) was found most suitable for porous substrates while the Krimesite<sup>™</sup> Imager was most beneficial for nonporous substrates. Further, the double-swab technique with 0.01% SDS provided higher DNA yields than all other collection methods tested. Based on these results, these lights used along with the double-swab technique (with 0.01% SDS) are recommended for future use when attempting to detect, locate, and collect touch DNA material from forensic samples.

Touch DNA, ALS, DNA Recovery

## A68 Optimizing DNA Typing From Fired Shell Casings

Alicya E. Orlando, BS\*, and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will have been informed about maximizing DNA quality and yield, minimizing PCR inhibition, and what DNA typing methods work best on spent shell casings

This presentation will impact the forensic science community by establishing how best to isolate, purify, and analyze DNA from fired shell casings that may have originated from criminal activity.

Firearms are commonly used in crimes in the United States, but when investigators arrive at a crime scene, often the only evidence of firearm use is empty shell casings. Upon collection, shell casings may be processed by the latent print unit, however, attempts to recover prints are generally unsuccessful. Because of this, in the past few years researchers have started to examine the utility of DNA profiling from spent shell casings.

Cells/DNA may be deposited on the surface of a cartridge as it is loaded into the chamber; however, a cartridge is likely to be handled for only a short amount of time resulting in limited DNA deposition. Previous research has shown that it is sometimes possible to obtain a partial STR profile from DNA found on fired shell casings, although success rates have been low, and PCR inhibition was often problematic. Given this, optimizing the methodologies for obtaining and analyzing DNA from fired shell casings is critical. Considering the minute amount of DNA left on a casing, and that such DNA is likely to be degraded due to the heat that is characteristic of firing a weapon, it is important to optimize the quality and quantity of DNA recovered, and to analyze it in such a way so as to maximize the chances of obtaining probative information.

In the research presented, conducted as a blind study, volunteers were asked to load a gun magazine, as well as provide a buccal swab. The magazine was then loaded and the gun fired by a trained professional until the magazine was empty. The shell casings were collected, swabbed, and DNA purified using multiple methods, including phenol-chloroform and commercially available extraction kits. DNA yields were compared via real-time PCR. DNA typing was performed using commercially available STR kits, as well as mitochondrial DNA analysis. Profiles were developed both singly and through a consensus profile technique, in which results from all casings from a gun were considered collectively. The accuracy of these results was then determined through comparison to the profiles obtained from the buccal swabs. Taken together, this research shows what methods are best used to retrieve DNA from fired shell

casings, along with what DNA analysis techniques produce the most data on the individual who loaded the weapon.

DNA From Fired Shell Casings, Mitochondrial and Nuclear DNA Analysis, Cartridge Casings

#### A69 Fabrication and Evaluation of Adhesive Coated Collection Swipes for Improved Particle Collection Efficiency

Jessica L. Staymates, MFS\*, Jessica Grandner, and Greg Gillen, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Mailstop 8371, Gaithersburg, MD 20899

After attending this presentation, attendees will learn about one of the ongoing projects occurring at the National Institute of Standards and Technology that is related to forensics.

This presentation will impact the forensic science community by providing information on improved trace contamination collection efficiency.

Sample collection can be considered one of the most important aspects of trace chemical analysis. A trace sample can be in the form of small particles or vapor, either of which can be detected with the appropriate instrumentation. However trace evidence can not be reliably analyzed without a robust method for collecting and transporting the sample to the chemical detector or to a microscope for proper identification. There are many methods used to collect trace contamination, including tape pulls, swiping a surface with a collection swab, and a vacuum with a filter attached. One collection method widely used in airport security today consists of swiping a surface, such as luggage and even people's hands, with a particle collection swab. These swabs are then placed in an ion mobility spectrometer (IMS), with the ultimate goal of detecting explosives or drugs. The swab material used can vary between different IMS instruments. In a previous study,<sup>1</sup> it was found that some of these swabs collected particles with a higher efficiency than others. The current study was designed to find a method to improve the collection efficiency of some commercially-available IMS collection swabs.

One type of collection swab used in many IMS instruments is a fiberglass woven swab with a proprietary polytetrafluoroethylene (PTFE) coating. This swab had relatively low particle collection efficiencies compared to a muslin woven cloth collection swab.<sup>1</sup>

This presentation will describe a simple modification used to improve the particle collection efficiency of the PTFE swab. In this work, the PTFE swabs were coated with a heat-resistant low out-gassing pressure-sensitive silicon adhesive to make the trap surface tacky, promoting increased adhesion of the particles to the trap surface. Initial studies with polymer test particles suggest that improvements in collection efficiency by factors of 14 are possible using this approach. However there are other important factors to consider when using this adhesive material with IMS. One reason PTFE coated fiberglass swabs are used for IMS is because of the low background interference with the IMS chemical analysis. Adding an adhesive could potentially create more background noise and cause competitive ionization by depleting the reactant ions. These issues were tested during this study, and results revealed that the low-outgassing adhesive did not interfere with the ionization process, nor did it increase background noise. To examine the collection efficiency, polymer microspheres containing small amounts of explosives were placed on four types of surfaces using three different deposition methods.<sup>2</sup> All surfaces were swiped in a repeatable manner with both untreated and adhesive coated PTFE swabs. Both the particle collection efficiencies and IMS responses were recorded. IMS calibration curves with explosives solution deposition directly onto the untreated and

adhesion swabs were created prior to the swiping experiment for comparison of IMS results. Results, feasibility, and potential issues with using this method will be discussed, and videos and/or images from the swiping method will be presented.

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Trace, Ion Mobility Spectrometry, Particle Collection

#### A70 Effects of Temperature, Exposure Time, and Sample Size on the Recovery of Smokeless Powder Constituents From Car Fires

Dee A. Turner, BS\*, 4620 Nikki Drive, Apartment A, Indianapolis, IN 46237; Guihua L. Lang, PhD, ATF Forensic Science Laboratory-Washington, 6000 Ammendale Road, Ammendale, MD 20705; and John V. Goodpaster, PhD, Indiana University Purdue University Indianapolis, Forensic and Investigative Sciences Program, 402 North Blackford Street, LD 326, Indianapolis, IN 46202

After attending this presentation, attendees will understand the concept of the recoverability of the components of smokeless powder from a car bomb fire.

This presentation will impact the forensic science community by allowing explosives analysts to realize trends between recovery of the components in smokeless powder and the temperature of the fire, the exposure time of the sample to the fire, and the sample size.

The analysis and identification of the explosives used in improvised explosive devices (IEDs), such as pipe bombs, are an important part of bombing investigations. Intact particles from low explosives such as smokeless powder can often be found in post-blast debris. However, if the pipe bomb is placed inside a car or other areas containing combustible material, the explosion could cause the combustible material to catch fire. This may completely burn smokeless powder residues.

This work seeks to determine the temperature at which the chemical components of various smokeless powders burn off and how great of an effect exposure time and sample size have on the recovery of the constituents in smokeless powder. This work also seeks to make correlations between laboratory samples and real samples from car fires, in terms of the temperature and time that the smokeless powder residue was exposed.

Samples of single base and double base smokeless powder were heated in covered crucibles at varying temperatures, with a constant exposure time. The exposure time was tested by keeping the sample size and temperature constant, while varying the heating time. The sample size was tested by holding the temperature and exposure time constant while varying the mass of the sample. All samples were extracted with dichloromethane (DCM), sonicated for 30 minutes, and then filtered through a 0.45 micron PTFE filter prior to the analysis by GC-MS. DCM extracts of IMR PB, a single base smokeless powder, contain diphenylamine (DPA) and dibutylphthalate (DBP). DCM extracts of IMR 700-X and 800-X, double base smokeless powders, contain nitroglycerin (NG) and ethyl centralite (EC).

At higher temperatures the more volatile components will disappear while less volatile components will remain. This is shown by keeping the sample size and exposure time constant, while varying the temperature. For example, DPA and DBP were completely consumed at 160°C and 190°C, respectively. Also, EC and NG were completely consumed at  $150^{\circ}$ C and  $180^{\circ}$ C, respectively.

The amount of time that the sample is exposed to heat can also affect the recovery of smokeless powder components. Even though the temperature inside a pipe bomb can be extremely hot, intact particles can be found among the debris. This is due to the fact that the amount of time that the sample is exposed to the heat is very short. However, in a fire, the sample is exposed to the elevated temperatures for an extended period of time. For example, DPA was still present after exposure to 140°C for one hour but was completely consumed after two hours. DBP, on the other hand, was still abundant even after an exposure time of two hours. In addition, even after a two hour exposure time at 140°C, neither NG nor EC in the double base powder were consumed.

Car fire samples were also analyzed for comparison to these results to determine the temperature at which the fire was burning when it was extinguished. These results can be used to explain why all or some of the components of smokeless powder are not present or are significantly reduced in abundance.

**Smokeless Powder, Car Fire, Pipe Bomb** 

#### A71 Separation and Identification of Anions Using A Porous Graphitic Carbon Column and Electrospray Ionization Mass Spectrometry: Application to Inorganic Explosives and Their Post-Blast Residues

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After attending this presentation, attendees will learn the benefits of utilizing a Porous Graphitic Carbon (PGC) HPLC column coupled with Electrospray Ionization Mass Spectrometry to analyze inorganic low explosives.

This presentation will impact the forensic science community by introducing an efficient method for detection of inorganic low explosive constituent anions using readily available instrumentation.

Identification of the anions in inorganic explosives and their postblast residues using ion chromatography (IC) and/or capillary electrophoresis (CE) is well established. However, IC and CE instrumentation are not as common in forensic science laboratories. Furthermore, coupling IC and CE to a mass spectrometer can be challenging as volatile buffers are required or ion suppressors must be used. Porous Graphitic Carbon (PGC) stationary phases are a relatively recent column type that is available for High Performance Liquid Chromatography (HPLC). This stationary phase is known for its high retention of polar species and separation of anions using PGC has been demonstrated.

In this presentation, the application of a PGC column (100 x 2.1 mm, 5  $\mu$ m particles, 250 Å pores) to the separation of anions in low explosives will be discussed. The chromatographic method used a mobile phase of 0.5% formic acid in HPLC-grade water at 0.4 mL/min, a sample run time of 10 minutes, an injection volume of 1  $\mu$ L, and a column temperature of 30° C. The mass spectrometer cone temperature and voltage were set to 550° C and 75 V respectively.

The inorganic low explosive propellants analyzed were milligram quantities of American Pioneer Powder, GOEX Black Powder, Triple Seven, and Pyrodex. All powders were dissolved in 2 mL of HPLC grade water until completely dissolved. Burnt residues of the same powders were then analyzed by rinsing the residue several times with 2 mL of HPLC water from the watch glasses on which they were burned. A used piece of fire clay from a pyrotechnic device was also analyzed by rinsing with 5 mL of HPLC water. The samples were then filtered into 2 mL LC vials using a nylon syringe filter with a 0.2  $\mu$ m pore size. All samples were then analyzed at full concentration.

An HPLC water blank was run between each sample and selective ion monitoring was utilized to detect both isotopic forms of chloride <sup>35</sup>Cl<sup>-</sup> and <sup>37</sup>Cl<sup>-</sup> (m/z = 35 and 37 respectively); nitrate NO<sub>3</sub><sup>-</sup> (m/z = 62); both isotopic forms of chlorate <sup>35</sup>ClO<sub>3</sub><sup>-</sup> and <sup>37</sup>ClO<sub>3</sub><sup>-</sup> (m/z = 83 and 85 respectively); dichlorodiamide (DCDA) (m/z = 83); sulfate SO<sub>4</sub><sup>2-</sup> (m/z = 97); and both isotopic forms of perchlorate <sup>35</sup>ClO<sub>4</sub><sup>-</sup> and <sup>37</sup>ClO<sub>4</sub><sup>-</sup> (m/z = 99 and 101 respectively).

The major ions present in the propellants analyzed were consistent with their formulation. The results for black powder revealed the presence of nitrate. The results for American Pioneer Powder revealed the presence of nitrate and perchlorate. Analysis of Pyrodex and Triple Seven powders revealed the presence of nitrate, DCDA, and perchlorate. The water extract from the fire clay contained chloride, nitrate, sulfate, and perchlorate.

Inorganic Explosives, Porous Graphitic Carbon, HPLC

#### A72 Quantification of Inkjet Printed Ammonium Nitrate Test Materials by Ultraviolet Visible (UV/VIS) Spectroscopy

Marcela Najarro, MFS\*, Timothy J. Barr, and Greg Gillen, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Mailstop 8371, Gaithersburg, MD 20899

After attending this presentation, attendees will understand the feasibility of using UV/Vis to quantify ammonium nitrate.

This presentation will impact the forensic science community by introducing a simple analytical technique capable of accurately quantifying ammonium nitrate.

Ammonium nitrate (AN) is a white crystalline solid and strong oxidizing agent produced from the reaction between ammonia and nitric acid. Ammonium nitrate constitutes approximately 80 to 90% of the explosive used in the United States for industrial purposes (i.e. coal mining, metal mining, and civil construction). In addition, it is widely used as high-nitrogen fertilizer by the agricultural community. Even though ammonium nitrate alone is not an explosive, when mixed with fuel oil it forms a reasonably powerful commercial explosive. Due to its wide availability in the United States and worldwide, ammonium nitrate fuel oil (ANFO) has become the weapon of choice for domestic terrorism as well as devastating terror attacks worldwide. The Oklahoma City Bombing of the Murrah Federal Building in 1995 used about 5,000 pounds of ANFO.

ANFO is often encountered in the form of an improvised explosive device (IED) or car bombs. In 2009, ammonium nitrate fertilizer was used to make about 95 percent of the IED's in Afghanistan and accounted for most of the U.S. casualties. Therefore, analytical techniques such as Raman spectroscopy, Laser-induced breakdown spectroscopy (LIBS), and Infrared spectroscopy are of particular interest since they are capable of non-contact detection by using highly energetic lasers from varying distances. Test materials with known amounts of deposited ammonium nitrate are currently being developed to evaluate the capabilities of detection of a variety of techniques. The goal of this project was to determine the feasibility of using ultraviolet visible spectroscopy (UV/VIS) as a quantitative technique for the ammonium nitrate test materials (target accuracy and precision of 5% relative standard uncertainty). This goal will entail the characterization of UV/VIS, including determining its sensitivity, repeatability of measurements, and

linear dynamic range for the quantification of ammonium nitrate. Method validation will require the preparation of an ammonium nitrate solution gravimetrically and quantifying the solution by UV/VIS. The goal was to obtain measurement agreement between the two techniques of  $\leq 5$  % relative standard uncertainty. In addition, the use of inkjet printing systems for precision deposition of known quantities of the ammonium nitrate on a variety of surfaces will be evaluated.

Prior to analysis, the spectrophotometer was calibrated using standards in both the UV and Vis range to ensure wavelength accuracy. Preliminary results showed that UV/VIS spectroscopy can be used to detect and quantify ammonium nitrate from 275-196 nanometer (nm). Maximum absorbance was measured at a wavelength of  $201 \pm 3$  nm. Stability studies indicate that a sample remains stable for a period of at least nine days and that instrument drift is negligible (0.042% relative standard uncertainty) within a two hour period. Advantages of using UV/Vis spectroscopy to quantify ammonium nitrate are its ease in operation, rapid sampling time (~ 1 min/sample), and lack of sample preparation. A significant disadvantage of using UV/Vis for ammonium nitrate is the limited linear dynamic range  $(2 - 18 \mu g/mL)$  and the short wavelength at which it absorbs, leading to more specific solvent, cuvette, and spectrometer requirements. Gravimetric measurements of solid ammonium nitrate proved difficult given the hygroscopic nature of the compound in crystalline form. Results also indicate that inkjet printing is a suitable technique for precision deposition of ammonium nitrate. The main advantage of using inkjet printing systems to deposit ammonium nitrate is their good repeatability (< 4% uncertainty per run). Significant disadvantages of printing ammonium nitrate are consistent with standard inkjet printing systems, which include clogged tips and crystallization around the tip leading to inconsistent results.

Ammonium Nitrate, UV/VIS, Inkjet Printer

## A73 On the Origin of Volatile Compounds Emitted by Plastic Explosives

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After attending this presentation, attendees will learn about the chemical composition of plastic bonded explosives (pbx) as well as what volatile compounds are emitted by these materials. Data suggesting potential origins for these compounds and the implications for canine detection of PBX will also be discussed.

This presentation will impact the forensic science community by increasing the understanding of how explosive-detecting canines locate explosives and explosive devices.

One of the enduring riddles regarding the performance of explosivedetecting canines is their ability to readily detect samples of plasticbonded explosives (PBX) whose base explosive is essentially nonvolatile. For example, the headspace concentrations of PETN (the base explosive in Detasheet) and RDX (the base explosive in Composition C-4) are on the order of 10<sup>-12</sup> and 10<sup>-13</sup> M, respectively. Therefore, while canines have been shown to be extraordinarily sensitive, it is not unreasonable to hypothesize that perhaps other species that are emitted by PBX are responsible for canine alerts to these materials. Previous studies of the headspace above PBX using Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) have identified taggants such as dimethyldinitrobutane (DMNB), residual solvents such as cyclohexanone, and several other species – all of which are more volatile than the parent explosive. In particular, 2-ethyl-1-hexanol has been found in the headspace above Composition C-4 and butyl acetate has been found in the headspace above PETN-based flexible sheet explosive. Although the response of explosive-detecting canines to these compounds has been evaluated, response rates varied widely depending upon the amount of the chemicals used. Although not identified as such in the literature, it is hypothesized that these compounds can be formed by hydrolysis of the plasticizers used in PBX. These plasticizers include bis(2-ethylhexyl)adipate (DOA), bis(2-ethylhexyl)sebacate (DOS), and tributylacetylcitrate (citraflex). In addition, plasticizer hydrolysis would be catalyzed due to the natural formation of nitric acid by the base explosive as it degrades.

In this study, several samples of various PBX (Composition C-4, Detasheet, Shape Charge and a "Bubble Gum" Booster) were extracted with pentane, acetone, and water. All solvent extracts were prepared by placing samples of PBX in culture tubes, adding the appropriate solvent, and sonicating. The pentane extracts were analyzed by GC/MS to identify the plasticizer used in the formulation. The acetone extracts were analyzed by LC/MS to identify the base explosive. The water extracts were also analyzed by LC/MS for residual nitrate, which may indicate the formation of nitric acid. Finally, samples of PBX as well as the plasticizers DOA, DOS, and citraflex were analyzed by headspace SPME-GC/MS to identify any volatile compounds. The results of these analyses are summarized in the following table:

South .	VELATILE COMPONENTS	TRANSFERRAL (STATE	11,6:17(722245)	STEATS
Star Ga	(Archanol	21T	Cambre	38.5
Caspake.	entriences, Laboration, TAND	212	TEX.	62
Diated	Horard, cycleboards, 20184- Labrard, 12605	PETE	Confectinger)	712
Sheet Sharp	Default, cyclemases, Veteral Connect TWHA	WE's	DuStrians Dantes (dasa)	19.9
DOWNEDDS Studie	Setting	-	in the second	-

Several trends were noted following these analyses. As in previous studies, residual solvents and taggants were identified in the headspace of PBX. In addition, 2-ethyl-1-hexanol appeared in the headspace of all explosives that contained either DOA or DOS (both of which have 2-ethylhexyl side chains). It was also noted that DOS generated significantly higher levels of 2-ethyl-1-hexanol than did DOA. Although 1-butanol appeared in the headspace of all explosives that contained citraflex, it was present at low levels. In contrast, the citraflex plasticizer itself generated significant amounts of butyl acetate, which was not observed in any of the PBX samples. In all cases, the PBX samples did not exhibit any compounds in their headspace that could be associated with the base explosive under the conditions used for SPME-GC/MS. Finally, the levels of residual nitrate found in the PBX samples were significant and this could indicate that nitric acid is being generated and that it also catalyzes the hydrolysis of the PBX plasticizers.

Plastic Explosives, Canine Detection, Headspace Analysis

#### Validation of a A74 Prototype Surrogate Explosives Kit as Tool for a the Detection Enhancement of Explosive **Canine Training**

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After attending this presentation, attendees will understand the steps taken for the validation of the prototype surrogate explosives kit that provides a less hazardous and more controlled delivery of explosive odorants. Attendees will also learn that the validated prototype explosives kit provides an additional tool for canine handlers to use reducing the risk to the detector teams and increasing the number and consistency of target odors used for training.

This presentation will impact the forensic science community by providing a tool which gives access to more uniform training materials, improves the reliability of biological detectors, and allows for direct comparisons to other biological detectors as well as electronic detectors. Standardization of detection canine training aids will ensure that the maximum number of explosive odors are detectable in the most efficient and reliable manner.

Detection canines are commonly used in explosives detection and have been proven to be valuable assets for the rapid detection of an explosive odor. Detection canines are the most common and widely accepted biological detectors due to the ability of canines to quickly and reliably locate the source of an odor to which they are trained. The goal of this study is to outline the steps taken for the validation of a prototype surrogate explosives kit that provides a less hazardous and more controlled delivery of explosive odorants.

Recent research has been conducted in determining the dominant odor signatures of explosives resulting in the development of a prototype explosives kit containing a small number of proposed mimics that can be used to more consistently train a biological detector. The prototype explosives kit contains only non-controlled substance mimics previously demonstrated to be dominant odor compounds used by biological detectors to reliably locate the majority of target explosive materials.

The validation of the prototype explosives kit has several aspects which will be directly dealt with. The first is to prove that previously certified detector canines will alert to all of the compounds found within the prototype explosives kit. This step is necessary to validate the functionality of the kit as the kit contains only non-controlled mimics of the minimum set of mandatory explosives along with plastic and nitroglycerin containing explosives. Explosive canines with no previous exposure to the compounds in the kit should alert to all of the compounds as long as they were trained on the real explosives mimicked within the kit. To date, previously certified detector canines have had a positive range of alert rates to aids contained within the prototype surrogate explosives kit. The second aspect is to train green canines for explosives detection only using the mimics within the prototype explosives kit. Once successfully trained to the odors in the training kit, double blind field trials are used to test the handler/canine team's ability to alert to actual explosive material. After testing a small number of canines, a 100% alert rate has been achieved for the detection of real explosive material. The next aspect is to determine the efficacy of detection between explosive materials vs. commercial explosive materials vs. developed explosive mimics/pseudos. There has been long standing debate over the use of real explosive material versus mimics/pseudos. This experiment shows which, if any, of the materials are superior through double blind odor recognition tests with previously certified explosive canines. Testing materials include: real explosives, NESTT training aids, NIST pseudo training aids, and explosive mimics used in the prototype explosives training aid kit.

The validated prototype explosives kit provides an additional tool for canine handlers to use that reduces the risk to the detector teams and increases the number and consistency of target odors used for training. Access to more uniform training materials improves the reliability of biological detectors and allows for direct comparisons to other biological detectors as well as electronic detectors. Standardization of detection canine training aids will ensure that the maximum number of explosive odors are detectable in the most efficient and reliable manner.

This research was produced with partial funding from the Office of Standards, Science & Technology Directorate, and U.S. Department of Homeland Security.

**Detection Canines, Explosives, Training Aids** 

#### A75 Update on the Adoption of the Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG)

Kenneth G. Furton, PhD\*, International Forensic Research Institute, Florida International University, University Park, Miami, FL 33199; and Jessie Greb, BA, Florida International University, 11200 Southwest 8th Street, CP 330, Miami, FL 33199

After attending this presentation, attendees will have a better understanding of how establishing best practices for detection teams is improving interdiction efforts as well as courtroom acceptance of dog alert evidence by improving the consistency and performance of deployed detector dogs.

This presentation will impact the forensic science community by providing a better understanding of how improving the consistency and performance of deployed detector dog teams and their optimized combination with emerging electronic detectors improve the collection of evidence. This is done by maximizing the location of trace evidence in an efficient, cost effective manner while minimizing the collection of samples not relevant to an investigation.

The Scientific Working Group on Dog and Orthogonal detector Guidelines (SWGDOG) are being developed by a membership of respected scientists, practitioners, and policy makers representing diverse backgrounds. SWGDOG is cooperatively funded by the NIJ, FBI, DHS, and TWSG with general meetings held biannually since 2005. This project was undertaken as a response to concerns coming from a variety of sectors including law enforcement and homeland security regarding the need to improve the performance, reliability, and courtroom defensibility of detector dog teams and their optimized combination with electronic detection devices.

The approval of each subcommittee best practice document takes six months to complete including a two month period of public comments. The nine SWGDOG subcommittees and target timetable for posting of the best practice guidelines are as follows:

- Unification of terminology (Part A April '06; Part B October '06; Part C - March '07; Part D - August '07; Part E - March '08; Part F - September '08; Part G - March '09; Part H - September '09; Part I - March '10; Part J - September '10);
- General guidelines for training, certification, maintenance and documentation (April '06) - Publication in FSC October '06; First Revision (September '08); Second Revision (September '09);
- 3. Selection of serviceable dogs and replacement systems (October '06) Publication in FSC October '08;
- 4. Kenneling, keeping, and health care (October '06);
- 5. Selection and training of handlers and instructors (Part A October '06; Part B March '10);
- 6. Procedures on presenting evidence in court (October '06);
- 7. Research and technology (March '07; First Revision September '10);
- Substance dogs: Agriculture; Arson; Drugs; Explosives; (August '07) Chem./Bio.; Human remains (September '09);
- 9. Scent dogs: Scent identification; Search and Rescue; Trailing dogs; Tracking dogs (Part A March '07; Part B August '07; Part C March '08; Part D September '08; Part E September '09; Part F March '10)

Establishing consensus based best practices for the use of detection teams is providing a variety of benefits to local law enforcement and homeland security. Benefits include improved interdiction efforts as well as courtroom acceptance by improving the consistency and performance of deployed teams and optimizing their combination with electronic detection devices. An update of ongoing studies involving the identification of odorants used by certified law enforcement detector dogs and using these signature chemicals for instrumental detection to reliably locate forensic specimens including drugs, explosives and human scent is presented. The current success of SWGDOG is being manifest by a shift of some national canine organizations to adopt the approved best practice guidelines proposed. Though SWGDOG guidelines are not mandatory, this positive change is the ultimate goal of the working group. The continued approval and revision of SWGDOG documents has received an increased number of public responses and input which has shaped the documents making them publicly vetted. While it is not technically part of the scope of SWGDOG, a future accreditation, the International Commission on Detector Dogs (www.ICODD.org) will be formed with a mission to implement SWGDOG best practice guidelines through voluntary accreditation of certification bodies.

Detector Dogs, Evidence Recovery, Consensus Guidelines

#### A76 Liquid Chromatography - Tandem Mass Spectrometry Analysis of Organic Compounds in Soil

George Hupfer\*, 700 Forbes Avenue Apartment 1504, Pittsburgh, PA 15219; and Stephanie J. Wetzel, PhD, Duquesne University, Department of Chemistry and Biochemistry, 600 Forbes Avenue, Pittsburgh, PA 15282

After attending this presentation, attendees will gain a better understanding of soil analysis and the ability of a Liquid Chromatography - Tandem Mass Spectrometry instrument (LC-MS/MS) to analyze and differentiate the organic components of soil.

This presentation will impact the forensic science community by demonstrating the development of an objective method that may be used to analyze and discriminate obtained soil evidence.

Soil evidence can be important in forensic science cases as it contains many compounds that can be analyzed, including minerals and organic compounds. Unfortunately, most current analysis methods involve subjective analyses: comparisons of particle size, color, and type. The use of a more objective method, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), can eliminate the inherent bias present in those subjective methods and lead towards a more accurate and reliable soil analysis. Soil samples vary more from each other when comparing between two areas separated by a large distance. In this research, however, the sampling area was kept small and limited to the area of Allegheny County, Pennsylvania. Previous research was conducted analyzing the mineral composition of the soils from the same regions. This research focused on an analysis of the organic components of the soil and the variability of the organic composition in a small geographical area.

Soil samples were obtained from locations across Allegheny County. An organic solvent was used to extract the organic compounds from the soil, and the resulting extract was analyzed using LC-MS/MS. The mass spectrometer used was a Triple Quadrupole Mass Spectrometer (QqQ). By using the LC-QqQ instrumentation, tandem mass spectrometry experiments, like multiple reaction monitoring (MRM), could be developed to increase the sensitivity of the analysis.

Early experiments used acetonitrile as the extraction solvent and a 100% water to 100% acetonitrile mobile phase gradient with a C18 column. These results showed poor resolution and difficulty in distinguishing soil extraction samples from extraction solvent blanks. In later experiments, different organic solvents, (e.g. acetone, isopropanol, methanol, and tetrahydrofuran) were used as the extraction solvents. Different mobile phase compositions were also used, including a 100% methanol to 100% acetonitrile and a 100% methanol to 100% acetone mobile phase gradient. A more polar cyano column to improve the separation and analysis of the soil samples was also tested.

Preliminary results have shown an increase in resolution and peak differences between samples using the methanol mobile phase gradients and the cyano column. These methods will continue to be further researched and optimized. An optimized scanning method will be used to set up a precursor ion scan method, which will determine how the soil components are being fragmented. These results will then be used to set up an MRM method. The development of an MRM method for the analysis gives a discrete set of variables that can be used for the characterization of each individual soil sample. The optimized MRM method then allows for a comparison between all collected soil samples for each MRM parameter.

Liquid Chromatography, Mass Spectrometry, Soil Analysis

#### A77 Comparison of Four Commercially Available Portable Raman Spectrometers

Hillary Markert, MFS\*, Joan G. Ring, MS, Kirk M. Grates, BA, and Nicole Campbell, BS, National Forensic Science Technology Center (NFSTC), 7881 114th Avenue North, Largo, FL 33773

After attending this presentation, attendee shall be able to describe the operation of all the portable Raman instruments evaluated in this study, explain the strengths and opportunities for improvement of each device, and discuss the limitations of individual instruments and Raman technology in general, with regard to the analysis of controlled substances and explosives.

This presentation will impact the forensic science community by assisting prospective users of portable Raman devices to determine which device may best suit their agencies' needs.

In today's post 9/11 forensic environment, it has become increasingly important that civilian first responders, combat soldiers and forensic personnel have the tools necessary to quickly probe unknown bulk materials for the presence of explosive compounds, explosive precursors, controlled substances and other potentially hazardous materials while in the field. In response to this growing need, several manufacturers have produced portable Raman spectroscopy-based devices that may offer great potential for rapid, non-destructive sample analysis where this type of on-site chemical investigation is required. These devices can be used to identify unknown chemical materials at ordinary traffic stops, crime scenes, clandestine laboratories, airports, borders or on the battle field.

The National Forensic Science Technology Center (NFSTC); as part of its Forensic Technologies Center of Excellence (FTCoE) award from the National Institute of Justice (NIJ), evaluated four portable Raman spectrometers. The instruments included in the evaluation were the ICx Technologies Fido® Verdict<sup>™</sup>, the DeltaNu® ReporteR<sup>™</sup>, the Ahura Scientific Thermo Scientific FirstDefender RM and the Smiths Detection RespondeR RCI. This assessment was conducted to provide potential users of this type of portable Raman technology with independent and unbiased technical information on each of these devices. Each spectrometer was tested separately using the same standardized systematic evaluation scheme to assess individual strengths, areas for improvement, limitations, GUI interfaces, and safety issues, as well as the entire chemical characterization process involved from sample introduction through result output for each device. Representative samples of controlled and non-controlled drugs (standards and adjudicated case samples), drug diluents, ignitable liquids, explosives, explosive precursors, common household materials, and compounds sharing similar chemical composition were used to assess each unit for conformity, reproducibility, ruggedness, specificity, portability and mixture sensitivity. Samples were analyzed in triplicate and the resulting data and evaluator observations were recorded.

These rugged, portable field units are specifically designed to provide law enforcement, airport security, border patrol, military, emergency service personnel and other first responders with the ability to perform analysis on unknown bulk powders and liquids containing compounds such as illicit and pharmaceutical drugs, explosives, ignitable liquids, oxidizers, industrial chemicals and common household materials. Evaluation of these portable Raman spectroscopy instruments is critical to the advancement of forensic science, homeland security efforts, and military operations. These devices hold the promise of empowering first responders with crucial forensic intelligence, enabling them to make the best decisions to preserve public safety. They have further potential to help reduce the burden on overtaxed crime laboratories, by effectively screening out and prioritizing evidence before forwarding it to the forensic laboratory for additional testing.

Raman, Spectrometry, Portable

### A78 Room Temperature Fluorescence Spectroscopy as an Analytical Tool for the Forensic Examination of Textile Fibers

Krishnaveni Appalaneni, BS\*, University of Central Florida, Chemistry Department Building 5, 4000 Central Florida Boulevard, Orlando, FL 32816; Anthony F.T. Moore, BS, University of Central Florida, 4000 Central Florida Boulevard, Chemistry Building (CH) 117, Orlando, FL 32816-2366; Andres D. Campiglia, PhD, University Of Central Forida, Department of Chemistry, 4000 Central Florida Boulevard, Orlando, FL 32816; and Michael Sigman, PhD, University of Central Florida, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2367

After attending this presentation, attendees will be exposed to the application of analytical methods to forensic fiber examination. The analytical methods include high-performance liquid chromatography and fluorescence spectroscopy. The later technique will be introduced to the audience in the form of two-dimensional excitation and fluorescence spectra and excitation-emission matrices data formats.

This presentation will impact the forensic science community by introducing this technique to many practitioners in the forensic science field, as fluorescence spectroscopy has not been widely explored in the Forensic fiber evidence plays an important forensic science field. role in many criminal cases. Analytical techniques that can either discriminate between similar fibers or match a known to a questioned fiber are highly valuable in forensic science. When fibers cannot be discriminated by non-destructive tests, the next step is to extract the questioned and the known fiber for further dye analysis. Solvent extraction, enzymatic hydrolysis, and alkaline hydrolysis have been used to release dyes from the various types of fibers. Thin-layer chromatography (TLC), high-performance liquid-chromatography (HPLC) and capillary electrophoresis (CE) have been used to separate and identify colored dyes in fiber extracts. For the many hundreds of dyes used in the textile industry that appear to be the same color, that have highly similar molecular structures, virtually indistinguishable absorption spectra and identical or highly similar chromatographic retention times or electrophoretic migration times, the best approach appears to be the combination of mass spectrometry (MS) to HPLC (HPLC-MS) or to CE (CE-MS). Unfortunately, MS techniques destroy the fiber just like all the other methods that provide chemical information based on previous dye extraction. The main goal of this research is to provide the forensic scientist with nondestructive analytical methodology for textile fiber examination encountered as physical evidence in criminal investigations.

A different aspect of fiber analysis based on the total fluorescence emission of fibers was evaluated. In addition to the contribution of the textile dye (or dyes) to the fluorescence spectrum of the fiber, the contribution of intrinsic fluorescence impurities (i.e., impurities imbedded into the fibers during fabrication of garments) as a reproducible source of fiber comparison was investigated. This presentation provides conclusive evidence on the reproducibility of fluorescence patterns for forensic fiber examination.

Textile Fiber Examination, Non-Destructive, Fluorescence

#### A79 Instrumental Set-Up for the Collection A80 of Fluorescence Data Directly From Textile Fibers

Anthony F.T. Moore, BS\*, University of Central Florida, 4000 Central Florida Boulevard, Chemistry Building (CH) 117, Orlando, FL 32816-2366; Krishnaveni Appalaneni, BS, University of Central Florida, Chemistry Department Building 5, 4000 Central Florida Boulevard, Orlando, FL 32816; and Andres D. Campiglia, PhD, University Of Central Forida, Department of Chemistry, 4000 Central Florida Boulevard, Chemistry Building (CH) 117, Orlando, FL 32816-2366

After attending this presentation, attendees will be introduced to the optimization of an instrumental set up for the collection of twodimensional (2D) fluorescence spectra and excitation-emission matrixes (EEM) directly from a textile fiber. Published articles on fluorescence microscopy of fibers published have not taken full advantage of the information content that exists in the spectral signatures of textile fibers because measurements were made with excitation and emission bandpass filters. This presentation takes room temperature fluorescence (RTF) spectroscopy to a higher level of selectivity by optimizing the collection of 2D spectra and EEM directly from the fiber.

This presentation will impact the forensic science community by introducing instrumentation to forensic science practitioners that has the capability to explore the full potential of fluorescence microscopy not only for the analysis of textile fibers but also for other types of solid samples.

The nondestructive techniques currently available for comparing dyes in textile fibers; diffuse reflectance infrared Fourier transform spectroscopy and Raman spectroscopy have shown some promise. Unfortunately, the limited capability to detect small concentrations of dyes that could add valuable information to the signature of fibers certainly reduces their discrimination power for forensic fiber examination. A search of the literature has revealed that no efforts have been made to investigate the full potential of fluorescence spectroscopy for the purpose at hand. Articles on fluorescence microscopy of fibers took no advantage of the information content that exists in the spectral signatures of textile fibers because measurements were made with excitation and emission band-pass filters. This research takes roomtemperature fluorescence (RTF) spectroscopy to a higher level of selectivity by optimizing the collection of fluorescence data directly from the fiber. The work presented here deals with the optimization of an instrumental set up for the collection of two-dimensional (2D) spectra and excitation-emission matrixes (EEM) directly from the fiber. The instrumental set up interfaces an epi-fluorescence microscope for reflected light fluorescence measurements to a spectrofluorimeter via fiber optic probes. The excitation source of the spectrofluorimeter consists of a continuous 100 W pulsed xenon lamp with broadband illumination from 200 to 2000 nm. Excitation and fluorescence spectra are recorded with two spectrometers holding the same reciprocal linear dispersion (4.2 nm·mm<sup>-1</sup>) and accuracy (±0.5 nm with 0.3 nm resolution). Both diffraction gratings have the same number of grooves per unit length (1200 grooves mm<sup>-1</sup>) and are blazed at 330nm (excitation) and 500nm (emission). A photomultiplier tube (PMT) with spectral response from 185 to 850 nm is used for fluorescence detection operating at room temperature in the photon-counting mode. The sample compartment of the spectrofluorimeter is equipped with a fiber optic platform that optimizes collection efficiency with the PMT via two concave mirrors. An in-house made fiber holder facilitates the reproducible positioning of the microscope objective with respect to the fiber for reproducible collection of 2D spectra and EEM data formats.

Room Temperature Fluorescence, Microscopy, Textile Fibers

#### **Luminescence Studies of Feldspar Minerals** and Implications for Forensic Geology

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After attending this presentation, attendees will understand the principles of luminescence spectroscopy applied to forensic mineral analysis and its potential for enhanced discrimination of sediment sources and provenance determination.

This presentation will impact the forensic science community by illustrating the practical application of luminescence spectroscopy to forensic geologic examinations and its integration into techniques currently used in forensic sediment analysis. The additional discrimination among sources of feldspar minerals could provide a useful tool for the forensic comparison of geologic materials. Further, luminescence microscopy and spectroscopy, combined with traditional forensic geologic methods, may offer information for source determination by providing information about the conditions under which the mineral was formed.

Feldspar minerals are the most common constituents of igneous rocks on this planet and, as such, are usually encountered as constituents of sediment samples. Because feldspar minerals are ubiquitous, they may be underutilized in traditional forensic geologic examinations, such as simply providing mere mineral identification rather than yielding a provenance determination or source-level association. Complete mineralogical characterization of each feldspar grain can be used to help distinguish particular soil characteristics, but this process is tedious and expensive to perform and will not necessarily yield the provenance for each sample. One possible method to rapidly analyze large numbers of diverse soil samples involves measuring the luminescence of feldspar minerals among them, which could rapidly yield highly discriminating characteristics of the feldspars.

Alternatively, spectroscopic analysis of the feldspar luminescence that could be performed relatively rapidly could yield specific identifying characteristics of a soil sample that offers additional source discrimination instead of or in addition to commonly used methods. With this approach in mind, the UV-Visible-NIR luminescence of a wide variety of North American feldspar samples was measured and analyzed for distinguishing features that could be used in forensic provenance studies.

In this study, 44 feldspar samples of known provenance were obtained from the geology department of a reputable museum. These included 20 potassium feldspars (microclines, orthoclases, etc.), 8 albite specimens, and 16 plagioclase feldspar samples. These common feldspar mineral separates were examined by two ion beam analysis techniques, as well as by cathodoluminescence (CL) spectroscopy. Particle induced xray emission (PIXE) was used for elemental analysis and ion beam induced luminescence (IBIL) was measured spectroscopically and compared to CL. Previously reported luminescent centers (Mn<sup>2+</sup> and Fe<sup>3+</sup>) were observed and their UV-Visible peak positions vary with stoichiometric changes in the Na-K-Ca composition of the feldspars as expected. Similarly, Si-O and Al-O lattice defect luminescence in the UV-Visible spectra were observed; in addition, a previously unassigned IR luminescence peak was seen in some of the feldspar specimens analyzed. Analysis of the feldspar samples by x-ray diffraction, totalreflection x-ray fluorescence spectrometry, and laser ablation-inductively coupled plasma-mass spectrometry was performed in an attempt to determine the mechanism for this unassigned IR peak. An observed shift in specific luminescence peak centroids between IBIL and CL measurements is also reported. Experimental methods and preliminary results will be presented.

Luminescence, Forensic Geology, Feldspar

#### A81 FT-IR Microprobe Analysis of Suspected Bioterrorism Hoaxes in a Sealed Cell

Brooke W. Kammrath, MA, MS\*, and Pauline E. Leary, MS, The City University of New York/The Graduate Center, 899 Tenth Avenue, Room 636T, New York, NY 10019; and John A. Reffner, PhD, The City University of New York/John Jay College of Criminal Justice, 445 West 59th Street, New York, NY 10019

After attending this presentation, attendees will obtain knowledge about the interaction of light with barium fluoride in both the visible and mid-IR spectral regions and discover the optimal experimental conditions for collecting quality mid-IR spectra of suspected bioterrorism hoaxes from microscopic samples in a sealed cell.

This presentation will impact the forensic science community by demonstrating the optimal experimental conditions for analyzing suspicious powders in a sealed cell with an FT-IR microprobe.

Bioterrorism hoaxes involving the use of white powder or labels to falsely suggest the use of a biological agent are frequently reported in the United States. Following 9/11 and the subsequent anthrax attacks, hoaxes have increased despite no occurrence of a genuine bioterrorism attack. The FBI and U.S. postal inspectors have responded to thousands of white-powder events and targets have included government offices, U.S. embassies, banks ,and news organizations. Consequently, the analysis of suspicious white powders is of critical importance to both homeland security and public safety laboratories.

FT-IR microprobe analysis is a useful tool for the screening and identification of bioterrorism hoax powders. FT-IR spectroscopy can easily differentiate between powdered biological agents and many hoax powders due to the presence of protein in samples that contain biological material. There are two considerable advantages of using an FT-IR microprobe over traditional transmission FT-IR instruments: (1) the small sample size required for analysis; and, (2) the sample is directly viewed with a polarized light microscope with fluorescence capabilities. However, the analyst must be protected from potentially toxic samples, which prompted the development of the sealed cell. Sealed cells consist of an IR-reflective microscope slide with a barium fluoride cover slip attached with an impermeable adhesive, thus enabling the analyst to remain isolated and safe from the sample during FT-IR microprobe analysis. The use of barium fluoride as the cover slip is the best choice of material because of its resistance to chemicals, insolubility in water, and transparency in both the visible and mid-IR regions of the spectrum. However, barium fluoride disperses mid-IR radiation and it is important to understand this dispersive nature in order to maximize results. While the use of a barium fluoride cover slip introduces dispersion effects that are unavoidable, it is possible to adjust instrument settings when analyzing in the reflection-absorption mode of a FT-IR microprobe to almost completely compensate for dispersion and minimize its impact on the quality of the sample spectrum.

FT-IR Microprobe Analysis, Sealed Cell, Bioterrorism Hoaxes

## A82 Cathodoluminescent Signatures of Neutron Irradiation

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After attending this presentation, attendees will understand the principles of cathodoluminescence spectroscopy and the effects of neutron irradiation damage to minerals commonly encountered in soils and building materials.

This presentation will impact the forensic science community by illustrating the practical application of cathodoluminescence spectroscopy of commonly encountered minerals to the detection of proximate nuclear materials.

Nuclear proliferation and the potential threat to national security from unsecured special nuclear materials have renewed our national interest in not only detecting the presence of these materials, but also in detection of materials' pathways into this country. Currently, the only method to identify where special nuclear materials have been stored involves measuring induced radiation in adjacent materials, which is usually short-lived (n, gamma) radiation. Identified in this research is a permanent change to the luminescent properties of certain common minerals that is due to neutron irradiation that could potentially be developed into a nuclear forensics tool.

Feldspars and carbonates are ubiquitous minerals that are known to luminesce under electron bombardment. The UV-Visible spectra of hundreds of individual potassium feldspar and calcite grains were measured with cathodoluminescence (CL) spectroscopy before and after neutron irradiation. CL excitation uses an electron beam to induce fluorescence in certain minerals due to their chemical composition and defects in their crystal lattice structure; the resultant emission spectrum is acquired with a UV-Visible spectrometer.

The presence of ionizing radiation causes additional crystal lattice defects that leave a permanent CL signature. A spectroscopic signature is described that increases proportionately to neutron dose in both calcites and feldspars. Preliminary dose-response results from a neutron source study and a reactor study will be presented. There is also an orientation dependence in the luminescence measurement technique that complicates the analysis, but when fully understood could allow not only the total dose to be estimated, but also the direction of origin of the neutrons to be determined.

Cathodoluminescence, Nuclear Forensics, Minerals

# A83 Trace Analysis of Urea Nitrate as an Ion-Pair

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After attending this presentation, attendees will understand the potential new methods for the analysis and detection of urea nitrate at trace levels.

This presentation will impact the forensic science community by providing a better understanding of the development of reliable procedures for the detection of this energetic salt. This presentation will review a number of different methods for the analysis of this energetic salt.

Urea nitrate is a fertilizer-based explosive that has been used by terrorists in various bombings all over the world. One example of its use is the World Trade Center bombing in New York City in 1993. One interesting property of urea nitrate is that it is easily decomposed into urea and nitrate in the presence of water. As these two components may be ubiquitous in certain environments and not necessarily characteristic of the explosive, new methods are needed to characterize the urea nitrate salt and differentiate it from low level background ions.

This project aims at developing a reliable method to extract urea nitrate as an ion-pair from a variety of surfaces and analyze it at trace levels using spectroscopic (UV-visible and fluorescence) and/or mass spectrometry (MS) detection. The ultimate goal of this project is to rapidly detect urea nitrate as a non aqueous ion pair on a variety of surfaces. Therefore, the analysis has to be quick and very sensitive while interferences with urea, nitrate, and other matrix components should be as low as possible.

Urea nitrate is only slightly absorbent in the UV-visible range so derivatization is needed for spectroscopic analyses. In this study, the derivatization was performed with fluorescent compound xanthydrol, which has already been used successfully for urea detection. After the derivatization, the samples were injected on reverse high performance liquid chromatography (HPLC) coupled to UV and fluorescence detection to separate the different products. A gradient composed of sodium acetate or ammonium acetate and acetonitrile mobile phases permitted separation of all compounds in less than 20 minutes. Different parameters such as reaction temperature, solvent and time, xanthydrol amount and wavelength were optimized to achieve accurate quantification over the widest range of concentrations possible. Then extraction from both porous and non porous surfaces was investigated. Finally, potential interferences were added to the sample to determine how they affect the analysis. Preliminary results indicated detection limits around 0.05 mM or 6 ppm and the ability to differentiate urea nitrate from urea and nitrate samples. Thanks to addition of an internal standard, a quantification limit very close to the detection limit was achieved.

In parallel, mass spectrometry was used in an attempt to characterize urea nitrate as an ion pair without any derivatization. Electrospray ionization (ESI) and time of flight mass spectrometry were used in order to determine the exact mass of urea nitrate potential adducts. In order to separate urea nitrate from other compounds present in the sample, liquid chromatography has been coupled to the ESI-MS system and HILIC (Hydrophilic Interaction Liquid Chromatography) columns have been tested since they allow a good separation with only a small amount of aqueous phase (which can break the ion-pair).

These two methods should permit the detection of urea nitrate with high accuracy and permit its detection on a wide variety of surfaces. **Urea Nitrate, Fluorescence, Mass Spectrometry** 

#### A84 Development of a Chemical Fingerprint for Salvia Divinorum Using Liquid Chromatography-Mass Spectrometry for Association and Discrimination from Related Salvia Species

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After attending this presentation, attendees will learn how *Salvia divinorum* can be differentiated from related *Salvia* species using liquid chromatography-mass spectrometry (LC-MS) and multivariate statistical procedures. Differentiation of *S. divinorum* from other *Salvia* species will be demonstrated based on their non-volatile compounds, using principal components analysis (PCA) and hierarchical cluster analysis (HCA).

This presentation will impact the forensic science community by developing a LC-MS method for the analysis of non-volatile compounds in *S. divinorum*. Most forensic laboratories analyze *S. divinorum* submissions using gas chromatography-mass spectrometry (GC-MS); however, GC-MS is limited to the analysis of volatile compounds. Analysis of non-volatile polar compounds using LC-MS potentially offers more discriminating information to differentiate *S. divinorum* from other *Salvia* species.

Salvia divinorum is a hallucinogenic plant that has recently gained legislative attention due to an increase in its recreational use. S. divinorum and/or its active constituent, salvinorin A, are currently regulated in several states. Hence, the ability to identify S. divinorum and differentiate it from more than 900 other Salvia species is imperative in a forensic context. Salvinorin A is known to be found only in S. divinorum thus far. Therefore, the presence of salvinorin A, detected using GC-MS, serves as the current method for identifying S. divinorum. However, salvinorin A can be extracted from S. divinorum to prepare liquid extracts for recreational use. This can result in low levels of salvinorin A in the residual plant material that may or may not be detectable, causing possible difficulty with identification. Hence, an alternative procedure for identifying S. divinorum would be beneficial. Using LC-MS, nonvolatile polar compounds in plant material can be determined, generating a chemical "fingerprint" of S. divinorum. This fingerprint should be unique and complementary to that already available by using GC-MS.

The purpose of this research was to generate a chemical fingerprint of S. divinorum using LC-MS. This chemical fingerprint was then used to investigate differentiation of S. divinorum from other related Salvia species based on the non-volatile polar compounds. S. divinorum was extracted in triplicate using different solvents (e.g., acetonitrile, acetonitrile/water, and acetonitrile/water/isopropanol) and all extracts were analyzed by LC-MS. The optimal extraction solvent was determined based on the number of compounds extracted and the precision of the extraction, which was determined using Pearson product moment correlation (PPMC) coefficients. Using the optimal solvent, an additional four Salvia species (e.g., S. guaranitica, S. nemorosa, S. officinalis, and S. splendens) were then extracted in triplicate and analyzed by LC-MS. Resulting chromatograms were subjected to data pretreatment procedures (e.g., retention time alignment and normalization) to limit any sources of non-chemical variance. Principal component analysis was then used to visually associate and discriminate extracts. Extracts that were chemically similar, such as replicates of the same species, were clustered closely in the scores plot and separately from those extracts that were chemically different. In addition, the chemical compounds contributing to the variance described by the principal components were identified in the loadings plot. Hierarchical

cluster analysis (HCA) was performed based on the scores plot and used to statistically measure the extent of association and discrimination of the different *Salvia* species. The combination of PCA and HCA results in a statistical evaluation of association and discrimination, in accordance with recommendations put forward in the recent National Academy of Sciences Report, "*Strengthening Forensic Science in the United States: A Path Forward*."

Salvia Divinorum, Liquid Chromatography-Mass Spectrometry, Multivariate Statistical Procedures

#### A85 Method Development for the Rapid Separation and Detection of Organic Gunshot Residue by UPLC/MS

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After attending this presentation, attendees will become familiar with the key principals of ultra-performance liquid chromatography and the advantages of using this newer technique for analyzing gunshot residue.

This presentation will impact the forensic science community by providing new methods to law enforcement personnel for determining whether or not an individual has fired a weapon, which can link the individual to a crime scene, victim, or gun.

Upon firing a gun, a mixture of vapors and particulates are ejected from the weapon and deposited onto the shooter's hands and clothing. These particulates are referred to as gunshot residue (GSR) and the detection of this residue may assist law enforcement personnel in determining whether or not an individual has fired a weapon.

GSR is composed of inorganic and/or organic constituents that arise from the primer, propellant, bullet, and other sources within the gun. For the purpose of this research, we are primarily focusing on the analysis of organic GSR (O-GSR) resulting from smokeless powder by ultra high pressure liquid chromatography (UPLC). UPLC is a newer analytical technique which provides increased resolution and separation speed when compared to traditional HPLC. The advances are due to the smaller particle columns which help to minimize band spreading and the pumping system's ability to accommodate higher backpressures. For detection, a tandem MS was utilized for its sensitivity, selectivity, and fast acquisition speeds. With the tandem MS, both parent and daughter ions can be monitored for more accurate identification of the individual components.

The overall purpose of this project was to develop and optimize methods for the UPLC/MS analysis of organic gunshot residue. In this project, a total of 20 different smokeless powder additives were analyzed.

These included diphenylamine, N-nitrosodiphenylamine, 4nitrosodiphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenylamine, 2,4'-dinitrodiphenylamine, 4,4'-dinitrodiphenylamine, methyl centralite, ethyl centralite, dimethyl phthalate, diethyl phthalate, dibutyl phthalate, nitroglycerin, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 2,3dinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, and 3,4dinitrotoluene. These additives may act as a propellant, stabilizer, plasticizer, flash inhibitor, or as a combination of several of these functions.

The sample preparation process involved first preparing stock solutions of each explosive in organic solvent at 1mg/mL and then combining each one to form a mixture. Working solutions were prepared by diluting aliquots of the solution mixture to the appropriate concentrations using a 50:50 mixture of acetonitrile and water with 2mM ammonium acetate added to promote efficient electrospray ionization. A C8 reverse phase column (100mm length, 2.1µm i.d.) was evaluated for

its ability to separate the mixture of 20 standards. In order to optimize the separation, the mobile phase, gradient, and flow rate were examined at various combinations. Simultaneous positive and negative ESI was used along with APCI to detect all relevant compounds. Optimized analysis times were under 12 minutes with a gradient of 10%-80% organic at a flow rate of 0.500mL/min.

A variety of extraction techniques were then examined to permit optimum recovery of real and simulated GSR from several different types of handguns. The resultant method permits simultaneous and sensitive determination of a wide variety of organic compounds present in gunshot residue.

Organic GSR, UPLC, MS

#### A86 Physical and Chemical Description of Barnes XLC Coated X-Bullets

Klint R. Epperson, Paul B. Lawence, Josh Knapton, Amberlee R Neibaur, Gary H. Naisbitt, PhD\*, Forensic Science Program, Criminal Justice Department, Utah Valley University, Orem, Utah 84058

The goal of this presentation is to present a physical and chemical description of the Barnes XLC Coated X-Bullets before and after being fired. It employees several instrumental and microscopic techniques used in chemical and materials analysis, most of which are available in well equipped crime laboratories.

This presentation will impact the forensic science community by providing Information contained in this presentation can be used by firearms examiners as a reference standard to identify Barnes XLC Coated X-Bullets recovered at crime scenes.

Barnes XLC Coated X-Bullets are specialty bullets for the ammunition reloader enthusiast. The solid copper hollow point bullets are distinguished by their blue coating that acts as a lubricant to improve ballistic performance. Microscopic examination showed it to be a sprayed-on single layer coating similar to automotive paint finishes. Coating thickness was determined with a stereomicroscope equipped with imaging software capable of making measurements to be 24.2 microns with a standard deviation of 0.76 microns. The color spectrum with a maximal peak at 495 nm was obtained with a microspectrophotometer. Fourier transform infrared (FTIR) spectral analysis of the unfired bullet provided a reference spectrum for forensic identification. When the bullet is fired, the coating is lost from the surfaces that come in contact with the barrel, but because of mushrooming petals that form upon impact at the tip of the bullet, the coating is protected and remains intact. Heat, pressure, and impact due to firing do not change the IR spectrum of the coating, thus providing a comparison link between the unfired standard reference to bullets recovered at crime scenes.

Further chemical analyses were conducted to determine the lubricant, coloring agent, and binder. Starting with the reference IR spectrum, spectral matching techniques were used to identify polytetrafluoroethylene (PTFE) as the lubricant by its characteristic twin peaks at 1210 and 1150 nm. Its presence was confirmed by pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS). The pyrolysis product of PTFE appears early in the pyrogram as a single large peak of tetrafluoroethylene, and this results agrees with peer reviewed literature.

After subtracting the PTFE spectrum, searches for other matching spectra suggested methylmethacrylate, a group of "drying oils like tung oil and fatty acids, and cyclic compounds as the most likely components. Pyr-GC-MS analysis suggested methylester-2-methyl-2-propenoic acid, many cyclic compounds some of which containing nitrogen, fatty acids, and a small peak identified as Blue Pigment 15. Because mixtures of polymers, co-polymers and cross-linkers are specifically formulated to produce desired properties, exact identification is often not possible. However, taken together the binder composition is rationalized to be a mixture of methylmethacryate and alkyd drying oils.

Blue Pigment 15 is member of the copper cyanin dye family that is modified with slight variations to produce different shades of blue. Pyrolysis of these large heterocyclic compounds produces a large variety of smaller heterocycles, some of which are direct components of the parent molecule and others that are structural rearrangements. Exact reconstruction of a specific copper cyanin was not possible. However, Raman spectrometry supports the presence of a copper cyanin compound in the coating.

Reference Standard Barnes XLC Coated X-Bulltes, Blue Bullet, Chemical Analysis

#### A87 Preliminary Studies on the Chemical and Morphological Changes of Gunshot Residues Following Ingestion by Fly Larvae

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After attending this presentation, attendees will understand the importance of the differentiation between the presence of Pb, Ba, and Sb as pure chemical elements or as a GSR structure in the blowfly larvae tissue.

This presentation will impact the forensic science community by producing a series of different laboratory techniques to investigate in the presence of GSR in blowfly larvae tissues.

Medico-legal entomology is the use of insects and other arthropods in forensic investigation concerning decomposed bodies and it has become the gold standard for estimating time since death in many countries. In addition to this well-known use to estimate the postmortem interval (PMI), insects that feed on carcasses may also represent a reliable specimen for toxicological analyses (entomotoxicology) especially in the absence of tissues and fluids normally taken for such purposes.

The literature reports many studies involving the extraction of drugs and chemicals (morphine, cocaine, opiates, benzodiazepines, paracetamol, mercury, etc.) from blowfly larvae (Diptera: *Calliphoridae*) associated with a corpse. These analyses are useful in acquiring information about causes or circumstantial of death.

At present little research has focused on the analyses of gunshot residues (GSR) detection in blowfly larvae. The observation of the presence of GSR elements such as lead (Pb), barium (Ba) and antimony (Sb) in the tissues of larvae could help the interpretation of how a person died especially when bullets cannot be recovered in the body and the surrounding crime scene. Furthermore, on a highly decomposed body the larval activity could also easily lead to the destruction of gunshot wound diagnostic features but retention of GSR by the fly larvae will indicate a shooting crime.

Previous investigation involved in detection of GSR elements in blowfly larvae were conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Roeterdink *et al.* 2004; LaGoo *et al.*, 2010) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) (Swan, 2006). All these studies focused exclusively on the detection of gunshot residues in larvae as pure chemical elements.

However, due to the natural existence of these elements in the environment and in the fly larvae tissues, for a forensic purpose it's fundamental to differentiate the presence of natural Pb, Ba, and Sb from the presence of these elements as GRS specific morphological structure in the larva tissues.

The aim of this study is to determine if there are chemical and morphological changes of Pb, Ba and Sb following the uptake of GSR by larvae. This study was performed using the Scanning Electron Microscope (SEM), currently considered the most common technique for GSR detection analyses because it combines morphological examination with elemental analyses.

The fly larvae were fed meat that had been shot and then were formalin fixed and processed following basic histological methodologies. The resulting horizontal thin sections were examined by SEM.

This study allowed the identification of the presence of Pb, Ba, and Sb in fly larvae not just as chemical elements but as a complex gunshot residue structure helping in the interpretation of the causes of death in a highly decomposed body.

Gunshot Residues, Blowfly, Scanning Electron Micro

#### A88 Laser Induced Breakdown Spectroscopy (LIBS): Characterization of Bullets, Gunshot Residue, and Bullet Wipe

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After attending this presentation, attendees will have a basic understanding of Laser Induced Breakdown Spectroscopy (LIBS), the effects that distance has on the detection of gunshot residue and bullet wipe on clothing, cement block, wood, and drywall, and the general trends to observe when applying LIBS to the analysis of bullet fragments and bullet holes.

This presentation will impact the forensic science community by introducing LIBS as a presumptive test for the identification of bullet fragments, gunshot residue (GSR) and bullet wipe. It will demonstrate that LIBS, in comparison to established chemical testing, will be more useful in the detection of bullet wipe in the absence of GSR. The techniques developed in this project will have the potential to establish an area of bullet impact detection in the presence and absence of gunshot residue.

Laser Induced Breakdown Spectroscopy (LIBS) can be used to quickly determine the elemental composition of gas, liquid, and solid samples with minimal sample preparation. A LIBS instrument commonly incorporates a ND: Yag Laser and a CCD or Eschelle detector. The laser pulse ablates material from a sample, producing high temperature plasma.

The plasma emits light at wavelengths that are characteristic of the elements ablated from the sample. The emission of the plasma is collected and analyzed by a detector within the LIBS system. The advantages of LIBS are that the method is relatively non-destructive, very little sample preparation is required, and the spectra can be obtained instantaneously. The disadvantages are that the limit of detection is presently only 4-10 ppm and the percent composition of trace elements cannot be determined to the level of accuracy required for forensic analyses. However, as shown by this project, LIBS can be used as a quick test when the presence of specific elements can be used to identify a sample; lead (Pb) for a bullet fragment or lead (Pb) and barium (Ba) for a suspected bullet hole.

Gunshot residue can serve as a vital form of circumstantial evidence in crime scene investigations. Cases involving the use of firearms, traces of lead (Pb), barium (Ba), and antimony (Sb) can be detected on the victim, criminal, and other objects that have come in contact with the firearm and or fired projectiles. However, when the point of impact is more than three feet from the source of the gunshot, the GSR becomes less visible and the only detectable trace is a dark ring of lead or barrel residue formed from the bullet (lead or full metal jacketed) at the site of impact as it passes through the material. This is classified as bullet wipe in the firearms field. LIBS can be used to identify lead (Pb) and barium (Ba), the elemental components of bullet wipe and bullet fragments. This instrumentation has the potential to become an important tool in the analysis of trace evidence.

The LIBS System has been used to analyze four unfired bullets and a variety of materials shot from a range of one inch to twelve feet. Peaks at 280.16, 368.49, and 405nm indicated a positive test for lead and at 455.4, 493.4, and 553.5 nm for barium. The analysis of spectra for materials from a T-shirt with simple spectra and few elements will be compared to cement block, a more complicated material composed of several different minerals and numerous inorganic elements.

Laser Induced Breakdown Spectroscopy, Gunshot Residue, Bullet

#### A89 The Effects of Render Safe Procedures on Forensic Evidence From Improvised Explosive Devices

Chad Wissinger, MBA\*, and Sonja Rawn, JD\*, Ohio Division of State Fire Marshal Forensic Laboratory, 8895 East Main Street, Reynoldsburg, OH 43068

After attending this presentation, attendees will have a better understanding of the value of forensic evidence from rendered safe Improvised Explosive Devices (IEDs). Various render safe procedures (RSPs) will be examined to determine the impact each will have on the recovery of forensic evidence from (IEDs) and a recommended examination triage will be presented.

This presentation will impact the forensic science community by providing forensic examiners, investigators, and bomb squad personnel with a better understanding and awareness of the value of forensic evidence from rendered safe IEDs.

When investigating incidences involving Improvised Explosive Devices (IED); military Explosive Ordnance Disposal (EOD), civilian bomb technicians, explosive incident investigators, and forensic laboratory personnel need a better understanding of the effects render safe procedures (RSPs) have on forensic evidence. Most investigators, bomb technicians, and laboratory examiners question if valuable forensic evidence remains after rendering safe an IED. This project is designed to determine if valuable evidence does or does not exist and if any detectable contamination occurs form using render safe tools. With the wide range of RSP tools available to EOD and civilian bomb technicians, it is important for the personnel conducting the RSPs to understand these effects in order to choose the best tool to preserve the forensic evidence and render the device safe effectively. In addition, it is important for forensic laboratory analysts to be able to identify that an IED has been rendered safe so the analyst is alerted to any contamination that could have occurred from the RSP tools and apply the appropriate examination triage.

The objective of this program in combating terrorism is to determine the evidentiary value of IEDs after RSPs have been conducted. This study will result in recommendations that identify different RSPs, the effects on evidence, and provide laboratory personnel with procedures for analyzing post-RSP IEDs. The novel approach to be evaluated is:

- 1. Build multiple IEDs with forensic evidence applied;
- 2. Render them safe using multiple disruption techniques in real world environments;
- 3. Evaluate the effects that render safe procedures have on the value of the forensic evidence (trace evidence, explosives, fingerprints, tool marks, and DNA).

Because of the complex nature of designing, rendering safe and forensically evaluating IEDs, conducting this program requires a team of subject matter experts (SMEs) experienced in this real world process on a routine basis. The team consists of SMEs from the National Security Division at Battelle Memorial Institute, Columbus Division of Fire Bomb Squad, Ohio Division of State Fire Marshal Forensic Laboratory, Columbus Police Department Crime Lab, and Hamilton County Coroners Crime Laboratory.

The effects RSPs had on different IED designs and the results of forensic examinations including, DNA, fingerprints, trace evidence, tool marks, and explosives, will be presented. Suggested procedures and techniques used to collect evidence following a render safe procedure and the subsequent forensic analysis triage of the rendered safe IEDs will be included.

**Render Safe Procedures, IED, Forensic** 

#### A90 Ultra Rapid Separation of Cocaine and Cocaine Adulterants by Differential Mobility Spectrometry-Mass Spectrometry

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After attending this presentation, attendees will understand how differential mobility spectrometry (DMS) is similar to ion mobility spectrometry (IMS) as well as how it is different. Relevant applications to forensic drug analysis will be highlighted.

This presentation will impact the forensic science community by introducing practical applications of a new, ultra-rapid, separation technology to the field of drug analysis. This separation technology, DMS, is interfaced to an ion trap mass spectrometer for confirmation by MS-MS analysis.

Differential Ion Mobility Spectrometry (DMS) has been interfaced to nano-ESI-MS to provide an ultra rapid ion filtration technique for the separation of ions in gas phase media prior to mass spectral analysis. This technique as a forensic tool for ultra rapid separations and analyte quantitation with minimal sample pre-treatment in an effort to replace the necessity for lengthy GC or LC-based chromatographic separations is being evaluated. DMS affords an analyst the ability to selectively feed an ion trap with targeted analytes preventing saturation of the trap from unnecessary chemical noise. DMS-MS separation conditions were optimized and included modifier selection, desolvation gas temperature, and variance of trap fill time for the analysis of a variety of chemical species.

The aim of this work has been the rapid detection of analytes of interest to the forensic science community with the overall goal of reducing case backlogs. A planar, DMS-Ion Trap MS has been constructed in the laboratory. This system has been shown to be efficient at the rapid separation of ions under ambient conditions and has been utilized in the laboratory as an ion filtration technique prior to mass analysis. Compared to traditional LC-MS based techniques requiring 30-40 minutes of chromatography, DMS-MS is capable of separating a five drug mixture in 25 seconds. DMS allows for ion selection from a mixture by the application of dispersion (Rf) and compensation (Vc) voltages. Determining appropriate Vc's by scanning as a function of time is a critical step to isolating species of interest from a complex chemical matrix. Drug and drug adulterant mixtures were introduced to the DMS-MS system via infusion without sample pre-treatment or purification. MS-MS of the targets at fixed Vc's was then conducted, confirming the

presence of the analyte of interest from the background matrix. DMS-MS was shown to suppress chemical noise by approximately one order of magnitude allowing for increased signal-to-noise at increased trap fill times. Furthermore, use of DMS as an ion filtration technique afforded a nine-fold increase in S/N for the analyte of interest.

Drug Analysis, Separation Science, Differential Mobility Spectrometry

## A91 The Application of UV-VIS and Fluorescence Derivative Spectra in the Forensic Analyses of Illicit Drugs

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After attending this presentation, attendees can expect to have a more thorough understanding of the application of derivative Ultraviolet-Visible and fluorescent spectroscopy to the presumptive identification of controlled substances.

This research will impact the forensic science community by providing a method to improve the discriminatory power of Ultraviolet-Visible and fluorescent spectroscopy and evidence to consider upgrading the use of UV-VIS and fluorescent spectroscopy from Category C to Category B in the SWGDRUG guidelines. Furthermore, the ease of use and low cost associated with this method can provide smaller labs with an additional tool with which to analyze illicit drug samples.

Ultraviolet-Visible (UV-VIS) and fluorescent spectroscopy have long been used in forensic science due to the ease of operation and low start-up costs when compared to other instrumental methods, as well as the non-destructive nature of analysis. These methods have been used most frequently in the presumptive identification of illicit drugs due to their low discriminatory power. The current research provides indication that that these techniques may be underestimated in terms of their discriminatory power and therefore underutilized for this purpose. It has been shown that first and second derivative spectra can provide more discriminatory information than just the zero order spectra alone, especially when several different solvents are used. For this research, cocaine salt, crack cocaine, lidocaine, procaine, benzocaine, tetracaine, heroin, morphine, codeine, methamphetamine, 3,4methylenedioxymethamphetamine (MDMA), methylenediethylmethamphetamine (MDEA) methylenedioxyamphetamine (MDA), and lysergic acid diethylamide (LSD) were examined. Based on solubility, the drugs were analyzed in at least three of the following solvents: acetonitrile, 0.1M HCl, 0.1M NaOH, 0.1M phosphate buffer (pH 7), and acetonitrile. Samples were prepared to several concentrations ranging from 0.03 mg/mL to 0.0009375 mg/mL and analyzed from 200-400 nm. The resulting spectral minima, maxima, and crossover points for each derivative were tabulated along with the ratio absorbances at these wavelengths. The information gathered from the derivative data in the different solvents can, when used together, provide a much more thorough evaluation of each drug. In many cases, identification alone is not enough. This method has the potential to also be applied to estimating the quantity of illicit drugs in the presence of cutting agents. After spectral information was collected for each individual drug, spectral overlays were prepared for each drug-cutting agent combination to determine points of overlap. A blind study conducted by comparing solutions of unknown concentration containing both drug and cutting agent to a prepared calibration curve of the drug showed promising results. From this, an approximate concentration was calculated using Beer's Law.

Continued work in this area could provide substantial justification for the increased usage of UV-VIS and fluorescence spectroscopy in the analysis of illicit drugs. In the current SWGDRUG guidelines, UV-VIS and fluorescence spectroscopy are in Category C. Additional work in this area will continue to bring to light the abilities of UV-VIS and fluorescence spectroscopy, and may eventually present enough evidence to warrant the transfer from Category C to Category B. This shift, minor as it may be, could make adherence to the SWGDRUG guidelines more practical for smaller labs that are unable to purchase big-ticket items such as mass spectrometers or tandem mass spectrometers.

Ultraviolet-Visible Spectroscopy, Fluorescence Spectroscopy, Illicit Drugs

#### A92 Analysis of Illicit Tablets by Ultra-High Performance Liquid Chromatography

Emily C. Miller, BS\*, Drug Enforcement Administration Mid-Atlantic Laboratory, 1440 McCormick Drive, Largo, MD 20774

After attending this presentation, attendees will understand how Ultra-High Performance Liquid Chromatography (UHPLC) can be used effectively in the analysis of illicit tablets, pharmaceutical tablets, and drug mixtures of forensic interest. In particular, it will be demonstrated that those tablets and mixtures containing phenethylamines and other compounds which can be difficult to separate by Gas Chromatography (GC) can be easily analyzed using UHPLC.

This presentation will impact the forensic science community by highlighting some of the potential uses of this new technology in forensic drug analysis, including the ability of UHPLC to separate and fully resolve compounds that cannot easily be resolved by many of the commonly used GC methodologies. This allows for more efficient analysis, more accurate drug identification, and more precise quantitation.

Many phenethylamines and other compounds typically encountered in illicit tablets can be difficult to fully resolve using the GC screening methods typically utilized in forensic drug laboratories. High performance liquid chromatography (HPLC) provides alternate mechanisms of separation and selectivity than GC. However, HPLC's lower peak capacity makes the length of time needed to adequately separate all compounds too time-consuming to use for general screening. However, the development of UHPLC instrumentation and the availability of columns containing sub-2- $\mu$ m particles have made this technique more appealing for forensic drug screening. The number of theoretical plates achievable with sub-2- $\mu$ m particle UHPLC columns approaches those possible with capillary GC columns. UHPLC provides a complementary method for screening these types of tablets quickly and efficiently with a high degree of selectivity.

A UHPLC method will be presented which can separate many of the components of illicit tablets, including common 3.4methylenedioxymethamphetamine (MDMA) and related compounds (including MDA, MDMMA, and MDEA), methamphetamine, amphetamine, ephedrine, pseudoephedrine, N-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)-piperazine (TFMPP), and caffeine. Several other non-controlled adulterants which may be present in illicit tablets (including diphenhydramine, aspirin, guaifenesin, lidocaine, procaine, and others) will also be shown to be well resolved using this method. Analysis of tablets containing BZP, MDMA, and TFMPP by both GC and UHPLC will be compared. A normal-phase UHPLC screening method for BZP will also be introduced, as well as an ion-pairing UHPLC method for the quantitation of BZP. The benefits and limitations of each method will be discussed. The applicability of UHPLC screening to many compounds of forensic interest that are at least somewhat soluble in water and possess a chromophore will be shown. Examples of analysis of controlled and non-controlled pharmaceuticals by UHPLC will also be presented along with the analysis of illicit tablets containing unusual components (such as cocaine). The sensitivity of the technique (with ultraviolet (UV) detection) will be demonstrated, and sample preparation will be discussed.

UHPLC is a powerful new technique which can be of great value to a forensic drug analysis laboratory. Many mixtures which can prove difficult to analyze by traditional techniques benefit from the unique retention mechanisms in liquid chromatography and can be better resolved by UHPLC. In addition, exhibits containing multiple units (such as illicit tablets or pharmaceutical tablets) can be quickly and efficiently analyzed with no requirement for any additional sample preparation steps that might be necessary for GC analysis (such as derivitization, basic extraction, or further dilution). This technology has the potential to make forensic drug analysis laboratories more efficient through faster screening, easier sample preparation, and less reanalysis of multipleunit submissions.

UHPLC, BZP, Phenethylamine

#### A93 Identification and Determination of Opiates in Poppy Tea Preparations

Angela S. Mohrhaus, BS\*, Jill M. Gracie, MFS\*, Heather A. McCauley, BS, and Samuel R. Gratz, PhD, FDA Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237

The goal of this presentation is to present the forensic community with a survey conducted on various sources of Opium Poppy and Papaver somniferum. This presentation will also discuss the alkaloid profiles as well as the levels detected.

This presentation will impact the forensic science community by discussing a survey of *P. somniferum* obtained from various sources conducted on both intact poppy pods and the separated seeds. The alkaloid profiles obtained using GC-MS and the calculated levels acquired using HPLC-UV will be compared and presented.

The Opium Poppy, *Papaver somniferum*, is widely grown as an attractive flower throughout Europe, South America, Asia, and parts of the United States. Although it is prized as an ornamental plant in the garden and its seeds are used in the production of foods and oils, for many, *P. somniferum's* value lies in the opium. Opium refers to the dried latex that is collected from the plant by scoring the immature seed pods. It contains numerous opiate alkaloids including morphine, codeine, thebaine, papaverine, and noscapine. Morphine is the most prevalent alkaloid in opium comprising anywhere up to 20% of the total mass. Codeine is the second most common alkaloid making up 0.3% to 3% of the total.<sup>1</sup> Thebaine, although not used therapeutically, can be converted industrially into a variety of compounds including oxycodone and oxymorphone. It is the most poisonous opium alkaloid and often causes nausea and vomiting when ingested. All three of these compounds are Schedule II controlled substances as defined by the DEA.

Although the latex is the greatest source of opiate alkaloids, these compounds can be found in other parts of the plant as well. Poppy tea is a popular drink made from various portions of the dried poppy flower and induces a long lasting intoxication. The seeds and dried seed capsules are generally indicated for use in making the tea and due to the widespread availability of dried poppy pods on the internet, in floral markets, at craft stores, *etc*, this can be problematic. Over the last two years, multiple deaths attributed to acute morphine and/or codeine intoxication have been reported, with poppy tea being noted as the likely source of the opiates.<sup>2</sup>

The FDA's Forensic Chemistry Center first received dried poppy pods in May of 2009 after the death of a 20-year-old male college student. Several pods were received and analysis was conducted on the entire fruit (*i.e.* the dried pod capsule with seeds) as well as the seeds alone. Both samples were prepared by grinding portions with either a food processor or by hand using a mortar and pestle. The ground parts were then steeped in boiling water for 30 minutes, as per the tea recipe received with the pods. The extract was then filtered and analyzed using GC-MS analysis and HPLC-UV analysis. The early results showed a trace amount of morphine in the seed extracts. However, extracts examined from the entire pod demonstrated the presence of morphine, codeine, and thebaine. Subsequent quantitation by HPLC-UV confirmed an average of 18.5 mg per pod of morphine, 3.7 mg per pod of codeine, and 0.4 mg per pod of thebaine. Preparing the tea as described in the recipe yielded 92 mg, 18.5 mg, and 2.0 mg of morphine, codeine, and thebaine respectively per cup of tea. With the minimum lethal dose of morphine reported at 200 mg and cases of 60 mg causing sudden death in hypersensitive individuals, the levels detected are alarming.<sup>3</sup>

A survey of *P. somniferum* obtained from various sources will be conducted on both intact poppy pods and the separated seeds. The alkaloid profiles obtained using GC-MS and the calculated levels acquired using HPLC-UV will be compared and presented. **References:** 

- <sup>1.</sup> http://www.sciencedaily.com/articles/o/opium\_poppy.htm
- <sup>2.</sup> Bunch, Joey, "Boulder death could be second from poppy tea," The Denver Post, 7/21/2009.
- <sup>3.</sup> Macchiarelli, L., Arbarello, P., Cave Bondi, G., Di Luca, N.M., Feola, T., Medicina Legale (compendio)II edition, Minerva Medica Publications, Italy, Turin 2002.

Poppy Tea, Morphine, Opiates

#### A94 DART<sup>®</sup>-AccuTOF<sup>™</sup> as a Complementary Tool, or is it an Essential Tool in Managing Today's Complex Drug Chemistry Environment?

Erin M. Shonsey, PhD\*, Vaughn Barron, MEd, Andrea Headrick, BS, and Dale Carpenter, PhD, Alabama Department of Forensic Sciences, 2026 Valleydale Road, Hoover, AL 35244

After attending this presentation, attendees will learn the need for additional screening techniques in controlled substances analysis for robust case analysis.

This presentation will impact the forensic science community by investigating and filling a void in current controlled substances analysis, greatly reducing the risk of false negatives.

Whether we are cognizant of it or not, there exists a void in the classical screening capabilities of conventional drug chemistry laboratories. For the majority of cases encountered by forensic chemists, analysis with traditional methodologies is sufficient. However, the controlled substances world is not static, and the increasing complexity of not only controlled substances but also product tampering cases, is outpacing the capabilities of classical analytical techniques. Introduction of newer technologies into the classical workflow is essential to keep pace with the controlled substances world. The introduction of the DART<sup>®</sup>-AccuTOF<sup>TM</sup> has increased screening capabilities in a single analysis by orders of magnitude in a fraction of the time.

The DART<sup>®</sup> is one of the first open air, ambient pressure ion sources for a mass spectrometer. The design of the DART<sup>®</sup> opens the source for sampling substances in either solid, liquid, or gas states. This simplifies the sample preparation and extraction prior to analysis. In most instances, no sample preparation is required, and in fact, the raw sample is preferred for analysis. Analysis of a variety of substances including unknown powders, residues, organic liquids, plant materials, and aqueous liquids is possible without any sample preparation or extraction. The combination of the DART<sup>®</sup> with an AccuTOF<sup>TM</sup> mass spectrometer provides putative identifications of compounds based on molecular mass measurements. This process is targeted for analysis of protonated molecular ions, or  $[M+H]^+$  ions, resulting in a mass spectrum of the analytes that are capable of being ionized. This means that the intact mass of a compound must be measured within five millimass units (mmu) of the theoretical mass based on molecular formula for a positive screening result.

Drug Chemistry workflow in the laboratory consists of presumptive and confirmatory testing. Traditional presumptive tests include color tests, microcrystal tests, and gas chromatography or liquid chromatography analysis. Mass spectral identification is required for controlled substances confirmation in many laboratory systems. The void became readily apparent with the implementation of the DART<sup>®</sup>-AccuTOF<sup>TM</sup> into the screening process utilized in the author's laboratory. As a result the workflow was changed to require DART<sup>®</sup>-AccuTOF<sup>TM</sup> screening prior to a conclusion that no controlled substances are detected.

This applies to approximately 5% of the cases encountered in the author's laboratory system. Cases which screen positive by DART<sup>®</sup>-AccuTOF<sup>TM</sup> analysis undergo further testing to determine if molecular identification is possible.

Since the adoption of the DART<sup>®</sup>-AccuTOF<sup>TM</sup> screening, a suprising 40% of the cases (2% of the total) analyzed in the complementary workflow have screened positive. The cases that screened positive underwent additional analyses and mass spectral identification was obtained for controlled substances approximately 50% of the time or in 1% of the total cases analyzed. The ability to direct the analysis prior to extraction with a screening tool that covers the wide range of samples encountered in the laboratory has increased the ability to detect and confirm controlled substances. It has therefore been determined that the DART<sup>®</sup>-AccuTOF<sup>TM</sup> has become an essential tool for case analysis (demonstrating superiority to GC/MS in determination of true negatives), evaluation of extraction efficiency, and method development within the Alabama Department of Forensics Drug Chemistry laboratories.

Controlled Substances, DART®-AccuTOFTM, New Technology

#### A95 LC-MS/MS Analysis of Psilocybin and Psilocin in Mushrooms: SPE Approach

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After attending this presentation, attendees will learn about the extraction of psilocybin and its metabolite (psilocin) from seized mushrooms using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to provide data on both compounds in samples.

This presentation will impact the forensic science community by offering analysts in forensic facilities a method that permits samples of mushroom to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both SPE and LC-MS/MS.

This novel extraction (SPE) procedure was performed on a commercially available mixed mode column (C8/SCX) that had previously been conditioned with methanol, deionized water, and pH 6 phosphate buffer (0.1 M (3 mL, 3 mL and 1 mL, respectively)) prior to sample loading. The methanolic mushroom samples (1 mL) were adjusted to pH 6 with 0.1 M phosphate buffer (5 mL) and an internal standard was added (ethyl morphine) to the resulting solution. After loading the sample onto the SPE under gravitational flow, the sorbent was washed with deionized water and methanol (3 mL of each, respectively). Each SPE column was eluted with 3 mL of a solvent consisting of ethyl acetate containing 2% (v/v) ammonium hydroxide followed by 3 mL of methanol containing 4% (v/v) ammonium hydroxide. The individual eluates were collected in separate glass tubes under gravitational flow, evaporated to dryness (under nitrogen), and dissolved in a mobile phase (250 µL). These individual solutions were combined for analysis by LC-MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of psilocybin, psilocin, and ethyl morphine, respectively.

Liquid chromatography was performed in gradient mode employing a 50 mm x 2.1 mm C18 analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% acetonitrile for re-injection. The total run time for each analysis was less than five minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the

chromatography and analysis along with calibration curves and representative chromatograms of real mushroom samples.

**Results:** The limits of detection/quantification for this method were determined to be 50 ng/ g and 100 ng/ g, respectively for both psilocybin and psilocin. The method was found to be linear from 100 ng/ g to 2000 ng/ g (r2>0.999). Data is presented to show that recoveries of psilocybin and psilocin were found to be greater than 85% for both compounds. Interday and Intraday analysis of psilocybin and psilocin were found to be < 5% and < 8%, respectively. Matrix effects in this procedure were determined to be < 6%. Details of real samples (10) showing concentration of both compounds are given at the presentation.

**Conclusion:** The use of this new procedure for the analysis of psilocybin and psilocin using both SPE and LC-MS/MS will be of great use to analysts in the field of forensic drug analysis as the concentrations of both drugs can now be reported rather than just the psilocin value alone, as is currently provided by laboratories using gas chromatography coupled to mass spectrometry.

Hallucinogens, SPE, LC-MS/MS

## A96 Identification of Gamma-Hydroxybutyrate (GHB), Gamma-Butyrolactone (GBL), and 1,4-Butanediol (1,4-BD) Using Trimethylsilyl Derivatization

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After attending this presentation, attendees will learn how to trimethylsilyl derivatize GHB (gamma hydroxybutyrate), which is a controlled (Schedule I) drug and in turn positively identify it, separately from GBL (gamma-butyrolactone), which is not controlled, using a typical GC-MS (gas chromatograph-mass spectrometer). 1,4-butanediol (1,4-BD) is also included in the derivatization reaction, because 1,4-BD is used to manufacture GHB and sometimes comes in a mixture with GHB and GBL. Because these drugs usually come spiked in aqueous solutions like wines and soda, a simple extraction procedure is also included which precedes the actual derivatization reaction.

This presentation will impact the forensic science community by teaching the utility and versatility of the technique of trimethylsilyl derivatization of drugs like gamma-hydroxybutyrate (GHB) a Schedule I drug. From an identification point-of-view, this drug has become a headache for crime laboratories worldwide, because it converts to GBL (gamma-butyrolactone), which is not controlled, under high temperatures in a typical GC-MS (gas chromatograph-mass spectrometer). This problem can be easily solved by employing this simple, fast, efficient, and user-friendly technique.

GHB has been in literature since 1874. In the 1960's, it was developed as an anaesthetic in the field of medicine. In addition, this four-carbon molecule is purported to have anabolic properties and also induces sleep. This and several other reasons prompted the Food and Drug Administration in 1990, to issue a warning against the use of GHB. A decade later, the sale and synthesis of GHB was stringently controlled and the drug was placed in Schedule I by the Controlled Substances Act. This in turn led to an increase in the illegal synthesis of GHB. Recently, it has been increasingly used as a "date rape" drug. Cases of GHB use in the United States have gone from 56 in 1994 to 3,340 in 2001, especially among the youth.

For trimethylsilyl derivatization,  $\sim 2 \text{ mg}$  each of GHB, GBL, and 1,4-BD was mixed with 50 ml ethyl acetate. Then a 50 ml mixture of BSTFA+TMCS (in a ratio of 99:1) was added to it. The reaction mixture was incubated at room temperature for  $\sim 30 \text{ min}$ . Then 1-2 ml of the reaction mixture was subjected to GC-MS analysis. The excess derivatization reagents and ethyl acetate in the reaction mixture had retention times (RT) in the GC ranging from  $\sim 0.5$  to  $\sim 3.0 \text{ min}$ .
Trimethylsilyl-derivatized GHB appeared at ~6.0 min. Underivatized GHB, after converting to GBL at high temperatures has a RT of ~3.3 min. Due to its characteristic dragging on the column, the RT for underivatized 1,4-BD ranged between 3.6 - 4.0 min. Derivatized 1,4-BD had a RT of 5.5 min. The major ionic fragments of the derivatized GHB on the mass spectrometer (MS) fully corroborated with theoretical calculations, which are m/z 73, 117, 147, and 233. Underivatized GHB, after converting to GBL, had the following major ionic fragments m/z 42, 56, and 86. The major ionic fragments monitored for derivatized and underivatized 1,4-BD were m/z 73, 116, 147, 219, and m/z 42, 57, and 71, respectively.

To date, this is a straight forward, simple and accurate method for identifying GHB from GBL. Due to its closed ring structure, GBL is immune to derivatization. However, this technique can be effectively used to derivatize specifically GHB, even in a mixture of GHB and GBL. The mass spectra of derivatized and underivatized 1,4-BD is entirely different from that of GHB and GBL and the presence of 1,4-BD in the mixture does not hinder the identification of GHB. This technique of trimethylsilyl derivatization becomes more attractive in the fact that, after the derivatization reaction, there is no need to extract the derivatized or underivatized products from the finished derivatization reaction before the GC-MS analysis because the retention times in a GC and the resulting mass spectra of the derivatization reagent, BSTFA (N,Obis(trimethylsilyl) trifluoroacetamide) are quite different when compared to that of the derivatized and underivatized products. More over, the whole reaction and its analysis on a GC-MS takes less than an hour, which makes it quite attractive for a fast paced drug laboratory.

The law enforcement agencies submit cases containing wines and soda, purportedly spiked with these drugs whose usage is becoming more common in night club parties. Because the derivatizing agent is incompatible with aqueous solutions like soda and wines, a very robust, simple, and high recovery extraction procedure is also included, which delivers pure drugs that are derivatization ready before the actual derivatization reaction. This ultimately leads to the identification of these drugs.

Gamma-Hydroxybutyrate (GHB), Gamma-Butyrolactone (GBL), Trimethylsilyl Derivatization

#### A97 Is FEPAC Accreditation Opening the Employment Door?

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After attending this presentation, attendees will: learn about the relationship between FEPAC accredited program graduates and the hiring practices of some ASCLD-LAB accredited forensic laboratories; will learn that most laboratory directors know of FEPAC but do not consider it an advantage in the hiring process; and will learn that AAFS and FEPAC need to market the goals and significance of FEPAC accreditation to forensic laboratories.

This presentation will impact the forensic science community by presenting data that shows that FEPAC accreditation is not an advantage in the hiring process for accredited program graduates. Therefore, is it worthwhile for post-secondary educational institutions with forensic science degree programs?

The American Academy of Forensic Sciences (AAFS) developed the Forensic Science Education Programs Accreditation Commission (FEPAC) in 2002. FEPAC accredits forensic science programs located in regionally accredited universities, including undergraduate and graduate degrees, and as a result it is hoped that it encourages academic quality through the accreditation. Seventy-six randomly selected forensic laboratories from across the United States were chosen for the telephone survey from a list of American Society of Crime Lab Directors

- 1. Are you involved in the hiring process?
- 2. What is your title or position in the lab?
- 3. Have you heard of FEPAC accreditation?
- 4. Do your potential new hires receive any advantage in the hiring process if they have graduated from a FEPAC accredited program? What is it?

Overall, a total response rate of 42% was obtained for the telephone survey, generating 32 completed surveys from participating laboratories for qualitative data analysis. Of these participating forensic laboratories, 72% had heard of FEPAC education accreditation and of significance, 69% of these forensic laboratories believed that graduation from a FEPAC accredited program did not confer any type of advantage during the hiring process. When an actual forensic laboratory employee, including the head of the laboratory, was surveyed, the results showed that greater than 70% of the respondents had knowledge of FEPAC. However, when human resources personnel for the laboratory or appropriate administrative agency were contacted, the results showed only 25% even knew of FEPAC accreditation. When interviewing the head of the forensics laboratory, 40% of those individuals who were aware of FEPAC accreditation, stated a belief that the accreditation status should give an advantage during hiring. However, no human resources members believed in this advantage. Based on this project's data and interpretations, in today's job market, successful completion and graduation from a FEPAC accredited program does not confer a specific advantage during the hiring process. The cause for these results as shown by this project appears to be due to a lack of knowledge about the requirements of FEPAC accreditation, as well as, general knowledge as to the number and location of current FEPAC accredited programs. Although knowledge of FEPAC accreditation within the forensic science educational population is growing, the corresponding growth within the general forensic science community has yet to be seen. This presentation will suggest solutions to change the perception of FEPAC accredited programs. The American Academy of Forensic Sciences and the FEPAC committee itself needs to focus more on "marketing" of the goals, process, requirements, and standards necessary for FEPAC accreditation. If the marketing is successful, then the value for FEPAC accreditation will be shown in the form of an outcome that will give hiring advantages to graduates of FEPAC accredited programs. Certainly, the advantages they deserve in today's job market.

FEPAC Accreditation, Employment, Hiring Process Advantage

## A98 Online Laboratory Analysis Training: Feasible or Not?

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After attending this presentation, attendees will understand adult learning related to online courses, described as a theory called "andragogy." The andragogy hypothesis states that online adult learning should focus more on the application and less on the content being taught. Strategies such as case studies, tabletop application, role-playing, simulations, group projects and discussions, and self-group evaluations are most useful for adult learning.

This presentation will impact the forensic science community by demonstrating strategies such as case studies, tabletop application, roleplaying, simulations, group projects and discussions, and self-group evaluations are most useful for adult learning.

The University of Central Florida's National Center for Forensic Science and Institute for Simulation has developed two online courses.

One online training course is well suited for first responders to a Chemical, Biological, Radioactive, Nuclear, and Explosives (CBRNE) incident. However, the online training for laboratory personnel completely with "no hands-on" training for the examination and identification of high explosives may be another story.

Malcolm Knowles emphasizes that adults are self-directed - they expect to take responsibility for decisions about their own learning and professional development. Adult learning courses delivered online must accommodate this fundamental aspect. Andragogy makes the following assumptions about the design of learning: (1) adults need to know why *they* need to learn something; (2) adults need to learn *experientially*; (3) adults <u>approach</u> learning as *problem-solving*; and, (4) adults <u>learn best</u> when the topic is of *immediate value*.

Professional development has become a critical challenge across all federal, state, and local agencies. Research has consistently demonstrated that training which provides only information and theory-based content results in little more than an increase of knowledge–*not skills*. Simulations and advanced learning technologies provide an effective tool for the preparation and maintenance of competencies required for responding to the various dynamic and critical situations; they also provide greater knowledge retention and expand/refine skills, resulting in overall improved job performance, employee satisfaction, and employee retention.

Online Courses, Andragogy, Adult Learners

# A99 Continuing Education for Forensic Professionals Through Virtual Crime Scene Assessments

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The goal of this presentation is to demonstrate an interactive, virtual crime scene, as part of an online course and its validity for training. Discussion will relate to the benefit of training through an online medium and how such a training format gives the student a practical experience. While replicating onsite training and education is challenging, a thorough presentation of virtual crime scenes is a move toward the enhancement of online training.

This presentation will impact the forensic science community by demonstrating to working professionals the value of virtual crime scene training when it is used in addition to other training platforms.

Recommendations in the National Academy of Sciences (NAS) report address the needs of forensic professionals to have continuing education and training in forensic science. Included in the report is a recommendation given by the Technical Working Group for Training and Education in Forensic Sciences (TWGED). The efforts of TWGED discuss training content and how it should include a combination of essential topics and elements specific to forensic disciplines. One critical area requiring training is crime scene processing. Because evidence collection and preservation is central to the investigative process, it is beneficial for professionals across various disciplines to understand crime scene collection, preservation, and processing. Crime scenes in an academic context, however, are difficult to reproduce, manage, and distribute to students over wide geographical areas. A virtual crime scene provides students the advantage to further comprehend crime scene procedures in an accessible format. This is accomplished by demonstrating collection and preservation methods as they are published in crime scene literature as well as the National Institute of Justice (NIJ) and Federal Bureau of Investigation (FBI) research reports on crime scene investigation.

A panoramic video system captures a complete view of an area, such as a crime scene, without manually "stitching" individual photographs together. The 360° area can be viewed from above, below, and at eye level in relation to the viewer. After the scene is captured visually, the views are then combined with correlative software and links to evidence, objects, or locations can be embedded within the scene. Students choose collection and processing methods appropriate for the given evidence. For example, one of several presumptive tests can be selected for a suspected bloodstain. Other options include, but are not limited to, the Electrostatic Lifting Apparatus (ESLA), alternate light source (ALS), and camera setting adjustments for crime scene photographs. During the experience, the student maintains a crime scene notebook, which is embedded in the online course. The notebook is used to document their work, organize the crime scene, and maintain a chain of custody.

Certainly, some forensic professionals are skeptical when a virtual crime scene is used for training purposes. Academic programs, certification tests (i.e., IAI, ABC), and on-site trainings, such as National Institute of Justice (NIJ) sponsored programs, are the core of continuing education. Online courses and the virtual crime scene, much like on-site courses are only as good as the content and the depth of learning a student gains from the experience. Fortunately, the advancement of video and graphic design software gives online courses the potential to emulate training formats commonly used in forensic science. In addition, virtual, interactive training is an effective way to obtain low-cost continuing education while performing job related responsibilities and managing casework.

Training, Virtual, Crime Scene

# A100 Two Case Studies of Controlled Substance Laboratories Using Custom Macros and Commercial LIMS-Based Paperless Systems Under ASCLD/LAB©-International Accreditation Requirements

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After attending this presentation, attendees will understand the process associated with the implementation of a LIMS-based paperless system through custom instrument macros. Attendees will observe two approaches that comply with ASCLD/LAB-International accreditation requirements, particularly regarding security and documentation of technical review.

This presentation will impact the forensic science community by increasing efficiency while simultaneously reducing operational costs. A major challenge of controlled substance laboratories is managing high caseload submissions in an efficient, cost-effective manner. This presentation chronicles the implementation of two paperless systems using commercial LIMS-based systems. The purpose of this presentation is to provide a blueprint for laboratories seeking ways to implement costsaving measures and efficiency without expending a great deal of resources.

The Oklahoma State Bureau of Investigation (OSBI) initially created macros specifically for Agilent Chemstation<sup>™</sup> to automate sample analysis and report creation for Agilent<sup>™</sup> dual column GC-FID and GC-MS instrumentation. The Harris County Institute of Forensic Sciences (HCIFS) implemented these macros and further tailored the macros to

comply with agency-specific procedures. HCIFS also provided customization for the automatic dual creation of hard-copy and electronic report formats. All reports, regardless of instrument type, are automatically generated and display all relevant case information and instrument settings. The macros incorporate quality control data to ensure proper instrument performance in an easy to read format.

Macros are also used to add a layer of security. A ."txt" file is created that assigns a unique Validation Code to each sample. The ."txt" file contains the validation number, case number, item and analyst information; with the Validation Code also appearing on the report. The ."txt" files and sequence logs for each instrument run are archived in secure folders.

The OSBI and HCIFS have connected instrumentation computers to their individual laboratory-wide networks to allow for access of instrument reports at analyst workstations. After review by the analyst, the instrument reports are uploaded into the respective LIMS system and are time and/or date stamped with the user login information. The LIMS system in place at HCIFS is Justice-Trax<sup>TM</sup> whereas the OSBI uses the BEAST<sup>TM</sup>, both prominent, commercial LIMS systems.

Instrument reports are uploaded into the BEAST<sup>TM</sup> as PDF documents and all transactions are documented in an Audit Log. The Audit Log tracks all the details of the actions taken for individual cases. Administrative/technical reviews are performed before the BEAST<sup>TM</sup> releases a case.

Justice-Trax<sup>TM</sup> requires a technical review of these electronic documents which is tracked through a review history before the case can be released. Justice-Trax<sup>TM</sup> generates an electronic worksheet which documents the total number of technical pages uploaded into the electronic case file, complying with ASCLD-LAB/*International* requirements of documenting the review of each page in the official case record.

Other topics of discussion will include enhancements of the HCIFS paperless workflow including the implementation of additional commercial and/or in-house compiled software to document exhibit weights, the creation of custom barcodes for Agilent<sup>TM</sup> GC-FID and GC-MS and the use of Kováts retention index (RI) for GC-MS confirmation, in lieu of repeated injections of positive controls.

**Controlled Substances, Paperless, Macros** 

#### A101 The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

Scott R. Oulton, BS\*, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081

The goal of this presentation is to update forensic drug analysts on recent work products from SWGDRUG.

This presentation will impact the forensic science community by providing the current work products by SWGDRUG as it relates to the analysis of seized drugs.

Currently, SWGDRUG is working on the following topics:

- Examples for the estimation of uncertainty of measurement in purity determinations
- Developing standard training competencies
- New initiatives such as: providing a searchable mass spectral database; development of a measurement uncertainty calculator; new section on website documenting method limitations; etc.

In past presentations to the American Academy of Forensic Sciences, a synopsis of the history of SWGDRUG and goals of the core committee have been presented. This year's presentation will focus on the specifics described above. However, the following information is presented here for those unfamiliar with the SWGDRUG process. SWGDRUG has been in existence since 1997. The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance. The objectives of SWGDRUG are the following:

- Specifying requirements for forensic drug practitioners' knowledge, skill and abilities;
- Promoting professional development;
- Providing a means of information exchange within the forensic science community;
- Promoting ethical standards of practitioners;
- Providing minimum standards for drug examinations and reporting;
- Establishing quality assurance requirements;
- · Considering relevant international standards; and
- Seeking international acceptance of SWGDRUG recommendations.

The SWGDRUG core committee is comprised of representatives from federal, state, and local law enforcement agencies in the United States, Canada, Great Britain, Germany, Japan, Austria, Switzerland, Australia, the European Network of Forensic Science Institutes (ENFSI), the United Nations Drug Control Program (UNDCP), forensic science educators, the American Society of Crime Laboratory Directors (ASCLD), American Society for Testing and Materials (ASTM), and the National Institute of Standards and Technology (NIST). Published recommendations are available on the SWGDRUG website located at: www.swgdrug.org.

Criminalistics, SWGDRUG, Drug Analysis

## A102 Using Quality Control (QC) Data for Estimating the Measurement Uncertainty Associated With Purity Results

Sandra E. Rodriguez-Cruz, PhD\*, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081

After attending this presentation, attendees will learn how to use quality control analyses to estimate the uncertainty associated with purity determinations.

This presentation will impact the forensic science community by providing laboratories with easy to implement procedures to comply with accreditation requirements pertaining to the uncertainty of measurement.

Accreditation under the ISO/IEC 17025:2005 standard requires testing and calibration laboratories to have established procedures for estimating the uncertainty of their measurements. Forensic chemistry laboratories throughout the world provide law enforcement customers with a variety of analytical results, including weight measurements, controlled substance identification, and in many cases, results pertaining to the purity of the substances present. Purity determinations are not routinely performed by smaller, local, and state crime laboratories in the United States; however, such measurements are commonly reported during the analysis of controlled substances throughout the Drug Enforcement Administration (DEA) laboratory system. As a result, laboratory reports generated must also include the uncertainty of measurement associated with purity results.

The uncertainty associated with purity measurements can be evaluated using the strictly mathematical approach recommended by the ISO *Guide to the Expression of Uncertainty in Measurement* (GUM). However, for most forensic chemistry applications, such an approach is somewhat impractical due to the variability of samples and matrices encountered by analysts on a day-to-day basis. A more reasonable and applicable approach is necessary for measurements performed in forensic chemistry laboratories. Technical Reports published by the European Federation of National Associations of Measurement, Testing, and Analytical Laboratories have provided alternative methods for the evaluation of measurement uncertainty; among them, estimation of uncertainty using quality control data and inter-laboratory comparisons.<sup>1,2</sup>

This presentation will describe the use of quality control samples for the estimation of the uncertainty associated with purity measurements. The approach presented is applicable to multiple controlled substances and analytical techniques used throughout crime laboratories. This methodology represents a top-down approach for uncertainty evaluation, where the contributions of multiple factors can be considered at once, avoiding tedious uncertainty budget re-calculations resulting from variations in instrument performance, sample and matrix changes, and environmental conditions. The use and evaluation of quality control samples also provides a way for verification of uncertainty of measurement estimates previously obtained using different methods, such as collaborative studies or budget approaches.

Results from the analysis of four quality controlled samples will be presented and discussed. These samples were prepared using authenticated reference materials and were kept under controlled environmental conditions. Two of the samples contained cocaine HCl concentrations of approximately 25% (CocaineQC#1) and 75% (CocaineQC#2), respectively. The other two samples were prepared to contain methamphetamine HCl at 20% (MethQC#1) and 80% (MethQC#2), respectively. Multiple adulterants and diluents were also added to the samples, in order to mimic routinely encountered compositions. Quality Control (QC) solutions were prepared fresh prior to analysis using a laboratory-validated HPLC quantitative method.

Results from more than a year of QC analyses will be presented in the form of control charts. Statistical analysis of the data will demonstrate that the QC results represent a reasonable estimate for the uncertainty associated with the quantitation of cocaine and methamphetamine using the selected method and instrumentation. Results will also provide verification for previously reported uncertainty estimates obtained via the statistical analysis of proficiency testing samples.

This presentation is expected to be of interest to other forensic chemistry analysts and laboratory personnel involved in the evaluation of measurement uncertainty, as required for accreditation under the ISO 17025:2005 standard.

#### **References:**

- EUROLAB Technical Report 1/2006 Guide to the Evaluation of Measurement Uncertainty for Quantitative Test Results.
- EUROLAB Technical Report 1/2007 Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation.

Uncertainty, Purity, Controlled Substances

# A103 Forensic Science and the Concept of Relevancy

Durdica Hazard, MS\*, and Pierre A.J-L. Margot, PhD, University of Lausanne, Institut de Police Scientifique, Ecole des Sciences Criminelles, UNIL-BCH, Lausanne, 1015, SWITZERLAND

After attending this presentation, attendees should understand the concept of relevancy in forensic science and crime scene investigation. The aim is to spark off an interest in thinking about this fundamental notion in forensic science in attendees and arouse their interest for the study of basic principles of forensic science. These objectives embrace the following positive outcome: a better understanding should help the process of forensic science in its investigative dimension and should improve elementary actions and thought processes applied on investigative scenes

This presentation will impact the forensic science community by discussing a combination of factors to improve the detection of relevant traces, that should help design training and education schemes.

Over 50 years ago, Kirk (1963) highlighted a serious deficiency in theory and basic principles. This was a realistic and serious analysis of the Forensic Science situation, and it is still relevant today. Despite the small number of studies that have discussed principles attributed to Kirk

and Locard, even fewer (none in the authors' view) have been dedicated to the concept of relevancy of collected traces.

Although obvious, the criminalist's vocation is to find, collect, and analyze relevant traces. From crime scenes to laboratories, working with relevant physical traces is a leitmotiv, and belongs to the thought process governed by the above two mentioned forensic principles. Focusing on the relevancy concept in Forensic Science, it goes far beyond a simple question of definition and Inman and Rudin (2000) did formalize the current conundrum that criminalists have to deal with: the most difficult challenge is "the recognition of relevant physical evidence," whereas it may be questionable whether the capacity to recognize objects as evidence would not have limits. Those limits pertain to the framework of interventions of criminalists: crime scenes are consecutive but not alike, being peculiar to every criminal activity, where resources are always limited (whether material, time, etc.). This forces criminalists/investigators to adapt to places and cases in order to find what is relevant. But how does it work? Actually, what is a relevant trace? Do all crime scene investigators perceive relevancy in the same way? And what will influence their perception?

These questions raise issues that go back to the very foundations of the (forensic) investigative process. This means discussing the elementary and indispensable piece of the forensic puzzle, the trace, and investigating what parameters could act on the criminalist's thought process in its detection and recognition, taking into account the crime scene environment. Arising from a research dedicated to the relevancy concept in forensic science, this presentation will focus on the links between trace, clue, evidence, relevancy, and crime scene investigators. Semiotics, and a brief state of the art, will help define the relevancy concept and introduce the fundamental, sometimes fuzzy and tenuous distinction between trace and evidence. This will stress the point that sets apart trace from evidence notions.

According to semiotic views, the relevancy concept could be defined as a trace-object perception on investigative scenes conditioned by context and by what the criminalist decides to recognize and use as features from (relevant) trace, i.e potential for transfer and for discrimination (identification). The trace is understood as a "vestige or marks remaining and indicating the former presence, existence or action of something," without any given meaning at this point, except that it is perceived as a potential source of information to explain issues in the investigated case. The trace becomes a clue when the criminalist recognizes the information content. It allows the examiner to make inferences with different alternative causes that give different values and meaning to the sign-trace. It is relevant when it gives information to the case while taking into account the context. It becomes evidence that is understood as information coming from the trace-object that "raises or lowers the probability of a proposition," i.e., gives confidence to decision making in deciding probable cause.

These meanings insist on the need to use proper terminology specific to particular steps of the forensic reasoning and positioning within judicial proceedings. Such an attempt in this differentiation is expected to help in the understanding of the thought processes applied in forensic science and to crime scenes.

This research aims to define a basic question which is difficult to measure. Exploring the meaning of forensic notions, while using a semiotic approach, can be a useful tool to help understand a concept which plays a fundamental role in Forensic Science and is born out once you leave the crime scene.

**Relevancy Concept, Trace, Evidence** 

#### A104 Forensic Training and Development of the Erbil Forensic Laboratory, Iraq

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After attending this presentation, attendees will have an understanding of the state of the forensic laboratory system and training in Iraq. Topics addressed will include an overview of evidence collection at the crime scene, the laboratories' role in the Iraqi criminal justice system, and the United States and Coalition's continuing efforts to equip and train the laboratory system to full operational status. Cultural and historical background within the country as relevant for an understanding of the challenges faced by the laboratories will also be addressed.

This presentation will impact the forensic science community by describing the status of the Iraqi Forensic Laboratory System, as part of the overall nation-building process undertaken by the United States and Coalition. It will also give practitioners an understanding of the challenges encountered in setting up forensic laboratories under challenging conditions.

The U.S. and Coalition have worked since the beginning of the second Gulf War in 2003 to build and equip the forensic laboratory system of the country of Iraq and to integrate it into the existing criminal justice system. Despite significant challenges presented by terrorist activity, infrastructure, and logistics, the Iraqi Forensic Laboratory System, consisting of three full-service laboratories, one training and research laboratory, and seven comparative forensic laboratories, has been established and is operational. Analyst training has taken place both in-country as well as abroad over the past several years and has developed many well-qualified laboratory scientists.

This presentation focuses on the objectives and results of the mentoring and training contract of Iraqi analysts, which took place in Erbil in 2009 and 2010, and the challenges that the Iraqi Forensic Laboratory System faces as it moves forward. The project entailed mentorship of and advanced instruction in the major forensic disciplines within the Erbil Forensic Laboratory, located in the northern autonomous region of Kurdistan. Mentors worked with analysts in the various disciplines to develop written protocols, assisted in ordering of supplies and in arranging for equipment installations, as well as technical mentorship of validation processes. Instructors delivered advanced training in the major disciplines to analysts from all regional labs within Iraq, utilizing both theoretical and practical training.

The DNA and Chemistry section startups of the Erbil Forensic Laboratory were a point of focus within the project. Manufacturer's representatives traveled to Erbil, installed the instruments, and provided initial instrument analysis and maintenance training. Mentors and Instructors worked with the analysts to ensure that proper procedures were developed. During this time period, the first forensic cases in Iraq for Chemistry and DNA were analyzed and reported out.

The laboratory system has an ultimate objective of ISO accreditation and will continue to progress towards that goal. Major challenges faced by the Erbil Forensic Laboratory and the Iraqi forensic laboratories system-wide, include further advancement in power, temperature, and humidity regulation within laboratories for preservation of delicate instrumentation, the expansion of supply chain and delivery logistics for supplies and consumables, and hiring and training of more analysts, plus further training and development of the existing analysts. At the evidence collection and receipt level, increased training is necessary for judicial investigators, judges, and crime scene personnel, both in evidence collection and in education of the laboratories' capabilities. This holistic approach is currently being addressed and will need to continue as such. All sections will continue to require the assistance of external experts, acting as technical leaders or mentors, in the near future. The DNA unit specifically will address further challenges, including databasing within a tribal culture, the establishment of theta, and the development of DNA databank and search capabilities.

Iraq, Laboratory Startup, New Forensic Lab

# A105 The Effect of Accelerant and Passive Headspace Analysis on DNA From Simulated Arson Evidence

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After attending this presentation attendees will gain knowledge of the potential negative effects that passive headspace has on DNA present on evidentiary material taken from an arson investigation. Attendees will also understand the potential damage to DNA that can be caused by the accelerant and the burning of the material.

This presentation will impact the forensic science community by determining the optimal headspace analysis parameters of the detection of accelerants on simulated arson evidence that will maximize DNA typing results.

Fires are a very destructive force of nature. However, not all fires are naturally occurring. Many fires are deliberately set to cause damage to one's property or even to another individual. These types of fires are classified as arson. Unlike most crimes, in an arson case evidence is typically destroyed by the fire which leads to a more difficult investigation. Accelerants are generally flammable or volatile liquids used to aid the ignition of the fire and are therefore, commonly found at the scene.

DNA can be used to link a suspect or victim to the scene of the crime. DNA can be extracted from any type of body fluid, hair, and even skin cells. Studies have shown that by briefly touching an object a detectable amount of DNA is left behind. In order to deliberately start a fire one must typically be present at the scene of the fire. For instance, blood may be present if a suspect or victim is injured during the commission of the arson. Furthermore, items simply touched by individuals may transfer DNA to the object.

In an arson investigation, items of evidence may be collected to undergo accelerant analysis and then later sampled for DNA. A very common method for extracting accelerants from evidence is passive headspace. Heat is used to help speed up the volatilization process. By heating the evidence in a metal paint can, the accelerant volatilizes and becomes concentrated in the headspace of the can which can then be sampled. However, this method for accelerant analysis involves high temperatures, which could damage DNA, potentially impacting DNA typing methodologies.

A series of experiments have been carried out to simulate analysis of evidence that may be commonly collected at the scene of a fire and tested for both DNA and accelerants. Six porous substrates: 100% cotton, 100% nylon, wood, foam, carpet, and cardboard along with one nonporous substrate (glass) were used throughout all experiments. All substrates were stratalinked to crosslink any DNA that may have been present. Three different volumes of whole blood were added in triplicate to all seven substrates simulating high copy number DNA. This was to ensure that DNA extraction and downstream applications would have a higher success rate and differences could be seen.

Two different extraction methods, Chelex and DNA IQ<sup>™</sup> System (Promega), were utilized to extract DNA from the simulated evidence. A mock passive headspace procedure was followed to mimic the prolonged heat exposure to the DNA present in the blood. The maximum ASTM recommendations for time and temperature were used.<sup>1</sup> DNA extraction was performed on one set of samples prior to the mock passive headspace and one set of samples after the mock passive headspace.

Statistical analysis of quantitation results showed that DNA on simulated evidence was not adversely affected by passive headspace analysis. Two substrates, cardboard and carpet, yielded very low concentrations of DNA before and after passive headspace analysis, possibly due to the presence of PCR inhibitors. However, all samples produced a full genetic profile with 15 STR loci and amelogenin.

Results will also be presented on the effect of passive headspace analysis on samples of blood and touch DNA placed on the individual substrates mixed with accelerants. Optimal headspace analysis criteria maximizing both accelerant detection and DNA typing results will be determined. Substrates will also be burned with DNA sampled before and after passive headspace.

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Passive Headspace, Accelerant Analysis, DNA Quantitation and Typing

# A106 Recovery and Analysis of Nuclear DNA From Charred Muscle and Tendon Tissue Using White-Tailed Deer (*Odocoileus virginianus*) as an Animal Model

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After attending this presentation, attendees will be able to understand the recovery and utility of compromised DNA from charred muscle and tendon tissue burned with three of the most commonly used accelerants.

This presentation will impact the forensic science community by identifying the quantity of DNA available and also the quality of the DNA from charred muscle and tendon tissue obtained from white-tailed deer, which was used as an animal model. Both human and wildlife forensic investigators will benefit from the DNA analysis results from the charred soft tissue because perpetrators will often burn evidence in order to cover up a crime.

Among the top three reasons for arson is the concealment of crimes including homicides. A perpetrator will often burn their victim in order to make DNA identification more difficult. Historically, teeth have been used for the identification of badly burned remains largely due to the composition of enamel withstanding the burning process. However, dental records are needed for positive identification and are not always available. The proposed study examined the ability to recover nuclear DNA from both tendon and muscle tissue of white-tailed deer (Odocoileus virginianus) legs. It also examined the difference between the muscle and tendon samples in order to see if one of the tissues yielded higher DNA recovery. The white-tailed deer legs were subjected to one of the three accelerant treatment condition burns and a non-accelerant wood burn. Gasoline, kerosene, and lighter fluid were the accelerants chosen because they are frequently used to cover up a homicide in order to make victim identifications more difficult. The leg samples were obtained with a collection permit from the Pennsylvania Game Commission from road kill specimens found on Pensylvania roadways. One leg from each deer was incinerated with the same volume of the three accelerants and the fourth leg was burned without any accelerants as a control. Approximately ten deer were used for this study leading to a sample size of 44 deer legs, using each a muscle and tendon sample from each leg when discernable.

DNA was extracted from each type of tissue sample using a standard organic extraction protocol. Each extracted DNA sample was then

amplified using two in-house white-tail deer multiplexes optimized from the loci sequenced and cited from Anderson et al. (2002). The 11 individual primers in the multiplexes were separated into the two separate STR panels based on their base pair size range and fluorescent dye label on the forward primer. To date, DNA has been extracted, quantified, and amplified from the burned samples. Then, the samples were run on a genetic analyzer in order to obtain the genotype profile for each sample type. For the majority of samples, at least two or more loci were seen in the genetic profile, and the DNA concentrations obtained from the charred samples have been greater than anticipated. Also, the DNA recovery from the charred tendon samples was higher than that from the not charred tendon samples.

Even though this study uses white-tailed deer as an animal model, the application can be applied to charred human remains as well, leading to better identification of homicide victims that were burned by their perpetrators in order to destroy evidence. Overall, this study aims to illustrate that nuclear DNA can be extracted from remains in which the DNA in the soft tissue could be very degraded and very rarely used in the identification of badly burned remains.

**Charred Tissue, DNA Recovery, Accelerants** 

## A107 Enzyme-Based Preparation of PCR-Ready DNA From Semen and Mock Sexual Assault Samples

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After attending this presentation, attendees will have gained an understanding of the use of a neutral proteinase to extract DNA from sperm cells found in semen and mock sexual assault samples.

This presentation will impact the forensic science community by describing a novel method to extract male DNA without the use of a solid phase, which decreases analysis time and increases sample throughput.

Sexual assault samples often contain a mixture of sperm cells and female epithelial cells, typically on a cotton swab matrix. The most widespread method for the analysis of sexual assault samples, differential extraction, uses gentle lysing conditions to break open the female epithelial cells while leaving the sperm cells intact. The female DNA is removed and the sperm pellet is washed to rinse away excess female DNA. The sperm cells are lysed through the addition of a reducing agent, such as DTT, and both the male and female samples must undergo further purification to remove proteins, nucleases, and other inhibitory compounds before the DNA can be added to the polymerase chain reaction (PCR).

Typically, the samples are purified using a solid phase extraction (SPE) method, which utilizes a silica-based solid phase to reversibly bind DNA under high salt conditions while impurities are washed away. These extraction methods can have a number of sample transfer steps which may result in the loss of some DNA and reduce the probability of obtaining a full STR profile. Additionally, SPE is time-consuming, with many incubation and/or centrifugation steps and can take upwards of four hours to extract DNA from semen.<sup>1</sup> Finally, the chaotropic reagents used to load the DNA on the solid phase and the organic reagents used to wash away the impurities may inhibit PCR.

The issues that can hinder SPE methods can be nearly eliminated by switching to a method that does not use a solid phase. Liquid extraction has been used in the past in the form of a phenol-chloroform extraction, where proteins and lipids move to the organic phase and DNA moves to the aqueous phase; however, this method is time-consuming and hazardous. Recently, a liquid extraction procedure has been developed that uses a thermally-stable neutral protease to degrade cellular membranes, proteins, and nucleases.<sup>2</sup> The enzyme used (**Bacillus** sp. EA1) has optimal activity in buffers that are PCR-compatible, reducing the chance for PCR inhibition. This enzyme-based extraction method reduces the amount of sample transfer steps and, therefore, nearly eliminates any loss of sample that may occur, while yielding PCR-ready DNA after only 20 minutes. Commercially-available kits that utilize this enzyme can be applied to whole blood and saliva samples, but kits for semen sample analysis do not exist, primarily due to the incompatibility of the enzyme with most reducing agents.

The current work focuses on the development of a procedure for the liquid extraction of DNA from sperm cells. First, sperm cells are lysed during a brief incubation containing an alternative reducing agent. Then, an aliquot of the lysate is added to a solution containing buffer and enzyme and the sample is incubated for extraction of DNA in just 25 minutes. Using this method, DNA yield from sperm cells is significantly increased as compared to a traditional SPE method. Amplification results of genomic DNA from purified sperm cells show a full STR profile using this method. In addition, the use of this method towards sperm cells isolated from mock sexual assault samples will be demonstrated. Overall, this method represents an approximate three-fold reduction in average DNA extraction time, from 1.5 hours to 25 minutes as compared to conventional SPE methodologies.

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**DNA Extraction, Differential Extraction, STR Typing** 

## A108 Application of Pressure Cycling Technology (PCT) in Differential Extraction

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After attending this presentation, attendees will understand a new method for the differential extraction of DNA from sperm and epithelial cells in sexual assault casework.

This presentation will impact the forensic community by providing a better understanding of how pressure cycling technology can be used to speed up and simplify the extraction process.

One of the stumbling blocks in obtaining a successful male genetic profile in sexual assault cases involves the separation of the evidence left behind by the perpetrator from that of the victim. The conventional differential extraction methods used for the separation of DNA from sperm and epithelial cells is time consuming and requires expertise. It is imperative to develop a method that addresses the issues of time, efficiency, and ease of use.

Pressure cycling technology sample preparation system (PCT SPS) is a novel method that involves the use of pressure to disrupt tissues, cells, and cellular structures enabling the recovery of their components. In this research, the authors utilized a commercially available instrument from Pressure Biosciences with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 35,000 psi resulting in the lysis of cells. Sample cells were placed in liquid suspension in microtubes and subjected to a range of on and off pressure pulses in an

attempt to isolate and recover DNA. The microtubes were made from a fluoropolymer that renders them chemically resistant to improve sample recovery and limit adsorption.

The current study involves the application of pressure cycling technology in the extraction of nucleic acids from sperm cells and vaginal epithelial cells. The cells were suspended in 1X PBS buffer (pH 7.4) and subjected to 5,000 psi- 35,000 psi pressure in increments of 5,000 psi, accompanied by varying number of cycles in order to determine the conditions at which one type of cell could be lysed differentially over the other. Samples were placed in microtubes and introduced into the pressure chamber. This pressure treatment was followed by phenol chloroform isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with an Alu-based real-time PCR method using SYBR green.

The initial studies indicate the potential of PCT application in analyzing samples from sexual assault cases, in particular, indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells. Overall these results provide new opportunities to explore the ability to generate male DNA profile by selectively lysing sperm cells from mixtures.

Differential Lysis, Pressure, Sexual Assault

# A109 Choosing Relatives for DNA Identification of Missing Person Identification

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After attending this presentation, attendees will be informed of the information content that can be useful with different combination of relatives, which relatives would be most informative, and how many relatives are sufficient for DNA missing person and other kinship analysis identifications.

This presentation will impact the forensic science community by comparing the information content of different relative combination scenarios for kinship analysis and providing practical guidance to select the most informative relatives for missing person identification, especially when resources are limited.

DNA-based analysis is integral to missing person identification cases. When direct references are not available, indirect relative references can be used to identify missing persons by kinship analysis. The DNA Commission of the International Society for Forensic Genetics (ISFG) suggested calculating posterior odds for identification and setting 99.9% as a degree of confidence. There are two ways to increase the power of identification: (1) type more markers; and, (2) type more relatives. In many cases, the quality and quantity of DNA is poor. Thus the number of markers that can be typed will be limited by the quality and quantity of DNA derived from remains. Increasing the number of reference relatives can increase the chances of identifying remains and particularly for challenged samples. However, typing all relatives of a large pedigree can be costly and may not be necessary to reach a defined threshold for identification. At times decisions may need to be made on which relatives to type. Since there are information and cost factors regarding the selection and number of relatives, some selection criteria should be considered to guide identity testers.

In this study, the 37 most common relative combination scenarios (e.g., both parents, three children, one child plus one parent plus spouse,

one parent plus three fullsibs, one child plus one parent, two unrelated uncles, etc.) in missing person identifications were selected and using the 13 CODIS STRs as genetic profile data, large numbers of pedigrees (e.g., 1,000,000) were simulated for each scenario. The distribution of LRs for each scenario was evaluated to first confirm the well-known single relative reference scenarios and second to determine the most informative combinations of relatives for identifying an unknown person. Thus guidance is given on which and how many relatives should be selected and typed for kinship analyses for identification so that efficiency can be optimized under the constraints of limited resources. The LRs were calculated by a software program MPKin, which jointly considers DNA profiles from all available family members and missing persons/remains.

Based on the simulation results, the following guidelines are recommended in choosing relatives for missing person identification.

Parents are the preferred relatives and both parents of the missing person should be typed when possible. The expected LRs of single parent and both parents are about 104 and 1010, respectively. If both parents are typed, all other relatives, including fullsibs, may not be necessary.

Children are the second preferred relatives. Type as many children as possible or until the unknown genotype of the missing person can be reconstructed. The expected LRs of two, three, or four children are about  $1 \times 10^7$ ,  $1 \times 10^9$ , or  $2.5 \times 10^{10}$ , respectively. In absence of parents, if the missing person is male, sons are preferred because of the same Y-chromosome shared between father and sons; otherwise, sons and daughters are equivalent.

Even if a child is available, the spouse of the missing person (i.e., the father/mother of the child) should be considered for typing, if he/she is available. The LR can increase more than 100 fold, if the spouse of the missing person is further typed, for a scenario with a single child already available.

Fullsibs are the third preferred relatives. A single fullsib may not be informative, but typing three or four fullsibs with expected LRs  $3.3 \times 10^{7}$  or  $3.5 \times 10^{8}$  are sufficient for reliable identification. If the missing person is male, brothers are preferred compared to sisters, because the missing person and brothers can be tested for both Y-chromosome and mtDNA, and it is reasonable to type less relatives with the same discrimination power due to economical reasons in some conditions; otherwise, brothers and sisters are equivalent.

All other distant relatives, such as grandparents/grandchildren, halfsibs, uncles/aunts, and cousins, only provide limited identification capabilities based on autosomal markers, but their Y-chromosomes and mtDNA can be used to increase the LR or filter out false relationships.

Less genetic dependence between reference relatives provides a higher LR on average. This is practiced routinely for standard paternity cases where two unrelated parents are sought. But the concept can be applied to extended pedigrees as well. For example, two biologically unrelated uncles (with an expected LR = 100) can be more informative than two related uncles (with an expected LR = 28).

With limited number of relatives, type as many markers as possible. Kinship Analysis, Missing Person, Pedigree Likelihood Ratio

# A110 Concordance Testing Comparing STR Multiplex Kits With a Standard Data Set

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After attending this presentation, attendees will understand the value of concordance testing using commercial forensic DNA short tandem repeat (STR) multiplex kits to detect allelic dropout or "null" alleles present in a standard data set.

This presentation will impact the forensic science community by demonstrating that null alleles do occur within a standard data set when comparing STR multiplex kits with different primer sequences. These "null" alleles have been sequenced to confirm the results and determine the cause (primer binding site mutations) and are reported to the forensic community.

Concordance evaluations are important to detect allelic dropout or "null alleles" present in a data set. These studies are performed because there are a variety of commercial STR multiplex kits with different configurations of STR markers available to the forensic community.<sup>1</sup> The electrophoretic mobility of the markers can vary between kits because the primer sequences were designed to amplify different polymerase chain reaction (PCR) product sizes. When multiple primer sets are used, there is concern that allele dropout may occur due to primer binding site mutations that affect one set of primers but not another.<sup>2</sup> These null alleles become evident only when data sets are compared. Null alleles are a concern because this could result in a false-negative or incorrect exclusion of two samples that come from a common source (only if different PCR primers are used). A base pair change in the DNA template at the PCR primer binding region can disrupt primer hybridization and result in a failure to amplify and detect an existing allele.<sup>2</sup>

Multiple concordance studies have been performed at NIST with a standard sample set (~1450 in-house U.S. population samples) using various STR multiplex kits including Applied Biosystems Identifiler<sup>®</sup>, MiniFiler<sup>™</sup>, NGM<sup>™</sup>, SGM Plus<sup>™</sup>, and Profiler Plus<sup>™</sup> kits, as well as Promega PowerPlex<sup>®</sup> 16, ESX 17 and ESI 17 Systems.<sup>3-8</sup> Various discordant results have been identified using concordance software developed at NIST, confirmed by DNA sequencing, and reported to the forensic community on the null allele web page of STRBase.<sup>9</sup>

To test for concordance between data sets, the current strategy at NIST is to use standard samples, software, sequencing, and STRBase, or the four "S"s of concordance. Ultimately, the information can be used by kit manufacturers when designing new STR multiplexes to either add an extra (degenerate) primer or redesign primers away from primer binding site mutations in the final kit configurations. Some kit manufacturers decide not to change the primer sequences and rely simply on the documentation or publication of the reported null alleles.<sup>3</sup>

Even though concordance studies are important to find null alleles between data sets, discordant results rarely occur for most primer sets. At NIST, concordance evaluations have been performed for over 150,000 allele comparisons. In the MiniFiler concordance study,<sup>4</sup> 99.7% full concordance was observed and in the PowerPlex ESX 17/ESI 17 study,<sup>5</sup> full concordance was seen for >99.8% in all comparisons performed.

In addition, comprehensive STR multiplex evaluations to determine and characterize kit performance were completed with a subset of the aforementioned kits, including NGM<sup>™</sup>, SGM Plus<sup>™</sup>, Profiler Plus<sup>™</sup>, PowerPlex<sup>®</sup> ESX 17, and ESI 17 Systems. The thorough examinations include heterozygote peak height ratio and stutter percentage calculations, as well as the determination of allele frequencies and population statistics (i.e. heterozygosities and probability of identities).

A summary of the results, including discordance and kit performance results, will be shown in order to help assess the benefits of performing concordance testing using a standard data set with STR multiplex kits that have different primer sequences for the same markers.

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Forensic DNA, Concordance, STR Multiplex Kits

# A111 The Mythical "Exclusion" Method for Analyzing DNA Mixtures — Does it Make Any Sense at All?

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After attending this presentation, attendees will be properly sceptical of the popular "exclusion" paradigm for analyzing DNA mixtures because the usual claims in its favor do not survive careful analysis.

This presentation will impact the forensic science community by warning attendees of the weakness of one popular approach for the specific question of DNA mixture analysis. More generally a collection of disconnected plausible-seeming intuitions are not a sufficient basis for any analytic method. Instead there should be a clearly stated foundation and chain of reasoning to support it.

Two different approaches to DNA mixture analysis and computation are commonly called the "exclusion" and "likelihood ratio" methods. These are fundamentally different paradigms. Though the so-called likelihood ratio method is agreed to be more flexible and theoretically grounded, the exclusion method is often touted as adequate in principle, and preferable for a variety of practical reasons including ease of use and understanding. But critical consideration shows that none of the claimed advantages of the exclusion method hold much water.

1. The claim that it requires **no assumption about number of contributors** is mostly wrong. For example consider a mixture with three or four obvious and prominent alleles at each locus. A suspect who has other alleles can be excluded of being a conspicuous contributor, but not of being a negligible contributor based on the DNA alone. The standard but unstated assumption that only "conspicuous" contributions are probative is valid if only two contributors can be assumed, but is not valid absent any such adjunct assumption.

- 2. Similarly the supposed **ease of understanding** by judge or jury is really an illusion; the method is deceptively easy with emphasis on deception. The ease in apparent understanding rests on overlooking the ambiguous and uncertain nature of DNA evidence, especially mixtures. The basis of the "exclusion" method is a negative evidence paradigm, that the evidence against a real or hypothetical suspect is the lack of alleles *not* present in the unknown mixture. In practice, for the hopelessly elusive concept of "not present" the analysis substitutes a criterion such as "not present above 100 RFU" which is measurable but not logically probative. The audience who accepts the substitution has been hoodwinked.
- 3. Ease of use is claimed to be an advantage particularly for complicated mixture profiles, those with many peaks of varying heights. The truth is the exact opposite. The exclusion method is completely invalid for complicated mixtures. The only recourse in such cases is a proper likelihood ratio analysis however difficult that may be. It is only the clear and straightforward cases that might permit logical application of the exclusion method. But those cases are easy by any method.
- 4. The common belief that the **exclusion method is conservative** is usually true, but not for the expected reason. Calculating examples with moderate peak-height variation shows that against innocent but included suspects, it would usually unfairly exaggerate the strength of the evidence. Since innocent suspects, especially ones who are not excluded, are somewhat rare, this circumstance is unusual. Still, it would not sound good for a supposedly neutral DNA expert to admit in testimony to using a method whose validity depends on the suspect being guilty.

All of the above problems are manifestations of one basic error: the method rests on the false premise that alleles are "in" a mixture when a donor "contributes" them and that we can tell which alleles are "present" and which are not. Of course allele detection is really not so binary. No amount of ad hoc finagling about dropout repairs the fundamental shortcoming that those words in quotes are undefined. Without clear and explicit definitions as a foundation, any analytic method can't be more than guesswork. Certainly no one has laid out an explicit and rigorous chain of reasoning from first principles to support the exclusion method. It is at best guesswork.

**DNA Mixture, Exclusion, Logical Foundation** 

# A112 Utilizing Ultra-High-Density SNP Arrays to Analyze Forensic Mixtures: An Operational Assessment

Kevin C. McElfresh, PhD\*, Kristin M. Stanford, BSc, and Ron G. Sosnowski, PhD, Casework Genetics, LLC, 13580 Groupe Drive, Suite 301, Woodbridge, VA 22192

After attending this presentation, attendees will learn about direct solutions for multiple source forensic DNA samples.

This presentation will impact the forensic science community by providing a precise solution to forensic DNA mixture samples.

The use of Ultra High Density SNP Arrays (UHDSAs) for forensic investigations has transitioned from proof of concept to an accomplished tool for fighting crime. Much like Moore's law for computing, during the last two years, the UHDSAs have increased in size from several hundred thousand SNPs to 2.5 million, while the cost per SNP has decreased. In fact the power of this tool is approaching the capability to analyze all the known variations in the human genome. Technically, the UHDSA provides a precise measure of the genetic variation which allows for a more direct assessment of differences between individuals and populations than is possible using current sieving technology (capillary electrophoresis).

The use of UHDSA was validated according to the ISO17025 guidelines. As part of those validation studies population databases for Caucasians, African-Americans, Hispanics, and Asians were generated for the greater than 1 million SNP loci in the Illumina HumanOmni1-Quad system. Like STRs there are allele frequency differences between populations. But because the loci are bi-allelic those differences can be pronounced. For example, in the Caucasian population the B allele frequency for locus rs1002005 is 0.032 while in the African-American population the frequency is 0.985. While more extreme, it none the less is reflective of the population differences found in the frequency databases of STRs. Second, the mixture interpretation algorithm used to assess membership in a forensic mixture is sensitive to the frequencies of the genetic variants in the reference population. The data show that this sensitivity is especially important when less than 1,000 SNPs are used but negligible when greater than 500,000 SNPs are used.

The real test of the power of SNPs is their operational use in deciphering forensic mixtures. Comparison of STR results using sieving technology and SNP results utilizing ultra-high-density SNP arrays demonstrates that the UHDSAs provide a much more precise manner of mixture interpretation. Included in that precision is a statistical assessment of identity as compared to a CPE interpretation.

A review of mixtures from forensic samples using both STRs and UHD SNP analysis demonstrates that the power of 1.4 million SNPs provides a decisive and precise method of mixture analysis that is impossible with STRs.

Algorithms, data, and forensic interpretation methods are proprietary.

SNPs, Forensic Mixtures, DNA Analysis

# A113 Likelihood Ratio Statistics for DNA Mixtures Allowing for Drop-Out

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After attending this presentation, attendees will understand how the likelihood ratio can be used to assign statistical weight to comparisons between DNA evidence profiles and profiles from known individuals. This method is appropriate for low or high template samples, for single source samples or mixtures, for degraded or pristine DNA, and for single, duplicate, or triplicate measures.

This presentation will impact the forensic science community as the analytic method presented allows quantitative comparison between evidence and exemplar profiles when degradation and/or allelic drop-out may have occurred.

The standard statistic calculated when evidentiary and exemplar STR profiles are identical is the random match probability (RMP). The RMP can be used for single source evidentiary profiles and for mixtures when individual contributors' profiles can be deconvoluted (deduced). Two methods, Random Man Not Excluded (RMNE) and likelihood ratio (LR), are commonly used to quantify the statistical weight of mixed DNA profiles when contributors cannot be deduced. The DNA commission of

the International Society of Forensic Genetics recommends the LR (Gill *et al.* Forens Sci Int 2006 160:90-101),<sup>1</sup> as it uses more of the available data than RMNE and parameters for allelic drop-out and drop-in can be incorporated into the LR. A likelihood ratio method and software for analysis of single source or mixed low template or high template evidence samples has been developed and validated. This method, the Forensic Statistical Tool (FST), is modeled after existing methods (Curran *et al.* Forens Sci Int 2005 148:47-53; Gill *et al.* Forens Sci Int 2007 166:128-138).<sup>2,3</sup>

The method incorporates drop-out and drop-in parameters in the LR calculated by FST, using empirically determined rates from single source buccal swab samples containing 6.25 pg to 500 pg of template DNA and from mixtures of two or three contributors containing a total of 25 pg to 500 pg of template DNA.

FST computes the LR for pairs of prosecutor and defense hypotheses and for sample characteristics specified by the user. The user must select the number of contributors to the sample and then upload or enter an evidence profile and comparison sample profile(s), such as those from suspect(s) and victim(s). In addition, the amount of template DNA used for each amplification must be specified. Evidence profile data from up to three amplifications may be considered simultaneously. Finally, if the evidence profile represents a mixture of DNA from two or more contributors, the user must specify whether or not the profile of the major contributor can be deduced. FST calculates the LR according to the sample specifics and generates a PDF file that includes the LR, as well as the evidence and comparison profiles and the sample characteristics entered by the user. The LR is computed using allele frequency estimates in four New York City populations: Asian, Hispanic, Caucasian, and Black. These are the same samples that the Office of Chief Medical Examiner of the City of New York uses to calculate random match probabilities for single source or deduced sample profiles.

FST can also compute the LR using each individual in a LabTypes database as the test sample, rather than a profile from a comparison sample, to check for evidence sample contamination. This capability and a database of over 1,200 population samples were used to develop a null distribution for the LR for single source, two-contributor, and three-contributor scenarios. The main objective of this exercise was to determine the range of LR values that can be expected when the calculation is performed using individuals who did not contribute to the evidence sample. This will help to ensure that use of the program will not result in fortuitous matches between evidence profiles and non-contributors to the sample.

The performance of the program was evaluated with hundreds of profiles generated from a variety of sample types, including blood and buccal samples, purposefully degraded buccal samples, and touched items with one, two, three, and four known contributors. In this presentation, the analytical strategy and validation of the software will be presented.

#### **References:**

- <sup>1.</sup> Curran JM, Gill P, Bill MR (2005) Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure. *Forens Sci Int* 148:47-53
- <sup>2</sup> Gill P, Brenner CH, Buckleton JS, Carracedo A, Krawczak M, Mayr WR, Morling N, Prinz M, Schneider PM, Weir BS (2006) DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forens Sci Int* 160:90-101
- <sup>3.</sup> Gill P, Kirkham A, Curran J (2007) LoComatioN: a software tool for the analysis of low copy number DNA profiles. *Forens Sci Int* 166:128-138

DNA Mixtures, Likelihood Ratio, Allelic Drop-Out

# A114 High Sensitivity Detection and Typing of Mixed Contributor DNA Samples Using Massively-Parallel Deep Amplicon Pyrosequencing

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After attending this presentation, attendees will be introduced to an emerging massively-parallel DNA sequencing technology that is capable of detecting, typing, and resolving mixed contributor samples of human DNA. The presentation reviews the basic methods of preparing and analyzing samples using the Roche 454 sequencing system protocol.

This presentation will impact the forensic science community by demonstrating how massively parallel deep amplicon pyrosequencing can be used to resolve complex mixtures of human DNA. This technology can be used to resolve STR and mtDNA mixtures of one to five individuals at low template DNA concentrations.

The project tests the hypothesis that 454 deep sequencing of individual polymerase chain reaction (PCR) amplicons yields far higher sensitivity of detection for minor contributor's short tandem repeats (STRs) and mitochondrial DNA in a mixed sample than conventional methods based on gel electrophoresis and dye terminator sequencing. Introduction of such technology thus has the potential to profoundly impact human DNA forensics, particularly in cases where the DNA of interest (e.g., perpetrator) is present in a mixed sample well below the detection threshold of conventional methods.

The presented data demonstrate highly sensitive detection of low copy contributor STRs well below the 1-10% level, which is the approximate limit for gel electrophoresis. Dilutions of human DNA in mixtures from one to five different contributors at different relative concentrations were prepared for PCR amplification and subsequent analysis by the 454 system. In addition, samples were also prepared after DNA extraction from objects touched by multiple individuals. Deep amplicon sequencing with the 454 system requires that the PCR primers used for loci of interest contain special fusion sequences appended to their 5' primer ends. The project used "fusion primers" for eight loci drawn from the standard CODIS STRs used in routine forensic casework. Following PCR amplification, a process known as emulsion PCR is performed, where individual amplicons in the sample are attached to a bead (one per bead) and clonally amplified in micro-reactors in a massively-parallel fashion. Each of the beads is then loaded onto a glass picotiter plate, where at most one DNA-linked-bead is captured into each well. Flow-based pyrosequencing is then performed to determine ~500 bp of the sequence of DNA coupled to each bead. Each bead sequence correlates to one single molecule of DNA, and thus to one individual contributor. The ratios of the major to minor contributor sequence reads at each locus enables linkage of detected alleles across different loci to form the respective genotypes. A typical 454 sequencing run yields up to one million reads, thus offering minor contributors detection thresholds greater than one part per thousand. Up to 16 different samples can be analyzed simultaneously in a single run, with trade-off between time/cost vs. sensitivity.

The demonstrated detection sensitivity is well below conventional methods using off-the-shelf Roche 454 protocols. Reagent costs are modest per sample (less than \$500.00) when used in a multiplexed fashion. While pyrosequencing is not error-free, with an error rate of  $\sim$ 0.005 per base read, the impact of these errors on tandem repeat analysis is minimized through modest bioinformatics analysis of sequences against all known alleles. The system also offers the potential to both detect and sequence new alleles in the course of routine forensic casework

and thus can be an important future tool in the broader forensics community.

DNA Mixture Analysis, Pyrosequencing, STR Analysis

# A115 Evaluation of "Non-Toxic" Cleaners' Effects on Presumptive Blood Tests — Can "Green" Products Make a Difference?

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After attending this presentation, attendees will understand the effects of "green" cleaners on two commonly used presumptive blood tests which may lead to a change in protocol of the testing of suspected bloodstains at a crime scene or in the laboratory setting.

This presentation will impact the forensic science community by demonstrating that visual characteristics of blood may not necessarily be relied upon when deciding to test for blood. Important evidence may be missed if appropriate tests and collections are not attempted. This presentation will be of use to crime scene first responders and laboratory personnel who work with these presumptive blood tests on a daily basis and who may come across evidence that has been treated with a "green" cleaner. Many stains at a crime scene or in the laboratory may be dismissed from testing due to the physical characteristics of the stain.

This study demonstrates that visual characteristics of blood may not necessarily be relied upon when deciding to test for blood. Important evidence may be missed if appropriate tests and collections are not attempted.

The presumptive blood tests Kastle-Meyer (phenolphthalein) and Hemastix<sup>®</sup> are commonly used at crime scenes and in crime laboratories to determine if blood may be present. However, an attempt to clean up a crime scene may yield negative or false-negative results possibly based on the cleaner used to dispose of the biological material present. Non-toxic or "green" cleaners are becoming a more common product on the market and may be in more households than ever before. Experiments were conducted to determine what, if any, effects "green" cleaners had on the ability of the Kastle-Meyer and Hemastix<sup>®</sup> tests to detect the presence of blood on common substrates.

The data presented supports the hypothesis that the more environmentally-friendly cleaners did not have a negative effect on the Kastle-Meyer or Hemastix<sup>®</sup> tests' ability to detect blood under different experimental conditions on common substrates encountered in the field and in the lab. Blood was still detected using both tests on substrates treated with the cleaners. Positive results were also obtained on substrates where the blood and cleaners had been mixed and the stains were left to dry for a determined amount of time. Blood was also detected on a cleaned substrate, which was previously stained with blood and then cleaned with a test cleaner, which had no visible blood staining.

This study can lead to further experiments on the ability of the presumptive blood tests to detect blood from a substrate cleaned with a "green" cleaner, and thus leaves no visible blood staining. Studies using DNA analysis on the blood:cleaner mixtures to determine if the "green" cleaners have a negative effect on retrieving a usable, forensic DNA profile using common forensic laboratory protocols would also greatly add to the information already gathered regarding the effects chemicals (i.e., cleaners, forensic presumptive tests, latent fingerprint tests) have on forensic DNA analysis.

**Blood, Green Cleaners, Presumptive Tests** 

# A116 Comparative Evaluation of Manual Extraction Methods for the Biology/DNA Unit of the Las Vegas Metropolitan Police Department Forensic Laboratory

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After attending this presentation, attendees will understand the strengths and weaknesses of three extraction methods as they apply to desirable forensic outputs, such as high quantitative yields, robust and discriminating STR profiles, and time- and cost-effective methods. The methods evaluated included phenol-chloroform organic extraction, Applied Biosystem's PrepFiler<sup>TM</sup> Forensic DNA Extraction Kit, and Qiagen's QIAamp® DNA Investigator Kit.

This presentation will impact the forensic science community by alerting casework laboratories to the benefits of alternative commercial extraction kits in the analysis of important forensic samples, including low level types, in order to assist them in making a more informed decision when selecting an extraction method for future work.

An extraction method for forensic DNA casework must produce a high quantitative yield as well as a robust STR profile free from artifacts across all sample types, including low level samples such as touch DNA. The Las Vegas Metropolitan Police Department DNA Laboratory has previously relied on phenol-chloroform organic extraction for forensic casework. In order to expedite the extraction process and foray into automation instruments, a comparison study was undertaken with Applied Biosystems' PrepFiler<sup>TM</sup> Forensic DNA Extraction Kit and Qiagen's QIAamp® DNA Investigator Kit for the purpose of determining a manual extraction chemistry to replace organic extractions and to be automated in the future.

The kits were evaluated on the basis of: (1) contamination issues; (2) quantitative yield; (3) STR profile quality; (4) future automation potential; (5) time consumption of the method; and, (6) cost of the method. Based on the results of this study, LVMPD chose to validate the PrepFiler<sup>TM</sup> Forensic DNA Extraction Kit, citing higher quantitative yields for low level samples and better detection of minor contributors in mixture samples than the other methods, along with STR profile quality comparable to an organic extraction.

**DNA**, Extraction, Comparison

# A117 Effects of Degradation on Single-Source and Mixture Profiles Generated Using Traditional and Mini STRs

Jonathon S. Dunn, BS\*, Ruiyi Ren, MS, Robin W. Cotton, PhD, and Catherine M. Grgicak, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806B, Boston, MA 02118

After attending this presentation, attendees will become aware of the effects of degradation on mixture interpretation when utilizing both traditional- and mini- STRs.

This presentation will impact the forensic science community by characterizing the way in which degraded samples amplify in mixtures. This will be accomplished by determining important mixture characteristics such as drop-out rates and heterozygous imbalance for degraded mixture samples. Many samples submitted for DNA analysis from crime scenes are often degraded or contain mixtures from two or more individuals. Degradation of DNA in these samples presents a challenge as larger molecular weight STR markers are inefficiently amplified, if they're amplified at all, resulting in a loss of information when attempting to analyze and interpret samples of interest. Additionally, complexities associated with mixture interpretation can be further exacerbated when one or more of the contributors of a mixture exhibit degradation.

Commercially available multiplex amplification assays utilizing primers that amplify mini-STRs have been developed in order to gain information from degraded or inhibited samples that may otherwise be lost. For example, the AmpFℓSTR MiniFiler<sup>TM</sup> kit allows for the amplification of the eight largest loci from the AmpFℓSTR<sup>®</sup> Identifiler<sup>®</sup> kit (plus the Amelogenin locus) and has been shown to increase the likelihood of obtaining genetic information from compromised samples.<sup>1</sup>

The goal of this research is to evaluate the amplification success of both types of assays by comparing the MiniFiler<sup>™</sup> and AmpFℓSTR® Identifiler® kits when amplifying both degraded and non-degraded single source samples and mixtures. The objectives of this work include: (1) comparing peak height ratios of heterozygous markers and drop-out rates of the single source samples between kits; (2) determining whether these ratios are maintained in mixtures, and, (3) to examine whether the DNA within a mixture amplifies independently.

DNA from one male and one female was degraded by incubating the sample at 37°C for ten minutes in the presence of 15 units/ml of DNase I. The DNase I was then inactivated by adding EDTA and incubating at 75°C for ten minutes. Agarose gel electrophoresis with GelStar® staining was used to visualize the level of degradation which ranged from nondegraded (>1500 b.p.'s) to very degraded (<100 b.p.'s). Both the male and female samples were run as single-source samples in both the degraded and non-degraded forms in quadruplicate using both kits with targets of 2, 1, 0.5, 0.25 and 0.125 ng. Mixtures of the two samples where both were non-degraded and both were degraded were then run using each kit with female to male ratios (in ng) of 2:0.125, 2:2, 1:0.125, 1:1, 0.5:0.125, 0.5:0.5, 0.25:0.125, 0.25:0.25, and 0.125:0.125, respectively. Finally, mixtures using the same female to male ratios (with the exception of the 2:2 ratio) were run using the degraded female sample and the nondegraded male sample. All mixture samples were also run in quadruplicate.

This represents a detailed study aimed at determining the effects of degradation on mixture interpretation for both traditional- and mini-STRs. Emphasis will be placed on determining whether drop-out rates, heterozygous imbalance and amplification anomalies increase with degradation, and if so what laboratory tools may be used to determine which downstream PCR-STR kit to utilize prior to amplification. **Reference:** 

<sup>1.</sup> Mulero, Julio J., et al. "Development and Validation of the The AmpFℓSTR® MiniFiler<sup>TM</sup> PCR Amplification Kit: A MiniSTR Multiplex for the Analysis of Degraded and/or PCR Inhibited DNA." Journal of Forensic Sciences 53 (2008): 838-52.

**DNA Mixtures, Degraded DNA, Mini-STRs** 

# A118 Postmortem DNA Persistence in Soft Muscle Tissues in Relation to Accumulated Degree-Days (ADD)

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After attending this presentation, attendees will have a better understanding of the likelihood of successfully retrieving DNA from postmortem muscle tissue in relation to accumulated degree-days (ADD). Attendees will also receive information about the rate of DNA degradation in whole carcasses, fragmented muscles, and suspended muscle tissue in relation to ADD when all these tissues put in direct contact with the ground subjected to natural environmental conditions.

This presentation will impact the forensic science community by providing a new insight to access the patterns of DNA degradation using animal model based on both time and temperature. In mass disaster situations, scientists will be in a better position to have an idea about DNA persistence in soft muscle tissues in decomposing bodies at some specific time point under particular environmental conditions; otherwise they can collect hard tissues such as bones and hairs instead of soft muscle for mass disaster victim identification.

After the death of an organism, as the cells breakdown nucleases cause DNA degradation; the action of microorganisms also contributes to the DNA degradation. As the postmortem interval (PMI) increases, DNA continues to degrade until no high molecular weight DNA (HMW-DNA) remains. There is an inverse relationship between DNA yield and PMI with degradation accelerated by increases in temperature. Accumulated degree-days (ADD) provide a measure of time and temperature and have been used to assess DNA persistence in soft muscle tissues. The conclusions, however, are based on a very limited number of observations.

A series of experiments were conducted using 66 rabbit carcasses. Thirty-six rabbit carcasses, in three replicates sets, were placed in direct contact with ground and covered by a wire cage to prevent scavenger access. Thirty-six rear legs were cut from 18 rabbits and put alongside the whole carcasses. Muscle tissues were collected from the remaining six rabbits, were suspended inside 36 50-ml polypropylene tubes and left alongside the carcasses. The environmental temperature and humidity were recorded every hour using a data-logger. Muscle tissue samples were collected in triplicate starting from day zero until no soft tissue remained. Samples were stored at -20°C before processing.

DNA extraction was carried out using a blood and tissue kit according to the manufacturer's instructions. Two nuclear genes (Connexin 43 and RAG-1) were aligned to identify conserved regions for primer design to amplify 194 base pairs (bp), 305 bp, 384 bp, and 500 bp amplicons from pig, rabbit and human. Following DNA extraction PCR analysis was performed using a multiplex that has been developed to simultaneously amplify genomic DNA amplicons of 194 bp, 305 bp, 384 bp, and 500 bp.

500 bp to 122.75 ADD in whole carcasses and fragmented muscle tissues were amplified; however, the drop-out in amplification of 194 bp and 500 bp was found at 55.75 ADD and 122.75 ADD. A complete failure in amplification success of DNA for whole carcasses and fragmented muscle tissues occurred at 161 ADD and one of the replicates at ADD 122.75. In case of suspended muscle tissue samples, successful amplication of PCR multiplex was obtained up to 161 ADD. Further experiments are ongoing to assess how variable this value is; repeating the experiments with pigs and rabbits at different times of the year.

The future work will focus on analyzing quantitative DNA degradation in relation to ADD by quantitative real-time PCR using both pigs and rabbits and examine the relationship between decomposition and ADD at different times of the year.

Persistence, Accumulated Degree-Days, Postmortem Interval

# A119 Useful Methods to Overcome the Interference of Humic Acid With STR Typing

Seung Bum Seo, BS\*, Ai Hua Zhang, MD, Hye Young Lee, BS, Nam Yul Kim, BS, Hye Yeon Kim, MS, and Soong Deok Lee, PhD\*, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul, KOREA

After attending this presentation, attendees will learn useful methods to overcome the negative effect of humic acid (HA) on STR typing for bones buried in the soil.

This presentation will impact the forensic science community by offering useful methods to improve purification and STR amplification of DNA in samples containing HA.

It is important for a successful STR amplification to remove PCR inhibitors as well as obtaining appropriate amounts of DNA. DNA samples obtained from forensic evidence may contain PCR inhibitors such as HA, hematin, and urea. These PCR inhibitors can interfere with PCR amplification and thus may inhibit the detection of STR loci. One of the main PCR inhibitors is HA. This substance is frequently observed when DNA is extracted from old bone exposed to the soil, because HA is easily co-extracted and purified with DNA.

An attempt to increase STR amplification of DNA samples containing humic acid (HA) was conducted. This study was conducted with two purposes. One purpose was to find a useful purification method through the comparison of three commercially available DNA isolation kits, and the other purpose was to overcome the interference of residual HA with DNA STR amplification. For purification tests, 0.25 ng/µl DNA samples containing different concentrations (10-1,000 ng/µl) of HA were used. The efficiency of three commercial kits (QIAquick<sup>®</sup> PCR Purification kit (Qiagen), QIAamp<sup>®</sup> DNA Investigator kit (Qiagen) and Prepfiler<sup>™</sup> Forensic DNA Extraction kit (Applied Biosystems) were compared to the amplified STR loci using a AmpFISTR<sup>®</sup> Identifiler<sup>®</sup> PCR Amplification kit (Applied Biosystems). Sixteen full loci were identified by the QIAquick<sup>®</sup> kit at 76 ng/µl HA (final concentration in the Identifiler), 11.5 loci by the Prepfiler<sup>™</sup> kit and no loci identified by the QIAamp<sup>®</sup> kit at 38 ng/µl HA.

The interference of residual HA with STR amplification was overcome by the use of TaKaRa Ex Taq<sup>TM</sup> Hot Start Version (Takara) versus of AmpliTaq Gold<sup>®</sup> DNA Polymerase (Applied Biosystems). Eilert and Foran have shown that Ex Taq<sup>TM</sup> HS has advantages in assaying skeletal remains.<sup>1</sup> The usefulness of the STR amplification by different units (1-5 U) of both polymerases was also examined. Furthermore, 400 ng/µl bovine serum albumin (BSA) was added to the STR amplification. The Ex Taq<sup>TM</sup> HS was more resistant to HA than the AmpliTaq Gold<sup>®</sup>. Samples containing 19 ng/µl HA resulted in seven loci identified using one U Ex Taq<sup>TM</sup> HS, while no loci were identified using one U AmpliTaq Gold<sup>®</sup>. Full loci were identified using increasing the Ex Taq<sup>TM</sup> HS unit from one to two U. Full loci were also identified when BSA was added to the STR amplification containing one U Ex Taq<sup>TM</sup> HS. An increase in units of Taq and the addition of BSA improved the usefulness of STR amplification when assessed by using the AmpliTaq Gold<sup>®</sup>.

In summary, the QIAquick<sup>®</sup> PCR Purification kit appears to be more useful in removing HA than the QIAamp<sup>®</sup> DNA Investigator and Prepfiler<sup>™</sup> Forensic DNA Extraction kits. TaKaRa Ex Taq<sup>™</sup> Hot Start Version appears to be more resistant to HA than AmpliTaq Gold<sup>®</sup> DNA Polymerase. An increase in units of Taq and the addition of BSA may help to overcome HA interference.

**Reference:** 

<sup>1.</sup> Eilert KD, Foran DR. Polymerase resistance to polymerase chain reaction inhibitors in bone. J Forensic Sci. 2009;54:1001-7.

Forensic DNA, Humic Acid, STR Amplification

# A120 Modified Protocol for Extracting DNA From Bones and Teeth in Cases With Low Expectations for Success: Reliability and Validity

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After attending this presentation, attendees will be able to use a modified DNA extraction protocol useful on degraded specimens of bones, teeth, and other various tissues.

This presentation will impact the forensic science community presenting factual details that ancient DNA research shares a common problem with forensics and other approaches requiring analyses of museum and non-invasively collected specimens; the amount of endogenous DNA available in the samples is often limited. Thus, extraction techniques that retrieve as much DNA as possible from a specimen are of crucial importance.

A wide range of techniques has been published to date, all of which aim to maximize DNA yields, while minimizing the co-extraction of PCR inhibitors. Due to low levels of endogenous DNA, environmental, bacterial, and postmortem DNA damage, as well as the potential presence of environment-borne inhibitors that co-extract with DNA, the recovery of DNA data from degraded specimens can still pose a significant challenge.

Previously, DNA extraction from the dental pulp samples was performed following a modified protocol of a Total RNA isolation system, suitable for DNA extraction from samples containing only a small number of nucleated cells. The same method was used for the bone samples. The protocol was partially modified by lengthening the incubation time of the cell lysis step: each sample of dental pulp was placed, overnight, at room temperature, in a single microtube containing 350 L of SV RNA Lysis Buffer. These protocols were applied to five skeletons discovered in Canosa di Puglia (Bari, Italy), during the archaeological excavations of tombs. These protocols do not allow for a complete characterization of genetic systems; however, even though the results obtained were satisfactory considering that the bones were ancient dated between the sixth and seventh centuries.

The extraction method on bones, teeth and various tissue fragments of human remains, making some changes to previous protocols used were tested. Following this, the success of amplifying ancient DNA was estimated.

Five cases are presented: In the first case, human remains were found in the Apulian countryside in 2002. They most probably belonged to a man who disappeared in 1989 according to the results of parentage testing by forensic hemogenetic investigations performed on the remaining members of the alleged missing man's family. In the second case, human remains were found in 2006 on an Italian highway which probably belonged to a man reported as missing. Identification was made by comparing the DNA of the remains to a blood sample taken from a brother. The third case involved human remains, discovered in 2009 near a rest home for the elderly in the province of Bari, most likely belonging to an 84-year-old man who disappeared in 1995. In this case identification was carried out by comparing the genetic profile of the remains to a blood sample taken from the son of the missing man. The fourth case involved the remains of two unknown skeletonized individuals discovered two meters underground. They were discovered in the small town of Marsicovetere, in southern of Italy where a trench for an oil duct was being excavated. In this case, the remains consisted of two full human skeletonized bodies that were highly fragmented. They were presumed to be from a prehistoric period because of their extreme lightness and porosity: they were extremely fragile. The skeletons were

removed from the trench by digging around the remains and taking them out along with the soil. The last case concerned remains found in the attic of a church in Potenza in March 2010. DNA extracted from the human remains was compared with the DNA extracted from the blood of members of the missing girl's family in order to establish identification.

The modified method for extracting the DNA genome, followed by the amplification reaction has allowed for the identification of four cadavers and the typification of the fifth. Each of these cases had low expectations for success. These scenarios involved cadavers or remains of unknown origin, which were discovered many years following the time of death. Each was discovered under conditions that did not favor the maintenance of the integrity of nucleic acids.

**DNA**, Skeletal Remains, Bones

# A121 An Analysis of Binding Mechanisms for Real-Time Polymerase Chain Reaction (PCR) Inhibition Using Efficiency and Melting Curve Effects

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The goal of this presentation is to inform the forensic community on how inhibitors affect qPCR results and ways in reducing the effects of PCR inhibitors.

This presentation will impact the forensic science community by assisting DNA analysts with means of better interpreting results of inhibited samples.

Real-time PCR, also known as quantitative PCR (qPCR), is a relatively new method that allows determination of the amount of amplifiable DNA in a forensic sample. qPCR can also be used to detect inhibition through the monitoring of internal control sequences. The procedure is based upon the incorporation of a fluorescent dye during thermal cycling, therefore monitoring a change in fluorescence that correlates with the accumulation of amplified product. There are different approaches used for fluorescence-based detection assays. Two of these chemistries, Plexor HY and TaqMan Systems, incorporate internal control sequences to detect inhibition. Alternatively, inhibition may be detected through the use of melt curve effects. Such analyses are possible with Plexor and SYBR Green assays.

Previous work using SYBR Green intercalation for qPCR detection has demonstrated that inhibitors can affect melt curves differently depending on their structure and mode of action. Inhibitors binding DNA can cause melt curve shifts while those affecting Taq polymerase may not. Similar but distinguishable effects are seen when using Plexor based melt curves. Unlike qPCR procedures using SYBR Green, Plexor dyes are fluorescently linked to the modified base (5¢-methylisocytosine (iso-dC)) adjacent to the 5¢ end of the dsDNA. As a result, the Plexor HY System produces minimal interference in the dsDNA structure and therefore is an ideal procedure for measuring these effects. In this study, inhibition of qPCR was evaluated by observing the effect of various inhibitor concentrations and amplicon lengths on the amplification of forensic biological samples.

Based on the preliminary results, humic acid, calcium, collagen and phenol show concentration dependent shifts in melt curves for inhibitors suspected of DNA binding. These data tend to show utility in careful analysis of melt curves and the data obtained can provide complementary information with that produced by the amplification of internal control sequences. The inhibitory affects of other common PCR inhibitors (e.g., urea, bile salts, guanidine, hematin, tannic acid and melanin) are currently being evaluated. STR results performed in concert with these studies indicate that inhibition can lead to a generic loss of alleles from the larger STR loci; effects are commonly seen in all inhibitors. However, in addition to these generic effects, sequence specific allele dropout may also be observed, producing loss of smaller loci (e.g., amelogenin and D3S1358).

The goal of this research is to provide the forensic community with information on how inhibitors affect qPCR results and means of mitigating these effects prior to STR amplification. DNA analysts will be able to better interpret results and take corrective action to minimize inhibition based allele dropout in their results by monitoring qPCR efficiency and melt curve effects.

DNA Analysis, Real-Time PCR, Inhibitor Binding

## A122 Differential Extraction Conditions: Effects of Dehydration on DNA Mixture Quantification and Amplification

*Elyse S. Cooper, BA\*, Robin W. Cotton, PhD, and Catherine M. Grgicak, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806B, Boston, MA 02118* 

After attending this presentation, attendees will be able to discern the effects Proteinase K concentration, SDS concentration, incubation duration, and temperature have on differential extraction efficiencies and the premature lysis of spermatozoa of dried samples.

This presentation will impact the forensic science community by aiding in the determination of appropriate differential extraction conditions that should be utilized to ensure efficient separation of the nonsperm and sperm fractions during a differential extraction.

Biological mixtures comprised of multiple individuals' tissues or bodily fluids are commonly encountered and gathered during crime scene evidence collection; however, the unraveling and distinguishing of individual contributors in mixed samples continues to be a difficult and complex facet of DNA analysis and interpretation. Determination of the total number of donors to the sample, the relative amount of DNA from each respective contributor, whether or not all data is accounted for, and the ultimate inclusion or exclusion of a known individual to the mixture are all complications that increase the intricacy of forensic DNA testing.

If the sample is comprised of sperm and epithelial cells, the DNAs can theoretically be successfully separated through differential extraction. This method relies on differences in cell membrane composition of epithelial cells and sperm heads. The first step of the differential extraction process typically involves incubation of the sample and the addition of Proteinase K and a surfactant (e.g., Sodium Dodecyl Sulfate (SDS)) to lyse epithelial cells. Because sperm heads contain a rigid network of protein disulfide bonds in their outer membrane, they are resistant to treatment with Proteinase K and surfactant and are left intact. After epithelial cell lysis, the sperm is pelleted by centrifugation and the supernatant, which contains DNA from the epithelial cells, is removed. This is referred to as the "non-sperm fraction." Following removal of the non-sperm fraction, a second cell lysis is performed to extract the DNA from the sperm cells. In addition to Proteinase K and SDS, dithiothreitol (DTT) is added to reduce the disulfide bonds in the sperm head, thereby lysing it. The release of the sperm's DNA results in the "sperm fraction."

Work previously performed investigated how various differential extraction conditions affected the method's ability to yield two single source DNA extracts. It was concluded that only the presence of Proteinase K significantly affected the extraction. Additionally, the male contribution in the non-sperm fraction did not exceed 9% for any of the conditions tested.<sup>1</sup> This project is a progression of the aforementioned research and, in similar fashion, explores and advances upon those same parameters. In contrast to the aforementioned results, it has previously been reported that a significant portion of the sperm fraction is lost during the differential extraction process.<sup>2</sup> One of the differences between the two studies was whether or not the samples were dried. Since the

majority of samples received by crime laboratories are dried stains, it would be most beneficial to optimize the yield of DNA from the sperm fraction for these types of samples. Therefore, this study set out to discern the effects Proteinase K concentration, SDS concentration, incubation duration, and temperature have on the differential extraction efficiencies and the premature spermatozoa lysis of dried samples. The effect was quantified using qPCR and STR analysis and the concentrations of male and female DNA in the non-sperm and spermfractions were compared.

When comparing dried and wet samples, the results indicate that dried samples exhibit significantly more sperm lysis in the absence of DTT at all conditions analyzed. Preliminary results suggest the concentration (e.g., 0-300  $\mu$ g/ml) of Proteinase K does not have a significant impact on the quantity of male DNA in the non-sperm fraction of dried samples. Likewise, SDS concentration had minimal impact on the male contribution in the non-sperm fraction when measured with qPCR.

Furthermore, modifications to storage conditions and rehydration steps typically associated with differential extractions were examined. If simple rehydration techniques aid in the reconstitution of destabilized cell membranes (thereby negating the premature lysis of spermatozoa during the extraction process), then such action would be advocated in the preservation and storage of biological mixture samples.

**References:** 

- <sup>1</sup> Hennekens, Catherine, et al. Differential Extraction Conditions and the Premature Lysis of Spermatozoa: Effects on DNA Mixture Quantification and Amplification. *Proceedings of the 62<sup>nd</sup> Annual Meeting of the American Academy of Forensic Sciences*, Feb 22-27, 2010, Seattle, WA. Colorado Springs, CO: American Academy of Forensic Sciences, 2010.
- <sup>2.</sup> Norris, Jessica V., et al. "Expedited, Chemically Enhanced Sperm Cell Recovery from Cotton Swabs for Rape Kit Analysis." <u>Journal</u> <u>of Forensic Sciences</u> 52 (2007): 800-5.

**DNA**, Differential Extraction, Sperm Lysis

# A123 Do You Know How Much DNA You Really Have?

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The goal of this presentation is to provide attendees with insights including expectations of the consistency of DNA standards over long term use, data on the performance of different standards using the same kit chemistry, information that will show analysts how differences in standards affect quantitation results, and techniques for checking quantitation results and making corrections to them.

The presentation will impact the forensic science community by demonstrating the inaccuracies of current DNA standards being used to determine quantitation results and providing methods to correct for these inaccuracies.

Quantitation of DNA is a quality assurance step that: (1) ensures there is enough DNA in a forensic sample to provide the best possible results; and, (2) ensures the analyst has data to select the most appropriate amplification method. In fact, quantitation is such a crucial step that is mandated by the FBI. The Quality Assurance Standards for Forensic DNA Testing Laboratories, standard 9.4, states "The laboratory shall quantify the amount of human DNA in forensic samples prior to nuclear DNA amplification." Apart from this requirement, more recently, rules on what is considered low copy number analysis have been defined based on the quantity of DNA present in a sample.

However, testing has shown that the quantitation kits used do not always provide accurate results. Furthermore, these tests have found the DNA standard used in these quantitation kits can over- or underestimate the quantity of DNA in a sample by two to three fold.

To assess commercially available DNA quantitation standards, a study was designed to explore the variance in performance between several different quantitation kits. The study also examined if there was any difference between lot numbers of kits from the same manufacturer. To achieve this, only one kit chemistry was used but the standards were changed in the course of the experiment and results evaluated by slope,  $R^2$  value, Y intercept and resulting quantities of a serial dilution of blood samples. The results show a direct correlation between Y intercept value and the quantity of DNA in the sample.

This presentation will provide forensic scientists with insights including:

- Expectations of the consistency of DNA standards over long-term use.
- Data on the performance of different standards using the same kit chemistry.
- Information that will show analysts how differences in standards affect quantitation results.
- Techniques for checking quantitation results and making corrections to them.

Accurate and reliable quantitation results affect all aspects of the DNA process. Quantitation has a definite impact on all DNA validation studies conducted by laboratories especially when attempting to evaluate the sensitivity of a method. Accurate quantitation results will also ensure laboratories do not waste time and resources repeating processes because the results were not what were expected based on the DNA quantitation. As laboratories implement measures that provide for more accurate quantitation results of the samples they are processing, they can correct any inconsistencies and optimize their methods.

Quantitation, DNA, Standards

# A124 Comparative Analysis of Quantifiler® Duo and Plexor®HY DNA Quantification Systems

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The goal of this presentation is to educate attendees on the similarities and differences between Quantifiler<sup>®</sup> Duo, and Plexor<sup>®</sup>HY DNA quantification systems. In addition attendees will be informed of special characteristics unique to each system of which the attendees should be aware.

This presentation will impact the forensic science community by allowing laboratories to view a side-by-side comparison of the included system or systems and decide which will best meet their needs for specific tasks they may have.

A comparative analysis of Quantifiler<sup>®</sup> Duo and Plexor<sup>®</sup>HY DNA quantification systems was conducted to assist forensic laboratories in choosing the DNA quantification method that best suits the needs of their lab as well as some potential outcomes and consequences of choosing each system.

Quantifiler<sup>®</sup> Duo is a DNA quantification system manufactured by Applied Biosystems and is capable of simultaneously quantifying both total human and human male DNA present in an extracted sample. Quantification was carried out via Real-Time PCR and data analysis was completed in Sequence Detection System software v1.2.3. The human DNA target is Ribonuclease P RNA Component H1 and the human male DNA target is the Sex Determining Region of the Y chromosome. An increased fluorescence of the solution correlates directly with DNA concentration in this system.

Plexor®HY is a DNA quantification system manufactured by Promega Corporation and is capable of simultaneously quantifying both total human and human male DNA present in an extracted sample. Quantification was carried out via Real-Time PCR and data was analyzed in Plexor<sup>®</sup> Analysis Software v1.5.4.18 for analysis. The human DNA target is on chromosome 17 and the human male DNA target is on the Y chromosome. A decrease in fluorescence of the solution correlates directly with DNA concentration in this system.

The systems analyzed in the study were internally validated. Studies completed with each system include standard curve quality metrics, sensitivity, precision, reproducibility, contamination, and mixture analysis. The results of the internal validation studies from both systems were compiled and analyzed for comparison.

The standard curves quality metrics study assessed the slope, Yintercept, and R<sup>2</sup> values of the standard curves generated during data analysis and their correlation to final sample quantity. The sensitivity study assessed the sensitivity of the systems by reviewing quantification data from a range of dilutions with known DNA quantities. A log-linear relationship was expected between the C<sub>T</sub> and quantification values. This relationship was used to establish the limit of detection for the quantification system. The precision study assessed the precision of the systems through analysis of known standard quantification data generated. The reproducibility study assessed the reproducibility of the concentration data for various samples after repeating the quantification assay multiple times with those samples. The contamination study checked for the presence of contaminant DNA in negative control samples and demonstrate the levels at which contaminant DNA can be detected. The mixture study assessed the ability of the systems to discern male from female DNA in simulated mixtures.

**DNA Quantification, Comparison, Validation Studies** 

# A125 Increasing the DNA Yield of Biological Samples Stored on Membranes

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After attending this presentation, attendees will have an overview of the yield performance of different extraction methods applied on biological material stored on paper and FTA cards; attendees will also be able to evaluate side-by-side the performance of different methods of whole genome amplification (WGA) in terms of DNA yield and quality.

This presentation will impact the forensic science community by proposing the widespread use of a sample storage method which consumes less energy, space, and minimizes sample collection discomfort.

Storage of biological samples on membranes has traditionally been used in forensics and neonatal registries for enzymatic testing. It is a very advantageous form of storage, reducing the amount of space needed, easing transportation, and collection of biological material for DNA analysis where regulations and cultural issues prevent autopsies. Furthermore, for FTA cards, it is possible to reduce energy consumption as they do not require refrigeration for storage. If the preferred collection method is a buccal swab, sample collection discomfort is also minimized. Different extraction methods have been tested, both automated and manual in order to obtain the highest DNA yields; WGA has also been performed, as applications such as postmortem genetic testing require large amounts of DNA.

**Materials and Methods:** Blood and buccal samples were immobilized onto FTA cards (GE Healthcare) and 903-cards (GE Healthcare). For DNA extraction, a piece of the card containing biological material was removed and placed into a fresh sample tube. DNA was extracted using the recommended (GE Healthcare) protocol, the EZ1 DNA Investigator Kit (Qiagen), an EZ1Advanced XL robot (Qiagen) or the Generation Capture kit (Qiagen). Following DNA

extraction, quantification was done using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems - AB) on the 7900 HT Real-Time PCR System (AB). WGA was performed using Repli-G (Qiagen), Genomiphi (GE) and Genomeplex (Sigma-Aldrich). WGA amplification products were quantified using the Quantifiler® Human DNA Quantification kit (AB). The quality of the extracted DNA for forensic genetic applications was evaluated by conventional STR analysis using the AmpF{STR® Identifiler® PCR Amplification Kit (AB). Detection of the amplified STR fragments and subsequent genotyping was carried out by capillary electrophoresis on AB 3130xL Genetic Analyzers (AB). Results were analyzed using Genescan Analysis version 3.7 (AB) and allele calls were made using Genotyper version 3.7 (AB) macros.

Results and Discussion: One of the limitations for widespread use of membranes as storage medium for biological material is the low DNA recovery efficiency, particularly in the growing field of postmortem genetic testing. Some of these genetic analysis methods require high quantities of DNA (up to 3 µg genomic DNA). Using the Generation Capture kit, it was possible to obtain comparable yields as when extracting the same amount of biological material directly. Furthermore, Repli-g WGA amplification resulted in high quality DNA in quantities sufficient for genetic analysis with the highest DNA requirements. This indicates that the membranes may be used for storing biological material to be used in genetic testing protocols requiring large amounts of DNA.

Forensic Genetics, DNA Extraction, WGA

#### A126 Validation Studies of Allele Drop-Out and Heterozygote Peak Imbalance of Single and Mixture **Profiles** Generated With **AmpFlSTR® Identifiler**® PCR Amplification Kit and Analyzed With Gene-**Mapper IDX**

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After attending this presentation, attendees will better understand various critical features of allele drop-out and heterozygote peak imbalance in single source DNA samples and samples containing mixtures.

This presentation will impact the forensic science community by providing validation data and practical guidance to support understanding of stochastic effects.

Reduction in the amount of DNA template below optimal levels results in loss of genetic information observed in the corresponding DNA profile. The information loss begins as heterozygous peak height imbalance and ultimately results in allele drop-out. This process is generally attributed to stochastic effects occurring during the primer binding steps in the early cycles of the polymerase chain reaction (PCR). Stochastic effects are generally defined as intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles from low quantity DNA template.1 The effects may be locus and/or kit specific and the exact extent to which an evidence profile is effected cannot be known.

Data loss is commonly addressed through the use of a single stochastic threshold which defines the relative fluorescence units (rfu) value above which a single peak at a locus may be assumed to be homozygous. However, papers by Gill et.al. 2009 Forensic Science International and T. Tvedebrink et al. 2009 Forensic Science International provide methods which can be used to describe the

distribution of rfu values where drop-out is unlikely to be observed to values where drop-out is likely to be observed.<sup>2,3</sup> The probability of allele drop-out can be characterized and used to develop laboratory interpretation guidelines which do not rely on the definition of a single stochastic threshold (in rfu) which is unlikely to be sufficiently predictive. This study investigates the characteristics of drop-out in samples having low template DNA in single source and multiple source samples. This is accomplished experimentally by steadily decreasing the amount of DNA template added to the PCR followed by careful characterization of both heterozygous peak imbalance and allele drop-out. Equivalent observations are made in profiles from single source samples and sample mixtures of known proportions.4

In this study, six different amounts of template (2, 1, 0.5, 0.25, 0.125) and 0.06ng) for four single source samples A, B, C, and D were amplified in quadruplicate using the AmpFlSTR® Identifiler® PCR Amplification Kit. Additionally, combinations of A+B and C+D at nine different contributor ratios (1:19, 1:9, 1:4, 1:2, 1:1, 2:1, 4:1, 9:1, and 19:1) and the same six amounts of template were amplified using the AmpFlSTR® Identifiler® PCR Amplification Kit. Procedures were used to ensure that each of the quadruplicate amplifications would have the minimum possible variation. Each sample was at injected for 2, 5, and 10 seconds.<sup>5</sup>

Various features of allele drop-out and peak height ratio differences from all the above profiles were analyzed and interpreted using the GeneMapper® ID-X program from Applied Biosystems. Specifically, characterization of the change in heterozygote peak imbalance vs. the amount of DNA template as well as comparison of drop-out in the two person mixtures to drop-out in the single source samples of the same template mass was analyzed. Furthermore, the recovery of alleles falling below the analytical threshold when injection time was increased was also considered.

This data lays a foundation for better understanding the properties of stochastic effects which occur in the PCR. The data illustrate how careful characterization of a process can provide information which allows for improved analysis and interpretation procedures. **References:** 

- <sup>1.</sup> SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. SWGDAM Interpretation Guidelines for Autosomal STR Typing SWGDAM APPROVED 1/14/10
- <sup>2</sup> P. Gill, R. Puch-Solis and J. Curran. (2009) Forensic Science International: Genetics 3; 104-111.
- <sup>3.</sup> T. Tvedebrink, P., S., Eriksen, H., S., Mogensen and N., Morling (2009) Forensic Science International 3;222-226
- <sup>4.</sup> P. Gill, C.H. Brenner, J. S. Buckleton, A. Carracedo, M. Krawczak, W. R. Mayr et al. DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. 2006 Forensic Science International 160: 90-101
- 5. NIJ Award Number: 2008-DN-BX-K158

Stochastic Effect, Heterozygous Peak Imbalance, Allele Drop-Out

#### A127 Validation and Comparison of Four **Commercially Available STR Kits**

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After attending this presentation, attendees will gain insight into the benefits and limitations of four STR amplification kits based on our comparative analysis for application to forensic casework.

This presentation will impact the forensic science community by showing a direct comparative analysis of four commercially available kits, including two of the newest kits available in the forensic community.

The study includes the validation and comparative analysis of four STR amplification kits used in forensic casework: AmpFℓSTR<sup>®</sup> Identifiler<sup>®</sup>, Identifiler<sup>®</sup> Plus, and MiniFiler<sup>™</sup> (Applied Biosystems) and PowerPlex<sup>®</sup> 16 HS (Promega) run on the AB 3130 Genetic Analyzer. All kits were run using the same sample sets and included the analysis of sensitivity, precision, reproducibility, non-probative casework, stochastic issues (drop-in and drop-out), peak height ratios (PHRs), stutter percentages, calling thresholds, and mixture interpretation. Additionally, all kits were used to amplify Promega's four PowerPlex<sup>®</sup> 16 HS Challenge samples. These kits have been validated according to the FBI/National Standards and SWGDAM guidelines.

The results demonstrate significant performance improvements with Identifiler<sup>®</sup> Plus, and PowerPlex<sup>®</sup> 16 HS in respect to PCR inhibition and lower template amounts compared to the AmpF $\ell$ STR<sup>®</sup> Identifiler<sup>®</sup> kit. In addition, the data supports the assertion that the use of the MiniFiler<sup>TM</sup> kit can increase the likelihood of obtaining additional STR information from forensic samples in situations in which standard STR chemistries fail to produce complete profiles. The benefits and limitations of each kit were reviewed. Based on the results obtained, a new standard kit was chosen and new guidelines for the interpretation of DNA STR profiles were drawn up for use in forensic casework for the Utah Bureau of Forensic Services.

Validation, PowerPlex<sup>®</sup> 16 HS, Identifiler Plus

#### A128 Dscrimination of Architectural Paints: Single White Layers

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The goal of this presentation is to disseminate information to attendees about a recent study conducted by the FBI Lab in the discrimination capability achieved for single, white paint layers.

This presentation will impact the forensic science community by describing the steps taken to discriminate unrelated single, white paint layers in order to assess how successful this process was using light microscopy, FTIR, SEM/EDS, and py-GC/MS.

This presentation reports on a recent FBI Laboratory project designed to assess the discriminating power of physical and chemical comparisons of single-layer, white architectural paints.

In contrast to automotive finishes, assessing the significance of consistent findings between architectural paint samples can be problematic given their ubiquity. Moreover, composition cannot be used as an indicator of the manufacturer, in that different brands can be indistinguishable. Few studies have been conducted to assess the significance of architectural paint comparisons, particularly of single-layer, white paints. Thus, this study has been undertaken in an attempt to evaluate the extent to which randomly collected samples of single-layer, white architectural finishes can be discriminated as well as the probative value of conducting this type of examination. The methodology for this study utilized the FBI Laboratory's standard operating protocols for paint analysis.

Approximately 250 white architectural paint layers were selected as a subset of a larger study that involved 960 architectural paint samples. The original sample set was collected and submitted by FBI Laboratory and FBI field office personnel, as well as forensic scientists from a variety of law enforcement agencies within North America. Collection sites included interior and exterior surfaces such as walls, doors, and trim areas of private homes or commercial buildings. Therefore, the total sample set was considered representative of architectural paint systems that would be submitted as evidence to a forensic laboratory for analysis.

In order to concentrate this follow-up study on the discrimination capability of the FBI Laboratory's current paint protocols on single-layers

of white paint, the sample set was accumulated as follows. Any specimen with a surface layer characterized as "white" formed the basis of the sample set. In addition to these 197 samples, 56 samples with an off-white surface layer were considered to be viable candidates. This approach yielded a sample set of 253 "single, white-layer" samples, in that, the only evaluations being made involved intercomparisons of the physical characteristics and chemical formulations of the topmost layer.

In keeping with the format established in the FBI Laboratory's previous architectural paint study, the samples were first evaluated by Fourier Transform Infrared Spectroscopy (FTIR). From these analyses, samples were placed into one of four categories based on the filler pigments present. As the primary pigment used in architectural paint formulations, titanium dioxide was not considered to be a discriminating factor for classifying these groupings. Therefore, the four categories were: calcium carbonate; kaolin; both calcium carbonate and kaolin; and, neither calcium carbonate nor kaolin. Spectra of samples within each category were then subjected to pairwise comparisons.

Subsequently, any undifferentiated pairs or groups of samples were then macroscopically and microscopically compared side-by-side under the same lighting conditions to document physical characteristics, such as surface finish (glossy, matte, or eggshell) and "color." Chemical analysis of any remaining undiscriminated samples involved the use of scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM/EDS) and pyrolysis gas chromatography with mass spectral detection (Py-GC/MS).

The objective of this project was to evaluate the probative value of conducting comparisons of single-layer, white architectural paints, as well as to determine the ability of the FBI Laboratory's overall analytical scheme to distinguish between these types of samples. The results of this study will be the subject of the presentation.

Architectural Paint, White Paint, Single-Layers

# A129 Evaluation of Six Methods to Extract DNA From Chewing Gum Simulated Forensic Samples

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After attending this presentation, attendees will learn the best methods for extracting DNA from chewing gum of the six methods evaluated. This presentation will also include results with a previously unpublished extraction method.

This presentation will impact the forensic science community by providing systematic data that can be used by the analyst in selecting a method for extracting DNA from chewing gum especially when there is limited sample.

DNA evidence recovered from a crime scene is known to be a valuable tool in criminal investigations and a number of methods to extract DNA from a wide variety of substrates have been employed. However, there is very little information on the use of chewing gum as a source of DNA in the literature though this type of evidence is known to be encountered occasionally at crime scenes. Bond and Hammond<sup>1</sup> have noted that, though chewing gum is rarely encountered at crime scenes, it is a very rich source of DNA. They also point out the difficulty with which this type of evidence may be attributed to a suspect due to the location of recovery, which may be outside of the immediate crime scene and/or in a communal area. The condition of the chewing gum sample has also been shown to have little effect on the ability to obtain a DNA profile.<sup>1,2</sup> Thacker et al.<sup>2</sup> were able to obtain full profiles from chewing gum following incubation at room temperature or in a humidity chamber or after 30 hours of exposure to sunlight. Of the extraction methods they

used, Chelex®100 (Sigma) was found to be the most effective for recovering DNA from degraded samples. This presentation presents results of six DNA extraction methods on chewed chewing gum samples. A chewing gum sample from a crime scene can be divided into a small number of samples (e.g., approximately three replicates). Samples not tested initially are often saved for retesting or use by the defense. The method employed should be known to have produced useful results in the past. In this study, the methods that would yield the most DNA with the greatest efficiency and with consideration to cost were evaluated.

The research described in this presentation includes both the detailed systematic methods constructed in this study and answers to the question posed by concluding the results of each simulated forensic sample including non-destructive methods agarose gel (1%) electrophoresis, UV-Vis spectroscopy (260/280 nm ratio), and real-time PCR. The six methods employed were Chelex-100 (Sigma), Phenol-Chloroform-Isoamyl Alcohol (PCIA), DNA IQ™ (Promega), Silica G gel (Merck), Silicycle (TAAcONa, Silicycle Inc.), and EDTA dialysis (Spectra-Por). All extraction methods were validated using buccal cells. The simulated forensic samples were obtained in triplicate for each extraction method to demonstrate reproducibility. Six pieces of Trident Watermelon Twist with xylitol were chewed for thirty minutes each on separate days. Each chewed piece of gum was divided into three approximately equal pieces that were weighed in tarred spin baskets (Fitzco) or 1.5-mL microcentrifuge tubes prior to extraction. Extracted samples were stored at -20°C. Real-time PCR was conducted using undiluted DNA extracts, iQ SYBR Green Supermix, and forward and reverse TPOX primers3 on a BioRad iQ5 instrument. Negative controls were prepared using nucleasefree water in lieu of extracted DNA and a K562 DNA standard (Promega) was used for the positive control. The melting temperature of the expected 64-bp amplicon was calculated to be 78°C by both the basic and nearest neighbor methods using Northwestern University's online biotools software.4 DNA was successfully extracted from buccal cells using all six extraction types. Preliminary results of real-time PCR of the chewing gum extraction products indicated that the PCIA method yielded the highest quantity of DNA, the DNA IQ system yielded the second highest DNA quantity, dialysis the third, and Chelex the fourth. The K562 DNA amplified while the negative control did not amplify.

#### **References:**

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DNA Extraction, Chewing Gum, Real-Time PCR

# A130 Comparison of Different Methods for DNA Isolation

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After attending this presentation, attendees will learn about the performance of different methods for DNA isolation and comparisons by application of test parameters such as yield, quality, cost, and time taken to complete the isolation process.

This presentation will impact the forensic science community by proving it is useful to periodically assess the performance of different forensic science techniques as new ones become available. The present study provides knowledge on the outcome of testing and comparing different products for DNA isolation that may aid practitioners' evaluation of options for different analytical needs.

In forensic DNA testing, a crucial first step is the isolation of DNA from evidence samples for additional procedures. The performance of this first step enables the final results. Different methods and kits for the isolation of DNA are commercially available and may vary in different characteristics that are of importance to analysts such as time for processing, yield of DNA, integrity, purity, cost, and simplicity of process. Consequently, it can be useful to measure such parameters in comparison of different commercially available kits for DNA isolation. DNA analysts and educational institutions would thus have more bases for the selection of kits for particular needs.

Three different DNA isolation kits were initially obtained for study with others being later tested. The kits were QIAamp DNA Investigator from Qiagen, ZR Genomic DNA from Zymo Research and AccuPrepGenomic DNA Extraction kit from Bioneer. The kits were evaluated according to the following criteria: (a) purity of isolated DNA; (b) integrity of isolated DNA; (c) yield; (d) time taken to process; and, (e) cost per sample. Purity and yield were assessed by using a Nanodrop spectrophotometer and integrity of DNA was assessed by agarose gel electrophoresis. DNA was isolated according to the different manufacturers' instructions for three different samples of whole blood for each kit.

The results showed differences in performance among the kits. Agarose gel electrophoresis of products isolated by each kit showed bands at the positions expected for genomic DNA in each case and no signs of degradation. Staining intensity was strongest for the DNA isolated by the Qiagen kit. Based on measurement of ultraviolet wavelengths absorbance by Nanodrop spectrophotometry, the yield of DNA from 200 microliters of blood was highest for the Qiagen kit (5.6  $\mu$ g) followed by the Bioneer kit (2.74  $\mu$ g) and the Zymo kit (2.39  $\mu$ g). In regard to purity as measured by A260nm/A280nm ratio, the DNA isolated by the Qiagen kit had a ratio of 1.85 and was closest to the ideal ratio of 1.80, whilst the ratios were 2.0 and 1.03 for the isolates from Zymo and Bioneer kits. Assessment of costs gave values of \$3.60/sample for Qiagen, \$1.50 for Bioneer and \$0.72 for Zymo kits.

Comparison of the time taken to isolate DNA by the different manufacturers' kits showed that the shortest time taken to isolate DNA was used by the Zymo kit which took 25 minutes. The Bioneer kit took 35 minutes for the isolation of DNA and the method that took the longest was the Qiagen kit which took 50 minutes. During the process of isolation, samples were transferred among three tubes for the Qiagen and Bionner kit while the Zymo isolation involved one tube.

In conclusion, it was found that each kit had advantages and disadvantages. While the Qiagen kit gave the highest yield and quality, it was also the most expensive and took the longest to complete, in addition, the Qiagen filtration columns used for DNA binding and the proteinase K used in the procedure must be stored at -20°C. The Bioneer kit was less expensive and took less time than the Qiagen kit for the completion of DNA isolation, but the yield of DNA was less and the products were more contaminated with ultraviolet light absorbing material. In addition, the procedure uses proteinase K which must be stored at -20°C. The Zymo kit was the least expensive and took the least time to complete DNA isolation. The DNA isolated by the Zymo kit was not as pure as that isolated by Qiagen based on ultraviolet light spectrophotometry. All the components of the Zymo DNA kit can be stored at room temperature.

These findings may be exploited by individual laboratories to select kits for DNA isolation based on the type of sample from which DNA is being isolated, the subsequent analytical steps for the DNA and the numbers of samples, and economic considerations.

The various parameters for the efficacy of DNA isolation will also be compared for additional kits and for other types of samples of forensic interest such as saliva, semen and hair.

**DNA**, Spectrophotometry, Electrophoresis

#### A131 Improved Isolation of DNA From Forensic Dental Specimens

Allison E. Reklaitis, MS\*, and Chenique Sanders, BS\*, Office of the Chief Medical Examier, New York City Office, 421 East 26th Street, Department of Forensic Biology, New York, NY 10016; My K. Tran, DDS, and Amr M. Moursi, DDS, New York University College of Dentistry, Pediatrics Department, 345 East 24th Street, New York, NY 10016; and Sheila E. Dennis, MS, Office of the Chief Medical Examiner, New York City Office, Department of Forensic Biology, 421 East 26th Street, New York, NY 10016

After attending this presentation, attendees will know a different approach to the examination of forensic dental specimens and also the effect of specific environmental conditions on the ability to extract DNA from these specimens, specifically teeth.

This presentation will impact the forensic science community by giving those who work on forensic dental specimens and other specimens, that are compromised by the environmental conditions they are exposed to, another approach to examination and knowledge that the three specific environments that these teeth were exposed to do have an impact on the quality of DNA obtained.

The DNA Missing Persons Unit within the Department of Forensic Biology at the New York City Office of Chief Medical Examiner performs nuclear and mitochondrial DNA testing for the purposes of identification, re-association of body parts, and upload into the Combined DNA Index System (CODIS). While success rates for postmortem samples such as blood, tissue and bone were as expected, dental specimens failed to give consistent results. This may have been caused by exposure to detrimental environmental conditions. These environments may vary from bodies found in water, to remains buried in soil, or encased in cement. The goal of this study is to evaluate how exposure to the following conditions: water, soil, and, cement, affects the ability to extract nuclear DNA from dental samples; and determine what improvements can be made to the examination and extraction protocols.

One hundred-twenty adult teeth collected as dental waste from private dental offices were examined and categorized by a dentist according to tooth type (molars, incisors, premolars, and canines) and any abnormalities such as dental caries (cavities), open apices, calcification, and discoloration were noted. The teeth were incubated in soil, cement, water from the East River in New York City, or physiological saline from one to 48 weeks. Two specimens from each environmental condition had the pulp tissue extracted at Weeks one, two, three and four and every four weeks thereafter using a dental pulpectomy procedure. The pulpectomy involved removing tissue from the pulp chambers using nerve broaches utilized by dentists when performing a root canal. A phenol chloroform isoamyl alcohol (PCIA) extraction was performed on the pulp tissue. Each DNA sample was quantified, amplified with the Applied Biosystems (ABI) Identifilerä kit (28 cycles), and alleles detected using the ABI PRISM 3130xl Genetic Analyzer.

The current method used to extract DNA from teeth involved grinding the entire tooth using a freezer mill. This destroyed the morphology of the tooth and successful DNA results fluctuated and were unpredictable. Results from this study greatly improved examination procedures; the morphology of the tooth structure was maintained while examination efficiency increased by accessing the DNA in the tooth through pulpectomies.

By week 40, 30.8% generated full profiles and 16.7% generated high partial profiles all eligible for entry into CODIS. Overall by week 40, the number of identified loci decreased over time in most conditions but DNA extracted from dental specimens buried in soil showed the strongest reduction in identifiable loci.

Prior caries status of a tooth was not a factor in DNA extraction as those teeth yielded sufficient amounts of DNA. Conversely, teeth with open apices, that were calcified, or had no visible pulp tissue did not yield DNA or yield sufficient amounts of DNA for concentration or amplification. Overall, the aims of this study thus far have been met. Improvements to the examination of dental specimens through pulpectomies require less examination and hands on time compared to the conventional freezer mill method. Pulpectomies preserve the morphology of the tooth and yield similar DNA results. The dental specimens exposed to cement, soil, water, and even physiological saline show degradation over time as evidenced by the decrease in DNA concentration and quality of DNA profiles obtained.

**DNA**, Extraction, Identification

# A132 Optimizing Sperm Cell Recovery From Cotton Swabs Prior to Christmas Tree Staining and p30 Test

Laura L. Perrella, BS\*, 1310 Westmeadow Drive, Beaumont, TX 77706; Jordan L. Williams, BS, 110 Milo Street, Dayton, TX 77535; and Jennifer N. Watson, MS, and Andrew P. McWhorter, MS, Texas Department of Public Safety, 12230 West Road, Houston, TX 77065

After attending this presentation, attendees will be able to utilize the method presented in order to recover an abundant amount of sperm cells from cotton swabs for the Christmas Tree stain and p30 test.

This presentation will impact the forensic science community by enabling analysts to recover an ample amount of sperm cells from cotton swabs to perform the Christmas Tree stain, and therefore, confirm the presence of sperm.

The Texas Department of Public Safety Houston Crime Laboratory's confirmatory test for the presence of semen is microscopic visualization of the sperm after an extraction and staining. Many laboratories require this confirmatory test before sending evidence onto DNA analysis; otherwise, it is under the serologist's discretion to send the sample for further testing. Therefore, an optimal recovery method must be utilized prior to staining and microscopic viewing.

Previous studies performed by others suggest using different detergents to remove sperm cells from the substrate. Other detergents considered are sodium dodecyl sulfate (SDS) solution, a sarkosyl solution, and a Triton-X 100 solution. Garvin, et al. (2009) found Triton-X 100 to be the best detergent for DNA extraction. According to Norris, et al. (2007) 2% SDS was best for cell recovery. Current methods at the Texas Department of Public Safety Houston Crime Laboratory use ultrapure deionized (DI) water for removal of sperm from substrates.

A recovery method for sperm cells on a cotton swab was developed by determining the best detergent and the optimal working concentration of the detergent. The detergents used were an SDS solution, a sarkosyl solution, and a Triton-X 100 solution. The concentrations ranged from 0.5% to 20%. After determining the optimal concentration of each detergent, diluted semen samples subjected to the removal process to determine the best detergent. 0.5% SDS yielded the most sperm cells recovered at all diluted concentrations (lowest being 1:1000). The extraction method was performed according to the Texas Department of Public Safety Houston Crime Laboratory Standard Operating Procedure for all analyses. Following the extraction, the supernatant was removed for p30 testing and replaced with DI water; this reduced any soapy residue. The pellet was introduced onto a microscope slide and Christmas Tree stained. The slides were evaluated microscopically (400x) and the sperm present were manually counted.

The supernatant is used for p30 testing on an ABA card at the Texas Department of Public Safety Houston Crime Laboratory. Although SDS recovered the most sperm cells, a positive p30 was not obtained. If SDS is to be used as the recovery method for sperm cells, then two separate extractions would have to be conducted (one for the microscopic examination with the SDS solution and one for the p30 test with DI water).

Sperm Cell Recovery, Christmas Tree Stain, Sperm From Swab

# A133 Comparison of Collection Devices and Commonly Used Human Identification Kits for Forensic DNA Profiling of Soil-Inhibited Saliva-Skin Samples

Pamela J. Staton, PhD, Dishari Mukherjee, MBBS\*, Justin Godby, MSFS, and Tiffany Paugh, MS, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701

After attending this presentation, attendees will be able to make an informed decision as to protocols best suited for casework involving soil contaminated saliva-skin samples as the main DNA source.

This presentation will impact the forensic science community by significantly reducing the waste of and processing time associated with the analysis of soil-inhibited saliva samples by identifying the combination of analytical procedures that will generate the best DNA profile. This presentation will help to solve cases involving violent crimes and sexual assault with a positive influence on the conversion of soil contaminated saliva-skin samples into a DNA profile. This study can also provide a basis for future studies to be conducted involving similarly inhibited samples.

Human body fluids such as blood and saliva are common biological materials frequently encountered in Forensic DNA Investigations. Saliva plays an important role as evidence in cases involving violent crimes through bitemarks and in addition through kissing and licking in sexual assault cases where neither semen nor blood is found, or where neither gives conclusive results. Saliva which has come in contact with intact skin remains stable and can be recovered at least 60 hours after deposition, but it is typically overlooked as a potential source of DNA as it is difficult to visualize. However, when bitemarks or bruises caused by kissing or sucking the skin are observed, saliva is likely to be present. Obtaining DNA profiles from saliva stains can be challenging because there is always the possibility of the evidence becoming contaminated with environmental inhibitors such as soil, which is both ubiquitous and abundant in nature. Soil contains powerful Polymerase Chain Reaction (PCR) inhibitors like humic acid and fulvic acid which can make obtaining a DNA profile difficult. Data from the U.K.'s National DNA Database (NDNADB, 2006) shows that saliva as a DNA source constitutes 33.3% of total samples recovered, making it statistically the largest source of DNA recovered from crime scenes. Blood, however, constitutes only 17.4% of total samples recovered. Of the 33.3% total saliva samples, only 43.1% generated DNA profiles suitable for loading on the NDNADB, which is in vast contrast to 92.8% of the 17.4% total blood samples that generated DNA profiles suitable for loading on the NDNADB. An optimum procedure is therefore required to maximize the probability of DNA recovery and genotyping from saliva samples. The goal of this study was to establish the best methodologies for collecting and profiling of soil contaminated saliva stains on skin using commercially available kits and supplies common in Forensic DNA laboratories. Saliva obtained from male donors was deposited on a measured area of a female volunteer's skin surface, keeping with the amount of saliva generally associated with human bite marks (250-300µL). The testing samples were collected after allowing the saliva stain to properly dry. Two collection devices were used: polyester tipped sterile swabs (FisherBrand) and sample collection popules (Puritan). The double swab technique (wet and dry swabs, respectively) was used for collecting saliva samples with the swabs to maximize cell collection. Varying concentrations of soil were added to a portion of the saliva-skin samples after collection. Two magnetic particle based DNA extraction kits: Promega's DNA IQ<sup>™</sup> and Applied Biosystem's (AB) Prepfiler<sup>™</sup> were compared on the basis of their performance in DNA recovery. Quantification was performed using AB Quantifiler® Duo DNA Quantification Kit on the AB 7500 Sequence Detection System v1.2.3. PCR amplification was conducted with two multiplex STR systems: AB's and Promega's PowerPlex® 16 System, on the AB GeneAmp®

PCR System 9700. The amplified products were separated on the AB 3130xl Genetic Analyzer. The data generated was analyzed using AB's GeneMapper® ID Software v3.2.1. Sample peak height, allelic dropout, and artifacts such as pull-up were taken into consideration when making a determination of the best set of kits for generating DNA profiles of the aforementioned samples. Mixed profiles were not observed across most samples with only some profiles showing a few alleles from the female volunteer. This could be a result of DNA shedder variability. The project is ongoing, but statistical analysis between swabs and popules thus far show no significant difference between the two collection devices. Preliminary testing based on peak heights revealed that PowerPlex® 16 outperformed Identifiler® while DNA IQ™ proved to be better than Prepfiler<sup>™</sup> on saliva-skin samples with soil. Additional testing and analysis will be required to make a determination of the ideal combination of kits for maximizing DNA recovery and generating reportable genetic profiles from said sample types. This study was conducted after IRB approval for human research was obtained.

Soil, Saliva, DNA Profiling

#### A134 Application of Clostridiopeptidase A for DNA Isolation of Bone Specimens

Richard Li, PhD\*, John Jay College, CUNY, 445 West 59th Street, New York, NY 10019

After attending this presentation, attendees will understand the principle of this method to process bone samples prior to DNA isolation.

This presentation will impact the forensic science community by developing a method to potentially increase the yield of DNA isolation from bone samples.

Bone is difficult to process for isolating DNA, which presents one of the greatest challenges when attempting to identify victims through the analysis of DNA from bones. In addition, quantities of samples recovered may be too small to properly isolate sufficient amounts of DNA. Strategies to improve the yield of DNA isolation are needed to obtain an adequate quality and quantity of DNA templates.

The bone osteocytes containing DNA are embedded in a calcified matrix, which is a barrier preventing the isolation of the DNA from the osteocytes during the extraction process. Therefore, it is necessary to remove the matrix to improve the yield of DNA. The application of proteinase K is one approach to digest this matrix barrier.

Identifying other proteinases for digesting the matrix of bone tissue and optimizing proteinase treatment were of interest. Collagenases, in particular, are known for playing a role in the degradation of bone matrix proteins. Clostridiopeptidase A is one of the most potent collagenases; thus it was chosen for this study. In this study: (1) the characterization of the effect of clostridiopeptidase A on bone degradation have been carried out; and, (2) the characterization of the effect of clostridiopeptidase A treatment on DNA isolated from bones have been conducted.

This study revealed that clostridiopeptidase A is potent for bone degradation. The application of clostridiopeptidase A can achieve speedy and effective bone degradation. Thus, this method reduces digestion time. The STR analysis detected no adverse effects on DNA profiles after the clostridiopeptidase A treatment. The potential application of clostridiopeptidase A to the isolation of DNA in bone will be presented. The quality of the isolated DNA will be assessed.

Bone, Clostridiopeptidase A, Forensic DNA

#### A135 mRNA Decay in Biological Fluids in Order to Establish an Estimation of How Long a Stain Was Deposited at a Crime Scene

Kimberly Moran, BS\*, Cedar Crest College, 450 South Wood Street, Middletown, PA 18104; and K. Joy Karnas, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 18104

After attending this presentation, attendees will understand the molecular basis of mRNA degradation in blood and saliva stains, specifically in regard to 5'- or 3'-end degradation.

This presentation will impact the forensic science community by confirming the stability of mRNA in body fluid stains and substantiating its utility in identifying the source of those stains. The major objective of the study was to quantify the mRNA degradation in body fluid stains, particularly 5'- and 3'-end degradation. Not only does this allow researchers to select the most stable portion of the message for identification, particularly useful in highly degraded samples, but the patterns of postmortem RNA degradation might also help in determining how long a stain has been present at a crime scene. By studying the dried stain on a molecular level, it may be possible to estimate the time frame of when a stain was deposited at a crime scene.

Classical forensic analysis relied on serology and biochemical tests to identify body fluids left at a crime scene. More recently; however, fluid identification has been abandoned as the focus has shifted to the use of DNA analysis for identification of the individual who deposited the fluids. Choosing one type of analysis over another is necessitated by the fact that forensic samples are usually small in volume and size and, therefore, can only be subjected to one test. DNA may be useful at determining who left a biological stain, but gives no indication as to what the origin of that stain was. More and more scientists are seeing the value in body fluid identification and are looking at ways to revitalize this aspect of crime scene analysis. Due to this, a mRNA profiling method has been developed and can be carried out in conjunction with DNA profiling so both the individual and the body fluid can be identified simultaneously. mRNA is useful in body fluid identification because it is expressed in a tissue specific manner, with a different subset of mRNAs produced in each body fluid.

The initial phase of this study used primers designed specifically for housekeeping and fluid-specific genes to ascertain mRNA decay in blood and saliva stains incubated at various temperatures and for various periods of time. The majority of the genes studied were detected in both blood and saliva for up to one month at 37°C, although housekeeping genes appeared to be more stable than fluid specific genes. The followup study focused only on degradation over time and used real-time PCR and primer sets designed to amplify the ends of the mRNA to assess the amount of 5' and 3' message remaining in deposited samples. It was found that end stability also appears to be gene-specific and similar in both body fluids studied. All housekeeping genes showed 5' stability, while all fluid-specific genes showed 3' stability.

mRNA, mRNA Stability, Real Time PCR

# A136 Reflectance Spectroscopy for Recognition and Age Determination of Blood Stains

Rolf Bremmer, MSc\*, Academic Medical Center, Meibergdreef 9, Amsterdam, 1105 AZ, NETHERLANDS

After attending this presentation, attendees will understand the basic principles of reflectance spectroscopy and its applicability for recognition and age determination of blood stains. Non-contact reflectance spectroscopy can be used as a rapid, non-destructive identification test for blood and can be used for the determination of hemoglobin derivatives in a blood stain, which are related to the time the blood stain was deposited at the crime scene.

This presentation will impact the forensic science community by introducing a new method for rapid, non-destructive identification, and interpretation of blood stains in the original context of the crime scene.

Blood detection and identification at crime scenes are crucial for harvesting forensic evidence. Unfortunately, most tests for the identification of blood are destructive and time consuming. A fast and non-destructive identification test for blood, using non-contact reflectance spectroscopy will be presented. Investigated in this experiment is whether blood can be discriminated from other body fluids and substances visually mimicking blood, based on the correlation coefficient between the reflectance spectrum and a blood-component fit. To test the sensitivity and specificity of this method, two sets of samples were analyzed: a set of 40 blood samples and a set of 35 red/brown colored substances and various body fluids. Discrimination is possible between blood and non-blood on white cotton with a specificity of 100% and a sensitivity of 98%. In addition, an example is given of a recent forensic case, in which non-contact blood identification was applied.

The age of blood stains can be crucial in reconstructing crime events; however, no reliable methods are currently available to establish the age of a blood stain on the crime scene. It will be shown that the fractions of three hemoglobin derivatives in a blood stain at various ages can be related to the age of the blood stain. Upon blood exiting the body, hemoglobin, the main chromophore in blood, transits from oxyhemoglobin into met-hemoglobin and hemichrome. Analysis of the blood stains with light transport theory allows for determination of the amount of the three hemoglobin derivatives. Observations in twenty blood stains show that the chemical composition of the blood stain exhibits a distinct time-dependent behavior, with a unique combination of the three hemoglobin derivatives at all moments in time. The ageing of the blood stain does not depend on the size of the blood stain and no variation was found in ageing of blood stains among eight donors. Finally, the previously obtained hemoglobin reaction kinetics were employed inversely to assess the age of twenty other blood stains studied over a time period of 0-60 days and stored under laboratory circumstances. The precision of the age estimation depends on the age of the blood stain, e.g., a stain of two weeks old can be estimated correctly within an uncertainty margin of four days.

In conclusion, discrimination is possible between blood and nonblood on white cotton with non-contact reflectance spectroscopy. The high sensitivity and specificity indicate that this optical test is close to confirmative. The non-contact reflectance spectroscopy setup is portable, low-cost, non-invasive, and non-destructive, all favorable properties when measuring in a forensic setting. Additionally, determination of hemoglobin derivatives allows for age estimation of the blood stain and assists in reconstructing the time line of the crime scene.

Blood Stains, Spectroscopy, Age Determination

# A137 The Utility of Raman Spectroscopy of Blood Samples for Forensic Applications

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After attending this presentation, attendees will understand the biochemical principles of Raman Spectroscopy for biological fluids, as

proposed for use in forensic body fluid detection and identification. This will include a review of the current and relevant literature, as well as results of recent research.

This presentation will impact the forensic science community by providing a balanced exploration of the utility of Raman Spectroscopy in forensic serological analysis, in the field for crime scene applications, as well as in the laboratory.

Recent reports have shown that Raman Spectroscopy can be used in forensic science to identify blood and other body fluids. Raman Spectroscopy is a very sensitive technique that is often used in forensic laboratories, mostly to analyze textiles and paints, but it has been seldom used to analyze blood and body fluids for forensic applications. Very recent experimental work, however, has shown that Raman Spectroscopy may be able to identify and discriminate between body fluids, to distinguish colored stains from blood residues, and to possibly discriminate between blood of different species. Because of these findings and to the recent development of hand-held and portable Raman Spectrometers, Raman field analysis of body fluids could soon become a reality.

To move the field out of the experimental arena and into validation for use in casework, several parameters must be investigated in order to establish reliable protocols. Raman scattering from human blood was investigated as a function of parameters that are relevant for field forensic analysis, such as excitation wavelength, age of samples, influence of substrates, and sample dilution. The scattering probability of green light (532.1 nm) was found to be higher than that of red light (632.8 nm) and UV light (325nm He-Cd laser). Additionally, the relative intensities of peaks arising from oxyhaemoglobin and ferrous ions that depend on sample age, fluorescence, or Raman scattering from fabrics prevent meaningful analysis of blood samples in certain instances. Relative differences in the amount of peaks can be found between fresh venous blood and the same sample retaken an hour after drying. Samples can be measured with a maximum dilution of approximately 1:250 (for an excitation power on the order of 2 mW measured at the sample plane). The sensitivity of Raman scattering to diluted blood allowed for measurement of blood reconstituted from fabrics, thereby alleviating issues related to fabric luminescence and scattering.

Thus, Raman scattering has a sensitivity comparable to many presumptive tests for blood, such as luminol, phenolphthalein, leucomalachite green, blood strips, and the forensic light source tests. These tests are highly sensitive (up to 1:5,000,000 dilutions can be measured in forensic laboratories), but in the field a cotton swab test is generally employed, which can only measure dilutions on the order of 1:100-1:250. Because of the sensitivity of Raman scattering to diluted blood, a protocol for the reconstitution of blood from fabrics can be established. Contrary to other published reports, results in this laboratory indicate that reconstituted blood alleviates issues related to fluorescence and scattering from fabrics and is vastly preferable to analyzing the stain *in-situ*. These findings will prove important in determination of utility and in the preparation of protocols for the field analysis of samples.

Blood Detection, Raman Spectroscopy, Biospectroscopy

#### A138 On-Site Body Fluid Identification

Stephanie T. Young, BA\*, and Clifton P. Bishop, PhD, West Virginia University, Department of Biology, 53 Campus Drive, PO Box 6057, Morgantown, WV 26506

After attending this presentation, attendees will understand the development and design of short, fluorescently-labeled, single-stranded DNA probes, known as molecular beacons to detect tissue-specific ribonucleic acids as a possible on-site confirmatory test for human body fluid type.

This presentation will impact the forensic science community by outlining a universal, cost-effective, confirmatory assay to determine the body fluid content of the three most common biological stains (blood, saliva, and semen), as well as determining if the stain is human in origin, while still processing a crime scene. With such an assay in use, exclusion of non-evidentiary specimens from collection, processing, and DNA profiling can represent a significant savings in terms of both time and money.

Collection and processing of potential evidentiary samples at a crime scene is critical to the successful resolution of an investigation. At present, various presumptive and confirmatory tests are available to assist the crime scene investigator in selecting which specimens to process. Most presumptive tests can be performed at the crime scene itself, but many of the confirmatory tests must be performed at the crime laboratory.

There is a great need for a universal confirmatory test for biological evidence left behind at the scene of a crime. Such an assay would assist crime scene investigators to determine whether to collect a specimen for further processing or ignore the stain as being non-evidentiary, thus saving time and money. The success, sensitivity, and popularity of DNA profiling has frequently resulted in the collection of a large number of samples at many crime scenes, resulting in increased costs to investigate a crime and often a considerable backlog in the processing of such samples. The presented assay has the potential to reduce the number of samples collected at a crime scene and could eventually have a positive impact on reducing evidence backlogs.

It was hypothesized that by designing molecular beacons to hybridize with RNAs that are specific for blood, saliva, or semen, a multiplex reaction could be created to determine the presence of each type of body fluid, or combination thereof, in a biological stain. Within this multiplex reaction, human-specificity is also determined by probing human-specific RNA sequences. With advances in technology that allow for fluorescence spectrophotometers powered by laptop computers and short RNA extraction methods that can be performed at room temperature, the advantage of portability to a crime scene is gained.

After an extensive literature search, candidate RNAs were identified and confirmed to be tissue-specific. Traditional PCR primers that encoded areas of interest on chosen RNA molecules were used to validate the candidate sequences as either tissue-specific or human-specific. The forward primer was then used to create the molecular beacon hairpin probe. The molecular beacons were then tested on biological samples of different ages.

Aspects of the development of the tissue-specific molecular beacons will be discussed in detail for the body fluids of blood, saliva, and semen. Various parameters of the assay will be discussed including detection limits (both in terms of minimum sample size, as well as over what time frame the assays are valid) and intrapersonal variability. The design of future experiments will be discussed.

Whether used in the field as originally intended, or within a crime laboratory to replace multiple current confirmatory tests, the assay has great promise. Once additional research and validation studies have been performed, the presented technique would provide a less time-consuming and more cost effective assay for confirming body fluid type.

Body Fluid Identification, Tissue-Specific RNA, Molecular Beacons

# A139 Phenolphthalein False-Positives: What's Buried in Your Garden?

Daniel J. Petersen, PhD\*, Oregon State Police Forensic Laboratory, 13309 Southest 84th Avenue, Suite 200, Clackamas, OR 97015

After attending this presentation, attendees will be familiar with several common garden plants which consistently yield false-positives when screened using phenolphthalein as a presumptive test for the presence of blood.

This presentation will impact the forensic science community by alerting investigators of materials and conditions which can produce misleading results when screening evidence for blood. This presentation will assist attendees in recognizing which plants yield phenolphthalein false-positives and alternative testing methods to prevent misidentification.

The presence of bloodstains on items of evidence is often critical to how a criminal investigation proceeds. A positive presumptive test for blood is a determining factor of how an item of evidence is prioritized and can even be decisive as to whether or not the evidence is further analyzed. Presumptive testing is typically performed using one or more colorimetric tests as a screening tool. These tests are inexpensive and rapid, allowing screening of potential bloodstains even while at a crime-scene. One very commonly used test is the Kastle-Meyer test, which is based on the catalytic activity of the heme group on hemoglobin. This test is often referred to as the "phenolphthalein test," as it involves the oxidation of reduced the phenolphthalein substrate to produce a rapid color change after the addition of hydrogen peroxide. DNA profiles extracted from phenolphthalein-positive stains are presumed to be from the blood on the evidence.

While certain substrates have previously been shown to occasionally yield false-positive reactions, this presentation describes a previously unreported class of plants, including *Pisum sativum*, which consistently yields phenolphthalein false-positive reactions. The particular plant tissue further mimics true aged bloodstains by causing brown or redbrown staining on cloth, swabs, and other materials. Longitudinal studies have demonstrated that these stains retain their phenolphthalein falsepositive reactivity after three years. Similarly, plant tissue which has been frozen for three years after harvesting continues to demonstrate the phenolphthalein false-positive reactivity which is indistinguishable from true bloodstains both in color quality and developmental time-frame.

The widespread prevalence of these plants in gardens across the United States adds to the deleterious potential for mis-interpretation of phenolphthalein testing results when screening evidence which has been in contact with these common plants. A proposed mechanism accounting for the false-positive reaction will be presented. This information can serve to further identify which plants are prone to yield phenolphthalein false-positive reactions.

Bloodstain, Phenolphthalein, Hemoglobin

# A140 Sex Crimes in Colombia From the Perspective of the Forensic Biology Laboratory

#### Luz A. Garcia, BSc\*, Medicina Legal, Calle 4B N° 36-01, Cali, COLOMBIA

After attending this presentation, attendees will understand that the integral analysis of the criminal context of sex crimes requires the involvement of various disciplines. All of these disciplines must work together to reconstruct the offense, based on the physical evidence collected from the crime scene and from the bodies of the victim and possibly the perpetrator. This presentation will describe the efforts of the Forensic Biology Lab of the National Institute of Legal Medicine and Forensic Sciences, Southwestern Regional Office, concerning sex crime management. The advances made since the opening of the Forensic Genetics Lab at the Southwestern Office, particularly since the implementation of the sex crimes management protocol and the creation of the Elite Sex Crimes Team (GEDES), will be highlighted.

This presentation will impact the forensic science community by describing the significant efforts made by the Biology Lab in the area of preliminary analyses. Several unknown perpetrators were identified in specific rape cases. The above, together with the interdisciplinary approach, contributed to obtaining genetic profiles from the evidence processed in search for sperm. The profiles were subsequently uploaded into the Combined DNA Index System (CODIS) for future comparison.

The primary basis of the integral management of sex crimes victims is to bring together all the law enforcement agencies and health care institutions involved (i.e., Colombian National Police, Law Enforcement Agencies, Attorney General's Office, National Institute of Legal Medicine and Forensic Sciences, both private and public Hospitals and Health Care Centers, Family Advocacy, Colombian Institute of Family Welfare, and others). These agencies must effectively communicate and coordinate their efforts, bearing in mind that the first 72 hours after the fact are critical to the collection of biological evidence that may have been left behind. Therefore, immediate attention to sex crime victims is a key factor that will allow medical examiners to obtain genetic profiles of potential sex offenders after preliminary evidence analysis.

This presentation will describe a case received by the Forensic Biology Lab, where current analytical methods, together with inadequate collection and sample delivery methods, jeopardized the investigative process due to the deterioration of physical evidence. The presentation will also describe cases where the laboratory has received evidence that, according to the victim's account, is not associated with the crime and does not contribute to the clarification of the facts.

• The following statistical information will be provided in order to give the audience an overview of the current situation in Colombia:

- National statistics on expert reports of alleged sex crimes
- Case characterization according to perpetrator typology
- · The number of cases received by the Forensic Biology Lab
- The number of cases related to alleged sex crimes
- The number of cases where analyses yielded positive results
- The number of cases referred to the Forensic Genetics Lab by the Criminalistics area
- The number of cases referred to the Forensic Genetics Lab by the Elite Sex Crimes Team
- The number of expert reports required for public hearings

Finally, this paper is intended to show the importance of the implementation of the GEDES project in the city. This is a valuable sex crimes investigation tool where unknown perpetrators may be identified, frequently, as serial rapists.

Sex Crimes, Physical Evidence, Elite Sex Crimes Team GEDES

## A141 Distinguishing Identical Twins With Antibody Profiling

Vicki Thompson, PhD\*, Jeffrey A. Lacey, PhD, Elizabeth Taylor, BS, Karen Delezene-Briggs, and William A. Apel, PhD, Idaho National Laboratory, PO Box 1625, Idaho Falls, ID 83415

After attending this presentation, attendees will gain an understanding of how antibody profiling can be used to identify individuals and an assessment of the ability of antibody profiling to distinguish between identical twins.

This presentation will impact the forensic science community by introducing attendees to the science behind a recently available identification technology, AbP-ID<sup>™</sup> and its ability to distinguish between identical twins. Distinguishing blood samples from identical twins is virtually impossible with other identification technologies. This technology provides a tool for investigators to use in cases where identical twins are suspects.

Antibody profiling examines individual specific autoantibodies (ISAs) that are produced by every individual; and, since ISAs are produced by the immune system through a number of random events such as error prone homologous recombination, mutations, multiple coding segments, and heavy chain/light chain re-association, the antibody profiles of identical twins should differ.

The antibody profiling technology was developed for forensic analyses at the Idaho National Laboratory (INL). This technology has been licensed to Identity Sciences, LLC who has developed a prototype test kit, AbP-ID<sup>TM</sup>. The INL is currently working to scientifically validate performance of the AbP-ID<sup>TM</sup> kit under a variety of conditions. The results reported from this study represent efforts to determine if twins can be differentiated by antibody profiling. Antibody profiling is an alternative technique for identification of individuals. Antibody profiling is based on the presence of ISAs in the body fluids of individuals. Infants have an ISA pattern that is identical to their mother's and develop their own unique pattern over the first two years of their lives. This pattern does not appear to be affected by environmental or disease exposures. After the age of two, the ISA pattern is set and does not change over the individual's lifetime.

In this study, ten sets of identical twins were identified to test this hypothesis. Sera and swabs were collected from each twin. The swabs were sent for twin zygosity testing while the sera was analyzed in duplicate with a prototype antibody profiling kit, AbP-ID<sup>TM</sup>. The AbP-ID<sup>TM</sup> kit instructions recommend using between six and 15 microliters of sera which was added to a buffered solution and incubated with test chips for 15 minutes. The chips were thoroughly washed and a detection reagent was added for a ten minute incubation. The chips were again washed and a developing reagent was added for 20 minutes for a total assay time of approximately 100 minutes. After development was complete, the chips were dried at room temperature. Images of the developed chips were captured using a desktop scanner. ImaGene 8.0, a microarray image analysis program, was used for image analysis and data extraction. Extracted data from all twin sets was compared using a standard correlation analysis.

Results from DNA solutions, Inc. indicated that each of the ten twin sets were identical with 99.9% confidence. For the ten sets of twins, the AbP-ID<sup>™</sup> correlations between twins ranged from 0.25 to 0.75. Other testing with unrelated individuals showed similar ranges of correlation. In contrast, technical replicates of the sera samples all correlated greater than 0.9. This supports the hypothesis that ISAs in identical twins are made randomly and have no greater chance of matching than in unrelated individuals.

Antibody Profiling, Identical Twins, Identification

#### A142 Sherlock Holmes and the DNA Likelihood Ratio

Mark W. Perlin, PhD\*, Cybergenetics, 160 North Craig Street, Suite 210, Pittsburgh, PA 15213

After attending this presentation, attendees will better understand the principles of reporting DNA match results using a likelihood ratio (LR). By framing the apparently obscure notions of the LR in a more accessible literary setting, forensic practitioners can develop increased comfort with this important reporting and testifying skill.

The presentation will impact the forensic science community by enabling practitioners to more comfortably use the LR in their DNA match reporting. Mastery of the LR principle is critical for reporting out the most informative results from complex DNA evidence. Society depends on this accurate LR information for apprehending and convicting criminals.

The principles of Bayesian inference (Bayes, 1763)<sup>1</sup> were well known to the Victorians (Jevons, 1874).<sup>2</sup> One master practitioner was detective Sherlock Holmes, who routinely employed a "balance of probabilities" to solve his fictional crimes (Doyle, 1890).<sup>3</sup> Holmes' deft use of the LR for weighing evidence (Good, 1950)<sup>4</sup> regularly astounded his compatriots. His clarifying insights into applied inductive logic are particularly relevant to the modern reporting of DNA evidence.

Chapter IV of the *"Hound of the Baskervilles"* (Doyle, 1902)<sup>5</sup> finds Holmes and his companions breakfasting at a London hotel. They are pondering the origin of a cryptic letter that warns the new Baskerville lord to stay away from his ancestral home, lest he too share the fate of his prematurely deceased predecessor. Holmes examines the handwritten address and announces that the letter was written in a nearby hotel. "Guesswork!" scoffs a skeptic. "Rather," rejoins Holmes, "we balance probabilities." His ensuing explanation is a lucid gem of likelihood clarity. Sherlock Holmes' same Bayesian logic underlies the scientific reporting of DNA evidence. The identification hypothesis asserts that a suspect contributed his DNA to some biological evidence. The alternative hypothesis avers that someone else was the contributor. The LR balances the probability of the evidence assuming the identification hypothesis, relative to the data probability under the alternative hypothesis. The resulting weight of evidence provides an objective numerical LR match score that focuses on the data and factors away prior prejudices.

This presentation presents the LR concept through the investigative eye of Sherlock Holmes. The presentation will work through a literary case example, illustrating every step of the LR determination using words and pictures. These concepts will then be applied to the reporting of complex DNA evidence, such as DNA mixtures.

Forensic DNA is an information science, with the LR providing unifying information metric for all interpretation methods. Every valid DNA match score is a LR. This presentation advances practitioner understanding of the LR concept and facilitates its comfortable presentation in courtroom testimony.

#### **References:**

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- <sup>3.</sup> Doyle AC. The Sign of Four. London: Spencer Blacket, 1890.
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- <sup>5</sup> Doyle AC. The Hound of the Baskervilles. London: George Newnes, Ltd, 1902.

DNA Reporting, Likelihood Ratio, Testifying Skills

#### A143 Performance of Statistical Approaches to Measure the Strength of DNA Evidence Exhibiting Possible Stochastic Effects

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After attending this presentation, attendees will appreciate how statistical approaches to assess the weight of DNA evidence perform when applied to samples exhibiting possible stochastic effects manifest as allelic drop-out. These conditions are frequently found in low-template samples or low-template components of mixed samples. Attendees will understand the conditions under which certain statistical approaches can, for example, over-state the strength of the evidence or lead to a false exclusion.

This presentation will impact the forensic science community by objectively exploring how commonly used simplistic approaches for interpreting challenging DNA samples perform in comparison to more rigorous approaches. The work presented provides a means to increase the accuracy and objectivity in interpreting DNA profiles.

Many biological samples recovered from crime scenes contain a limited amount of DNA. Because such samples contain relatively few copies of each locus, the random sampling of DNA molecules during the typing process may result in the failure to observe some alleles that are actually present. This phenomenon is known as allelic drop-out. The possibility of allelic drop-out can severely complicate the interpretation of forensic DNA profiles—even those obtained using the standard number of PCR cycles.

Several statistical approaches exist to assess the weight of DNA evidence for samples exhibiting stochastic effects. One strategy used, when only a single peak is observed at a locus, is to multiply the probability of sampling the observed allele from the population by two. This is called the "2p rule" and assumes that any other allele could be paired with the observed allele, but that this other allele has dropped out. A second strategy uses the standard random match probability, but omits those loci where allelic drop-out may be possible. In practice, loci containing any peaks below a pre-set stochastic threshold are excluded from the calculation. A third approach assesses the strength of the evidence using a likelihood ratio (LR). Extensions of the LR approach allow it to explicitly model and account for allelic drop-out in singlesource and mixed samples.

While most published studies and a majority of statisticians favor the LR approach, many forensic laboratories in the United States still use one of the other two approaches described above when allelic drop-out is possible, presumably for the sake of simplicity. However, because no objective study has compared the performance of the three approaches on the same evidentiary profiles, it is unclear how much valuable information is discarded when using the simpler approaches and how often the simplistic approaches might be misleading. This study seeks to fill that void by comparing statistics calculated using each of the three approaches for low-template DNA profiles in mock evidence for which the contributors are known.

For mock evidentiary profiles, this study used 60 single-source DNA profiles generated using the IdentiFiler system by Dr. John Butler's group at NIST. This dataset contains profiles obtained by amplifying 100 pg, 30 pg, and 10 pg of DNA from each of two individuals. For each of these 60 DNA profiles, LRs were calculated using each of the three previously described statistical approaches when the mock evidentiary profile originates from the person who contributed the reference sample. A good statistical approach to assess the weight of DNA evidence should produce a large LR under this situation. LRs were also calculated using each of the three statistical approaches when the mock evidentiary profile originated from a person other than the suspected contributor. Here the profile for the reference sample of the suspected contributor was simulated from a population frequency database. For a high-quality single-source profile, a good statistical approach should produce a small LR in this situation, ideally substantially less than one, indicating that the evidence supports an exclusion.

This presentation will include a comparison of the performance of each of the three statistical methods to quantify the strength of lowtemplate DNA evidence. Situations where these methods have the potential to dramatically mis-state the strength of the evidence will be highlighted. For example, omitting loci containing peaks below a stochastic threshold frequently understates the strength of the evidence against the true contributor by many orders of magnitude relative to the approach that models allelic drop-out in a LR framework. However, the 2p rule performs similarly to the aforementioned LR approach when the suspected contributor was the true contributor.

DNA Interpretation, Allelic Drop-Out, Likelihood Ratio

## A144 Punch-Out: An Evaluation of Six Collection **Devices for Databasing**

Craig Nolde, BS, Timothy D. Kupferschmid, MFS\*, and Darren Warnick, PhD, Sorenson Forensics, 2495 South West Temple, Salt Lake City, UT 84115

The goal of this presentation is to provide a comparison of six buccal collection devices currently used for DNA databasing.

This presentation will impact the forensic science community by providing important details on the robustness and ability of each collection device to collect and preserve DNA for forensic testing and convicted offender databasing.

Uploading convicted offender DNA information into criminal databases is an increasingly important and useful aspect of forensic DNA casework. Many times important new leads are generated in "cold cases" and other scenarios when a DNA profile from a crime scene produces a "hit" when searched against a database of convicted offender DNA profiles. DNA isolated from oral buccal cells is the most widely used material employed to generate offender genotype profiles. A diverse range of commercial devices are available to collect convicted offender samples and capture the DNA on solid support matrices and many contain proprietary chemical formulas that assist in lysing cells, denaturing proteins, or otherwise preserving the DNA sample until it can be processed in the lab. Along with the wide variety of commercial devices available that can be utilized to obtain these samples, there is significant variability in the procedures used to collect and store them. Additional sample variation can result from the disparate capabilities and level of training of those who collect convicted offender samples. Because the samples themselves are usually high quality, single source material, various automated methodologies are employed to process them. As a result, it is imperative that the device used to collect convicted offender samples be able to obtain enough DNA to overcome the variability not only in the "shedding" characteristics of the donor but in sample collection techniques as well. It must also be able to preserve the DNA through different storage conditions, be amenable to various processing techniques, and release enough of the DNA to generate a good quality profile. This presentation describes a study comparing the quantity and quality of DNA obtained under various conditions from the Whatman EasiCollect, Bode Buccal Collector, foam applicator with FTA cards, foam applicator with 903 cards, OmniSwabs, and Puritan cotton swab collection devices. The study examined variability of total DNA yield in replicate samplings from the same donors over several days and reproducibility of those yields from each device. Each device was also evaluated in its ability to generate a suitable DNA profile that meets applicable CODIS Database loading criteria (FBI QAS, NDIS Guidelines, etc.) using traditional organic extraction, quantification, and STR amplification methods as well as direct STR amplification and "punch in" techniques. Known samples were collected using each device from 30 different donors to evaluate the ability to generate suitable DNA profiles from a variety of donor 'types'. Furthermore, in an attempt to simulate some of the variability encountered in normal storage, DNA quantity and quality from each device were examined after allowing the devices to air dry for various lengths of time before processing as well as storage for different time periods. Devices were subjected to accelerated aging conditions to simulate long term storage and UV exposure to measure resistance to DNA damage. In this study all DNA yields were determined using the Applied Biosystems (AB) Quantifiler<sup>TM</sup> Human real time PCR chemistry and profiles were generated using the AB Identifiler<sup>TM</sup> or Identifiler Direct STR amplification kits. This study provides important details on the robustness and ability of various commercial devices to collect and preserve DNA for forensic testing and convicted offender databasing and may assist laboratories in their evaluation of a collection device to employ in their databasing efforts.

**DNA**, Database, Collection

#### A145 Pyrosequencing Analysis of DNA-Tagged **Cash-in-Transit**

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After attending this presentation, attendees will understand how synthetic DNA is utilized in allowing valuable items or cash to be traced.

This presentation will impact the forensic science community by discussing a growing field with increased use in the security area that will become more important.

Valuable property is often security marked regardless of the material. Security marking is especially interesting when it comes to cash in transit and ATM machines, where a vast amount of money is handled. One method is to stain the money with either ink dye or dye and smoke. A further step is to add unique synthetic DNA tags to the dye. Stolen money that is DNA-tagged can be traced back to its original location. This system is already being used in many countries.

Trace Tag, Inc. assay has been used for analysis of tags from individual bank notes to assist the law enforcement in robbery investigations. In short, the extracted DNA is PCR amplified and the short DNA sequences are determined by pyrosequencing technology. The result from each bank note is sent to Trace Tag International (TTI) in the United Kingdom where a database is used for identification of the code and the serial number of the sequence. The code is thereafter submitted to 3SI security systems, in Belgium, which identifies the customer, the location, and the installation of the unique DNA tag.

The successful analyses of unique DNA tags and the identification through TTI and 3SI show that it is possible to trace the bank notes back to specific cash in transit bags, ATM machines, and their staining device. This identification will provide an asset to overall security and has the

potential to be applicable on a larger scale in the near future.

DNA Analysis, Pyrosequencing, Sequrity Labelling

#### A146 Expert System Rules for Mitochondrial DNA Sequence Analysis

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After attending this presentation, attendees will understand the need for increased automation and standardization in forensic mtDNA sequence analysis and how software programs can function as an expert system to facilitate objective, high-throughput sequence data analysis.

This presentation will impact the forensic science community by highlighting a need for increased consistency and throughput of mtDNA data analysis and demonstrating current advances in expert system development that will potentially offer a solution.

Mitochondrial DNA (mtDNA) analysis has proven to be an invaluable tool for victim identification in mass disasters, missing persons programs, and criminal casework. The University of North Texas Center for Human Identification, primarily funded by the National Institute of Justice (NIJ) for the Missing Persons Program, uses advanced DNA technologies to process unidentified human remains and the family reference samples from biological relatives for both nuclear DNA and mtDNA. Since most missing person cases rely heavily on mtDNA testing of skeletal remains, mtDNA testing of appropriate family reference samples is necessary for recommending familial associations. The resulting DNA profiles are uploaded to the Missing Persons Index database. In this database, mtDNA and nuclear DNA profiles from the unidentified remains can be searched against the biological family reference profiles and associations are recommended through kinship analysis testing. There are thousands of missing persons cases reported each year, with more than 14,000 unidentified human skeletal remains stored in medical examiners' and coroners' offices nationwide. These numbers alone demonstrate the throughput needs for DNA processing.

Sample processing methods are continually improving with advances in liquid handling robotics, chemistry, and instrumentation. Consequently, the rate of data generation exceeds that of data analysis, review, and reporting, hence creating a bottleneck in the final upload of data and reporting. The bottleneck in STR data analysis was specifically addressed by NIJ's Expert System Testbed Project, which emphasized the use of expert system technologies for STR data analysis. The resulting implementation of such software programs has augmented backlog reductions across the country. However, there currently is not a software program or package that is capable of automating the more complex process of mtDNA sequence data analysis. This presentation discusses the unique challenges of automating mtDNA sequence data analysis and the solutions that are in development at the University of North Texas Center for Human Identification.

Three primary challenges in analyzing mtDNA data are: (1) managing the volume of sequence data generated, which greatly exceeds that of STR analysis; (2) base calling, which is more subjective than allele calling; and, (3) assigning haplotypes, which by nature is subject to inconsistency between individual analysts as well as laboratories. Initial software package developments have been made to address each of the three challenges while providing a workflow interface which facilitates laboratory processing and data management. Expert system rules have been designed to automate analysis at the trace level, the contig level, and the haplotype level, including new advances in eFAST<sup>™</sup> Software (University of North Texas Health Science Center, Ft. Worth, TX), the front-end program designed to expedite trace quality assessment. Unique trace-level expert system rules such as High Baseline (HB), Mixture (M), Length Heteroplasmy (LH), Homopolymeric Stretch (HPS), and Low Signal (LS) have been programmed and tested to increase the consistency and throughput of trace quality assessment. For example, High Baseline alerts the analyst if a trace has underlying signal that exceeds a userdefined threshold. The Mixture rule fires to alert the analyst that a trace contains a number of mixed bases that exceeds the user-defined threshold. The Length Heteroplasmy and Homopolymeric Stretch rules fire when the indicated anomaly is encountered in the sequence trace and eFAST Software will then adjust the Contiguous Read Length expectation. Such rules not only increase the accuracy and resolution of the filter metrics, but also give the analyst valuable information at a glance to guide further sample processing. These developments are the first steps towards providing the forensic mtDNA testing community with a comprehensive expert system that seamlessly takes the analyst from data collection through haplotype reporting in order to increase the accuracy and efficiency of mtDNA data analysis and management.

mtDNA, Expert System, eFAST

#### A147 Development of a Model National Code of Ethics for Forensic Scientists

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The goal of this presentation is to present to the attendees a draft Code of Ethics for forensic scientists which has been developed by the California Association of Criminalists. It is hoped that the presentation will generate a discussion which will inform the development of the national code of ethics called for by the 2009 NAS Report.

This presentation will impact the forensic science community by disccussing the National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, which calls for the development of a national code of ethics for the forensic sciences. A draft of such an ethics code has been developed by an ad hoc committee of the California Association of Criminalists (CAC) from a review of a large number of ethics codes of various forensic science organizations. The draft code will be presented to attendees in order to further develop a consensus code of ethics which can serve as the national code of ethics called for in the NAS Report.

The National Academy of Sciences (NAS) report, *Strengthening Forensic Science in the United States: A Path Forward*, calls for the implementation of a process that would require certification of all forensic scientists who would then be subject to a national code of ethics which would be enforced as part of the certification process. The implementation of the NAS Report's recommendations would mean that "No person (public or private) should be allowed to practice in a forensic science discipline or testify as a forensic science professional without certification" (NAS Report, Chapter 7, Recommendation 7). And further, all practitioners in the forensic sciences would have to be certified by a professional organization which would "incorporate this national code as part of their professional code of ethics." The provisions of the ethics "code could be enforced through [the] certification process" (NAS Report, Chapter 7, Recommendation 9).

The development of a national ethics code for forensic scientists as called for by the NAS Report is a daunting proposition. To be useful, an ethics code must serve as a guideline to practitioners for appropriate action in various situations. In addition, it must serve as a document against which actions of a practitioner can be judged and, if found inappropriate, act as a basis for appropriate sanctions. Whether or not an ethics code that is acceptable to all individuals or professions involved in the practice of forensic science is practical remains to be seen. Such an ethics code must not only be based on principles that are generally agreed on by all, but must include specific provisions that serve as guidelines to appropriate professional behavior for all practitioners. A draft ethics code has been developed based on a review and comparison of a large number of ethics codes of various organizations (Gannett, Carolyn, "Survey: I Don't Need No Stinkin' Ethics Codes," CACNews, Second Quarter, 2009, pp. 23-28). This draft ethics code will serve as a basis for further discussion to determine if a consensus document can be developed which will be acceptable to all stakeholders, including practitioners in diverse areas of forensic science, users of the services of forensic science practitioners, laboratory and agency managers, and others. The purposes of this document are: (1) to provide principles and rules for one's own conduct; (2) To provide a template against which to evaluate others' professional actions; (3) to offer protection of the individual if asked to perform unethical acts; and, (4) to ensure the community (colleagues, the justice system, and the general public) of uniformity and quality of service. The draft ethics code consists of sections dealing with both the practice of forensic science and the management of forensic science operations. For the practitioner, specific guidelines for appropriate behavior are set forth under general principles such as honesty, confidentiality, fairness, or forthrightness, along with a general statement for each about why the general principles are important. For the manager, general responsibilities to employers, employees, agencies, the profession, and the public are set forth along with specific provisions for appropriate actions to be taken in each of these areas.

**Ethics, Professionalism, NAS Report** 

# A148 Effects of Matrix Interference, Weathering, and Thermal Degradation on the Association of Ignitable Liquid Residues to Neat Ignitable Liquids

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After attending this presentation, attendees will understand the use of objective multivariate statistical procedures for the identification of ignitable liquid residues (ILRs) in simulated fire debris. A combination of Pearson Product Moment Correlation (PPMC) coefficients, principal components analysis (PCA), and hierarchical cluster analysis (HCA) are used to demonstrate the identification of ILRs in the debris despite the presence of matrix interferences, weathering effects, and thermal degradation.

This presentation will impact the forensic science community by providing a more objective method for the analysis of fire debris, which will help to minimize the chance of misidentifying the presence or the absence of an ignitable liquid. This method also provides a statistical means of assessing the association of the ILR to the neat liquid. Such statistical associations are consistent with the recommendations made by the recent National Academy of Sciences report.

Gas Chromatography - Mass Spectrometry (GC-MS) is a very common technique used in the analysis of fire debris. This type of analysis aims to associate an ILR extracted from the debris to a neat liquid in a reference collection by a visual examination of the resulting chromatograms. However, the association of the ILR to the neat liquid can be affected by factors such as matrix interferences and weathering of the ignitable liquid. Thus, the interpretation of chromatographic data gained from GC-MS analysis can be both challenging and subjective.

This research aims to develop an objective method for associating the ILR to the neat liquid using three multivariate statistical procedures: (1) PPMC coefficients; (2) PCA; and, (3) HCA. PPMC coefficients are used to place a numerical value on the similarity of two chromatograms, while PCA is used to discriminate between samples by identifying the greatest source of variance among the samples, allowing for both discrimination and association. In this research, HCA is used to provide a statistical measure of the discrimination and association shown in the PCA data. The combination of several statistical procedures maximizes the potential of successful associations between ILRs and neat liquids, while enabling a statistical measure of the associations.

The first step of this research was to investigate interferences caused by common household matrices. Four matrices (e.g., nylon carpet, upholstery, denim, and glossy magazine paper) were charred using a propane blow torch for different amounts of time ranging from 10 to 120 seconds. The matrix was placed in a nylon bag and extracted using a passive headspace extraction procedure with activated carbon strips, which were then analyzed by GC-MS. The burn time for future experiments was chosen as the time that generated the maximum amount of matrix interference, based on a visual examination of the chromatograms.

Next, the effect of matrix interferences on the association of evaporated liquids to corresponding neat liquids was investigated. Three ignitable liquids (gasoline, kerosene, and diesel) were evaporated to 10% and 90% by volume. The evaporated liquids and the neat liquids were spiked onto separate burned subsamples of each matrix. Spiking the liquids onto the already burned matrix ensured that only the effects of weathering were being investigated, with no contribution from thermal degradation due to burning. A combination of the multivariate statistical procedures provided a statistical measure of the association and discrimination of the evaporated liquid with matrix interferences to the corresponding neat liquid.

In addition to the effects of matrix interferences and evaporation, the effects of thermal degradation on the ability to associate an ILR to the corresponding neat liquid were also investigated. Fire debris was simulated by spiking separate samples of each of the four matrices with each of the three neat liquids. The matrices were then burned to generate significant matrix interferences. The simulated ILR was extracted and analyzed as previously described. PCA, PPMC coefficients, and HCA showed that simulated ILRs can be associated to a neat liquid, despite thermal degradation and evaporation of the liquid, as well as the presence of matrix interferences.

Arson, Multivariate Statistics, Ignitable Liquids

# A149 Interdisciplinary Sampling and Analytical Methods Utilized for the Confirmation of Contaminants From Non-Routine Fire Debris

Kelly L. Wouters, PhD\*, Andrew T. Armstrong, PhD, Marion K. Armstrong, MSPH, and Jeremy D. Rummel, MS, Armstrong Forensic Laboratory, Inc., 330 Loch'n Green Trail, Arlington, TX 76012

After attending this presentation, attendees will have a better understanding of how alternative sampling and analytical techniques can be used to supplement the current established methods of fire debris analysis. Common validated sampling methods will be discussed as well as case studies demonstrating their applications.

This presentation will impact the forensic science community by illustrating how methodologies from other investigative fields can be adapted to forensic analyses. Such methodologies are well suited for the field of fire investigation.

The utilization of ASTM standard practices and methods for the analysis of fire debris has long been recognized as the method of choice for fire loss investigations. Standard fire debris loss investigations involving routine debris samples have been well served with these sampling and analytical methods. The confirmed presence of an ignitable liquid in the living room of a home can be an indication that the fire was not accidental. However, for a fire loss where source identification for the purpose of assessing liability is necessary, typical fire debris sampling and analytical techniques may not be sufficient to achieve the sensitivity and selectivity required. Such losses may require atypical sample collection and alternative analytical methodologies outside the discipline of fire investigation.

Ongoing research has shown that the application of analytical methods typically reserved for disciplines outside fire investigations (e.g., environmental and industrial hygiene chemistry) can provide additional information that may lead to confirmation of the presence of ignitable liquids or other relevant contaminants in atypical fire debris matrices.

In typical fire debris analyses, the presence of a product or chemical mixture is usually reported and it is not necessary to report the individual compounds identified in the debris. Moreover, the concentrations of the components detected in the debris are rarely addressed when reporting the results of fire debris analyses. When these data are relevant to the investigation, EPA and NIOSH analytical methods can be used to provide specific component identification and quantitative analytical results.

EPA methodologies including: 8015, 8021, 8260, and 8270 and NIOSH Methods 1501 and 1550 are well suited for the detection and identification of low-level concentrations of ignitable liquid components. Benzene, toluene, ethyl benzene, and xylenes (BTEX), as a group of components, are routinely analyzed through EPA and NIOSH methods from soil, water, and air for environmental and industrial hygiene evaluations. Each of the methods presented is a nationally recognized published and validated method.

The use of interdisciplinary analytical methodologies in the evaluation of evidence collected from the fire scene can provide additional insight into the presence of ignitable liquids or contaminants. The environmental and industrial hygiene professions have established analytical methods for recovery of hydrocarbons and other solvents from matrices typically considered non-routine on a fire loss including: water, concrete, soil, surfaces, and air. These matrices are often present at the fire scene and available for sampling by the investigator. The samples of interest may include air conditioner condensate, liquid runoff, floor drain liquids, soot deposits, and other bulk and surface samples.

The use of interdisciplinary sampling and analytical methods can significantly improve the information available to a fire loss investigator. The established environmental and industrial hygiene sampling and analytical methods, by design, can be utilized to detect a wide variety of compounds that may contribute to or enhance fires. Case studies where alternative sampling and analytical techniques were utilized to investigate fire losses will be presented. In these investigations, additional methodologies were utilized to identify contaminants and establish the source of combustible materials that were associated with fires and fire losses.

Fire Debris, Interdisciplinary Techniques, Analytical Methods

# A150 Evaluation of a Novel Methodology for the Recovery of Volatiles From Fire Debris Samples

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After attending this presentation, attendees will understand the difficulties of recovering low molecular weight volatiles from fire debris samples and the need for the development of a new method for detecting these products. Attendees will learn about a new technique which seeks to improve the recovery of volatiles from fire debris and may be used to compliment the carbon strip extraction method.

This presentation will impact the forensic science community by evaluating a new methodology for the recovery of acetone and low molecular weight alcohols from samples collected during arson investigations. This research study aims to optimize adsorption and desorption of volatiles in comparison to the commonly used activated carbon strip.

Within the field of fire debris analysis, one of the most common methods for the extraction of traces of ignitable liquids from evidence samples is the passive headspace technique. This technique, as outlined in ASTM Standard Practice E-1412-07, employs the use of a charcoal based adsorption medium to capture volatile components from the headspace above a heated sample. As this procedure is carried out over a given period of time, it allows for the concentration of a representative sample of volatile compounds within the sample. Once collected, the compounds are easily eluted with a suitable solvent and analyzed using gas chromatography/mass spectrometry (GC/MS) for the potential identification of any ignitable liquid residues that may be present. This is a very sensitive technique that is capable of capturing minute amounts of volatile compounds.

While this technique has been well accepted in the fire debris analysis community, the difficulty of recovering volatiles from fire debris samples is an area in need of improvement. Given carbon's higher affinity for heavy molecular weight products over low molecular weight products and hydrocarbons over alcohols, the detection of low molecular weight alcohols and acetone is severely compromised. Previous research has shown a reduction in the recoverability of acetone and low molecular weight alcohols in the presence of heavier molecular weight products. This study seeks to develop a new methodology for fire debris analysis that may compliment the carbon strip method by having a greater affinity for low molecular weight alcohols and acetone. This new methodology utilizes zeolites for the adsorption and identification of volatiles from fire debris samples. Zeolites are both naturally occurring and synthetically prepared crystalline aluminosilicate mineral structures with uniform molecular sized pores. They are stable at high temperatures and can be used as catalysts or molecular sieves due to their unique pore sizes, which determine their application. The pores are formed from the tetrahedral framework of Si, Al, and O atoms and allow molecules to adsorb on the internal surface area of the structure based on the size of the pores as well as the polarity of the zeolite. The objective of this study is to evaluate the application of zeolites for the adsorption of volatiles from fire debris samples and determine the ease with which any retained compounds can be subsequently desorbed with an appropriate solvent for analysis using GC/MS.

Forensic Chemistry, Fire Debris Analysis, Volatiles

# A151 The Application of Self Organizing Feature Maps (SOFM) for Lighter Fuel Classification

Wan N.S. MatDesa, MSc\*, Niamh NicDaeid, PhD, Dzulkiflee Ismail, MSc, and Kathleen Savage, PhD, Centre for Forensic Science, University of Strathclyde, Glasgow, G11XW, UNITED KINGDOM

The goal of this presentation is to introduce the application of selforganizing feature maps (SOFM), an unsupervised artificial neural network method to classify ignitable liquids from petroleum based products. This presentation will also demonstrate the powerful ability of Self Organizing Feature Maps (SOFM) to associate highly weathered samples to their associated un-weathered products.

This presentation will impact the forensic science community by providing an alternative to relatively more conventional chemometric analyses such as Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) for pattern recognition purposes. The presented method helps to alleviate subjectivity issues when analyzing ignitable liquid samples in forensic arson investigations and can be potentially used for possible source identification.

Ignitable liquids are commonly used as accelerants to intensify and accelerate the rate of fire development in cases of deliberate fire setting. At present, gas chromatography-mass spectrometry (GC-MS) is widely accepted as an effective tool for the analysis and identification of ignitable liquids. Interpretation of the instrumental data and sample classification processes are based mainly on visual comparison. These methods can be difficult, time consuming, highly subjective, and rely heavily on the skill and experience of the analyst. Exposure to heat or evaporation due to aging can result in substantial changes in the ignitable liquid's composition, which in turn greatly affects their chromatographic profile. Another common complication encountered in fire debris analysis is the appearance of hydrocarbon by-products from the combustion and pyrolysis of background matrices.

The applications of multivariate pattern recognition to discriminate and classify ignitable liquid samples are suggested as a means of facilitating the pattern matching process and rendering it less subjective. Pattern recognition techniques using unsupervised approaches have been utilized to establish the underlying relationships amongst variables within complex datasets and can be used to self-organize established groups within a given dataset.

This work starts with the optimization of GC-MS temperature programming as recommended by ASTM E1618. Various brands of lighter fuel were obtained from commercial markets. For each lighter fluid brand, a set of partially evaporated samples was generated at approximately 10, 25, 50, 75, 90, and 95 percent weathered (evaporated) sample. The lighter fluids were analyzed by GC-MS and the resulting total ion chromatograms (TICs) were investigated. Upon examination of the TIC profiles, components selected as variables from all samples were pre-processed to assess the effectiveness of the method. The data was then processed using various chemometric techniques in order to distinguish between the various lighter fluid brands and whether it was possible to establish a link between the pure and evaporated samples of a specific lighter fluid brand.

Successful brand identification for highly weathered samples was achieved using HCA and SOFM. However, SOFM proved to have a more robust sample linkage capacity and confirmed visual similarities and differences between the samples in evidence within the chromatograms. It is also important to note that data pre-treatment was essential in order to obtain accurate classifications. This has demonstrated a potential means whereby pure and evaporated ignitable liquid samples can be linked successfully by brand and, as such, presents a powerful new means of interpreting chromatographic data retrieved from fire debris samples. **SOFM, Ignitable Liquids, Pattern Recognition** 

# A152 Investigation and Separation of Interferences From Fire Debris Analysis by Solid Phase Extraction

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After attending this presentation, attendees will understand a novel approach to clean up fire debris analysis by removing polar interferences by the use of solid phase extraction after performing a passive headspace extraction

This presentation will impact the forensic science community by making identification of accelerant use in suspected arson fire by eliminating interferences that occur during the process of a fire. This method can be done easily and quickly after passive headspace extraction when analysts are confronted with complex chromatograms that are overwhelmed by interferences.

Arson investigation is one of the most difficult challenges that forensic scientists can encounter due to the destruction of forensic evidence. Interferences are often seen in analysis of such evidence due to substrate background, pyrolysis, and combustion products. These interferences result in rather noisy chromatograms when analyzed by gas chromatography (GC) or gas chromatography mass spectrometry (GC/MS) and if the interferences overwhelm the chromatogram, a proper identification of an ignitable liquid, if any is present, cannot be done. A new method has been developed to separate out these interferences by use of solid phase extraction (SPE). Through the implementation of SPE, an extracted arson sample could easily and quickly be run through a SPE cartridge, which will retain any polar interference compounds in the sorbent and elute any accelerant compounds present, resulting in a cleaner chromatogram.

This new method has been applied to sets of compounds representative of matrix interferences, pyrolysis products of man-made polymers, and petroleum distillates. Several Waters Sep-Pak® SPE cartridges were tested, specifically Silica, Alumina N, Aminopropyl, Cyanopropyl, and Florisil®. The Silica and Aminopropyl cartridges worked the best to separate out the representative interference compounds, as seen in analysis by gas chromatography flame ionization detection (GC-FID). Both cartridges successfully removed eight out of the nine representative interference compounds. Twenty ignitable liquids, representing a broad spectrum of petroleum based acceleratants and both locally obtained and selected from the National Center for Forensic Science, were also extracted and spiked on to the SPE cartridges. No significant difference was seen between the extracted accelerants before and after SPE. A Column Resolution Check Mix, as described in ASTM E1387, was also spiked onto the SPE cartridges. Subsequent analysis and comparison of the analytical data obtained from the neat check mix and the SPE treated check mix disclosed little variation. This method was also applied to ten common household items that were burned with and without an ignitable liquid. Based on the analytical results obtained thus far, it is believed that this method is desirable and could easily be employed routinely by fire debris analysts.

Fire Debris Analysis, Solid Phase Extraction, Interferences

#### A153 The Effects of Carbon Disulfide on the Elution and Solvation Phases of Light and Medium Range Ignitable Liquids

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After attending this presentation, attendees will learn that activated charcoal strip elution by  $CS_2$  occurs immediately once the  $CS_2$  comes in contact with the activated charcoal strip. Attendee will also learn that light and medium range ignitable liquids have a lifespan of one month when dissolved in the solvent,  $CS_2$ .

This presentation will impact the forensic science community by demonstrating that elution by  $CS_2$  occurs immediately upon contact with the charcoal strip. Also, after attending this presentation, attendees will know that neat ignitable liquids dissolved in  $CS_2$  are stable compounds for one month. Therefore, fire debris analysts will be able to improve their practice by keeping their standards for up to one month under proper conditions instead of remaking a new standard for every case. This will save supplies, time, and laboratory finances.

The objective of this research was to understand the effects of  $CS_2$  on the elution and solvation phases of ignitable liquids over time. All data was collected and analyzed by GC/MS. Statistics were used to determine differences and/or similarities among the samples over time. An internal standard, tetrachloroethylene at a concentration of 100 ppm, was used to compare samples.<sup>1</sup> This particular internal standard was used because: (1) its retention time falls within the middle of the ranges for all the ignitable liquid categories tested; and, (2) it allowed for normalization of the data.

It has been shown that  $CS_2$  is the universal solvent for activated charcoal strip elution.<sup>2</sup> The elution study conducted explored how well CS<sub>2</sub> completely extracts ignitable liquid residues (ILRs) from an activated charcoal strip. This study was comprised of a series of substudies: a vortex study, an elution period study, and a washing study. The first sub-study explored whether vortexing for one minute was necessary for lab procedures by varying the vortex time from no vortex to a one minute vortex. The second sub-study explored if a 15 minute waiting period was necessary for complete elution to occur by varying the waiting period after a one minute vortex from no waiting period to a waiting period of one hour. The third sub-study determined if CS<sub>2</sub> immediately extracted any ignitable liquid residue (ILR) from an activated charcoal strip and whether immediate extraction occurred for all ILRs or only certain types. This washing study was completed by rinsing three charcoal strips with varying amounts of CS2 with neither a vortex period nor a waiting period.

The second part of the time study, the solvation study, explored the evaporation pattern of ignitable liquids in CS<sub>2</sub> solution over a one month period. The ignitable liquids tested included light petroleum distillates (LPD), gasoline (GAS), medium petroleum distillates (MPD), and medium range isoparaffins (ISOP). Two milliliters of a 1% ILR solution v/v in CS<sub>2</sub> were prepared for each sample. The time intervals tested were immediate sampling of IL/ CS<sub>2</sub> solutions after preparation, and sampling of each of the solutions after one hour, one day, one week, two weeks, three weeks, and one month. Samples were stored in a -10°C freezer between removals for analysis.

Three different statistical tests were used to determine differences or similarities among the samples in each study over time. These tests included a Tukey Honestly Significant Difference (HSD) test, Principle Component Analysis (PCA) with cluster analysis, and Pearson Correlation with a Student's t-test and ROC plots. The Tukey test demonstrated that minimal differences occurred among samples in all Based on research thus far, a waiting period is not necessary for complete elution to occur;  $CS_2$  immediately extracts residue from the activated charcoal strip. From the solvation study, standard light and medium range ignitable liquids dissolved in  $CS_2$  can be stored under normal conditions in a freezer for up to one month without evaporating or chromatographic skewing.

**References:** 

- <sup>1.</sup> Lentini JJ. Scientific Protocols for Fire Investigation. Boca Raton: CRC Press, 2006. 150.
- <sup>2</sup> Dolan JA, Newman R, Stauffer E. Fire Debris Analysis. Burlington: Elsevier, Inc, 2008. 399-439.

**Carbon Disulfide, Elution, Solvation** 

# A154 Performance Testing and Comparison of Different Fire Debris Bags

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After attending this presentation, attendees will know that a good alternative polymer bag has been found for the collection of fire debris samples for ignitable liquid analysis, as a replacement of the Kapak FireDebrisPAK<sup>TM</sup> bag that was taken of the market several years ago.

This presentation will impact the forensic science community by teaching more about the performance (advantages/disadvantages) of different types of commercially available fire debris bags.

World-wide, different containers are used to collect and store fire debris evidence for ignitable liquid analysis. A questionnaire circulated among the forensic institutes in Europe revealed the use of metal cans, glass jars, and a wide variety of polymer bags. Previously published tests results showed that the polymer bag produced by Kapak was the best choice of all but because this bag was taken from the market several years ago, previous Kapak FireDebrisPAK<sup>TM</sup> users are searching for an alternative bag ever since.

A good alternative bag seems to have been found due to the release of a new fire debris bag in 2010. This bag has a similar polymer composition as the Kapak FireDebrisPAK<sup>™</sup> bag. The performance of this new bag material has been studied and compared to the performance of the most commonly used polymer bags in Europe today. These are nylon-11 bags and two multilayer bags (four layers or more) with different layer composition, one of these multilayer bags is aluminium coated. The bags were tested on the presence or absence of background interference and on the retention ability of ignitable liquids. The latter involved a study of potential leak rate, of potential cross-contamination, of potential adsorption ability, and of the recovery of the ignitable liquids spiked. For the initial tests, gasoline was used and the performance of the bags was monitored over a period of seven weeks. The test results were obtained after dynamic headspace sampling on Tenax, followed by TD-GCMS analysis. The results demonstrate that the newly released bags perform best in all tests: they exhibit lowest background, do not leak, show no cross-contamination, and do almost not adsorb the gasoline spiked. A full gasoline pattern is recovered from the spiked bag, even after seven weeks and even when the spiked concentration is high. The nylon bags also do not release background compounds, but started

leaking several days after spiking which results in cross-contamination of other nylon bags. Both multilayer bags release interfering background compounds when heated at 70 degrees Celsius and also leak, but no cross-contamination is detected. The nylon and multilayer bags all adsorb the gasoline to a certain extent.

The newly released fire debris bag was additionally tested with the oxygenated product denatured spirits. As this product mainly contains ethanol, the analysis did not involve Tenax TD-GC/MS, but involved direct headspace GC/MS instead. The test results showed that the bags also perform well for these types of ignitable liquids.

For the performance tests, the bags were closed by a heat seal. As heat sealing is not easy to perform on-site, other closing techniques using tape, tie-rap, and plastic clamp sealing strips have been studied. The results demonstrate that the clamp sealing strips are easy to use and only start to leak steadily about six hours after closing. This offers the fire investigators enough time to bring the samples either to their office or straight to the laboratory to be heat sealed prior to the ignitable liquid analysis.

Fire Debris Bag, Performance Testing, Ignitable Liquid Analysis

# A155 The Statistical Significance of Common Household Dust Specimens

Nicholas D.K. Petraco, PhD, John Jay College of Criminal Justice, Department of Science, 899 10th Avenue, New York, NY 10019; Nicholas Petraco, MS\*, 73 Ireland Place, Amityville, NY 11701; Craig L. Huemmer, MS, New York City Police Lab, 150-14 Jamaica Avenue, Jamaica, NY 11432; and Mary Eng, BS, and Kristy Sekadat, MS, New York City Police Department, 150-14 Jamacia Avenue, Queens, NY 11432

After attending this presentation, attendees will understand the significance of household dust specimens and its usefulness in enabling forensic investigators in making Locardian type associations between the people, places, and things involved in a crime.

This presentation will impact the forensic science community by helping to establish the statistical significance and error rate of a common form of trace evidential material often collected at scenes of crimes.

This paper discusses the statistical significance in comparisons of household dust specimens. The results of a microscopic study of over 300 household dust specimens obtained from homes and apartments encompassing the New York City Tri-State region are reported.

The procedure begins with a preliminary visual and stereomicroscopic examination of each dust specimen. The contents of each dust specimen are sorted with the aid of a stereomicroscope into groups of similar looking hairs, fibers, and particulate materials, depending on the composition of each dust specimen's composition. Next, each group is further subdivided into smaller subsets based on their macroscopic physical appearance, primarily color and morphology. Each fibrous and/or particulate subset is mounted on a 7.5 cm x 5 cm microscope slide in High Dispersion (HD) refractive index oil. Finally, each fibrous and/or particulate subset aliquot is characterized and identified utilizing stereo and polarized light microscopy (PLM). Three aliquots were examined from each household dust specimen. Particulates too large to be mounted for PLM examination were studied with other forms of analytical instrumentation, i.e., FTIR, XRF, XRD.

The resulting information for each mounted subset was collected on a dust tabulation sheet specifically designed for this study. Next, the data was subjected to principal component analysis, support vector machines and partial least squares discriminant analysis in order to build statistical models of the composition profiles. These statistical models were then subjected to a test set of randomly selected unlabeled dust samples in order to compute estimates of misidentification rates (error rates). The methods used to compute these error rates were hold-one-out cross validation and bootstrapping.

Statistical, Household Dust, Microscopy

## A156 Statistical Methods for Determination of Sample Sizes: The Bayesian Method

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After attending this presentation, attendees will understand the principles of the Bayesian method for determining the size of a sample from a population in the context of drug analysis. The drug analyst will be able to explain each of the variables in the Bayesian distribution, calculate the sample size required for a given confidence level and percent of positive samples, and relate the sample size calculated with the Bayesian method to those calculated by the binomial and hypergeometric distributions.

This presentation will impact the forensic science community by presenting an explanation of the Bayesian method for calculating the sample's size at a level appropriate for expert witness testimony in court, providing an understanding that will make it more easily accessible for use in laboratories.

The Bayesian Approach incorporates subjective logic, in which probabilities take into account subjective beliefs about the probability of events. The subjectivity aspect of the Bayesian approach is an advantage; smaller samples are calculated when the analyst's experience indicates that positives are likely. And the opposite is also possible. Larger sample sizes can be calculated when experience leads the analyst to believe that the unknown may not be an illicit drug. However, subjective methods in forensic science have lately come under fire and, finally, the relative complexity of the method itself may provide a barrier to use in the crime lab.

The basic equation for Bayes theorem is:  $P(A|B) = \frac{P(B|A)P(A)}{P(B)}$ 

Bayes theory uses condition probabilities, denoted by a vertical line. Therefore, the above equation states the probability that A is true, given that B is true, can be calculated if the probability that A is true, the probability that B is true, and the probability that B is true given that A is true are all known.

This is often rewritten as:  $P(\theta|\chi) \alpha L(\theta|\chi)p(\theta)$  where  $L(\theta|\chi)$  is the likelihood function (the binomial distribution in the case of drug sampling) and  $p(\theta)$  is the prior distribution. The prior distribution represents uncertainty about knowledge of  $\theta$ , the proportion of positives in the population. For the prior distribution, a beta function with parameters *a* and *b* are used. The beta distribution is used in subjective logic, in which probabilities take into account subjective beliefs about the probability of events. The parameters *a* and *b* represent the likelihood of "positive" (*a*) or "negative" (*b*) outcomes.

Guidelines set by the European Network of Forensic Science Institutes (EMFSI) Drugs Working Group recommend that the lower parameter should be set to one. If experience has shown that all 1.15 lb. bricks of plant material are marijuana, then b is set to one and a is given the value of three or even 10. When there is no prior knowledge, a and bare both set to one and the calculation equals the binomial distribution.

Plots of the sample size as a is held constant and b increases and as b is held constant and a increases, shows that the decrease in sample size with increasing a is linear, while an increase in sample size for increasing b is not. Finally, the sample sizes calculated by the Bayesian approach will be compared to those calculated by the frequentist methods: the binomial distribution and the hypergeometric distribution.

**Bayesian Approach, Statistical Sampling, Illicit Drugs** 

## A157 The Statistical Evaluation of Torn Duct Tape Physical Matches

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After attending this presentation, attendees will gain information on current research in the field of duct tape physical matching; this includes the uniqueness of duct tape end matches, statistical data and error rates associated with the analysis of duct tape end matches, the influence of various factors on false positive and false negative errors during duct tape end matching, and the development of a criteria for what constitutes a physical match.

This presentation will impact the forensic science community by establishing the reliability of duct tape end matching as an analytical technique. The statistical analysis on the error rates associated with duct tape end matching and the development of a criterion for what constitutes a physical match will prove valuable for criminalists both in the laboratory and during courtroom testimony. Finally, a recommendation as to a suitable training program for those analysts who participate in duct tape end matching will help create consistency in crime laboratories across the country.

Although the analysis of duct tape end matches is common in crime laboratories today, there is currently no objective criterion to support what constitutes a physical match, and there is a lack of significant statistical data as to the reliability of end matching and the error rates associated with it; therefore, this study was designed to examine duct tape physical matches in order to determine the statistical significance of an "end match" conclusion and attempt to develop some objective criteria of what constitutes an end match.

Several forensic science graduate student researchers (GSR) from the University of California, Davis were selected to participate as duct tape analysts during this study. All of the graduate students were extensively trained in the area of duct tape analysis and matching techniques using literature review, practice samples, and an intensive series of duct tape validation/ proficiency samples. The experimental design is a blind study using duct tape from two of the top manufacturers, which included two duct tape grades from each manufacturer and two colors from each grade. Two-hundred evidentiary and exemplar samples were created by tearing each of the eight different duct tapes, giving a total of 1,600 torn pairs of tape. Each of the evidentiary and exemplar samples was labeled with random numbers. The samples were then paired together with each pair containing one evidentiary sample and one exemplar sample. Half of the pairs were chosen at random to remain matching, while the evidentiary tapes in the other half of the pairs were randomly assigned not to match. Finally the pairs were randomized into 1,600 envelopes. Each graduate student analyzed all 1,600 envelopes of duct tape pairs in order from envelope #0001 through envelope #1,600; meanwhile, the analysts were blind of each other's results.

The data was subjected to statistical analysis to determine the false positive and false negative error rates for the various analysts and across a combination of factors. The statistical analysis was performed using analytical software.

Further testing involved the use of an Elmendorf tear tester on 200 duct tape samples. Like the previous 1,600 specimen, these samples were analyzed by the participating GSR and statistical analysis was conducted to determine if a more uniform tear has any effect in the overall error rates of the analysts.

This research will impact the forensic science community by establishing the reliability of duct tape end matching as an analytical technique. The statistical analysis on the error rates associated with duct tape end matching and the development of a criterion for what constitutes a physical match will prove valuable for criminalists both in the laboratory and during court. Finally, a recommendation as to a suitable training program for those analysts who participate in duct tape end matching will help create consistency in crime laboratories across the country.

This study was sponsored by NIJ Grant. **Duct Tape, End Match, Physical Match** 

# A158 Current Forensic Models May Make Inappropriate Statistical Assumptions

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After attending this presentation, attendees will learn the results of a study designed to show that the simple method of nearest neighbor classification performs comparatively with the current complex statistical models used in the evaluation of trace evidence such as glass and paint.

This presentation will impact the forensic science community by exposing attendees to systematic errors associated with standard statistical models, as they are applied to evidence evaluation.

Forensic interpretation models try to determine the extent to which trace evidence found at a crime scene and on a suspect could have come from a common source. A desired property of such interpretation models is stability; that is, minor changes to the data do not radically change the interpretation. If a model is unstable, then small errors in the data collection can confuse the interpretation. Stability is an important property since forensic data is collected in less-than-ideal situations.

It is shown that several well-known forensic glass fragment interpretation models (Seheult (1978), Grove (1980), Evett (1995), and Walsh (1995) are not stable. More precisely, they exhibit a *zig-zag* behavior: pushing it a little drives it one way and pushing it a little more drives it in the opposite direction. To test if a model zig-zags:

1. Select model inputs at random between their minimum and maximum values,

- 2. Record the output interpretation,
- 3. Sort the interpretations by the order of the inputs,

Look for small changes to inputs that lead to large changes in the interpretation. Specifically, look for two small successive increases in any input that leads to a large increase, then decrease in the likelihood interpretation.

Zig-zags were tested for in the Scheult, Grove, Evett, and Walsh models, coding up each model to include not just the crime scene data (for example, refractive index) but also the tuning attributes recommended by the authors of the models. These tuning values come from prior historical or laboratory studies. For example, the Evett and Walsh models derive these values via surveys of different laboratory results. While a useful product of prior research, these tuning parameters can critically and inappropriately affect the interpretation if the tuning values no longer apply in the new situation.

Many zig-zags were found in these models. For example, in the Seheult model, if refractive index of glass at a crime scene is measured at 1.49, a change to 1.50, and then to 1.52 changes interpretation dramatically from 2% to 86% then back to 28%. At four decimal places, the changes are no less dramatic:

<u>RI Ranges</u>	Interpretation%
1.4000 - 1.4236	36
1.4236 - 1.4437	87
1.4437 - 1.4623	22
1.4623 - 1.4803	100
1.4803 - 1.5028	75
1.5028 - 1.5254	36
1.5254 - 1.5441	16
1.5441 - 1.5656	75
1.5656 - 1.5818	22
1.5818 - 1.5999	22

These kinds of changes occur out to 16 decimal places of refractive index data. Similar zig-zags can be seen in the Grove, Evett, and Walsh models.

There are two disturbing aspects of these results. Firstly, it has not been previously reported, which raises the issue of whether or not forensic science research checks for model stability. Secondly, it is not true that more recent models (written in the 1990s) are more stable than the older ones (written in the 1970s). In this regard, it is not clear that forensic models are improving their stability over time.

The resolution of this model is the focus of the current research. The authors wish to understand the trade-off between stability and performance: if a model is too stable, it freezes up and cannot respond appropriately to new inputs. New classes of interpretation models that are *stability-aware* are currently being explored. Preliminary results with this approach are encouraging.

**Trace, Interpretation, Statistical Models** 

# A159 Analysis of Predictive Source Attribution Methods That are Based on Small Particle Traces

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After attending this presentation, attendees will understand, and be able to recognize applications of the major classifications of methods that have been used for predictive source attribution, along with the requirements for, and corresponding limitations of, their application.

This presentation will impact the forensic science community by organizing and specifying different classes of source attribution methods and providing an analysis of both their scope and limitations. The overall implication of this analysis is that specific applications can be addressed in alternative ways, but that general application requires a diversity of expertise and tools, along with a process that enables the facile choice among these in response to the particular investigative problem and the specifics of the samples that are available.

As part of ongoing research, a review and analysis was conducted of predictive source attribution efforts that are based on small particle traces. The goal of predictive source attribution is to use the results of analyses to make accurate inferential estimates of exposures to geographical areas, environments, activities, and processes. The discipline of predicative source attribution is distinguished from that of comparative source attribution, where the focus is on the degree of correspondence between two sources in relation to other possible sources.

Source attribution efforts in the forensic and broader scientific literature are conveniently grouped based on the type of material being analyzed. Major groupings are: foodstuffs, minerals (including soil and gemstones), illicit drugs, insects, plants (including pollen), bacteria (including viruses), and animal products. There are also some multidisciplinary case reports.

The review covered a large number of publications and studies relating to past and current source attribution efforts. A critical analysis showed, however, that nearly all of these were narrow in scope. There were four principle ways in which the scope was narrowed (with many of the efforts being limited in more than one of these ways). These categories for limitations in scope are: (1) those based on comparison; (2) those discriminating among members of a closed set of options; (3) those that employ only one method; and, (4) those that require a large sample size.

In contrast to these narrowed approaches, there were a set of individual source attribution case reports that had a microscopical, multidisciplinary perspective. Collectively, these cases are an excellent illustration of the potential of laboratory methods to provide information that either leads directly to source attribution or that does so when the results are integrated with other investigative information. Each of these cases used a multidisciplinary approach, but the particular methods that were used were determined by case parameters and the available expertise. Disciplines that have been applied repeatedly in these cases are geology, botany, pollen analysis, zoology, and forensic particle analysis. Three types of samples are also commonly represented: those from a particular location (as in a residence or business), those on clothing, and those on a motor vehicle.

The implications of the analysis of these cases are: (1) that a diversity of laboratory expertise and methodology is required in order for source attribution to be successful; (2) that different tools need to be applied in different cases; and, (3) that a process must be in place that allows a facile choice among this diversity of tools, in response to the particular investigative problem and the specifics of the samples that are available.

Source Attribution, Trace Evidence, Small Particles

# A160 Sex Differences From Ridge Density in Palm Prints: A Preliminary Study

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After attending this presentation, attendees will be able to recognize that the ridge density in the palm prints exhibit sexual dimorphism in Indian population.

This presentation will impact the forensic science community by recognizing the sexual dimorphism of the ridge density in palm prints that may be used in identification of dismembered human remains in cases where an individual hand is recovered and brought for examination. It can give vital evidence in identification of the perpetrator of the crime in cases where the prints left behind at a crime scene.

Although researchers have attempted sex determination from fingerprint ridge density, the sex differences from the ridge density in palm prints remain unreported. No systematic studies are available on the sex differences from ridge density in palm prints worldwide are available at this time. This preliminary study is done to evaluate the sex differences from ridge density in palm prints and study its usefulness in discriminating sex in Indian population using statistical considerations.

The present prospective research was conducted on 131 young adults (73 males and 58 females) at the Department of Forensic Medicine, Kasturba Medical College, Mangalore, India. Healthy individuals aged between 20-25 years were included in this study after taking informed consent. The subjects with any disease, deformity, injury, fracture, amputation, or history of any surgical procedures of the hand were excluded from the study. Each subject included in the study was asked to wash their hand clean with soap and water. A clean plain glass plate was uniformly smeared with black duplicating ink with the help of a roller. The subjects were asked to apply their hand on the smeared plate and then transfer them on to a white paper. Regular pressure was applied on the palm area to obtain the palm prints. A 5 mm x 5 mm square was drawn on a transparent film and placed on the obtained palm print samples in the areas to be analyzed. In order to measure ridge density, or the number of

ridges in a given space, the count was carried out diagonally on a square measuring 5 mm x 5 mm. The epidermal ridges were counted with the help of a hand lens. This count represents the number of ridges in 25 mm square area and reflects the ridge density value. The ridge density value was similarly obtained individually from the four designated areas on the palm prints that are commonly encountered at the crime scene. The four designated areas included; central prominent part of the thenar eminence (P1), parathenar region inner to the proximal axial triradius (P2), medial mount proximal to the triradius of the 2nd digit (P3), and lateral mount proximal to the triradius of the 5<sup>th</sup> digit (P4). The data obtained was analyzed statistically using SPSS (Statistical Package for Social Sciences, version 11.0) computer software. The male female differences in ridge density were analyzed individually for each of the designated areas from the palm prints. Ridge density was compared between right and left sides for each of the designated areas. Statistical significance was defined at the standard 0.05 level.

Mean ridge density was significantly higher in females than males in the designated areas except for P3 area in the right hand. No right-left differences were observed except for the P4 area in females. For all the areas taken together the mean ridge density was 11 ridges/25 mm<sup>2</sup> in males and 12 ridges/25 mm<sup>2</sup> in females for the right and left palm prints.

The likelihood ratio (LR) was calculated to obtain the probability inferences of sex, based on ridge density values. Posterior probabilities was calculated using Bayes' theorem and information obtained from both LR computations and posterior probabilities were used to show favoured odds. The present research reveals that females have greater ridge density hence, finer ridge details than males in Indian population. The mean ridge densities thus, can be used as a presumptive indicator of sex of an unknown print left at a crime scene.

Sex Determination, Ridge Density, Palm Prints

# A161 An Example Validation Procedure With Measures of Statistical Significance for Fingerprint Screeing Programs

Gary H. Naisbitt, PhD\*, Aaron Hall, Raymond Sievers, PhD, and Daniel Edwards, Utah Valley University, Forensic Science Program MS 286, 800 West University, Orem, UT 84058

After attending this presentation, attendees will be able to apply this validation model to their own fingerprint search software to determine the accuracy of its search performance and evaluate the statistical significance of the results.

This presentation will impact the forensic science community by presenting a model validation scheme for automated fingerprint screening and statistical evaluation of search results.

The 1993 Supreme Court decision, *Daubert vs. Merrell Dow Pharmaceuticals, Inc.* established quality control and scientific performance standards for the admission of evidence presented at court. After reviewing forensic science disciplines, The National Academy of Sciences in their 2009 Report, *Strengthening Forensic Science in the United States: A Path Forward*, concluded that forensic science disciplines needed a stronger scientific foundation. Both stated that error rate estimates and data reproducibility were needed to assess the strength of scientific findings. This was particularly true in pattern recognition disciplines such as fingerprint examination.

The learning objectives of this study are to present a model approach to verify that AFIS software is working as designed and present a statistical foundation of search performance.

The first step was testing the accuracy and reproducibility of the AFIS system by searching the database of known fingerprints with a duplicate print. This is a self-matches-self experiment with a match on every attempt. Experimentally, standard ten-print cards were scanned at 600 dpi into no-loss Tiff files, the subject print was cropped from the ten-

print card, the minutiae were extracted by the AFIS system, and the print was enrolled into the database of known prints. The same process was used for the latent print starting with the same cropped fingerprint. The expected result of 100% search accuracy was obtained and repeated searches established 100% reproducibility.

Note that this model starts with exactly the same print and only validates that the AFIS software is working to its designed specifications. Real world fingerprints are entered from scanners or ten-print cards, and are not exact duplicates of known prints in the AFIS database. To emulate real world prints, latent prints were cropped from ten-print cards for each replicate trial and searched against its duplicate in the AFIS database with the expected results of 100% accuracy and 100% reproducibility. Actual results were less than 100% and were similar to earlier findings that suggested the AFIS minutiae extraction engine was not consistent; however, the validation model presented above disproved that theory. Additionally, because the latent and database prints were exactly the same, with the same orientation, quality and distortion, the ridge detail is not at fault.

Instead, it is the outer boundaries where the print was cropped that cause a slight shift in position of the print when the minutiae are extracted. A common digital imaging approach is lay a grid over the print and each box in the grid is evaluated for the presence of a minutiae point. When the overlay is positioned by the image's outer boundaries, slightly different boundaries shift the grid causing different minutiae to be recognized. Therefore, repeated croppings of identical prints do not produce identical minutiae patterns. This more closely emulates the real world where the outer boundaries of latent prints are not precisely controlled when they are entered into an AFIS system. In this case, population distribution statistics were used to describe how variations in the number of minutiae affect search accuracy. Similar experiments were conducted using different images of the same finger in a database of thirty ten-print cards and in a second database of six hundred individual prints.

This model of using known prints with predictable outcomes can be used for whole prints, or parts of whole prints, to verify the accuracy and reproducibility of any AFIS system. This study can help fingerprint examiners validate their own AFIS software, explain sources of error, and evaluate efficiency of new software by measuring the ratio of true candidate to false candidate prints.

Fingerprint, Validation, Statistical Significance

## A162 Preparation and Evaluation of Nanoparticles for Latent Fingerprint Recovery

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After attending this presentation, attendees will learn how nanoparticles can be used for improving fingerprint development and visualization.

This presentation will impact the forensic science community by demonstrating the synthesis and use of nanoparticles for recovering and developing latent prints.

The identification of individuals using skin ridge patterns dates back almost 4,000 years and was first suggested for forensic use in the 19<sup>th</sup> century. Fingerprint identification remains a cornerstone of the forensic community. Recently, the literature has demonstrated that nanoparticles improve the developing and recovering of increasingly obscure latent fingerprints including enhancing currently used methods including the silver developer reagent and fluorescence detection. Nanoparticles are materials with dimensions less than 100 nm. Nanoparticles have unique absorbance and other properties as compared to the bulk material due to their small size. Applying nanoparticles to fingerprints begins with the successful creation of the nanoparticles; having control over the size, shape, and composition is crucial to successful functionality of any nanoparticle. This goal of this presentation is to present the synthesis and application of multiple nanoparticle preparations including gold, cadmium sulfide, and iron oxide (magnetite) nanoparticles in recovering latent fingerprints from a variety of materials. The presence of nanoparticles, as opposed to bulk material, was confirmed using color, UV-Vis spectroscopy, and scanning electron microscopy. The magnetite produced very fine dark colored particles which showed magnetic properties, as expected. The CdS solution turned pale yellow which indicated nanoparticle formation as cadmium sulfide precipitate produces an orange solution. The gold nanoparticle synthesis produced a deep red translucent solution. Varying the gold methods produced thick, opaque precipitates or no color change in the solution; both outcomes were strong indications that nanoparticles were not produced. Soaking a fingerprint in gold nanoparticles has been shown to increase the resolution and contrast of the silver developer. The silver developer is typically a "last resort" method due to its tendency to destroy the sample material. The silver developer works by reducing cationic silver (+1) to elemental silver (0) using a ferrous/ferric redox system and is often used with ninhydrin on porous surfaces such as paper and wood. The results of developing the fingerprints with the nanoparticles were compared those collected with traditional fingerprint dusting powders on a variety of porous simulated forensic surfaces including wood, paper, aluminum can, glass, and white cotton fabric. The nanoparticle production methods should hold broad appeal due to the ease of synthesis using very dilute solutions.

Nanoparticle-protein bioconjugates were also evaluated for applications in fingerprint detection and the detection of "lifestyle intelligence" or drugs or secondary metabolites found in fingerprints or other body fluids by attaching drug- or metabolite-specific antibodies. The antibody fluoresces if attached to the target metabolite, providing the ability to detect the drug and photograph the fingerprint under ultraviolet light. The fingerprint can also be developed with the silver developer solution. Testing fingerprints for drug metabolites shows promise in a variety of forensic applications including narrowing a list of suspects based on drug use, or in workplace or roadside drug testing. Drug or drug metabolite testing on fingerprints provides a unique identification method of the individual being tested, removing the possibility of samples being mixed. Initial experiments were performed using bovine serum albumin and ATR FT-IR and fluorescence analysis.

Fingerprinting, Nanoparticles, Drug Testing

# A163 Follow-Up Survey of Forensic Professionals on Key Issues Facing Friction Ridge Analysis

Samantha H. Neal, BS\*, West Virginia University Forensic Science Initiative, PO Box 6217, 208 Oglebay Hall, Morgantown, WV 26506-6217

After attending this presentation, attendees will have an increased understanding of the views, perspectives, and opinions of forensic practitioners on key issues facing friction ridge analysis.

This presentation will impact the forensic science community by highlighting current opinions and reactions of forensic science professionals in light of recent government publications. This data will create a deeper appreciation for the gap between perception and the reality of friction ridge analysis in forensic laboratories.

Since the National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, was released in February 2009, many forensic disciplines have been under increased scrutiny, including friction ridge analysis. As a followup to a "Science of Fingerprints" survey where 150 forensic practitioners were surveyed between 2007 and 2009, the same participants were asked the same eight questions with an additional four questions:

1. Do you conduct friction ridge analysis as part of your current position?

- 2. Do you believe that fingerprint identification is a science?
- 3. Do you feel that fingerprint identification should be admitted into courtrooms as expert testimony?
- 4. What type of research, if any, should be done to correct the misidentifications that occur?
- 5. Should there be a minimum number of points for identification? Why?
- 6. Do you feel that other misidentifications have occurred that have never been caught?
- 7. Do you believe that innocent people are in jail or on death row because of fingerprint errors?
- 8. What additional quality assurance steps should be taken for fingerprint examiners?
- 9. Do you or your laboratory perform blind verifications?
- 10. Do the Daubert factors apply to fingerprint identifications?
- 11. After reading the *NH v. Langill Opinion*, do you agree that the testimony should be inadmissible?
- 12. Has the NAS Report, *Strengthening Forensic Science in the United States: A Path Forward*, affected how you perform your job (whether it be daily routines, SOP, testifying, etc.)?

The majority of practitioners surveyed had five years or less experience in forensic science and represented local, state, and federal agencies across the United States. Consensus in the field of forensic science can be difficult given the variance in jurisdictional requirements; however, trends on this topic were significant. While the majority of practitioners surveyed believed that fingerprint identification is a science, this follow-up survey demonstrates that a clear, coherent message as to what constitutes that science is yet to be achieved.

Friction Ridge, Fingerprint Identification, NAS Report

# A164 Effects of Competitive Adsorption on Interpretation of Ignitable Liquid Residues in Fire Debris Analysis

J. Graham Rankin, PhD, and Amanda Heeren, BS\*, Marshall University Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701

After attending this presentation, attendees will gain an understanding in the effects of competitive adsorption among various substrates and the relationship it poses on fire debris analysis data interpretation. Also, attendees will better understand the methods commonly used in fire debris analysis to date.

This presentation will impact the forensic science community by discussing the possible misinterpretation and classification of the results, especially at low residual levels and possible pyrolysis products from the substrate.

Charred and un-charred substrate will be compared in the classification of ignitable liquids according to American Society for Testing and Materials (ASTM) method E1618. This presentation will impact the forensic community by demonstrating and providing an explanation for distorted chromatograms obtained from fire debris samples collected on a gas chromatography mass spectrometer (GC/MS). This research project is important to the fire debris analysis community due to the possible misinterpretation and classification of the results, especially at low residual levels and possible pyrolysis products from the substrate. This work will help to validate the methodology currently in use under a variety of conditions.

This presentation aims to show how various charred substrates may cause misinterpretation of the E1618 class of ignitable liquids in samples from fire scenes due to competitive adsorption. Also, it aims to demonstrate that amount the substrate is charred may have an influence on the relative height of certain key components in ignitable liquid chromatograms.
Understanding the key aspects of fire debris analysis can aid in isolating areas that potential problems can and do arise from when interpreting data. Ignitable liquids are the most common form of accelerants found at fire scenes across the United States. Most importantly gasoline and kerosene are the two most commonly recovered and identified. The common practice utilized today for analysis of these ignitable liquids is the ASTM E 1412 method, passive headspace concentration using activated charcoal strips. Though a reliable method, there are areas for potential problems when performing this technique. It has been previously reported that competitive adsorption of the charred wood present in the samples may affect the interpretation of the ignitable liquid class. The work presented here is an in depth study of the effects of substrate and amount of charring on the recovered ignitable liquids using E1412 and E1618 methods.

Several different substrates were used to demonstrate that competitive adsorption could lead to misinterpretation of results, as well as to describe the differences in peaks obtained in the chromatograms. Different wood samples were used at a set amount of charring on the wood to demonstrate the actual effects of competitive adsorption. Percent weight loss was how the degree of charring was calculated throughout the experiment. Samples were then spiked with gasoline and kerosene, as well as other ignitable liquids to test the effects of the solvents. Carbon disulfide was added to samples and analysis on a GC/MS were performed using an E1618 standard and method blank with each set of samples. Uncharred substrates, with and without ignitable liquid spikes, were also used for comparison and to identify any background interferents.

Though most samples exhibited some form of decrease in liquid present on the charred substrate in the chromatograms, some were more pronounced than others. Whether the main reason for the loss in area is the competition on the carbon strip or from retention of the liquid on wood was the main cause, conclusions that the difference was caused by competitive adsorption were made. Initial percent charred studies (by weight) demonstrated that the more charred the surface, the smaller the peaks on the chromatogram compared to those that were not charred as significantly. Overall, further development and studies should and will be performed to see how varying the amount of charring could also be a factor in explaining the difference in the chromatograms and mass spectra. This work was also used to test the robustness of the target compound ratio method for the further discrimination of gasoline and kerosene residues.

Fire Debris Analysis, Competitive Adsorption, GC/MS

#### A165 Migration of Ignitable Liquids in Pour Patterns on Carpet

Meggan L. Macomber, BA\*, Marshall University Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV; 25701; J. David Tebow, BS, Minnesota Bureau of Criminal Apprehension, Forensic Science Lab, 1430 Maryland Avenue East, Saint Paul, MN 55106; and J. Graham Rankin, PhD, Marshall University Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701

After attending this presentation, attendees will understand the migration of gasoline in carpet during a fire, and where the best place is to sample when a pour pattern is evident.

This presentation will impact the forensic science community by providing arson investigators with the knowledge of where to sample a suspected pour pattern in order to obtain the best results possible concerning the use of an ignitable liquid.

When an ignitable liquid is used as an accelerant in a fire, a pattern is created where the ignitable liquid is poured. Only the edge of the pour pattern burns in a fire due to that being the location where the ignitable liquid turns to vapor and mixes with the air, the mixture being what actually burns. Arson investigators differ in opinion as to whether chances are better to detect any ignitable liquid at the center of a pour

\* Presenting Author

pattern or at the edge of the pour pattern where the fire is burning. Sampling the edge of the pour pattern involves sampling across the edge to obtain both burned and unburned material. Investigators who believe the edge of the pattern is better base their belief on the assumption that the ignitable liquid migrates to the edge of the pattern as the fire burns, which causes there to be a greater concentration of the liquid at the edge. The theory that the center is the best place to sample is based on the assumption that the edge burning causes the fire to move slowly inward and that too much of the ignitable liquid burns off at the edge to get conclusive results, therefore the center should be sampled.

In order to test the theories of edge versus center sampling, an experiment was designed to sample from the center to past the edge of a pour pattern in carpet samples that varied from unburned to burned until self-extinguishment. One quart of gasoline was poured on each of five two-foot by two-foot squares of carpet. One square remained unburned, one square was allowed to burn itself out (approximately ten minutes), one was burned for two and a half minutes, another was burned for five minutes, and the last was burned for seven and a half minutes. Samples were taken in four directions, labeled north, south, east, and west.

Solvent extraction with n-pentane was used to extract any remaining gasoline from the carpet samples for analysis by GC/MS. The peak area of 2-methylnaphthalene, a compound found in gasoline, was compared with the peak area of 3-phenyltoluene as an internal standard in order to find the ratio between the two. The ratios were then compared to ratios of 2-methylnaphthalene/3-phenyltoluene for known concentrations of neat gasoline in order to approximate the amount of gasoline in that sample.

The pour pattern for the gasoline appeared to be about 25-30 cm (10-12 in.) in diameter on the surface of the carpet; however, when lit the pour pattern appeared to roughly double in size due to gasoline spreading out on the bottom of the carpet due to gravity. The lack of pattern in the unburned carpet square is most likely due to the pattern in the carpet allowing gasoline in some areas to flow farther than in others when poured. The trend lines for each of the carpet squares suggest that the best place to sample is from the center out to approximately 10 cm (4 in).

Gasoline, Fire, Arson

#### A166 National Center for Forensic Science and Technical Working Group for Fire and Explosions Databases

Michael Sigman, PhD, and Mary R. Williams, MS\*, National Center for Forensic Science University of Central Florida, PO Box 162367, Orlando, FL 32816-2367

The goal of this presentation is to inform attendees of the valuable fire debris and explosives analyses resources available in the National Center for Forensic Science and Technical Working Group for Fire and Explosions (NCFS/TWGFEX) databases.

This presentation will impact the forensic science community by providing information on the analytical data and product information contained within three databases: Ignitable Liquids Reference Collection Database, Substrates Database, and Smokeless Powders Database.

In 2000, the Ignitable Liquids Reference Collection (ILRC) and Database were established as a joint project between the National Center for Forensic Science (NCFS) and the Technical Working Group for Fire and Explosions (TWGFEX). The need for a collection of reference ignitable liquids with associated GC-MS analysis data was confirmed by fire debris analysts in a 1998 national survey of forensic laboratories.<sup>1</sup> The collection of reference ignitable liquids are housed at NCFS. The ILRC committee, a committee within TWGFEX, reviews all of the data and classifies each reference ignitable liquid based on the American Society for Testing and Materials (ASTM) E1618 – 06 classification scheme.<sup>2</sup> The ILRC database contains product information, classification information, and GC-MS data. The

ILRC database became accessible to the public with 100 entries in January 2002 and now contains over 500 entries.

In 2007, NCFS and the ILRC committee began investigating the feasibility of establishing a substrate database. Substrates are materials which undergo pyrolysis and combustion processes during a fire and are constituents in fire debris collected at a fire scene. These materials may produce compounds that can interfere with the identification of ignitable liquids in fire debris. A modified destructive distillation method is utilized in producing burned substrates for analysis. The Substrate Database is a compilation of headspace GC/MS data from burned and unburned materials that are common to fire scenes. The database can assist fire debris analysts by demonstrating the types of compounds and chromatographic patterns that may be produced by these commonly encountered materials. The Substrate Database became accessible to the public with 60 entries in July 2010.

In 2009, NCFS and the explosives database committee of TWGFEX began developing a database for smokeless powders. The Smokeless Powders Database will be a compilation of product information, physical descriptions and analytical data on smokeless powders from three main sources. The FBI and Orange County Sheriff-Coroner Department in California will provide historical information and data on smokeless powders preceding the development of the database. NCFS will continue to populate the database with information and data on newer smokeless powders. A prototype of the database has been approved by the TWGFEX explosives committee. It is anticipated that the Smokeless Powders Database will be accessible to the public with 100 entries by January 2011.

Over the last decade, combined efforts from the National Center for Forensic Science and the Technical Working Group for Fire and Explosions have developed three databases; the Ignitable Liquids Reference Collection Database http://ilrc.ucf.edu, the Substrate Database http://ilrc.ucf.edu/substrate, and the Smokeless Powders Database. The databases are designed for the user to search product information, classification information, physical descriptions and analytical data rapidly. These databases are valuable tools in the investigation and analysis of fire debris and explosives.

This work was supported in part 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:**

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- <sup>2</sup> ASTM International, ASTM E1618-06 Standard test method for ignitable liquid residues in extracts from fire debris samples by gas chromatography-mass spectrometry. Annual Book of ASTM Standards, Volume 14.02, ASTM International; West Conshohoken, PA, June 2006

#### **Databases, Fire Debris, Explosives**

## A167 Preventing Ignitable Liquid Degradation Using Antimicrobial Agents

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After attending this presentation, attendees will understand the concept of microbial degradation of ignitable liquid residues and what can be done to preserve fire debris evidence.

This presentation will impact the forensic science community by detailing means for delaying degradation, which will allow forensic analysts to determine if an ignitable liquid is present on soil or other organic substrates.

The analysis of fire debris evidence is important in the investigation of arson cases. Although most of the ignitable liquid is burned as fuel in a fire, its residue is often collected in conjunction with substrates such as soil and wood. However, certain organic compounds present in the residue evidence that are necessary for their positive identification are also food sources for bacteria commonly found in these environments. It is believed that if degradation can be delayed or eliminated completely then forensic analysis can accurately identify the types of ignitable liquids used in arson cases.

Based on literature review and work previously done at Indiana University Purdue University Indianapolis (IUPUI), it is hypothesized that the antimicrobial agents triclosan, benzethonium chloride, and benzalkonium chloride could be effective at slowing or preventing degradation. Also, certain species of soil amoeba are being explored as a possible means of eliminating or delaying microbial degradation because the main food source for these organisms is bacteria.

In a preliminary qualitative investigation, culturing studies were carried out using solutions of triclosan in varying concentrate ions and solvents. Solutions were made accordingly: 0.1%, 0.5%, and 1.0% triclosan in methanol and 0.1% and 0.5% triclosan in glycerin. Water was not used as a solvent because of triclosan's low solubility. After preparing these solutions, 5.0 mL of each type were mixed with 2.0 g of two types of soil: potting soil and lawn soil from the IUPUI campus. Plastic culture tubes housed these mixtures, and they were stored in a cool, dry area in the lab. After specific time points (one hour, one, two, and seven days) inoculating loops were used to spread 10 mL of the liquid onto agar plates.

The 1% triclosan solutions (in both methanol and glycerin) proved to be most effective, preventing growth for up to seven days. Although both solutions successfully delayed biodegradation, a water-based solution is needed. For this reason, culturing studies were ultimately completed using a 1.0% solution of triclosan, benzethonium chloride, and benzalkonium chloride in a 5.0% glycerin in water solution. Research has been conducted using both benzethonium chloride and benzalkonium chloride as biocides and antimicrobial agents. These chemicals also have surfactant properties making a mixture using them potentially doubly effective for this study as triclosan might have greater solubility in their presence and antimicrobial action may be increased. Glycerin was added to this mixture to increase the solubility of triclosan, but the solution still needed to be sonicated in a heated bath for at least 45 minutes before the triclosan was mostly dissolved. The previously described procedure was repeated with these solutions. Except, for this study all of the agar plates were sealed with parafilm after inoculation to mimic an airtight situation found in real life scenarios. This solution was seen to inhibit microbial growth for up to two days.

During a passive headspace study, cans with mixtures of soil, gas, and antimicrobial solution were analyzed via GC/MS. Based on the results, the degradation of gasoline on potting soil was more pronounced than on lawn soil. Also, degradation is only noticeable on soil when

smaller volumes of gasoline are present (20mL). Furthermore, the recovery of gasoline was not adversely affected by the antimicrobial solution. Finally, residues of gasoline were preserved on potting and lawn soil for time periods of up to seven days.

Ignitable Liquid, Microbial Degradation, Fire Debris

#### A168 Chemometric Assisted Detection and Classification of Ignitable Liquids in Fire Debris

Erin Waddell, BS\*, Caitlin Rinke, BS, Mary R. Williams, MS, and Michael Sigman, PhD, University of Central Florida, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2367

The goal of this presentation is to describe the use of chemometric techniques to aid in the detection of ignitable liquid residue in fire debris, and to assist in the correct classification of ignitable liquids residues that may be present.

This presentation will impact the forensic science community by introducing an objective method to be applied to ignitable liquid and pyrolysis product classification in fire debris analysis. The methods investigated are intended to provide statistical support for current laboratory practices.

This research will examine the use of principal components analysis (PCA), discriminant analysis, and soft independent modeling of class analogy (SIMCA) for the classification of fire debris samples containing ignitable liquids and/or pyrolysis products. Ignitable liquid classes followed the American Society for Testing and Materials (ASTM) E 1618 standards.

This presentation will focus on the development of two models from a library of ignitable liquid GC-MS data and a library of pyrolysis product GC-MS data. The models were developed for: (1) the classification of the total ion spectrum, TIS, from a single sample as an ignitable liquid, IL, or a substrate pyrolysis, SP, product; and, (2) classification of a single sample TIS as an SP product or an IL from a specific ASTM classification. One data set was developed for ignitable liquids falling into all ASTM classes and substrates based on 452 ignitable liquids and 60 substrates, DS 1. Another data set incorporated ignitable liquids in ASTM classes other than the broadly defined miscellaneous and oxygenate classes based on 288 ignitable liquids and 60 substrates, DS 2. The influence of including the miscellaneous and oxygenate classes was evaluated. The models were tested on samples of ignitable liquids and burn samples created in a laboratory and field trials.

The first step of model development was to reduce the complexity of the dataset by selecting a group of ions from the total ion mass spectra that had the largest ratio of between group variance to within group variance (the largest F values). Modeling then proceeded by further reducing the dimensionality of the data through the use of principal components analysis (PCA) to construct a set of latent variables that explained known amounts of the variance in the data. The pretreatment of the F-selected data (prior to PCA) was varied by mean centering and autoscaling (mean centering with standardization of the variance of each variable).

**Model 1:** Initial results for DS 1 show that 98% of the autoscaled samples can be correctly classified as IL or SP, using 65 high-F ions and reducing the data dimensionality to 27 latent variables required to account for 95% of the variance. DS 2 results show that 98% of the autoscaled samples can be correctly classified as IL or SP, using 67 high-F ions and reducing the data dimensionality to 24 latent variables required to account for 95% of the variance. Reducing the number of latent variables to three for DS 1, which only accounts for 47% of the variance in the data, results in 86% and 70% correct classification into only the IL and SP classes, respectively. Reducing the number of latent variables to three for DS 2, which only accounts for 49% of the variance in the data, results in 92%

and 72% correct classification into only the IL and SP classes, respectively.

Model 2: DS 1 ASTM classes include aromatic; gasoline; heavy, medium and light petroleum distillates; isoparaffinics; naphthenic paraffinic; normal alkanes; miscellaneous; and oxygenates. DS 2 uses the same classes but excludes miscellaneous and oxygenates. Subclassification of the DS 1 IL samples into their ASTM classes, using 65 high-F ions and three latent variables, gave 55% correct classification for SP and 82% correct classification for all of the IL ASTM classes with the exception of the miscellaneous and oxygenate classes which were correctly classified for 17% and 56% of the samples, respectively. The lower correct classification rate for the miscellaneous and oxygenate IL classes is seen to result from the broad definitions of these classes and the resulting population of the classes by ignitable liquids having diverse properties. Subclassification of the DS 2 IL samples into their ASTM classes, using 67 high-F ions and three latent variables, gave 66% correct classification for SP and 89% correct classification for all of the IL ASTM classifications with the absence of the miscellaneous and oxygenate classes. Increasing the number of latent variables will increase the overall percent correct classification.

These results will be compared to other hard and soft chemometric techniques.

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.

Fire Debris, Principal Component Analysis, Chemometric

#### A169 Identification of Ignitable Liquid Classes by Target Factor Analysis of Total Ion Spectra

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The goal of this presentation is to describe a chemometric approach to identifying American Society for Testing and Materials (ASTM) E1618-06 defined classes of ignitable liquids in the presence of pyrolysis products in fire debris.

This presentation will impact the forensic science community by presenting a supplementary data analysis method that uses the total ion spectrum (TIS) method and target factor analysis (TFA) to identify classes of ignitable liquids in fire debris samples.

Current methods in ignitable liquid identification and classification from fire debris rely on pattern recognition of ignitable liquids in total ion chromatograms (TIC), extracted ion profiles, and target compounds as described in ASTM E1618-06. The total ion spectra method takes advantage of the reproducibility among sample spectra from the same ASTM class and is independent of chromatographic conditions which affect retention times of target compounds, aiding in the use of computerbased library searching techniques.1 The total ion spectrum was obtained by summing the ion intensities across all elution times. The TIS from multiple fire debris samples were combined for target factor analysis. Principle components analysis allowed the dimensions of the data matrix to be reduced prior to TFA, and the number of principle components retained accounted for approximately 95% of the variance of the overall sample set. The latent variables were rotated to find new vectors (resultant vectors) that were the best possible match to spectra in a reference library of over 450 ignitable liquid spectra (test factors). The Pearson correlation between target factors and resultant vectors were used to rank the ignitable liquids in the library. Ignitable liquids with the highest correlation represented possible contributions to the sample. The technique of receiver operating characteristics (ROC) was used to evaluate the likelihood of identifying a specific ASTM-designated class of ignitable liquid as a contributor to the sample set.

Tests included small-scale laboratory burns of substrates with ignitable liquids as well as large scale burns in 20'x8'x8' containers complete with furnishings and flooring. Large scale burns were designed to test the identification limits of the chemometric approach to ignitable liquid identification in the presence of background pyrolysis products. Burn conditions were controlled by adjusting the volume of ignitable liquid used, the fuel load, ventilation level, and the elapsed time of the burn. Samples collected from the large scale burns were analyzed using passive headspace adsorption with activated charcoal strips and carbon disulfide desorption of volatiles for analysis using gas chromatographymass spectrometry.

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.

#### **Reference:**

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Chemometric, Fire Debris, Factor Analysis

#### A170 Clustering of Medium Petroleum Distillate Products: A Self Organizing Feature Map (SOFM) Approach

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The goal of this presentation is to discuss an artificial neural network technique to differentiate medium petroleum distillate samples. Attendees will understand the basic idea of self organizing feature map (SOFM), an unsupervised pattern recognition neural network most commonly used for revealing natural clustering within multi-variables dataset.

This presentation will impact the forensic science community by introducing a new approach to classify and discriminate ignitable liquid samples within the same class. This presentation proposes an alternative to "conventional" multivariate analysis for example principal component analysis and hierarchical cluster analysis.

SOFM can aid the investigator in the assignment of weathered (evaporated) medium petroleum distillates to their source.

This work involves the GC/MS analysis of medium petroleum distilates and the chemometric analysis of the derived data.

The identification of liquid accelerant is a complex problem in fire debris analysis. Many techniques can be utilised in order to obtain the ignitable liquid residue from the submitted debris. It is also of increasing importance that the analysing laboratory compiles a reference collection of relevant ignitable liquids. The reference collection should also contain weathered or evaporated samples of ignitable liquids scene these are more commonly encountered in debris. The comparison of ignitable liquid residues extracted from fire debris samples is generally based on class characterisation, hence a systematic grouping of ignitable liquids has been established by the American Standard Testing and Material (ASTM) International which in principle, groups ignitable liquids on the basis of hydrocarbon compositions, boiling point, and raw material source.<sup>1</sup> Medium petroleum distillate (C8-C13 hydrocarbon range) includes products such as paint thinners, white spirits and paint brush cleaners are quite common in arson cases, although they may not be as frequently encountered as petrol and kerosene.

Chromatographic profiles and mass spectral data of medium petroleum distillates, in fact most ignitable liquids from the same

classification class, often have high degree of similarity in terms of their chromatographic pattern. Therefore, examination by visual comparison of chromatographic profiles for classification and identification purposes can be very challenging, and sample individualization, arguably, unachievable.

A number of multivariate analysis techniques have been applied to differentiate ignitable liquids. These types of analysis enable more than one variable to be measured and the conclusions about these data can be presented as 2D or 3D graphical trends. This work details the mechanism by which SOFM can be utilized for chromatographic data analysis. SOFM is an artificial neural network computation which performs unsupervised multivariate analysis. Among its many applications, it is used for dimension reductionality but at the same time preserves the original data and is applicable with linear or nonlinear datasets.<sup>2</sup> It is perhaps the most popular unsupervised pattern recognition ANN.

A reference collection from various brands of MPD products was compiled. MPD samples were analyzed using GC-MS and their chromatographic data was examined. Automated comparison between data derived from different brands of MPD was carried out using a som software. The outcome from SOFM provided a topological map for easy visualization; hence the interpretation of the outcome is straight forward. Furthermore, the dataset variables could be studied closely from within the SOFM structure (known as component planes) to gather information about the clusters proposed. Results revealed that SOFM was capable of objectively discriminating between product brands using chromatographic patterns from unevaporated and evaporated MPD samples.

#### **References:**

- <sup>1.</sup> Stauffer, E.; Lentini, J. J., *Forensic Science International 2003*, 132, 63-67.
- <sup>2.</sup> Zupan, J.; Gasteiger, J., Neural networks in Chemistry and Drug Design. Second Edition ed.; Wiley-VCH: Weinheim, 1999.

SOFM, Medium Petroleum Distillates, Pattern Recognition

#### A171 Multivariate Pattern Recognition Analysis of Petroleum Based Accelerants

Wan N.S. MatDesa, MSc\*, Niamh NicDaeid, PhD, Dzulkiflee Ismail, MSc, and Kathleen Savage, PhD, Centre for Forensic Science, University of Strathclyde, Glasgow, G11XW, UNITED KINGDOM

After attending this presentation, attendees will be introduced to the application of statistical and mathematical tools to interpret gas chromatographic patterns derived from petroleum based products commonly employed in arson.

This presentation will impact the forensic science community by demonstrating the feasibility of utilizing multivariate pattern recognition techniques to discriminate petroleum based ignitable liquids by type and brand. This study reveals that the ability of some multivariate technique to link evaporated petroleum products back to unevaporated constituents.

In fire investigations, petroleum distillate products are commonly used as liquid accelerants by the arsonist to initiate and promote the spread of fire. The use of such products is reported in many arson cases, thus discrimination and identification of these samples are of forensic interest. At present, sample classification is based on a visual comparison of a given sample to reference standards. The application of multivariate pattern recognition to discriminate and classify liquid accelerants sample has the potential of making this process rapid, less subjective and more conclusive.

Application of GC-MS for ignitable liquid analysis provides very large datasets from chromatographic and mass spectral profiles. In their original form, this information is hard to relate to; however, these data can be more easily comprehended when processed using multivariate analysis such as the Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Self Organizing Feature Map (SOFM). These computerized pattern recognition methods generally improve the visualization of large numerical datasets by presenting them in a simpler graphical form. The use of these techniques can be extended to compare, classify, and possibly link samples to potential common origins because they are able to establish underlying discriminating relationships within a complex dataset.

A set of petroleum based ignitable liquid samples from various classes designated by the ASTM classification scheme were obtained. These samples were evaporated to different evaporation degrees (from 10% weathered to 95% weathered) in order to build a database that captured the compositional changes of the samples. Samples were analysed by Gas chromatography-mass spectrometry (GC-MS) and characteristic peaks from the total ion chromatograms (TICs) were selected as variables for data processing.

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed. Both of these multivariate techniques are commonly reported in studies relating to classification and discrimination of ignitable liquids. Data were also subjected to artificial neural network analysis, using Self Organizing Feature Map approach and the results were compared.

This work has shown that the the best overall discrimination within and between the samples was achieved when chromatographic data was pre-processed using the normalised fourth root power transformation. Results obtained with SOFM, when compared to PCA and HCA provided more useful and distinctive where the weathered and unweathered constituents from the same product were grouped together. Visualisation of SOFM results are also easier to digest than PCA and HCA.

Pattern Recognition, Ignitable Liquid, Arson

#### A172 Surfactant Pyrolysis Products: Implications for Fire Debris Analysis

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The goal of this presentation is to discuss pyrolysate of surfactants, specifically those based upon linear alkylbenzene sulfonates, and how these interferences can affect interpretation in fire debris analyses.

This presentation will impact the forensic science community by providing additional insight into the affects of materials and pyrolysates for interpreting results of ignitable liquid analyses.

Determining substrate interferences in ignitable liquid investigation is an ongoing concern for analysts performing laboratory analysis of fire debris samples. Previous studies have shown interferences for substrate materials such as asphalt, wood, and polymers. This presentation; however, will discuss the potential that substances used in the extinguishing of a fire may complicate the interpretation of ignitable liquid analyses.

Observed in a recent case sample was a distribution of linear alkylbenzenes, with a linear chain of ten to twelve carbons. Linear alkylbenzenes are used in the manufacture of surfactants through aromatic sulfonation to yield linear alkylbenzene sulfonates, which are used in many common household soaps and detergents. Liquid detergents, possibly containing linear alkylbenzene sulfonates, were utilized in the suppression of the fire in question. This was done by aspirating the liquid detergent into the water hose to produce fire suppression foam, as a substitute for sodium perfluorinated alkylsulfonates (AFFF). Desulfonation of the linear alkylbenzenes is hypothesized as the origin of the compounds detected due to aromatic desulfonation which can occur at high temperatures and in the presence of water.

Aromatic compounds such as ethylbenzenes, propylbenzenes, and naphthalenes were also observed in the case sample. These compounds can be indicative of an ignitable liquid, such as gasoline. The objective of this study was to determine the origin of these aromatic compounds, whether from an ignitable liquid or degradation from the linear alkylbenzenes. It is proposed that decomposition of the carbon chains in the linear alkyl benzenes, similar to what is observed in the pyrolysate of polyethylene, contributed to the observed aromatics in the sample. A series of trials were performed by pyrolyzing various liquid detergents in order to replicate compounds and patterns to those observed in the case sample. Several mediums, such as terry cloth, cotton, and aluminum foil, were tested in an attempt to replicate pyrolysis conditions without contributing additional interferences in the resulting chromatograms. Samples were extracted using passive headspace and analyzed by gas chromatography/mass selective detection in accordance to ASTM 1618-06. Preliminary results indicate the presence of light aromatic compounds when linear alkylbenzenes were also detected in the sample. Light aromatic compound ratios observed in the presence of linear alkylbenzenes; however, do not correspond to light aromatic compound ratios observed in gasoline. Subsequent tests are being conducted to provide further interference information on additional mediums and detergents.

This presentation will discuss and compare the test methods utilized, as well as compare the results from the different trial conditions. Summed ion profiling will be utilized to aid in distinguishing pyrolysate interferences from linear alkylbenzes and ignitable liquids, such as gasoline.

Fire Debris Analysis, Surfactants, Pyrolysis

### A173 Microcrystal Analysis of Methamphetamine in the Presence of Added Adulterants

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After attending this presentation, attendees will have a basic understanding of microcrystal tests, the effect of adulterants on methamphetamine microcrystal morphology, and the primary characteristics to monitor when performing these tests.

This presentation will impact the forensic science community by providing a new approach for interpreting the changes in the crystal morphology of methamphetamine when diluted and mixed with common adulterants and the reagents used in the manufacture of methamphetamine. A comparison of the changes shows that the microcrystal test is specific enough to be used to not only identify methamphetamine, but also the contaminant. The procedures developed in this project also have a potential application in drug profiling on street samples.

In 2008, there were 95,000 new users of methamphetamine 12 yearsof-age and older. While this number is quite large, it is significantly lower than earlier estimates; however, the number of meth lab incidents has risen to 996 in the month of March 2009, up from 756 in March of 2008.

The methamphetamine submitted into evidence is generally 70% pure. Common adulterants such as lidocaine, sugar, caffeine, and levamisole are added to pure methamphetamine to add bulk and increase the street value. The purpose of this project is to document the changes in the crystal morphology of methamphetamine in the presence of common adulterants as table sugar, caffeine, ephedrine, D-

pseudoephedrine, procaine, lidocaine, levamisole, methyl sulfone, Linositol, D-inositol, and baking soda. Samples of methamphetamine with the above adulterants at 10, 20, and 50% concentrations were prepared and examined under a polarized light microscope and the changes in the crystals' growth were observed and photographed.

The advantages of using microcrystal tests as a presumptive test for illicit drugs in crime labs are the low cost, the minimal amount of sample required, and that the only instrumentation required is a polarizing optical microscope. The most common microcrystal test for methamphetamine is a hanging drop test that uses gold chloride reagent and acetic acid to form distinctive elongated, bar-shaped crystals with regular edges and varied colors that run perpendicular to the long axis of the crystal. By using the hanging drop method, the methamphetamine is purified in the same step. While this is generally an advantage, in this study it was necessary to do the test on a microscope slide so that the effect of the adulterants on the crystal morphology could be observed.

The tests were first done on aqueous solutions of the drug and adulterants. This was followed by tests on powdered samples that more closely matched samples analyzed in a crime lab.

The observed changes in the morphology of the methamphetamine crystals were unique to both the specific adulterant and the concentration of that adulterant. Similar trends were seen for both the aqueous and powder samples. For example, a methamphetamine/caffeine mixture can be identified by the appearance of fine needles in the form of rosettes. The degree of formation of needle rosettes increases with caffeine concentration. However, at 50% caffeine, short needles in the form of rosettes appear in a barbed wire fashion. The crystal formation was also delayed in the presence of caffeine.

The results show that changes in the crystal morphology of methamphetamine does exhibit trends that can be linked to a specific adulterant. Distinctive trends were observed with each adulterant at each concentration.

Methamphetamine, Microcrystal Test, Adulterants

#### A174 Methamphetamine Via High Temperature A175 Spectral Analysis of the Interconversion of Dry Cook: Thirty Second Meth Gamma-Hydroxybutyrate (GHB) and

Monica S. Price, BS, and Harry F. Skinner, MS\*, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081

After attending this presentation, attendees will understand a new manufacturing process for methamphetamine (meth), as well as the overall process, by-products, impurities, hazards and dangers, and yield.

This presentation will impact the forensic science community by presenting a new variation of meth manufacturing, as well as the hazards and dangers of these clandestine laboratories.

The chemicals and procedures used by the clandestine laboratory operator have evolved through the years. These changes typically have been made in response to the availability of precursors and chemical reagents used in the clandestine manufacture of many drugs including methamphetamine. Methamphetamine has been synthesized from phenyl-2-propane (P2P) since the 1970's. Once phenyl-2-propane was controlled in 1980 and became very difficult to obtain, the clandestine laboratory operator adapted by synthesizing the P2P from an easier to obtain precursor, such as phenylacetic acid or benzyl cyanide.

In 1982, a new clandestine method to manufacture methamphetamine was encountered. This method very quickly replaced the P2P method with no controls over the precursor or reagents. Over time, as controls were implemented, the clandestine laboratory operator adapted. Initially the synthesis was done using ephedrine, hydriodic acid, and red phosphorus. Pseudoephedrine quickly became the precursor of choice when ephedrine became regulated. Hydriodic acid was manufactured from iodine and red phosphorus. Hypophosphorous acid was first substituted for red phosphorus, and later by phosphorous acid. In each case, the manufacturing process involved adding water or using a

reagent that contained water, producing an aqueous liquid mixture which was then heated in some cases to reflux temperatures.

The latest variation of the method now involves the manufacture of methamphetamine caused by heating a mixture of pseudoephedrine, red phosphorus and iodine, typically in a "closed" glass vessel, to a very high temperature with a propane torch. The cook has been dubbed by various names including "Flask to Glass Meth," "Volcano Meth," "Quick Meth," or "Thirty Second Meth;" however, a better description or name is "High Temp Dry Cook." This technique is very notable in that there is no water added to the cook. The combination of the pseudoephedrine, iodine, and red phosphorus produces a dark colored solid mixture. Information obtained from clandestine "cooks" suggests that although the process can be very dangerous, methamphetamine is produced. To assess the veracity of these claims, many time-controlled, trial "cooks" have been performed. These tests demonstrate that the solid mixture melts and vaporizes during the reaction and methamphetamine is produced within five seconds. Phenyl-2-propane and amphetamine are also produced during the cooking process, as well as the major impurity propylbenzene. After the short cooking process, "when clear or white vapors are obtained," water is added and the reaction is processed in the typical clandestine laboratory manner by basifying with sodium hydroxide and extracting with organic solvents such as camp type fuels or ether. The organic layer is then "gassed" with hydrogen chloride gas and the methamphetamine HCl is filtered using common items such as coffee filters. The finished product contains methamphetamine HCl as well as some amphetamine HCl. Yields of methamphetamine HCl are about 50%. However, a lot of the cooks fail because of heating the mixture too long. In some cases, a piece of a magnesium fire starter is also added "to make the reaction hotter." All the aspects of manufacturing methamphetamine via a high temperature dry cook, including the overall process, byproducts, impurities, and yields will be discussed.

Thirty Second Meth, High Temperature Dry Cook, Clandestine Manufacture

#### A175 Spectral Analysis of the Interconversion of Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) Using Near Infrared Spectroscopy

Kristen J. Johnson, BS\*, and Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 10104

After attending this presentation, attendees will have a better understanding of the use of Near Infrared Spectroscopy (NIRS) to study the chemistry and equilibrium between the cyclization of gammahydroxybutyrate (GHB) to gamma-butyrolactone (GBL).

This presentation will impact the forensic science community by serving to provide a critical understanding of the rate of interconversion of GHB to GBL, and vice versa, at different matrix conditions of GHB in evidential samples.

GHB is a central nervous system depressant classified as a sedativehypnotic and abused for it's euphoric, sedative, and anabolic effects. GHB and its many derivatives can be studied using the spectroscopic technique of NIRS because it is a quick non-destructive technique that allows for the measurement of organic compounds with vibrational overtones between 700nm and 2500nm. GHB is classified as a federal Schedule I illicit substance, but can also be obtained in prescription form. GHB commonly cycilizes into a lactone, GBL, and the equilibrium depends upon the matrix conditions. The equilibrium ratio between GHB and GBL can vary with the pH of the medium and the temperature of the matrix. The equilibrium reactions can be measured accurately using NIRS in the transmittance mode. Due to the solutions being clear and colorless, the transmittance mode is most appropriate for this equilibrium study. The unique changes in the carbon groups can be used as the main parameter of comparison in shape, size, and intensity of each spectrum with the intention of understanding the rate of interconversion at different specific conditions. All samples were diluted in deionized water, measured in the same cuvette (2mm) using NIRS, while varying the pH with a phosphate buffer. Thirty-two scans for each run were taken and the interconversion was measured as it occurred at a given time interval. To standardize the spectral data, all spectra were analyzed using the multivariable mathematical software provided with the instrument. Using a specific time interval, the conversion rate can be seen at the various pH levels using the spectra generated by the NIR software. The fanning of the spectra over the time interval clearly demonstrates the conversion of the alcohol to the lactone or the lactone to the alcohol depending on the pH tested. The fanning demonstrates a reaction rate which can be measured using a regression plot.

The results in this study agree with that as measured by Ciolino et al.<sup>1</sup> When GHB is exposed to a basic solution with a pH = 12, the solution is stable. If GBL is added to this basic medium instead, a rapid conversion takes place with 100% completion to form GHB. The basic medium de-cycilizes the GBL and forms GHB due to the introduction of more free OH- molecules in the medium. The reaction also goes to completion very rapidly due to the amount of free OH- groups present at this pH. The reaction time for 100% GHB at pH=12 is approximately 15 minutes. Once the pH begins to lower toward the acidic region, the reaction rate of GBL to GHB drops off significantly and the reaction no longer goes to 100% GHB. The pH has a drastic effect on the reaction rate as well as the equilibrium concentrations of the two drugs. At an acidic pH, the GBL is stable, but upon addition of GHB to a solution with a pH of two, the reaction rate of conversion to GBL is very fast and the relative concentration of GBL to GHB is much greater. At a pH of seven, the reaction is very stable, amounting to a longer period to equilibrate and relatively equal concentrations of GHB and GBL in an aqueous solution. **Reference:** 

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Forensic Science, GHB, Near Infrared Spectroscopy

#### A176 Investigation Into the Stability and Storage Conditions of Benzylpiperazine (BZP) for Chemical Impurity Profiling

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After attending this presentation, attendees will have an increased understanding of the storage and analysis of benzylpiperazine for drug profiling. Beneficiaries will include those in the law enforcement agencies involved in the seizure and analysis of drugs of abuse.

This presentation will impact the forensic science community by increasing the understanding of drug profiling. This presentation will provide a general discussion on handling, storage, and analysis of seized drugs focussing on the latest research and development in this area relating to Benzylpiperazine (BZP).

Since the 1990's a new group of synthetic drugs called "piperazines" have gained popularity among those who use amphetamine type stimulants (ATS) (Staack, 2007).<sup>1</sup> N-benzyl piperazine (BZP), an example of a piperazine type drug was initially synthesized by Wellcome Research Laboratories in the United Kingdom in 1944 as an antiparasitic drug. Its popularity in the illegal drug market is attributed to the physiological effects similar to those of ATS such as "ecstasy" (de Boer et al., 2001) as well as tighter regulatory control on ATS type drugs.<sup>2</sup> The

number of clinical reports relating to BZP abuse continues to rise, making the development of an analytical method to profile the drug and its clandestine impurities not only necessary but imperative. Until recently, BZP was perceived as a safer legal alternative to ATS. However, since December 2009, BZP has been controlled in the United Kingdom under the Misuse of Drugs Act 1971, Amendment Order 2009. It is also controlled as a Schedule I drug in the United States. There is very little data on optimized analytical and profiling methods for this drug class. This project provides data on the storage conditions and introduction of BZP to an HPLC instrument so that BZP may be successfully analyzed and profiled in support of law enforcement activities.

High performance liquid chromatography (HPLC) was used in this study (Smith et. al., 2008).<sup>3</sup> A Shimadzu liquid chromatograph (LC-10AD VP) with a diode array detector (SIL-10AD VP) fitted with a normal phase silica column (SphereClone 5µm Silica gel, 250 mm x 4.60 mm i.d.) with the detection wavelength of 254 nm was used. The injection volume was 10 µl at 20°C with a flow rate of 1 mL/min and isocratic elution using methanoic HC1 (methanol, ammonia, and hydrochloric acid at the ratio of 1000: 9.2: 2.9 v/v), giving a total analysis time of 15 min.

A linear detector response range was established for a drug concentration range between 0.1- 1 mg/mL. The coefficient of correlation ( $r^2$ ) was found to be 0.99. The limit of detection and limit of quantitation were calculated as 0.426 and 1.15 µg on column respectively.

In order for the data to be valid, the drugs must be stable in the system in which they are introduced to the HPLC. The stability of BZP (0.5 mg/mL) in the solvent used to introduce the drug to the HPLC (methanoic HCl) was investigated in light and dark over a period of 72 hrs. The stability of the BZP solution in the autosampler was also explored. This study shows that the BZP in methanoic HCl is stable at room temperature in conditions of light and dark only up to 22 hrs. The study of the BZP stability in an autosampler revealed similar results.

Having established the analytical conditions necessary for accurate determination of BZP, the next stage was to identify the storage conditions prior to its analysis. BZP was stored under four different conditions: light and dark at room temperature (~20°C), refrigerator (4°C), and freezer (-18°C). Samples were analyzed at time intervals of 0, 24, 48, 72, and 96 hours and the significance of any observed changes were investigated using the Kruskal Wallis non-parametric test at a 95% significance level. The results demonstrate that there was no significant difference between the bench light and dark conditions for 96 hours. However, when samples were stored in an incubator in the dark or in a freezer, analysis showed there was evidence of sample instability.

The conclusions from this study are that BZP samples should be analyzed as soon as possible after seizure and can be stored on a bench in the light or the dark. This study also shows that as an analytical method, the HPLC method presented is suitable for quantitative determination of BZP.

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- <sup>2</sup> de Boer, D., Bosman, I.J., Hidvegi, E, Manzoni C, Benko, A.A., dos Reys, L.J. and Maes, R.A., (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic science international*. 121(1-2), pp.47-56.
- <sup>3.</sup> Smith E. C., Lawther, G., and Cole, M., (2008). Analysis of piperazines by high performance liquid chromatography. *Global Forensic Science Today*. 7: pp14-22.

BZP, HPLC, Profiling

### A177 Detection of Anabolic Steroids in Dietary Supplements

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After attending this presentation, attendees will become aware of the various steroids that have been detected as active ingredients in over-thecounter nutritional supplements. In recent years, the use of performance enhancing dietary supplements has become popular. A particularly dangerous class of synthetic steroids has been created for athletes and has no approved medical use.

This presentation will impact the forensic science community by discussing analytical profiles of some of the most recently encountered steroids in dietary supplements.

In recent years, a number of designer anabolic steroids have surfaced as active ingredients in dietary supplement products. Designer anabolic steroids are synthetic modifications of testosterone, sharing the same four-ring core structure of the molecule. Today, many athletes have turned to these products to improve their performance and increase their muscle mass. As such, these supplements are marketed as muscle building, performance enhancing, and weight loss over-the-counter (OTC) products.

Recent investigations of OTC dietary supplements have indicated that often, the information on the label is misleading, incomplete, or simply absent. Chemical analysis has revealed that many of these supplements are contaminated or spiked with low concentrations of anabolic steroids and contain undeclared prohormones. A prohormone, such as 4-androstene-3,17-dione can be converted in the body into testosterone, a natural male hormone. The use of these substances has been prohibited by the World Anti-Doping Authority (WADA) in order to achieve fair play and to prevent cheating in athletic competitions.

Most of the designer steroids have surfaced either as esters, ethers, chlorine, bromine, or nitrogen derivatives. Newer submissions of dietary supplements have indicated the presence of other steroids and prohormones that have not yet been classified as controlled in the U.S. Controlled Substances Act (CSA). Once ingested, they can produce similar anabolic effects on the body with a chance of not being detected in routine doping tests. These supplements have been found to contain complex matrices that make their analysis challenging, time consuming, and expensive.

This presentation will discuss the analytical profiles of some of the most recently encountered steroids in dietary supplements. Prohoromone and hormone derivatives of testosterone such as nandrolone, drostanolone, dihydrotestosterone, methyl-1-testosterone, and 4- androstene-3,17-dione will be discussed. Other designer steroids such as prostanozol, dimethazine, madol, methoxygonadiene, 4,9(10)-estradiene-3,17-dione, and 1,4-androstadiene-3,17-dione will also be covered. The steroids were characterized by gas chromatography mass spectrometry (GC/MS), electrospray ionization (ESI) liquid chromatography mass spectrometry (LC/MS), and LCMS/MS. Steroid detection by high resolution accurate mass spectrometry will also be covered along with some of the limitations and challenges of this type of analyses will.

Dietary Supplements, Anabolic Steroids, Mass Spectrometry

### in A178 Identification of Synthetic Cannabinoids in K2 Herbal Incense and Drug Paraphernalia by TLC, GC/MS, LCMS/MS, and LCTOF

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After attending this presentation, attendees will be able to discuss the rise in popularity of a number of synthetic cannabinoid agonists currently subject to abuse in the United States, and the products that contain them. Attendees will be able to select appropriate analytical techniques for their forensic identification and confirmation.

This presentation will impact the forensic science community by preparing forensic laboratories to identify these drugs which are increasingly being outlawed and scheduled on a state by state basis throughout the United States.

A variety of therapeutic drug candidates have been developed since the 1970's, with activity at the cannabinoid CB1 receptor, responsible for the psychoactive effects of tetrahydrocannabinol (THC), the active component of marijuana. These compounds include a series developed at Hebrew University, the HU-series, drugs developed by Pfizer, the CP Series, and drugs developed at Clemson University, the JWH Series. As investigational compounds never developed for therapeutic trials, they were never scheduled or prohibited in the United States. Beginning in 2007, these drugs began to appear in a smokeable form in Europe, doped onto benign herbal material and sold as "Spice," a herbal incense containing HU-210, a potent CB1 agonist. Subsequently a variety of products appeared on the recreational drug market in Europe and the United States, including a variety of "K2" blends - Strawberry, Citron, Blueberry, Blonde, Summit, etc - other brands include Yucatan Fire, Space, and K3. A variety of these products have been characterized through the use of various chemical color tests, thin layer chromatography (TLC), gas chromatography mass spectrometry (GC/MS), liquid chromatography tandem mass spectrometry (LCMS/MS), and liquid chromatography time of flight mass spectrometry (LCTOF). Quantitative analysis was performed by high performance liquid chromatography (HPLC) and GC/MS.

The primary identification technique was GC/MS, identifying compounds by library matching. Compounds not identified through library matching were identified by molecular formula through analysis by accurate mass LCTOF. Reference standards for JWH133, CP47,497 (C=7), CP47,497 (C=8), JWH-250, CP55,940, JWH-015, HU-210, HU-211, JWH-073, JWH-018, JWH-018, JWH-019, JWH-200, and WIN55,212 were obtained for confirmation of drugs identified in the herbal material and they have been characterized using a selection of the techniques described above.

Quantitative analysis by GC/MS and HPLC showed significant variability in concentration of the active drugs within a single sachet and also between different sachets with the same name, purchased from different suppliers.

The most frequently identified synthetic cannabinoids were JWH-018 and JWH-073, either together or alone, which had concentrations in the range of 7 to 20mcg/g of herbal material. Less frequently occurring were JWH-200, JWH-250, JWH-175, CP47,497 (C=7), and WIN55,212, but generally in the same concentration range, with the exception of WIN55,212 which was present in trace amounts and identified only by LCMS/MS.

Water pipes used for smoking K2 blends were also tested and the drugs and degradation products were identified in the bowl, screen, and stem of the smoking apparatus.

Routine color tests (Dille-Koppanyi, Scott, Duqenois-Levine) were not found to be useful for screening herbal material. TLC has some value, but has limited ability to resolve closely related compounds in the same mixtures and inadequate sensitivity for low levels of drugs present in some blends.

As more states adjust their scheduling for these emerging recreational drugs, criminalistics laboratories will need to adapt to use non-traditional analytical methods to identify new analogs and add these to their analytical menus.

Synthetic Cannabinoids, K2, LCMSMS

#### A179 The Characterization of a Series of Synthetic Cannabinoid Reference Standards Followed by the Analysis and Comparison of Various Herbal Incenses and Unknown Powders

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After attending this presentation, attendees will have a firm grasp on the properties and characteristics of synthetic cannabinoids, understand their relationship to herbal incenses or "legal marijuana," understand how to effectively analyze such samples, and finally, understand why such information is becoming increasingly important to forensic drug chemists.

This presentation will impact the forensic science community by informing attendees on the emerging synthetic cannabinoid or "legal marijuana" phenomenon among drug users and educating drug chemists on the proper analysis of such compounds.

Recently, herbal blends marketed as "legal marijuana" have become increasingly popular on the streets due to their ability to produce a marijuana-like high.<sup>1,2</sup> Although declared as an incense and not for human consumption, these blends are consumed as herbal drugs via inhalation, much like cannabis.<sup>3</sup> In the United States, these products are largely an internet phenomenon,<sup>2</sup> but are also readily available at smoke shops across the country.

One particular brand of herbal incense that has emerged from Europe within recent years is Spice. The list of ingredients on the packaging of Spice indicates a mixture of plant components, such as Indian Warrior, Lion's Tail, and Blue Lotus.<sup>3,4</sup> The overall blend of these herbs was claimed to be responsible for the cannabis-like intoxication produced upon consumption.<sup>3,4</sup> However, there was strong suspicion that synthetic cannabinoids not reported on the label were added to Spice in order to produce the described pharmacological effects.<sup>3,4,5</sup> Upon scientific analysis by various laboratories, Spice and other herbal blends were found to contain several synthetic cannabinoid compounds, which supported prior suspicions.<sup>1,5</sup> These compounds include the following: HU-210; HU-211; CP 47,497; JWH-018; JWH-073; JWH-398; JW-250; and the fatty acid oleamide.<sup>1,2,3,4,5</sup> Of these, only HU-210 is controlled federally in the U.S. (schedule I), but states such as Kansas, Missouri, and Illinois have already or are in the process of enacting laws to outlaw one or more of the above mentioned compounds.

There is no evidence that JWH, CP, and/or HU compounds are present in all herbal incense products or even batches of the same product.<sup>4</sup> Various concentrations and diverse combinations of these substances have been used in different herbal products to produce cannabis-like effects.<sup>4</sup> This poses an obvious danger to consumers. It also expresses the need to identify precisely what is contained within the herbal blends, which can be difficult. According to Elizabeth Williamson, professor of pharmacy at the University of Reading and a specialist in herbal medicines, "Unless researchers know precisely what they are looking for - and have the "fingerprint" of the compound that they are trying to find with which to compare their results - it can prove almost impossible to produce the necessary breakdown."<sup>6</sup> Currently, there are more than 100 known compounds with cannabinoid receptor activity, and it is plausible that additional substances from different chemical groups will soon be discovered.<sup>4</sup> Any of these compounds could one day be added to any of the existing herbal blends, which poses a challenge for future identification, monitoring, and risk assessment of this "legal marijuana" phenomenon.

As word publicly spread that herbal blends were found to contain synthetic cannabinoids capable of producing effects comparable to or more potent than those of  $\Delta^9$ -Tetrahydrocannabinal, the popularity of smoking herbal incenses has risen significantly. Now, online websites have begun selling what they claim to be pure synthetic cannabinoid powders to anyone willing to purchase them, no questions asked. Since concentrations and identities of synthetic cannabinoids vary greatly between brands of herbal incenses and even within batches of the same brand, purchasing pure powders is more beneficial for users because they can be sure of what they are receiving and one gram of powder can be purchased for approximately the same price as one package of herbal incense. With the powder in their possession, users have a wide range of options. For example, comments on online drug and toxicology forums such as Toxicology Synchronium indicate that users have begun to make their own herbal blends by preparing a solution of one or more synthetic cannabinoids and spraying it onto a mixture of plant material.<sup>7, 8</sup> Users have also indicated that they have begun experimenting with various routes of administration, such as insufflation.<sup>7,8</sup> With new trends such as these, it is highly plausible that synthetic cannabinoid powders will soon end up in forensic science labs. Therefore, to be able to conclusively determine the identity of these substances, it is essential that scientists know how to analyze such compounds.

In order to build a synthetic cannabinoid library to aid in the future analysis and characterization of various brands of "legal marijuana," various synthetic cannabinoids already found to be present in herbal blends, as well as those that could possibly be found in future blends, were obtained from Cayman Chemical of Ann Arbor, Michigan, and subsequently analyzed by gas chromatography/mass spectrometry (GC/MS) and fourier transform infrared (FTIR) spectroscopy. The reference standards analyzed include the following: JWH-018; JWH-019; JWH-073; JWH-200; JWH-250; HU-210; HU-211; HU-308; HU-331; (±)-CP 47,497; and 9-octadecenamide. The data gathered from the reference standards were applied to the analysis of various brands of herbal incenses obtained from laboratory submissions and smoke shops as well as synthetic cannabinoid powders obtained via the Internet. The powders and herbal incenses were quantitated using gas chromatography/flame photometric detection (GD/FPD) to (1) determine the purity of the JWH powder samples relative to the reference standards; and, (2) determine the percentage of drug present in each of the herbal incense extracts relative to the corresponding powder samples. GC/MS, FTIR, and GC/FPD data are presented for all reference standards and samples analyzed.

Synthetic Cannabinoids, JWH-018, Herbal Incense

## A180 Identification of Small Mineral Grains Separated From Soil Samples Using MicroRaman Spectroscopy

Howard A. Harris, PhD\*, University of New Haven, Forensic Science Program, 300 Boston Post Road, West Haven, CT 06516

After attending this presentation, attendees will become aware of the possibility of a simple preparation of mineral grains from small soil samples suitable to obtain Raman spectra and the potential of using such spectra in the examination and comparison of forensic soil evidence.

This presentation will impact the forensic science community by discussing how soil mineral grain comparison using Raman spectroscopy should become a simple and useful part of forensic soil examination and comparison. Analysis and comparison of soils can be greatly aided by identification of mineral grains present in the soil. However, mineral identification using polarized light microscopic methods, as currently done, requires considerable specialized training and skill and can be time consuming. Many forensic laboratories do not have an examiner with such training. There exist on the internet several quite large databases of Raman spectra of minerals. Instrumental analysis followed by the use of a suitable Raman database<sup>1</sup> should provide a less time consuming and more easily implemented method of soil mineral analysis.

Reffner et al.<sup>2</sup> have recently published a small database of known mineral spectra using attenuated total reflectance (ATR) to obtain infrared spectra by crushing mineral grains against the diamond ATR crystal. Raman spectroscopy has the advantage of allowing spectral data to be captured in the 600 to 50 cm<sup>-1</sup> region, where many minerals have strong absorption; this region is not available with most infrared detectors. Since it has been found that many dark colored mineral grains give very weak Raman absorption, infrared might be very useful for improving the identification of such mineral grains.

The researchers have developed and tried out a simple method for recovering two different sized fractions of sand-sized mineral grains. If a small (generally two grams or less) sample of soil is deflocculated by stirring the soil overnight in a 4% solution of sodium metaphosphate followed by agitation and rinsing first with fresh deflocculating solution and then several times with distilled water, material suitable for size separation is obtained. Three sieves were chosen to do this separation, numbers 60 (250  $\mu$ m), 120 (125  $\mu$ m), and 170 (90  $\mu$ m). The material is wet sieved through this set of sieves with distilled water and then alcohol and the materials in the 120 and 170 sieves are dried in a warm oven. This produces two fractions of small mineral grains that are quite clean and are suitable for direct Raman analysis. Excellent Raman spectra have been obtained on many of these grains that are suitable for identification using a large Raman mineral database (RRUFF) compiled jointly by Caltech and the University of Arizona.<sup>1</sup>

Approximately 15 soil samples were collected over about a five mile stretch of Connecticut route 34 by sampling from intersections with easily available soil. Additional suburban soil samples were collected from several different locations also in Connecticut. These samples were processed as described above to produce two sand grain-sized mineral fractions each. Samples were examined by spreading a small amount of mineral grains on a cleaned piece of aluminum sheet cut to microscope slide size. The aluminum slide had a small grid scratched onto the surface to allow a systematic search of the sample. Mineral grains were then examined to produce Raman spectra that were then searched against the RRUFF Database. In several cases grains were found with what appeared to be good quality spectra, but they did not match any of the minerals in the Database. Grains of five to ten different types, some identified as a particular mineral or as from a mineral family, were found in each sample. Those that did not match any of the spectra in the Database but provided distinctive Raman spectra were likely grains with a mixture of two or more different minerals.

Over twenty soil samples were examined to determine if this technique can provide useful data toward differentiating these samples. The data developed data in this way was found to be consistent and reproducible.

#### **References:**

- <sup>1</sup> B. A. Weinger, J. A. Reffner, P. R. DeForest; J Forensic Science; Vol. 54; No. 4; pp851-856; 2009
- <sup>2.</sup> http:minerals.caltech.edu/FILES/raman/

Raman Spectroscopy, Minerals in Soil, Soil Comparison

#### A181 Liquid Chromatography/Mass Spectrometry Investigations of Dye and Fiber Degradation Resulting From Environmental Exposure

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The goal of this presentation is to describe characterization changes that occur in textile fibers as a result of exposure to environmental conditions including laundering and outdoor exposure to sunlight, heat, and moisture.

This presentation will impact the forensic science community by providing trace evidence examiners a better understanding of textile physical and chemical changes and by accounting for these effects through laboratory comparisons of fibers.

Textile fibers have become an important aspect of forensic science due to their abundance at crime scenes. However, fibers are rarely found in pristine condition. The degradation of fibers and dyes can complicate the forensic comparison between questioned and known fiber samples, particularly if only one sample has been weathered. Although ultraviolet (UV)/visible and fluorescence microspectrophotometry allow direct and nondestructive analysis of a fiber of a few millimeters in length, a more selective and sensitive technique, such as liquid chromatography/mass spectrometry (LC-MS), is required to characterize diminutive amounts of dye (2-200 ng) present on forensically relevant analytes.

In this study, dyed fabric samples of the most commonly used fiber types (cotton, polyester, nylon, and acrylic) were used with the most commonly used dyes (reactive, disperse, acid, and basic) and subjected to a variety of environmental conditions (washing, bleaching, sunlight, heat, accelerated weathering, and natural weathering). Fabric samples were exposed to outdoor weathering (Arizona and Florida) and accelerated outdoor weathering (EMMA and EMMAQUA equivalent to three, six, nine, and twelve-months in hot-dry and hot-wet environments). Samples were also laundered with various commercial detergents, each alone; with chlorine bleach; and with peroxide bleach. Textile samples were retired from exposure at predetermined time intervals of exposure and screened by UV/visible and fluorescence microspectrophotometry for degrading effects. Microextractions of dyes and fluorescent brighteners (FBs) from fibers were subsequently performed on small scale threads and single fibers (1 mm-5 cm) followed by reconstitution of the dyes in liquid chromatography-compatible solvents. Fiber extracts were then analyzed using an Agilent 1100 Series LC system interfaced to an AB SCIEX 3200 O-Trap mass spectrometer. The dyes and other traces associated with the manufacturer and/or weathering conditions present in the fibers were qualitatively identified and their relative quantitative composition was estimated.

The analysis of environmentally exposed samples by LC/MS allows investigators to examine the loss of dyes from textiles and the addition of extraneous contaminants from the environment, as well as to profile degradation products from both fibers and dyes. Finally, the experimental design approach used in these studies permits assess of the nature of changes in those profiles that occur as a function of time. This wealth of information could contribute to the interpretation of environmental effects on fiber evidence and determination of their forensic relevance.

Textile Fibers, LC/MS, Dyes

#### A182 Use of Surface Enhanced Raman Spectroscopy (SERS) Applied to the Study of Fluorescing Pigments and Dyes

Jennifer N. Herb, MS\*, and Christopher S. Palenik, PhD, Microtrace, 790 Fletcher Drive, Suite 106, Elgin, IL 60123-4755

After attending this presentation, attendees will know a limitation of Raman spectroscopy (fluorescence) and learn a technique to overcome the limitation–Surface Enhanced Raman Spectroscopy (SERS).

This presentation will impact the forensic science community by demonstrating a technique that may be useful in overcoming a limitation of Raman spectroscopy and may be applied to samples in both civil and criminal cases.

Raman micro-spectroscopy provides a means to classify and identify colorants *in situ*. The laser-based analysis method affords a small analytical volume (as small as a few cubic micrometers), requires minimal sample preparation, and is generally non-destructive. Past presentations have illustrated the ways in which Raman spectroscopy can be advantageously applied to the study of pigments in automotive and architectural paint samples. The most significant limitation to Raman spectroscopy is sample fluorescence. Fluorescence can easily overwhelm a Raman spectrum. While there are various analytical approaches to minimizing or avoiding fluorescence (*e.g.* different laser wavelengths, fluorescence bleaching, etc.), the issue remains a major limitation, in many instances. Surface Enhanced Raman Spectroscopy (SERS) offers another alternative to overcome this problem.

SERS is based upon enhancing the Raman scattering from a particle through resonance effects created by the addition of a metal colloid. In practice, preparation of a SERS sample is relatively straightforward. A capillary drop of a metal colloid solution (typically gold or silver), which can be prepared in a laboratory or purchased, is applied to an area of interest on the sample. Once the colloid solution has dried, the sample is analyzed by Raman spectroscopy. The technique can be extremely sensitive (spectra have been obtained by researchers from a single molecule) and due to the strong resonance enhanced scattering, fluorescence can be overcome. The downsides to this technique are that: (1) not all molecules resonate with all metals and so a metal colloid must be matched to a molecule; and, (2) the SERS spectrum can show peak shifts and different peak intensities than a corresponding Raman spectrum. The latter point makes identification of unknown samples by SERS a difficult prospect (since a regular Raman spectral database is not applicable).

The research into forensic applications of SERS that was conducted and will be presented here consisted of several components. First, colloidal solutions of gold and silver were precipitated. Reference dyes known to produce SERS spectra were analyzed to ensure that the effect was being observed. Various colloid application techniques were evaluated. Once a suitable technique had been developed, numerous reference spectra were collected from common dyes. Many of the dyes examined (e.g., erythrosine B, methyl violet, and pararosaniline), which have virtually no Raman spectrum due to fluorescence, produced excellent SERS spectra. Pigments studied, in general, were less amenable to producing a useful SERS spectrum. Still, certain pigments produced SERS spectra when a corresponding traditional Raman analysis showed only fluorescence. A small database of SERS spectra was developed.

Over the course of twelve months, SERS was applied to various casework samples in an attempt to find practical applications of this method as a forensic technique. In one case, requiring the identification of an unknown solid floating in a liquid product, FTIR analysis suggested the presence of a dyestuff. Analysis of the solid by Raman spectroscopy produced only fluorescence. Application of a gold colloid solution to a small, isolated fraction of the unknown material produced an intense SERS spectrum. The SERS spectrum was identified, by comparison to reference spectra, as erythrosine, a food dye that has been largely phased out of U.S. usage. This identification was supported by elemental data obtained by EDS analysis.

The successful application of SERS to a civil forensic matter illustrates the potential value of this technique in specific circumstances. Although SERS will never be used as commonly in the forensic field, it deserves consideration and use as a valuable micro-analytical accessory to Raman spectroscopy in specific instances.

Pigment, Raman Spectroscopy, Surface Enhanced

## A183 Development and Validation of AccuTOF-DART(TM) as a Screening Method for Analysis of Bank Security Device and Pepper Spray Components

Robert Steiner, MS, Virginia Division of Forensic Sciences, 700 North 5th Street, Richmond, VA 23219; and Allison Pfaff, MS\*, 4369 Quail Creek Road, Martinez, GA 30907

After attending this presentation, attendees will be familiar with the results of the validation for a method of screening for pepper spray and bank security device components using Direct Analysis in Real Time (DART<sup>TM</sup>) ionization coupled with Accurate Time-of-Flight (AccuTOF) mass spectrometric detection.

This presentation will impact the forensic science community by providing a much more efficient procedure for processing evidence of this type by decreasing the time spent analyzing negative samples and by quickly identifying areas of interest on positive samples.

Currently at the Virginia Department of Forensic Science, cases involving pepper spray and bank security devices are analyzed similarly. An initial visual screen is performed to locate noticeably stained areas. The visual screen is more effective in the case of bank security devices due to the red dye, 1-methylaminoanthraquinone (MAAQ), which is contained in these devices. However, a visual scan may not locate stains on darkly colored clothing and can not determine the source of the red color. Pepper sprays can be difficult to locate visually due to capsaicin's, the main component in such sprays, lack of color. Visual screens can be aided by the use of UV light, but this method has no specificity and many stains can still be missed. Stained areas are extracted for confirmation of MAAQ, o-chlorobenzylidenemalononitrile (CS, a tear gas), or capsaicin by GC/MS. When no stains are visible, a random sampling of areas is extracted for GC/MS analysis, which can be very time-consuming.

This study consisted of five main steps: (1) Optimization of AccuTOF-DARTä parameters for each analyte; (2) Determination of the most efficient extraction solvent for each analyte; (3) Development of an appropriate technique for introducing fabric samples into the DARTä sample gap; (4) Development of an appropriate technique for extracting samples for GC/MS confirmation; and, (5) Validation of the final method by determining the lower limit of detection (LLOD) of the method, interferences, selectivity, reproducibility, and robustness.

For the optimization of parameters, each analyte was tested at a range of different temperatures and orifice 1 voltages to determine the most favorable conditions for ionization and detection. A gas temperature of 325°C was chosen for all analytes. The first voltage provides a spectrum showing protonated molecules while the second voltage provides additional spectral information via collision induced dissociation. For MAAQ and CS, orifice 1 voltages of 30V and 85V were chosen, while 20V and 50V were best for capsaicin. To determine the appropriate extraction solvents, each analyte was spiked onto several different fabrics and extracted with hexane, dichloromethane, and methanol and analyzed on the GC/MS. Hexane was found to be iDrug Enforcement Administrationl for MAAQ and CS, while methanol was best for capsaicin. The main focus of this research was the determination of an appropriate sampling technique for fabrics on the AccuTOF-DARTä. Inserting the stained fabric directly into the DARTä sample gap

was the simplest and most efficient technique, but several problems arose with some fabrics melting in the high gas temperature or introducing lint into the ion optics of the AccuTOF. The best technique was found to be extraction through the fabric onto cardstock followed by direct insertion of the stained cardstock into the DARTä sample gap. Methanol was chosen as the extraction solvent for all analytes due to its added screening ability of maintaining the red color of MAAQ, while hexane produced a pale yellow extract that was not readily visible on the cardstock. After AccuTOF-DARTä screening, stains were cut from the cardstock and extracted in hexane for MAAQ and CS or methanol for capsaicin, followed by concentration of the extract prior to confirmation by GC/MS. The LLOD of the method was determined by spiking fabric samples with serial amounts of each analyte and analyzing with the final method. No spectral interferences were found for any of the analytes. No potential selectivity issues were determined for MAAQ or CS. Pepper sprays were tested alongside hot sauces, which also contain capsaicin, to determine a method for differentiation between the two. Differences were found in the relative abundance of the ion peak representing linoleic acid vs. the ion peak representing capsaicin. The linoleic acid peak is relatively large compared with the capsaicin peak in hot sauces, whereas it is insignificant compared with the capsaicin peak in the pepper sprays tested. Reproducibility was determined by repeating the LLOD studies ten times each on two different days. Robustness was shown by having three separate analysts repeat the LLOD determination for each analyte.

The combination of sample extraction onto cardstock followed by analysis using AccuTOF-DARTä can provide an efficient method for analyzing evidence for the presence of bank security device components and pepper spray components, saving time both in the location of such stains and in the early elimination of negative samples. This study validates the use of the AccuTOF-DARTä in such an analysis scheme.

Direct Analysis in Real Time, Pepper Spray, Bank Dye

#### A184 The Use of SEM/EDS and FT-IR Analyses in Identification the of Counterfeit **Pharmaceutical Packaging**

S. Frank Platek, MS\*, Sara E. Andria, PhD, Moseley Fulcher, and Mark R. Witkowski, PhD, U.S. Food & Drug Administration, Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237-3097

After attending this presentation, attendees will have an understanding of a method to isolate and examine pharmaceutical packaging using scanning electron microscopy (SEM), energy dispersive x-ray spectrometry (EDS), and Fourier transform infrared (FT-IR) spectroscopy. The method allows for the detection of counterfeit pharmaceutical packaging and characterization of the counterfeit packaging itself.

This presentation will impact the forensic science community by providing knowledge of a method for the analytical identification and characterization of pharmaceutical packaging components.

The forensic analysis of counterfeit pharmaceuticals often focuses upon the analysis of the dosage forms with minimal analysis being performed on packaging components. The dosage form analysis is of primary importance due to the potential danger to public health. Counterfeit pharmaceuticals may be dangerous and possibly lethal if taken by a consumer as they may contain little or no active ingredient, undeclared drugs of uncertain potencies and possibly dangerous excipient materials. Counterfeit drugs without active ingredients could compromise an individual's health as they are not receiving the required medication for their health condition. In the area of counterfeit pharmaceutical analysis there has not been a great Drug Enforcement Administrationl of attention devoted to the analysis of the individual packaging components.

The packaging components used for a counterfeit pharmaceutical product can contain a wealth of forensic information which may help in a counterfeit pharmaceutical investigation. Packaging components may include, but are not limited to high density polyethylene (HDPE) bottles, bottle caps, safety induction seals, blister packages, cartons, paper intserts/outserts, and adhesives used on the carton closures and outserts. Each of these packaging components can be effectively analyzed using SEM/EDS and FT-IR spectroscopy. The chemical information derived from the materials can then be used to determine commonality and potentially be used to source counterfeit pharmaceutical products.

The examination of pharmaceutical packaging includes using alternative light sources and stereoscopic light microscopy (SLM). Both of these techniques allow for enhancing visual differences between the suspect packaging and the authentic. The use of SEM/EDS and FT-IR analysis provide unique chemical information about the suspect packaging, which enhances the forensic information provided by the visual examination techniques. The method the authors will present details the identification and sampling of regions of interest from pharmaceutical packaging for examination by SEM/EDS and FT-IR spectroscopy. Sample preparations are made from the same regions of interest on both suspected counterfeit packaging and authentic packaging. The study presented will detail printed regions of blister-style packaging from a variety of tablet and capsule products. The results of the elemental analyses of both top and bottom package surfaces will be covered in this study. SEM/EDX analyses will include backscattered electron imaging and elemental analyses of single features and full field analyses, as well as x-ray mapping of the region of interest, with the results reported as a comparison of suspected counterfeit and authentic pharmaceutical packaging. The FT-IR spectroscopic data will highlight the different polymer materials used in the blister packages. The SEM/EDX and FT-IR data are used to build data libraries of authentic and counterfeit products, which can be used in the analysis of future sample analyses.

Counterfeit, SEM/EDS, FT-IR

### A185 Bullets as Trace Evidence

Peter J. Diaczuk, BS\*, 555 West 57th Street, New York, NY 10019-2925; and Jack Hietpas, BS, 226 Fall Street, Apartment 3, Seneca Falls, NY 13148

The goal of this presentation will be to discuss issues concerning shooting reconstructions. More specifically, attendees of this presentation will learn how recovered bullets from crime scenes as well as from victims may provide significant information, beyond "traditional" firearm examination, for the reconstruction of an event.

This presentation will impact the forensic science community by providing a broad perspective concerning the value of bullet evidence in shooting scene reconstructions. This presentation has important implications for trace evidence and firearm examiners as well as those specializing in crime scene reconstruction.

The complex nature of shooting incidents may generate a variety of firearm-related evidence, such as the firearm itself, cycled or discharged ammunition components, gunshot residue, trace evidence embedded in/transferred to a bullet, or impact sites with traces of the bullet's prior location. Firearm-related evidence is traditionally viewed as the comparison of ammunition to other ammunition (bullet to bullet or case to case) or ammunition to a firearm. However, another area of firearm evidence is trace evidence on bullets and the interpretation of the condition of the recovered bullet itself (deformed, flattened, expanded, broken, destabilized prior or subsequent to impact, etc.). Whether considered firearm or trace evidence, this information, to be most beneficial, must be integrated with the scene reconstruction.

When a shooting incident takes place and firearm evidence is recovered, whether in the form of cartridge casings or bullets, it is likely

that an examination of these ammunition components will be undertaken using the well-established methods of comparison microscopy. There are occasions; however, when 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, or the sequence of the shots that were fired. Information that can shed light onto these types of questions may be gleaned from detailed non-routine microscopical examination of the bullet.

Pulling the trigger of a firearm initiates a series of events that culminates with the discharge of a bullet with considerable energy. The bullet may not only impact its intended target but it may perforate or be deflected by an intermediate object(s) on its way to the target, or it may pass completely through the intended target and retain sufficient energy to continue downrange and impact another object.

These types of interactions invariably impart information about the event onto the bullet, in effect serving as a tape-recorder, documenting prior substrate interactions and thus may provide spatial-chronological information. If information from these secondary impacts is recognized, examined, and interpreted in the context of the case, it may provide extremely valuable data for developing more accurate shooting scene reconstructions.

This presentation will discuss the transfer of material from the substrate to the bullet and the overall change to the bullet morphology due to the physical properties of the substrate materials, as well as the angle of incidence of the bullet to the substrate.

This project investigated predominantly 9 mm full-metal jacketed and jacketed hollow point ammunition with a variety of common substrate materials. Bullet-substrate interactions were documented using high-speed photography to capture the bullet impact and deceleration dynamics. After recovery, the bullets were examined microscopically for trace evidence transferred from the substrates and for morphological changes to the bullet surface or the overall shape. The results of these experiments will be discussed in detail.

The presentation will conclude with a case study of a 1979 capitaloffense appeal, where assessing and correctly interpreting bullet damage was absolutely critical to the accurate shooting reconstruction. The identity of the shooter was not disputed; however, the nature of the shooting act was the primary contended issue. The information learned from the detailed examination of the bullet and bullet fragments recovered from the shooting scene provided sufficient evidence to offer opinions about the series of events that led up to the fatal shot.

Shooting Reconstruction, Trace Evidence, Microscopy

### A186 Gunpowder Particle and Vaporous Lead Deposit Patterns on Fabric From Hand Gun Discharges III

## Kay M. Sweeney, BS\*, KMS Forensics, Inc., PO Box 8580, Kirkland, WA 98034

After attending this presentation, attendees will learn about the deposit patterns for gunpowder particles and varporous lead when selected hand guns are fired into clothing fabrics using different ammunition and at different distances.

This presentation will impact the forensic science community by demonstrating that the type of clothing fabric and the collection/manipulation history of clothing items exhibiting gunshot defects seized as evidence during shooting scene investigations are extremely important factors in determining muzzle to target distances.

Gunpowder particle deposit patterns on clothing fabrics, particularly in the region of a bullet penetration defect, provide interpretive opportunities for forensic scientists interested in establishing an intervening distance measurement between the discharging firearm and the target clothing fabric. The same can be said for vaporous lead deposit patterns. This presentation reports on the results of testing conducted thus far involving one 9mm semi-automatic pistol using 25 different rounds of 9mm luger ammunition representing fourteen manufacturers or brands.

In order to establish baseline information relating to the source of lead in gunpowder particle deposit patterns on clothing, the gunpowder, jacketed bullet, and cartridge case of one round representing each of the ten manufacturers were tested using x-ray fluorescence spectrophotometry (XRF). All gun powders were found to contain lead ranging from 25 ppm to 180 ppm.

All 25 different rounds of ammunition were used to fire into white 100% cotton t-shirt type fabric target panels and white 100% nylon ripstop type jacket fabric panels at a uniform muzzle to target distance of 10 inches. Then, one manufacturer's specific cartridge design was used in the 9mm pistol to fire into white 100% cotton t-shirt type fabric target panels and white 100% nylon rip-stop style jacket fabric at muzzle to target distances of 2," 4," 6," 8," 10," 12," 14," 16," 20," 24," 30," 36," 42," and 48." Again the same 9mm caliber pistol was used for all test firing exercises. Results relating to firearm discharge into 100% cotton targets were originally reported on in 2007 for a 9mm caliber firearm and in 2008 for a .40 caliber firearm and the current presentation relates to results from 9mm firearm discharge into 100% nylon targets and contrasts those results against the cotton target results.

A template of concentric circles drawn at one inch, two inches, three inches, and four inches from the center point was prepared on clear plastic sheet stock and this was used as an overlay on top of the test fire panels with the center point placed Drug Enforcement Administrationd center on the bullet defect in the panels. The circles were scribed into quarters and during microscopic examination, counts for gunpowder particle deposits in each one quarter of the circle were recorded for each zone, or order, from one inch through four inches out from the bullet defect center point.

The counts, for purposes of this presentation, are reported in three ways. One unit used is the number of gunpowder particles counted in a particular quarter circle area. The gunpowder particle count for the area ranging from the circle center point out to the quarter arc at one inch from the circle center is recorded as the "First Order Quarter-Circle Gunpowder Particle Count" and the number for the "Second Order Quarter-Circle Gunpowder Particle Count" is the number of gunpowder particles counted in the quarter of circle area ranging from the circle center point out to the quarter arc at two inches from the circle center, and so on. Another unit used is the calculated density of gunpowder particles in a particular designated quarter of a circle area and that figure is recorded using the appropriate quarter-circle reference as "First Order Quarter-Circle Density," "Second Order Quarter-Circle Density," and so on. The third unit is the gunpowder particle count for a particular "Ouarter-Arc Band" in which gunpowder particle deposits were found. For instance, the "First Order Quarter-Arc Band" is the same space as that designated by the "First Order Quarter-Circle" area and the "Second Order Quarter-Arc Band" is the area between the quarter circle perimeter at one inch from the bullet penetration center and the quarter circle perimeter at two inches from the bullet penetration center, and so on. Gunpowder particles were found on the test panels out to a muzzle to target firing distance of 48 inches.

Gunpowder particle counts for 100% cotton targets were significantly higher than those for the 100% nylon targets in the same order of quarter circle areas. The lowest range of particle counts for the nylon targets is 6% of the cotton target counts using the same ammunition with an identical muzzle to target distance of eight inches (76 particles on the cotton target in the 3<sup>rd</sup> order quarter circle area and five particles in the same zone for the nylon target) and the highest range for the nylon targets is 28% of the cotton target counts with a common muzzle to target distance of 12 inches (25 particles on the cotton target in the 1<sup>st</sup> order quarter circle area as opposed to seven particles in the same zone for the nylon target).

Selected test fire panels of both 100% cotton and 100% nylon were subjected to XRF analysis for the presence of vaporous lead deposits and

four out of five corresponding panel pairs demonstrated higher levels of lead for the cotton panel over the nylon panel for the same firearm, same ammunition, and same firing distance in the first order quarter circle area. Vaporous lead concentrations were found to drop off dramatically in the  $2^{nd}$  order quarter circle band.

Clothing items with bullet holes and gunpowder deposits as recovered from shooting homicide victims can be examined and analyzed for their gunpowder particle deposit patterns and vaporous lead deposits for the purpose of muzzle to target distance determinations, however in doing so one must make every effort to establish gun powder particle counts out to at least the 4<sup>th</sup> order in short distances (from 2 to 14 inches in muzzle to target distances).

Gunpowder Pattern, Gunpowder Particles, Vaporous Lead

## A187 A Comparison of Lead, Barium, and Antimony Isotope Concentrations in Gunshot Residue Using ICP-MS and SEM

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After attending this presentation, attendees will understand the necessary elements that make up the unique composition of gunshot residue and the the most advantageous combination of analytical techniques used for the detection of gunshot residue on different materials.

This presentation will impact the forensic science community by serving as a quick alternate method of detection for gunshot residue on a particular sample of interest.

Gunshot residue (GSR) evidence is of utmost importance in the investigation of violent crimes involving firearms as it may directly link an otherwise unknown subject to an environment of weapon discharge. A unique GSR particulate of interest is composed of heavy metals in varying proportions that include lead, barium, and antimony. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is an analytical technique that will yield the overall concentration of lead, barium, and antimony present in a sample suggested to contain GSR. Some environmental sources, including vehicle brakes and fireworks, have been shown to resemble GSR, which could yield false positive confirmations of the presence of GSR on the particular sample of interest. Therefore, a need exists for an analytical technique that will thoroughly and quickly analyze a piece of fabric or carbon-coated adhesive stub suspected to contain GSR. Manual Variable Pressure Scanning Electron Microscopy (VP-SEM) allows the specific identification of a unique GSR particulate that contains lead, barium, and antimony embedded within a charging piece of fabric or on the surface of the carbon-coated adhesive stub. It was hypothesized that the advantage of SEM over ICP-MS is the ability to distinguish Pb, Ba, Sb, Pb-Ba, Pb-Sb, and Ba-Sb particles from a Pb-Ba-Sb particulate, thus yielding a more accurate representation of whether or not GSR is present in the sample or not. Acetate, cotton, nylon, polyester, and rayon fabrics were wrapped around the wrist of the shooter that fired a 9mm handgun. Carbon-coated adhesive stubs were then dabbed against the back of the hand and fingers and the palm of the shooter. A bulk analysis of Pb-208, Ba-137, and Sb-121 concentrations in acetate, cotton, nylon, polyester, and rayon fabrics containing GSR was done by ICP-MS.

Then a total evaluation of all the particulate present in the fabrics was done by manual SEM to determine if the potential particles were consistent with GSR. ICP-MS results showed that acetate and nylon retained the lowest amount of GSR, while rayon, cotton, and polyester retained the most GSR. Contrastingly, SEM results indicated that acetate had a multitude of GSR particles embedded within the fibers, whereas polyester had numerous Pb-Ba particulates, but only a single unique GSR particulate. The Pb-Ba particulates found in polyester using SEM could have contributed to the higher intensity of Pb-208 and Ba-137 isotopes found in ICP-MS, leading to a false indication that a high amount of GSR was retained. ICP-MS was a valuable tool used to search for GSR on fabrics, but SEM was able to indicate the elemental composition of each particulate, whether it be GSR or not, on the fabric for a more accurate representation of the amount of GSR present. A quick backscatter method for the detection of GSR on fabrics using SEM was also developed during analysis of the different types of fabrics, and easily applied to the analysis of the carbon-coated adhesive stubs. The methodology has been used in other scientific applications, but it has not been applied in the forensic science field to the detection of GSR. This method could be a valuable tool to the forensic science field as it can quickly detect the presence of GSR on fabrics to directly link someone to a firearms crime scene.

Gunshot Residue, Inductively Coupled Plasma-Mass Spectrometry, Scanning Electron Microscope

### A188 Determination of Ancestry in Forensic Skeletal Cases From Haplogroup Assignments Based on Hypervariable Region Mitochondrial DNA Sequence Data

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After attending this presentation, attendees will understand the circumstances in which mtDNA can potentially help to determine the ancestry of skeletal remains and the limitations of any such determination.

This presentation will impact the forensic science community by informing forensic anthropologists and geneticists of how their disciplines can jointly inform the determination of ancestry of unknown skeletal remains.

In forensic casework, DNA is primarily used for the individuation of evidence or the identification of remains. This is especially true for autosomal DNA (auDNA), which can be used in a positive identification, but is generally the case for Y-chromosome (yDNA) or mitochondrial DNA (mtDNA) as well. While variation in yDNA and mtDNA actually only places an individual within one or more particular groups of paternally or maternally related individuals, it is generally used in conjunction with other circumstantial evidence (such as a passenger manifest) to determine individual identity. When individuation is not possible, either because references are not available for comparison or because no victim names are known, how well can DNA be used to assign an individual to a population group?

MtDNA is the type most frequently sequenced from skeletal remains, because of its high copy number and good preservation. Because of its haploid, or unilateral, inheritance, mutations in mtDNA over time have led to a branching tree of variation, where any specific haplotype, or genome, can be placed in relationship to all others. Branches of this tree are referred to as haplogroups, each of which groups various haplotypes united by common descent. While early studies of mtDNA variation at the population level focused on coding region mutations, using these to define the original haplogroups, forensic applications generally sequence the hypervariable region (HVR) and occasionally the broader control region. The high mutation rate across the HVR makes it iDrug Enforcement Administrationl for individuation, but also means that a particular mutation may well have occurred multiple times and not necessarily signify anything about relatedness. Individuals belonging to different haplogroups, and exhibiting different polymorphisms elsewhere in their mitochondrial genome, may bear the same sequence in the HVR. Some polymorphisms in the HVR are more stable and are associated with particular haplogroups, but most haplogroups are defined on the basis of markers that are not sequenced in standard forensic casework.

To use mtDNA for determining ancestry in a forensic setting, three questions must be addressed: (1) how accurately can a given sequence be assigned to a specific haplogroup; (2) how accurately can a given haplogroup be assigned to a specific ancestral population; and, (3) what is the correlation between that ancestral population and a modern "racial" category?

Comparison of HVR sequence data to the global mtDNA phylogeny allows many sequences to be accurately placed within broad haplogroups, and some to be much more specifically sorted. Others, particularly within the M and R macrohaplogroups, cannot be accurately assigned on the basis of HVR data alone. Even in such cases, however, the range of possibilities may be broadly restricted to a given continental origin.

Because of its haploid inheritance, mtDNA only reflects an individual's maternal ancestry through previous generations. Population genetic studies have found that it has a very weak correlation with geography on a local level, because of the movement of wives between communities. On a broader, continental level, it does have a high correlation with geography. That is, most haplogroups, or groups of sequences or haplotypes united by shared descent, tend to be found in populations originally from one continent or another.

Historically, most gene flow occurred at the sub-continental level. Over the past few centuries, individuals and entire populations have moved over much broader distances, creating a higher degree of gene flow. In a forensic context, this means that any ancestral determination based upon genetics has to take the context of the remains into consideration and has to explicitly address the question of mixed descent and cultural classification.

As an example, the very diverse macrohaplogroup L, aside from its descendant clades M and N, is restricted in distribution to African populations. In forensic casework, an individual of haplogroup L can be reliably stated to be of African maternal ancestry. But does this always correlate with Black, or African-American? In a sample of U.S. military casework, most occurrences of haplogroup L were indeed individuals who were racially classified as Black; however, several were White. It is most likely that these were not "mixed race" individuals, defined as those with parents or grandparents of distinct ancestry; rather, they were individuals descended, as far as they knew, from uniformly White ancestors, but who had a distant maternal ancestor who was African-American. These same individuals were classified as White by physical anthropologists, in accordance with their own self-categorization.

Another example is provided by World War II-era skeletal remains recovered from the Pacific theater. Because there was minimal historic gene flow between Japan and Europe, while the limited migration from Japan to the United States had occurred within one or two generations before the war, in most cases the haplogroup determined from a given mtDNA sequence allows a set of remains to be assigned to either Japanese or American origin.

Because race is a cultural category, albeit one based upon perceived biological differentiation, it will never perfectly correspond with determinations of ancestry based upon biological variation. Nonetheless, mtDNA variation can help in hypothesizing the ancestry of a given set of remains and what race their owner might have been classified in, if its limitations are clear.

The views expressed herein are those of the author and not necessarily those of the Joint POW/MIA Accounting Command or the U.S. Department of Defense.

Mitochondrial DNA, Ancestry, Human Skeletal Remains

#### A189 Validation of SNPs to Predict Pigment-Related Features in Diverse Populations

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After attending this presentation, attendees will understand some principles of pigmentation, including some components of its pathway. Attendees will be aware of how single nucleotide polymorphisms (SNPs) may affect eye, hair, and skin color.

This presentation will impact the forensic science community by describing pigment-related features based solely on DNA analysis.

An essential component in the identification of human remains is documenting the decedent's visible characteristics, such as eye, hair, and skin color. However, if a decedent is decomposed, or only skeletal remains are found, critical, usually externally visible information is lost. It is presently not possible to use genetic information from recovered, fragmented DNA samples to reveal such visible characteristics due to the lack of markers that are significantly correlated with visible traits.

Human genomes demonstrate 99.9% identity, while the remaining 0.1% accounts for variations between individuals, which include single nucleotide polymorphisms (SNPs). Based on sequencing results, it has been estimated that the human genome contains at least 11 million SNPs, of which most are silent and do not contribute to a phenotype. However, some have functional consequences. Since there are so many SNPs, it is challenging to find those which are directly responsible for human traits.

Melanin is the main pigment of eye, hair, and skin color and its synthesis depends on multiple genes and factors, such as age, drugs, diseases, and environmental conditions. Pigmentation is therefore considered a complex trait. Furthermore, only minimal correlation exists among eye, hair, and skin color, within the European population, where blue and brown-eyed individuals can have all shades of neutral hair colors. In other geographical regions; however, populations with darker skin tones tend to have darker eye and hair colors. To reduce complexity, previous studies focused on a single trait in one population, such as the eye color in Europeans, or on few genes, including MC1R, SLC45A2, OCA2, HERC2, ASIP, and SLC24A5, whose products are associated with melanin synthesis or its localization, to identify SNPs that correlate significantly with eye, hair, and/or skin color.

Presented at this meeting is the validation of several SNPs for their predictive value to describe eye, hair, and/or skin color of individuals. A predictor, comprised of several SNPs that were found to correlate well with these traits is created and applied on over 600 samples. Importantly, these samples were donated from non-related individuals of various populations, including European descendants, African-American individuals, Asian dark (Indians), Asian white (Japanese and Chinese descendants), and mixed populations and are thus well representative among the various populations. The authors will present the outcome of this validation for the pigmentation traits by focusing on accuracy and call-rate as well as applicability to the diverse populations.

The ability to utilize DNA analysis to predict visible pigment-related features of unidentified human remains has the potential to assist in the identification of missing persons and unidentified human remains by increasing the amount of information available which can be entered into the National Missing and Unidentified Persons System (NamUs) and the National Crime Index System.

Pigmentation, SNP, Identification

#### A190 Evaluation of Deparaffinization Techniques and DNA Extraction Methods for Formalin-Fixed Paraffin-Embedded Tissue

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After attending this presentation, attendees will gain an understanding of the best extraction method for DNA recovery from formalin-fixed paraffin embedded tissues. Attendees will also learn which organs or tissues are optimal for DNA recovery from paraffin embedded tissues from decomposed and non-decomposed human remains.

This presentation will impact the forensic science community by better defining which tissue type(s) is (are) the best for extraction of DNA from paraffin blocks and which type of DNA extraction method produces the highest quantity and best quality DNA. With this knowledge DNA analysts will be able to make an informed decision as to which organs or tissues to select for DNA testing in order to yield the best DNA profile possible.

Although infrequent, there has been a growing increase in requests to use formalin-fixed paraffin embedded tissue (FF-PET) as reference samples for DNA testing. Re-examination of old Drug Enforcement Administrationth investigation cases in the form of post-conviction review or law enforcement cold case initiatives and cases of unidentified human remains often have no other samples available for testing from the complainant/decedent. Formalin solution is often used in the fixation of human tissue samples. It is an excellent preservative and fixative that enhances the integrity of the tissue for histological sampling. Once the tissue is fixed in formalin, it is embedded in paraffin for long-term storage. In certain instances, such as cold cases, formalin-fixed and paraffin-embedded tissues (FF-PET) may be the only specimens remaining from a decedent for DNA testing.

Although formalin, an aqueous solution of 10% formaldehyde, is an excellent fixative, it cross-links proteins and nucleic acids within a tissue sample, so DNA extracted from formalin fixed tissue is generally fragmented to less than 300 base pairs in length. This study investigated the effect of the time since death and the time of fixation in formalin on the fragment sizes of the DNA purified from various tissue samples.

Four different tissue specimens (heart, liver, muscle, and spleen) were fixed in formalin at three different intervals. These were day one, day five, and day twelve after the autopsy was performed on three different individuals. Once fixed, the tissue specimens were paraffinembedded in the Histology Laboratory. Since the tissue type and time to fixation can affect the DNA quality, the muscle from day one of one individual was chosen to begin and sizes of three, ten, and thirty microns were cut using the microtome.

Deparaffinization involves the separation and removal of the paraffin wax from the tissue specimen. Two different techniques were evaluated and compared: solvent wash and melting. The solvent wash method involves washing the specimen with varying amounts of xylene and ethanol. The melting method involves melting away the paraffin by the use of a heating block set at 98°C.

Two different extraction methods were also evaluated and compared. The first method used was the organic extraction, followed by Microcon purification and concentration. The second method involved the use of a robot that performs automated extractions. Each procedure was performed according to the standard operating procedure of the laboratory.

Once the extractions were complete, the tissue specimens were quantified, amplified using Identifiler, and run on the 3130xl by capillary electrophoresis. The electropherograms were analyzed and the results were tabulated. Of the tissue specimens tested, most gave full profiles. A few gave partial results with just one or two loci showing drop out.

Based on the results, the melting deparaffinization technique was chosen to be superior over the solvent wash. Thus, for future samples, only the melting method will be performed. A size of ten microns will be used for future samples and both extraction methods will continue to be performed on all tissue samples.

Future samples will include tissue specimens from the heart, liver, and spleen of each of the three individuals from the different fixative times.

The methods employed by the laboratory for deparaffinization and extraction of FF-PET samples have proven to work thus far with the type of samples available. However, the laboratory is interested in using these methods on tissues from decomposed individuals and is currently working on compiling tissues from decomposed bodies to further evaluate these methods. The laboratory also has several older "cold" cases in which tissue specimens are the only sample left for identification. It is the hope to have a method for FF-PET samples validated soon to address these cases.

**FF-PET, DNA Extraction, STR** 

## A191 Reliable, Accessible Dental Database Coding and Quality Assurance

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After attending this presentation, attendees will understand some principles of flawed dental database coding, comparisons and exclusions for missing (MP) and unidentified persons (UP) and how they can be made reliable via access to volunteer forensic odontologists, trained in the use of the NCIC and NamUs systems.

This presentation will impact the forensic science community by stressing the importance of using forensic odontologists trained in NCIC and NamUs dental coding to compare missing persons' and unidentified persons' dental records, x-rays, and data for medical examiners/coroners and law enforcement investigators across the country.

Logically, only subject matter experts would collect/process/interpret/etc information/evidence germane to their training and experience. Counter intuitively and since the inception of the Missing/Wanted Persons and Unidentified Persons files of NCIC (and more recently NamUs), much of the dental data is not being coded and entered by odontology subject matter experts. Instead, some law enforcement, medicolegal death investigators, missing persons clearing house personnel and non-forensic "family" dentists who willingly volunteer for their local community, and for the "novelty" of the event, interpret forensic odontology evidence. Therefore, it should be expected that the foundation of missing and unidentified persons' dental data is frequently flawed with a wide range of misinterpretations.

Realistically, odontologists do not expect this non-forensic dental coding/data entry situation to change unless there is an interdisciplinary effort to share methods and pathways of improving the accuracy and precision of evidence development, as well as the collection and interpretation of the dental data.

Criminalists may not be aware of trained, volunteer forensic odontologists networked in local communities across the country. It is the authors' objective through this presentation to advise a large segment of the criminalists about these vetted odontology subject matter experts' availability to assist collection, processing, and interpretation of dental evidence, pivotal to identification at the outset of criminal investigation and ultimately in judicial proceedings.

The woven complexities of dental coding and comparison, with flaws and error rate as well as human fallibility are examined with remedies for each. Standards of reliable, forensic best practices, quality assurance as second opinions, and patient care are examined for relevancy and to address the National Academy of Science Report recommendation for medicolegal death investigation.

Examples of forensic odontology technique, translation, interpretation, imaging, and second opinion within the discipline will be demonstrated. This will heighten the attendees' appreciation of forensic odontology services in medicolegal death investigation.

Missing and unidentified persons' investigators, with their knowledge of any given case, are most likely the first to inquire and perhaps find dental information. The next step is critical to the coding of sound dental evidence, for case resolution, and subsequent man-hours invested into case development. Odontologists and the community of auxiliary personnel recognize the need for interdisciplinary team building. Trained and vetted forensic odontologists' resources, where all outcomes have quality assurance reviews, are available to the criminalists within their states at no cost. This presentation seeks to inform criminalists of these techniques/services and ease the odontology burden from the criminalist, enabling them to re-focus their experienced analysis on other elements of the case and also receive quality dental data delivered into case development.

During the conclusion, two access pathways will be presented for law enforcement, medical examiner/coroner personnel, and medicolegal death investigators to elevate the accuracy and precision of missing and unidentified persons' dental databases by seeking trained, volunteer, dental x-ray and records interpretation, and coding within their state and adjacent states, by email to these resources:

- FBI/CJIS/NCIC: NDIR@leo.gov Subject box: Dental coding/comparison needed
- NamUs: namus.02@findthemissing.org Subject box: Dental coding/comparison needed

Dental Identification, Quality Assurance, Best Practices

## A192 You Can Wash, But Can You Hide? Generating DNA Profile From Low Template DNA

Reena Roy, PhD\*, Pennsylvania State University, Forensic Science Program, 325 Whitmore Lab, University Park, PA 16802; and Stephanie H. Nickolas, MPS, and Lauren B. Williams, Pennsylvania State University, Eberly College of Science, University Park, PA 16802

After attending this presentation, attendees will be able to optimize the generation of DNA profiles from low template DNA. When a stain has been washed and no longer visible, a profile from the stains can be obtained using a combination of various methods including touch evidence and low copy number DNA analysis.

This presentation will impact the forensic science community by providing information on bloodstains that have been washed with detergents containing a bleach alternative as well as stains that have been washed with regular detergent and chlorine bleach. The presentation will include the impact on the DNA profile using different materials and the methods used to obtain STR DNA profiles from these washed stains.

Forensic analysts often encounter situations in which perpetrators wash their clothing in an attempt to destroy blood and body fluid stains that may implicate their role in a particular crime. Victims of sexual assault may wash their clothing immediately following the attack, deciding days or weeks later to report the crime. Obtaining DNA profiles from these blood and body fluid stains, which have been subjected to washing and drying, will allow the forensic scientists to link the perpetrator to the crime. The aim of this research was to use different procedures, including LCN methodology, to generate DNA profiles from bloodstains that have been deposited on various types of fabrics and subjected to different conditions of washing.

Known quantities of blood from living and deceased donors were deposited onto swatches of several types of fabric. Each swatch of bloodstained fabric was allowed to dry for approximately 24-hours at room temperature. The stained pieces of fabric were placed into lingerie wash bag and then subjected to a hot wash cycle for 29 minutes using an automatic, commercially available washing machine with 45 mL of detergents containing a bleach alternative. Another set of washing was conducted on bloodstained fabrics using the same wash cycle, but with 45 mL of regular detergent and 90 mL of regular bleach. After washing, all of the fabric swatches were dried in an automatic, commercially available dryer for 60 minutes, using a high temperature cycle. Along with the stained fabrics, two sets of negative controls, or substrate controls, were created. The first set was not washed, but it was processed to determine if any DNA may have been introduced into the fabric during the manufacturing or handling processes. The second set was washed to determine if any DNA may have been introduced into the fabric during the washing and drying process. All washed pieces were tested for the presence of blood using presumptive tests. Confirmatory tests were then performed on the samples which previously reacted positively with presumptive tests.

DNA extractions from the washed, bloodstained fabrics were performed following a conventional organic extraction procedure, a robotic extraction procedure, and also by a touch evidence extraction procedure. Extracted DNA was quantitated, followed by amplification with parameters recommended by the manufacturer. Some of the extracted samples with low template DNA were amplified using a reduced reaction volume and an increased cycle number. Analysis of the amplified products was carried out by capillary electrophoresis injection and the generated profiles were analyzed using appropriate DNA analysis software.

Using the organic and robotic extraction procedures, it was possible to generate autosomal STR and Y-STR DNA profiles from the cotton fabric swatches, washed with detergents containing a bleach alternative. Using the touch evidence extraction procedure, it was possible to generate autosomal STR DNA profiles from some of the bloodstained cotton swatches washed with regular detergent and regular bleach. No autosomal or Y-STR DNA profile could be generated from some of the washed bloodstains, particularly those deposited on polyester fabric. None of the negative control samples showed any DNA profile.

The results indicate that cotton is able to retain bloodstains better than other types of fabric and allows the generation of better quality STR DNA profiles. When examining the type of detergent and its ability to generate a DNA profile from the washed bloodstains, the detergents with a bleach alternative did not wash away the bloodstains as effectively as when the stains were washed with regular detergent and regular bleach. Sodium hypochlorite and sodium hydroxide are two of the components found in the regular bleach that are not included in the detergents containing a bleach alternative and it is possible that these two components cause the DNA to degrade completely.

This study indicates that DNA profiles can be generated from bloodstains that have been subjected to washing and drying in automatic machines. Obtaining DNA profiles from this type of evidence can help in the investigation of a crime.

LCN, Low Template DNA, Touch Evidence Extraction

### A193 Optimization of DNA Extraction Buffer Used in Conjunction with DNA IQ<sup>TM</sup>

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After attending this presentation, attendees will become familiar with the possibility of modifying a current DNA extraction buffer by substitution of an anionic detergent that has indicated superiority over another. Attendees will also benefit from knowing that this modification can be implemented into automation on robotic platforms for use in conjunction with the DNA  $IQ^{TM}$  system.

This presentation will impact the forensic science community by suggesting the anionic detergent, SDS, is more effective than sarkosyl in DNA extraction buffer and is able to be incorporated into a modified automated extraction method with the DNA IQ<sup>™</sup> system. A more effective DNA extraction buffer yields more DNA for analysis, especially helpful for low level samples or difficult sample substrates.

Forensic casework samples often contain low levels of biological material (low template) and are frequently deposited on difficult substrates. Challenging samples such as these have received a great deal of attention, in both research efforts and the court room. These samples, which are commonly derived from "touch" evidence, typically require optimized methods for efficient recovery of biological material. For this study, proteinase-K extraction buffers used in conjunction with the DNA  $IQ^{TM}$  System on an automated platform were experimented upon, with the main focus on varying concentrations of sodium dodecyl sulfate (SDS).

Automation is now extensively used in forensic laboratories; however a means for more efficient DNA extraction is desired since problematic samples constitute a large percentage of all casework samples. Organic extraction continues to be considered the "gold standard," as it is capable of isolating DNA from difficult samples. However, this method is time-consuming and non-automatable. At the Virginia Department of Forensic Science (VDFS), Promega's DNA IQ™ system is utilized in conjunction with a robotic platform for the automated extraction of DNA from forensic samples. However, it has been observed that low template samples and samples deposited on various substrates can sometimes prove problematic and produce lower than expected DNA yields using this automated approach. Thus, keeping in mind the high performance ability of the organic extraction method, a buffer similar to the stain extraction buffer used with organic extraction procedures is being evaluated. In particular, proteinase K buffers were tested for the effectiveness of the anionic SDS detergent compared to the anionic sarkosyl detergent; sarkosyl has traditionally been utilized since it is miscible with the DNA IQ<sup>™</sup> Lysis buffer.

Although SDS has been demonstrated to be superior to other detergents in extraction buffers, use of 1% SDS (the concentration used in stain extraction buffer) with the automated DNA IQ<sup>TM</sup> method at VDFS has resulted in an inconsistent performance, particularly with undiluted, low template samples. This is likely due to precipitation of guanidinium salt from the DNA IQ<sup>TM</sup> Lysis buffer, since concentrations at or above 1% are known to precipitate the salt. Thus, lower concentrations of SDS were experimented with in order to overcome this problem, as well as an extra step of plate heating during the robotic extraction process.

Experimentation with whole blood on various substrates has been successful, where nearly every sample incubated with an SDS-containing buffer yielded higher concentrations of DNA and higher average peak heights. Occasionally, the DNA concentrations obtained suggested a full profile should be generated, yet an incomplete profile resulted during STR analysis, suggesting that precipitation of the guanidinium salt or possible PCR inhibition continued to be a problem. Studies are ongoing utilizing mock casework samples in order to resolve the problem of precipitation or inhibition with the goal of ascertaining if indeed replacing the sarkosyl with SDS is possible and if it produces a more reliable yield of DNA from low template and problematic samples.

DNA Extraction, Low Level, Automation

#### A194 An Evaluation of Environmental Interference for Low Copy Number DNA Analysis of Non-Discrete Samples

## Crystal M. Czarnecki\*, and Kerry L. Opel, PhD, Upper Iowa University, Division of Science and Math, PO Box 1857, Fayette, IA 52142

After attending this presentation, attendees will gain an understanding of the quantity of background DNA found in a wide range of private and public access areas, as well as the areas which contain significant amounts of background DNA.

This presentation will impact the forensic science community by providing information on the levels of background DNA which may interfere with low copy number analysis.

New and improved technology in forensic DNA typing has allowed the detection and analysis of very low levels of nuclear DNA. However, for non-discrete samples, the ability to detect and analyze these low levels has been off-set by the potential for contamination. In non-discrete samples, the DNA of the contributor is in the form of a biological fluid stain deposited on a surface. Because there may already be skin cells and other sources of DNA left behind by previous contact, the collection of the stain from the surface has the potential to contain a DNA mixture. For large samples, this is generally not an issue, since the amount of DNA in the sample overwhelms the background DNA and interpretation is not difficult. For small and naturally low DNA concentration samples, which require specialized preparation and analysis, the presence of the mixture becomes much more obvious. Separation of the DNA profile of the contributor of the stain and interpretation is much more difficult, or potentially impossible.

An understanding of the normal background levels of human DNA which may be present, as well as knowledge of the surfaces and areas more likely to produce high levels of background DNA would be beneficial to those in the forensic community who perform low copy number analysis. These analysts would be able to better assess the potential for exogenous contamination in their samples once such data was available. Such data would also be useful in setting standards for the probative value of low copy number DNA for non-discrete touch samples. Similar studies for other fields have been conducted to determine background contamination of target analytes (e.g. ions associated with explosive residues that may also be found in the environment) and these studies have been used to direct the limits of detection for the analytical methods used to analyze them. Such a study has not been previously undertaken for human DNA.

In this study, replicate samples of background DNA were collected from a wide range of surfaces in private (residences) and public (government buildings, schools, malls, etc.) areas. These areas were designated as public or private, and high, medium, or low traffic, depending on the estimated number of people to come in contact with the surface per day. A minimum of ten different access areas were sampled for each category. Three samples were collected from each access area in three different locations. The samples were collected by swabbing a controlled area (one square inch) using a cotton swab moistened with distilled water. The swabs were extracted using a Chelex® resin extraction method, for which the average expected recovery of DNA was previously determined. The amount of DNA on the swabs was determined by real time PCR (rtPCR or qPCR) using an 82 bp amplicon Alu primer set. The average level of DNA was determined for each of the three swabbed areas in each location and a statistical comparison of the data was calculated to determine which areas are significantly more contaminated with exogenous DNA than others.

LCN DNA, rtPCR, Non-Discrete Samples

### A195 Minimum Distinguishable Signals and Limits of Detection: Applications of Uncertainty in Analytical Measurement for Determination of an RFU Threshold for Forensic DNA Analysis

Joli Bregu, BS, Danielle Conklin, MS, Elisse R. Coronado, MS, Robin W. Cotton, PhD, and Catherine M. Grgicak, PhD\*, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806B, Boston, MA 02118

After attending this presentation, attendees will be exposed to a number of analytical techniques that were used to determine the minimum distinguishable signal (MDS), the limit of detection (LOD), and the sensitivity of signals/alleles generated using STR typing.

This presentation will impact the forensic science community by illustrating methods commonly used to calculate and elucidate the minimum distinguishable signals, limits of detection, sensitivity, and signal to noise ratios. The assessment will allow the determination of an relative fluorescent unit (RFU) threshold that will represent a value which analytically distinguishes noise from "true allele" signal.

The amplification and subsequent fragment analysis of DNA recovered from crime scenes remains one of the most sensitive and powerful techniques available for the purposes of human identity testing. A number of technologies, methodologies, and chemistries have been introduced permitting amplification (and subsequent analysis and interpretation) of samples with low DNA concentrations. Such technologies include, but are not limited to, post-PCR clean-up, modifications to the cycle number and/or thermal cycling parameters, increased injection times, and optimized STR amplification kits/chemistries; however, as these advancements become more frequently implemented, it is necessary to determine their limitations. As such, it is imperative to determine the minimum target DNA concentration that can be detected (at a known confidence level) using these technologies.

The minimum mass of DNA that can be detected with confidence is known as the limit of detection. This amount not only concerns the signal of the target, but also the magnitude of the analytical signal in relation to the levels of fluctuation in the blank signal. Therefore it is dependent upon the laboratory's ability to calculate and elucidate the minimum distinguishable signals, limits of detection, sensitivity, and signal to noise ratios.

In this study a number of analytical techniques were used to determine: (1) the minimum distinguishable signal (MDS); (2) the limit of detection (LOD); and, (3) the sensitivity of signals/alleles generated using AB's AmpFlSTR<sup>TM</sup> Identifiler® kit.

The MDS was experimentally determined by running 33 blanks (formamide + LIZ600) using a 2, 5, and 10 s injection on a 3130 Genetic Analyzer (Applied Biosystem) using the manufacturer's recommended protocol. This is accomplished by using the following definition

$$MDS = \overline{S_{bl}} + 3SD_{bl}$$

where MDS is the minimum distinguishable signal,  $\overline{S_{bl}}$  the average of the blank signal, and  $SD_{bl}$  is the standard deviation of the blank signals. The MDS therefore represents the RFU value at which one cannot statistically distinguish between "true" signal and baseline noise of the instrument. However, since this only represents the expected MDS of the instrument - which is comprised of the noise from the electronics, detector, etc - it does not represent the MDS obtained from amplified blanks. Therefore the MDS for 86 amplified negatives run over a period of approximately one year were analyzed and it showed that the MDS was significantly larger than those calculated from the 33 run blanks (Table 1.). It was also observed that the MDS did not increase between injection times when calculated using the run blanks, but did significantly increase between 5

and 10 s injections when determined by utilizing the amplified negatives. This suggests a characteristic MDS is better calculated from a series of amplified negatives run over a period of time using representative injection times.

Lastly, determination of the sensitivity (i.e., slope) and y-intercept which are computed using linear regression ( $R^2 > 0.994$ ) of a calibration curve - which plots signal (RFU) versus target (0.0625 - 1 ng) - shows the y-intercept is less than the MDS, thereby suggesting: (1) the MDS should experimentally be determined using amplification blanks; and, (2) the limit of detection for the AmpFlSTR<sup>TM</sup> Identifiler® kit using these experimental conditions and defined by

$$LOD = \frac{\frac{3SD_{bl}}{m}}{m}$$

where LOD is the limit of detection, m is the slope, and is the standard deviation of the blank signals, is 42, 17, and 9 pg for 2, 5, and 10 s injections respectively.

Table 1. Minimum Distinguishable Signals for 2, 5, and 10 second injections calculated with run blanks and amplification blanks.

	MDS (RFU)					
Color	2s		5s		10s	
	Run	Amplification	Run	Amplification	Run	Amplification
	Blanks	Blanks	Blanks	Blanks	Blanks	Blanks
Blue	6	13	7	11	9	15
Green	9	15	9	15	10	19
Red	14	34	17	28	14	54
Yellow	15	17	14	18	14	25
Overall	11	23	12	22	12	35

Further analysis to determine the signal:noise, signal:amplification target and the LOD of a weighted regression were also analyzed and compared. Results show that as the signal increased, so did the level of amplified noise. These noise peaks were not consequences of bleed-thru, -A, positive/negative stutter, etc, but were amplified noise/artifacts. Analysis of the signal:noise and target:signal:noise shows that when analyzing data, the signal of the "major" peak(s) must be taken into consideration.

DNA Analysis, RFU Threshold, Minimum Distinguishable Signal

#### A196 Using the Autoclave for DNA Decontamination of Consumables for Use in Low Template DNA Testing

Michael A. Donley, MS\*, Dustin Foley, MS, and Mark Powell, MS, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054; and Lisa Gefrides, MS, Roger Kahn, PhD, Harris County Inistitute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will gain knowledge of different decontamination methods to remove consumable contamination, their application, and which method is the best approach to successful DNA decontamination.

This presentation will impact the forensic science community by providing information on three decontamination techniques and their effectiveness in a side-by-side comparison. This information will allow laboratories to make an informed decision when selecting a decontamination strategy with the intended result of minimizing consumable contamination in attendees' laboratories.

Technological advancements have greatly increased the sensitivity of DNA testing in recent years. Techniques for low template autosomal DNA testing focus on enhancing the amplification and the detection of the amplified product using capillary electrophoresis. Amplification is enhanced by increasing both the number of amplification cycles and the amount of polymerase in the reaction mix. Detection can be enhanced for low level samples by increasing injection times or by post-PCR treatment to allow more amplified product to enter the capillary.

As sensitivity increases so does the risk of detecting low levels of DNA from the laboratory environment during the testing process. Labs that perform extremely sensitive DNA testing, such as low template protocols (also known as LCN testing) and mitochondrial DNA testing, take great care to protect samples from contamination. For example, testing is generally performed in separate facilities from those used for routine autosomal casework. To combat low levels of exogenous DNA present in consumables or reagents, many laboratories UV irradiate consumables and reagents prior to use in casework. While UV irradiation is effective to some extent, it is not always able to remove all exogenous DNA present in a consumable item. Even after UV treatment, consumable contamination of casework samples may still be detected at a low rate. Recently, ethylene oxide (EO) treatment and autoclaving have been reported as alternatives to UV irradiation for treatment of consumables. Previous studies comparing autoclaving and UV irradiation of consumables for traditional STR typing have shown that autoclaving performs better than UV irradiation at eliminating exogenous DNA (Gefrides et al. 2010).1

The present study was undertaken to assess which method, UV, autoclaving, or EO, would perform best at removing contaminating DNA from consumables for low template testing. This study consisted of placing 50  $\mu$ l, 25  $\mu$ l, or 10  $\mu$ l of blood or saliva on cotton swabs and 2 ml tubes and allowing them to dry overnight. The samples were then treated in the autoclave for one, two, or three hours. Samples were quantified using real-time PCR and a commercial kit that detected human and male DNA at the same time. Following quantification, samples were amplified at 31 cycles using a single-amplification inhibition-resistant amplification kit. The amplicons were then injected into a 3130xl genetic analyzer for 10 seconds.

None of the treated samples obtained a quantification value after three hours of autoclaving. Only three out of the 12 samples obtained a quantification value after two hours of autoclaving. The general trend is consistent with previous studies (Gefrides et al. 2010) where, after two hours of UV or autoclave treatment, no alleles were detected following 28-cycle amplification.<sup>1</sup> In those studies, however, after two hours of UV treatment (~7000 mJ/cm<sup>2</sup>) alleles were still detectable with a DNA typing kit that uses 30 rather than 28 cycles of PCR amplification, suggesting that UV irradiation might not perform as well as autoclaving with increased numbers of amplification cycles.

Preliminary data for blood and saliva placed on cotton swabs indicate that a three hour autoclave treatment decontaminated consumables containing 10 - 50  $\mu$ l of dried blood or saliva even after 31 amplification cycles. A two hour treatment was effective at decontaminating swabs with 10 - 50  $\mu$ l of saliva or blood except for a single allele in one sample. After one hour of autoclave treatment, alleles were detected in all blood swabs while only one of the 50  $\mu$ l saliva swab obtained alleles.

To complete this study, 50  $\mu$ l, 25  $\mu$ l, and 10  $\mu$ l of blood or saliva samples on cotton swabs in 2 ml tubes will be treated with either two cycles of EO treatment or with one, two, or three hours of UV exposure in a crosslinker at a UV dose ranging from 3500 - 10,000 mJ/cm<sup>2</sup> (per Gefrides et al 2010). These samples will be subjected to low template amplification as well as mitochondrial DNA testing. Attendees will be presented with the findings of all studies and recommendations for the best methods for decontaminating consumables.

**Reference:** 

<sup>1.</sup> L.A. Gefrides, M.C. Powell, M.A. Donley, R. Kahn. UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumable. FSI:Genetics 4 (2010) 89-94.

DNA, Autoclave, Contamination

## A197 Demonstration of Rapid Light-Mediated PCR Amplification of STR Loci Using Polymeric Microfluidic Devices

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After attending this presentation, attendees will have learned about the development of expedited STR amplification using microfluidic devices and commercially-available STR kits.

This presentation will impact the forensic science community by demonstrating how the development of rapid multiplex PCR assays on microfluidic devices will reduce the overall time required for DNA typing.

The proven utility of forensic DNA evidence has increased the demand for DNA analysis services. Although conventional analysis techniques are effective, they are time-consuming and laborious, which has contributed to an overwhelming backlog of forensic casework samples with possible biological evidence. Conventional DNA typing steps include DNA extraction, quantitation, PCR amplification of multiple STR loci, and electrophoretic separation of the resulting STR fragments; of these steps, PCR amplification is the most time consuming, taking approximately three hours with standard thermal cycling protocols. Increasing the speed for PCR amplification of STR loci has the potential to not only help decrease the time required for the overall DNA typing process, but to improve the throughput of a crime laboratory. Previous expedited PCR studies have demonstrated the successful amplification of DNA in ~36 minutes using a commercially-available STR amplification kit, commercially-available polymerases that have been modified to have faster extension rates, and improved processivity over traditional polymerases and a conventional thermal cycler.<sup>1</sup>

Microchip technology offers the potential of a rapid, cost-effective alternative to conventional DNA analysis methods. Microdevices provide self contained, closed systems for analysis procedures, diminishing the potential for contamination or loss of sample. Techniques performed on microchips are advantageous because they can be integrated with upstream or downstream analytical steps on a single microfluidic device in the form of a lab-on-a-chip. These integrated microfluidic systems, which incorporate all of the sample processing steps required for DNA analysis, will reduce analysis times, and therefore, the forensic casework backlog.

PCR amplification on microfluidic devices is well-established and has been shown for a variety of applications, including human identification and STR analysis.<sup>2</sup> One example of PCR on a microfluidic device, infrared (IR)-mediated PCR, provides faster heating and cooling rates than can be achieved in a conventional thermal cycler, resulting in more rapid thermal cycling times. In addition, microchip PCR offers the distinct advantage of significantly decreasing hold times during cycling by reducing the reaction volume by an order of magnitude or more when compared with conventional thermal cycling protocols.

The work presented will highlight the development of expedited PCR amplification of STR loci using glass and polymer microfluidic devices. Unique in comparison to others exploring microfluidic PCR for STR profiling, PCR amplification is accomplished in sub-microliter reaction volumes using commercially-available STR kits and reagents. For the first time, the use of a non-contact temperature sensing method in tandem with IR-mediated heating for rapid thermal cycling and analysis is demonstrated. Results of separations of the STR fragments from DNA amplified from the microdevices are described. A rapid cycling protocol that amplifies 16 STR loci and the sex-typing marker amelogenin from commercially-available STR typing kits in as little as 25 minutes is demonstrated. The presented work represents the development of rapid multiplex PCR assays on microfluidic devices–a major step towards the development of a fully-integrated microdevice capable of total DNA analysis, as well as a reduction in overall time required for STR analysis. **References:** 

- <sup>1.</sup> Vallone, PM, Hill, CR, Butler, JM. FSI: Genetics 2008;3(1):42-45.
- <sup>2</sup>. Bienvenue, JM, Legendre, LA, Ferrance, JP, Landers, JP. FSI: Genetics 2010; 4:178–186.

DNA, Microchip, Rapid PCR

## A198 Demonstration of Rapid STR Separations on Microfluidic Devices With a Novel Detection System

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After attending this presentation, attendees will have learned about the development of rapid STR separations using microfluidic devices.

This presentation will impact the forensic science community by demonstrating how the development of rapid DNA separations on polymeric microfluidic devices will reduce the overall time and cost required for DNA typing.

The increase in demand for forensic DNA analysis services has led to a significant backlog of forensic casework samples due to timeconsuming and laborious conventional analysis techniques. This backlog is driving the development of new analytical techniques that will reduce the time and cost associated with forensic DNA analysis. Conventional STR analysis requires extraction and quantitation of the genomic DNA, multiplexed PCR amplification of the STR loci, and electrophoretic separation of the amplified STR fragments. Currently, the electrophoretic separation is performed on a large capillary electrophoresis (CE) instrument and requires up to 40 minutes or more to complete. Decreasing the time and cost associated with the process can increase the throughput of a crime laboratory.

Microfluidic devices have the potential to address these throughput issues by offering a low-cost analysis with significantly shorter sampleto-answer time. The advantages of microfluidic devices come from the ability to integrate the analytical steps into a single device. This single device will require less user interaction than conventional processes and more efficiently transfers the sample from one analytical process to the next.

Microfluidic chips can perform STR separations in significantly less time than conventional CE methods. Additionally, the chip must be made from a low-cost, single-use substrate to minimize the cost per analysis. The work presented here compares high-resolution DNA separations on multiple substrates using different laser-induced fluorescence (LIF) detection methods completed in less than 10 minutes.

STR separations on a microfluidic device require a robust detection system capable of performing multiple separations simultaneously. Here,

comparison of a detection system on which integrated STR analysis has previously been demonstrated (Proceedings of the Micro-Total Analysis Systems Conference, 2009) with the next generation system capable of simultaneous multi-channel detection. The systems are compared on the basis of sensitivity, data acquisition rate, and the flexibility to scale up to multiple microfluidic separations.

In addition to minimizing sample-to-answer time, decreasing the cost-per-run is critical for crime laboratories increasing throughput. The cost of the chip substrate can account for a significant portion of the analysis cost. Alternative polymeric substrates that can provide a low-cost, single-use alternative to conventional glass microfluidic substrates will be evaluated. The substrates are compared on a basis separation time and resolution of commercially available STR kits. The presented work on alternative substrates and scalable LIF detection systems represents significant progress toward an integrated microfluidic system capable of low-cost, simultaneous sample processing.

**DNA**, Separations, Microchip

### A199 Development of a Plastic Microdevice for Integrated Liquid DNA Extraction and PCR Focused on Forensic STR Analysis

Jenny A. Lounsbury, MSFS\*, and Natalie Coult, University of Virginia, Department of Chemistry, 409 McCormick Road, Charlottesville, VA 22904; Paul Kinnon, BS, and David J. Saul, PhD, ZyGEM Corporation, Ruakura Road, Hamilton, NEW ZEALAND; and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904

After attending this presentation, attendees will have gained an understanding of a disposable microdevice capable of extracting and amplifying DNA for forensic analyses.

This presentation will impact the forensic science community by demonstrating an integrated, plastic DNA analysis device that can reduce sample analysis time and increase throughput.

The current processes used to analyze forensic biological samples, namely DNA extraction and amplification, create a significant bottleneck, contributing to the ever-increasing size of the backlog, which has risen to over 70,000 cases as of January 1, 2008.<sup>1</sup> Microfluidic devices offer an alternative method to analyze biological samples and provide many advantages over conventional methods, such as decreased analytical time and a completely closed system to prevent sample contamination. Additionally, analyses performed on microchips have the potential to be integrated with upstream or downstream analytical steps within a single microfluidic device. A fully-integrated microdevice has been developed which is able to perform all DNA analysis steps on a sample of mouse blood and yield a positive result for *B. anthracis* in 24 minutes.<sup>2</sup> Using principles outlined in that work, a similar microdevice could be developed for forensic STR typing.

The most widespread method to extract DNA is solid phase extraction (SPE), which typically uses a silica-based solid phase to reversibly bind DNA, while impurities are washed away. This method has been successfully adapted to a microdevice, but can encounter problems with uneven packing of the bed or high backpressure.<sup>3</sup> The use of a liquid extraction method eliminates the need for a solid phase and, as a result, eliminates any issue associated with a packed bed. A recently developed liquid extraction method utilizes a thermally stable neutral proteinase to lyse cells and degrade proteins and nucleases, leaving highly pure DNA that is PCR-ready in only 20 minutes.<sup>4</sup>

After the DNA is extracted and purified, STR regions in the genome are amplified by performing the polymerase chain reaction (PCR). Through the use of modified polymerases, which have faster extension rates and improved processivity, the amount of time required for PCR can be reduced to as little as 36 minutes.<sup>5</sup> Concurrently, PCR has been

adapted to a microdevice using non-contact heating methods, such as infrared (IR)-mediated PCR,<sup>6</sup> which can significantly increase ramp rates and reduce thermal cycling time significantly. By using modified polymerases in conjunction with IR-PCR, the time needed for the amplification step could be greatly reduced.

The current work focuses on the development of an integrated plastic microdevice capable of accepting a fragment of a buccal swab and extracting and amplifying DNA from a swab. A fragment of a dried buccal swab was placed in the extraction chamber and the device was capped to prevent evaporation. The enzyme-based extraction solution was flowed into the chamber until the swab fragment was completely immersed and the sample was incubated for a short period of time. The extracted DNA was flowed through the device, mixing with PCR master mix, until the PCR chamber was filled and PCR was performed using the IR-PCR system. Results show that the DNA extracted and amplified with the integrated device was able to yield a full STR profile in ~1 hr, a reduction of three hours in analysis time when compared to conventional methodologies.

#### **References:**

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Liquid Extraction, STR Typing, PMMA

#### A200 HAIRbase: An Online Resource for the Forensic Analysis of Mammalian Hair

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The goal of this presentation is to introduce the professional trace community to HAIRbase<sup>TM</sup>; a valuable online reference tool for the discrimination of mammalian hair characteristics. As a result of attending this talk, potential users will have learned a fast and reliable method of accessing morphological information regarding the microscopic and macroscopic characteristics of the hair of many species across the class Mammalia.

This presentation will impact the forensic science community by explaining how HAIRbase<sup>TM</sup> offers a wealth of information regarding the structural characteristics of mammalian hair and goes beyond traditional reference atlases in its coverage of both species and individuals.

The microscopic examination of guard hairs is paramount to a wildlife forensic identification. Although primary guard hairs are often used, secondary guard hairs are more variable and, therefore, have the potential to be more diagnostic. Furthermore, the class Mammalia contains approximately 5,400 species that display incredible morphological variation. The range of variation displayed in available reference materials is lacking, because the species characterized are limited and coverage of hair grades, as well as variation on the specimen does not lend itself to forensic identification. HAIRbaseÔ, a digital database of mammalian primary and secondary guard hairs from three

different body regions (dorsal, ventral, and tip of tail), has been constructed using bright field and scanning electron microscopy images.

Animal specimens were obtained from the collections at the U.S Fish and Wildlife National Forensic Laboratory in Ashland, Oregon and the Biology Department at California State University, Fresno. Hair was collected from each specimen by either plucking or cutting as close to its base as possible with a sterile razor blade. Hair was collected from three body regions: (1) the dorsal region, between the shoulder blades; (2) the ventral region, on the midline between the forelimb and the hind limb; and, (3) at the tip of the tail. Approximately 20-25 hairs were collected from each body region of each animal, for a total collection of approximately 60-75 hairs from each animal. After the hair from each body region was collected, it was placed in separate sterile sealable bags. Approximately three to five primary and secondary guard hairs were selected from each collection bag. Several hairs of each type were then plated onto individual glass microscope slides using a commercial mounting medium with a refractive index close to that of hair. Each hair on each slide was examined and photographed in a manner that documented microscopic fields containing the most representative hair characteristics for the particular hair type and section under view. A transmitted light microscope coupled with a Camera was used to acquire digital images of the basal, sub-shield and shield portions of the hair of each specimen at 200-400 X magnification. Macroscopic and microscopic evaluations of each specimen were conducted. The macroscopic characteristics recorded included hair color, form, and banding pattern. Microscopic observations, such as medullar, cuticle, and cortex characteristics, were then recorded. A user interface was created that allows the publishing of website content quickly and easily.

HAIRbaseÔ will aid investigators by giving them a reliable reference that contains diagnostic information regarding the structure of mammalian hairs, such as traits of the hair shield, medullary configurations, and cuticle scale patterns that can be used for identification. Using the information contained in HAIRbaseÔ one can distinguish particular traits, thereby aiding in the generation of investigative leads and possible identifications in crimes involving animals. The potential user groups for such an atlas include wildlife forensic scientists, animal welfare investigators, trace analysts, ecologists, and food contaminant inspectors.

HAIRbaseÔ will be readily available on the Internet, allowing the addition of specimens and the accommodation of the needs of the forensic trace evidence community in real time. Currently, the database contains over 250 species from 16 orders and 62 families of the class Mammalia. Through the addition of relevant specimens and the ability to adapt to the changing needs of its user groups, HAIRbaseÔ will remain relevant and remain a valuable resource to investigators and researchers across multiple scientific disciplines.

Hair, Morphology, Trace Evidence

#### A201 The Microscopical Identification of Raccoon Dog (Nyctereutes procyonoides) Hair

Jason C. Beckert, MS\*, Microtrace, 790 Fletcher Drive, Suite 106, Elgin, IL 60123

After attending this presentation, attendees will understand some principles of animal hair microscopy, including the necessary techniques and terminology, and how it pertains to the identification of raccoon dog (*Nyctereutes procyonoides*) hair.

This presentation will impact the forensic scienc community by discussing how identification of raccoon dog hair has become increasingly relevant to the forensic science community as its use in the fur trade, mostly as trim on garments, has grown in recent years.

Native to eastern Asia, the raccoon dog was introduced to multiple areas of the former Soviet Union and Europe in the middle part of the last century. The raccoon dog, a basal canid, is commonly bred in captivity for its fur and goes by many common names including tanuki, Chinese raccoon, Asiatic raccoon, and Finnish raccoon.

Animal hair microscopy is an underutilized area of forensic science that requires only common laboratory reagents and basic microscopical instrumentation (generally, a stereomicroscope and a compound microscope). While the equipment may be commonplace, the required techniques are specialized and some, including scale casts and longitudinal cross-sections, are unique to animal hair microscopy. A brief description of these and other techniques, including transverse crosssections, will be discussed.

The identification of raccoon dog hair has become increasingly relevant to the forensic science community as its use in the fur trade, mostly as trim on garments, has grown in recent years. As such, raccoon dog hair is likely to be encountered in forensic casework as trace evidence. Identification of raccoon dog hair utilizes the amalgamation of macroscopic observations, including length and banding patterns, and a complete microscopical characterization, including scale patterns, crosssectional shape, and medullary form and structure. The ability of a forensic trace examiner to correctly identify its origin can provide investigative leads or aid in a comparative animal hair analysis.

In addition, the fur trade's use of raccoon dog hair in garments has its own legal implications. It has been demonstrated that raccoon dog hair is often incorrectly labeled as either faux fur or as the fur of other animals. This constitutes false advertising and is in violation of federal law as regulated by the Federal Trade Commission (FTC). A brief review of the

relevant federal legislation will be addressed. Raccoon Dog, Hair, Microscopy

### A202 Obtaining Investigative Forensic Information From the Analysis of Rodents in Food Products

Brendan D. Nytes, BS\*, Jason Beckert, MS, Christopher S. Palenik, PhD, and Skip Palenik, BS, Microtrace, 790 Fletcher Drive, Suite 106, Elgin, IL 60123-4755

After attending this presentation, attendees will learn how sound scientific methodology can be used to obtain investigative information on rodent food contaminates.

This presentation will impact the forensic science community by showing how firm scientific methodology can be used to investigate food contaminates.

The purpose of this presentation is to illustrate the means and extent to which firm scientific reasoning can be applied to the examination of deceased rodents associated with food products to provide investigative information. Due to the unusual nature of such examinations, observations, and a firm scientific methodology within a sample-driven (rather than protocol-based) approach are basic tenets of such an investigation. Cases involving rodents as foreign matter generally arrive with a question such as: when did this rodent make its way into the product? Initial observations, made as soon as possible, can be critical to the investigation. Documentation of how the sample arrived and its condition are facts that have made major impacts on the ultimate conclusions. The consumer's story and timeline can also serve as helpful guides and be used to form testable hypotheses during the sample evaluation.

Generally, submitted samples have undergone an initial "identification" by a non-scientist (typically a consumer). This can lead to misidentification as well as escalated concerns over health and/or safety. It behooves the scientist to start any analysis by confirming or disproving the initial identification. Once the presence of an animal or animal tissue has been confirmed, many avenues can be pursued to identify it as a rodent. If hair is present, it can be classified and sourced to a particular type of animal; however, animal hair identification is not

If the food container is received, the container and its contents can be examined. The container can be observed for scratches and bite marks that would indicate a live rodent was present. When possible, the food stuffs can be screened for hair, urine, and feces. These observations can all help to determine if an animal had been in the food container when it was alive.

As in a human autopsy, a necropsy can give valuable information about how and when the rodent died. The type of food stuff in which the rodent was found needs to be considered prior to necropsy in order to allow the veterinarian to consider the environmental conditions present when making their determination. If the rodent is alleged to be found in a liquid, the lungs need to be examined to determine if it drowned in the specific liquid. The cause of death can be a crucial aspect to understanding when or how the rodent came into contact with the product. Death by emaciation, poisoning, or compression (mouse trap) are facts that indicate the rodent was not in the product prior to opening. Following necropsy, the rodent's stomach can be generally excised and the contents are examined. An understanding of food structure and food microscopy can allow identification of the contents or, at least, a comparison to the product sample. The contents are also examined for the presence (or absence) of any other material that could help place constraints on the questions of interest.

Once the analyses and observations are completed, the facts are examined to determine what conclusions can be made.

Food Contaminates, Rodents, Scientific Methology

## A203 Evaluation of the Operational Parameters of a Non-Contact Airflow Dynamic Device for Collection of Scent Trace Evidence

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After attending this presentation, attendees will understand some of the main issues to be considered when using non-contact airflow dynamic systems for collection of scent trace evidence, as well as the scientific validation of the human scent collection system as a tool for non-contact sampling purposes.

This presentation will impact the forensic science community by providing a scientific approach to the instrumental evaluation of a novel non-contact scent collection device such as the Human Scent Collection System (HSCS) for trapping capabilities from target biological specimens.

The use of canines as evidence for judicial procedures in the United States dates back to the 19<sup>th</sup> century. Canine evidence was traditionally admitted in court as long as there was enough information as to the training, breeding, and handler experience with the case at hand. Toward the end of the 1800s, it was accepted that bloodhound trailing evidence could provide an association between the defendant and the crime of which he was accused. Centuries later the debate toward this type of evidence in court systems in the United States continues and is often challenged due to collection procedures and the scientific validation of

the definition of human scent via instrumental approaches. Field scent collection methodologies have entailed the use of direct and non-direct procedures to obtain scent pads from the scene and/or suspect in a particular criminal investigation. The use of non-direct methods, however, have emerged as the preferred choice for law enforcement personnel as it reduces contamination, eliminates the potential for destruction of other forms of trace evidence, and allows for a larger number of scent samples to be collected from a single target of interest. The already accepted Scent Transfer Unit (STU-100) provided a novel approach in non-contact scent collection procedures. The Scent Transfer Unit is currently the accepted method for the forensic collection of human scent evidence in the United States. It must be noted, however, that due to some drawbacks when operating the STU-100 in an instrumental setting, it has been shown that there is no reproducibility of the collected scent samples when utilizing the STU device. Therefore, in an effort to correct and improve some basic operation settings such as airflow and sampling times, a new non-contact device such as the human scent collection system (HSCS) was developed. This new device could potentially fix encountered instrumental challenges and provide a more efficient field-working device. Thus, the focus of the presented study was geared at a non-contact device performance comparison, evaluating both the STU-100 and the newly developed human scent collection system for the collection of volatiles in an instrumental setting.

The goals and significance of the present study were to validate the use of the Human Scent Collection System (HSCS) as a forensic tool for the collection of volatile organic compounds from various biological specimens. Through this evaluation, the operational conditions were monitored for the sampling of hand odor and buccal swabs as well as a standard mixture containing VOCs present in human scent using solid phase microextraction-gas chromatography/mass spectrometry (SPME-GC/MS) as the instrumental technique. Important operational parameters included airflow speed and collection sampling time with each biological specimen. By using a standard mixture of previously reported human scent compounds as well as actual individuals, the three distinct airflow settings of the device (low, medium, and high) were monitored as well as the duration of sampling by running the device at the two time settings, 30 seconds and 60 seconds, respectively. The collection of hand odor and buccal swab samples was conducted at indoor laboratory conditions from both female and male subjects to compare specimen and gender differences. Factors such as the number and amount of VOCs collected from a sample and reproducibility within consecutive samples taken were closely evaluated to monitor the efficiency of the device for sample collection and consequent instrumental analysis as measured by the obtained chemical profile.

The instrumental validation of the HSCS as a collection tool from possible scent evidence sources such as different biological specimens can improve and further validate the use of this trace evidence collection device and help establish the scientific foundation of canine work within the criminal justice system as it relates to scent evidence as a form of evidentiary tool.

Human Scent Collection System (HSCS), Scent Transfer Unit (STU-100), Non-Contact Methods

## A204 Comparison of the Instrumental and Canine Evaluations of the Chemical Composition of Biological Specimens for Human Scent Discrimination

Jessica S. Wirks-Brown, BS\*, Florida International University, 11200 Southwest 8th Street, CP 345, Miami, FL 33199; and Kenneth G. Furton, PhD, International Forensic Research Institute, Florida International University, University Park, Miami, FL 33199

After attending this presentation, attendees can expect to have a better understanding of the utilization of chemical profiles emanating from biological specimens for the differentiation of individuals both with the use of analytical instruments and human scent discriminating canines. As a result, attendees will become better acquainted with human scent and its role in forensic science.

This presentation will impact the forensic science community by providing the essential scientific foundation that is required for the use of human scent evidence in the court of law.

Human odor can be considered a biometric measurement with emanating chemicals particular to each person. Research has been done to substantiate this concept and many have shown the individualistic properties derived from chemicals present on the body. Human odor is comprised of volatile and non-volatile constituents arising from internal (genetic), external (diet and environment), and cosmetic origins. Human odor components can be detected through olfaction and instrumental analysis. Chemicals emanating from hands, axilla, and feet have been explored and now work is being done to evaluate novel biological specimens.

The detection and discrimination of human scent has found usefulness in the forensic sciences through the deployment of canines. Dogs have been used for their ability to detect minute concentrations of human scent and match that odor to the person it originated from. Studies have shown that canines are able to distinguish related and nonrelated people whether they live together or apart in addition to being able to match odors from the same person collected from different areas of the body. American law enforcement agencies are slowly integrating human scent discrimination evidence in U.S. court proceedings demands that it adheres to guidelines set forth by rulings such as *Frye* and *Daubert* and detailed in the Federal Rules of Evidence. Consequently, research must be conducted to validate the distinctiveness of human scent and demonstrate the ability for trained canines to be able to discriminate this odor among individuals.

This presentation will explore the human odor emanating from a novel biological specimen, saliva collected onto a buccal swab, and make comparisons to hand odor. Instrumental evaluation of the volatile compounds present from both specimen types will be performed on samples collected from twenty male and twenty female subjects using solid-phase microextraction (SPME) followed by gaschromatography/mass spectrometry (GC/MS) analysis. The array of chemical constituents from each biological specimen type possesses similarities such as a high percentage of hydrocarbons and aldehydes; however, hand odor contains a greater number of alcohols than saliva while saliva contains a greater number of acids than hand odor. Multivariate, non-parametric statistical tests reveal that the chemicals present from each specimen make the two specimen types distinct.

To further expand the laboratory component of this work, a canine field test was conducted to test canine response to saliva, collected onto a buccal swab, as a human odor source. Previous testing conducted by the laboratory has demonstrated the ability for canines to differentiate people based on their hand odor regardless of the chemistry of the collection medium (i.e., cotton, polyester, wool, etc.) used to collected the odor. For this work, field tests were performed with five canine teams on both hard and soft surfaces to test saliva, as an odor source. Saliva was evaluated

in reference to hand odor, which for testing purposes served as a standard odor source. Hand odor was collected from the palms of the hands utilizing a dynamic airflow collection device, the Scent Transfer Unit 100 (STU-100). Odor from saliva samples was collected from the buccal swab onto cotton gauze with the use of the STU-100. The results of these double blind field experiments demonstrate that canine teams responded similarly to both hand odor and saliva samples. Overall, this study confirms that individual hand odor and saliva samples were distinct chemically however the canines were able to identify persons using either hand odor or saliva samples.

**Biological Specimens, Volatile Analysis, Canine Discrimination** 

#### A205 Computational Pattern Recognition of Striation Patterns on Fired Cartridge Cases and Chisel Striation Patterns

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After attending this presentation, attendees will be able to appreciate the need for establishing a rigorous scientific basis for impression evidence comparisons.

This presentation will impact the forensic science community by giving a better understanding of how statistical pattern recognition techniques can be applied to striation and impression patterns left by firearms and tools and how these methods can be used to establish error rates of identification for evidence collected from crime scenes.

Forensic tool mark comparisons have received much attention in the last decade, especially since the publication of the National Academy of Sciences 2009 Report, "*Strengthening Forensic Science in the United States: A Path Forward.*" One of the major criticisms is that there is no accepted methodology to generate numerical proof that independently corroborates morphological conclusions in questioned tool mark impression examinations. This researched focuses on answering that criticism by developing standardized methodologies to study and critically evaluate impression evidence.

Primer shear marks from forty-five, 9 mm cartridge cases fired from four Glock model 19 pistols (Glock 19s) have been collected along with fifty striation patterns made in lead with five consecutively manufactured chisels. Three-dimensional surface topography data from these collective striation patterns was obtained with high-resolution white light confocal microscopy using a 50x objective (0.95 NA) for the primer shear marks and a 20x objective (0.6 NA) for the chisel striation patterns. All topographies were preprocessed with outlier and form removal. Filtration into "waviness surfaces" extracted the essential "line" information familiar to forensic firearms and tool mark examiners. A cubic spline filter (ISO/TS 16610-22 standard) with a 0.08 mm wavelength cutoff was used to extract all waviness surfaces.

The primer shear topographies were summarized by taking the mean of all the profiles that made up the surface (i.e., mean waviness profiles of the primer shear marks were obtained). The profiles typically consisted of upwards of 2,500 points, which statistically were treated as random variables in a very high dimensional data space. In order to reduce the dimensionality of the data set, the profiles were subjected to principal component analysis (PCA) to obtain "synthetic" features of much more manageable size, but which still contain most of the topographical information in the original profiles. Next, support vector machine (SVM) algorithms were used to build a supervised learning model for the classification of each profile to a Glock. Effectively, this computational procedure testably "identifies" a primer shear mark as having been made by a particular gun. Both PCA and SVM methods were chosen for the statistical analysis because they are relatively free of assumptions on the statistical distribution of the data on which they are used and have been extensively applied with success in many industries requiring robust statistical discrimination systems. Also, they have a long peer-reviewed history in the scientific literature and produce easily testable (i.e. falsifiable) predictions, both of which become important issues when these methods are applied to evidence in a case subject to the Daubert standard. Finally, the statistical analysis programs written and used in the study are all open sources and available to anyone.

Using 200 bootstrap resampling iterations, PCA-SVM required only five "synthetic" features (5D) to produce an estimated identification error rate (refined bootstrap method used) of 0.2% on a larger data set of assumed similar statistical properties. The more conservative .632 bootstrap estimate yielded a 0.4% identification error rate estimate on a larger dataset (same assumptions). Pattern recognition results from chisel striation patterns treated in the same way as the primer shear marks will also be presented. The preliminary results of this research lend strong credibility to the fundamental principle of firearms and tool mark identification: that the striations imparted by the action of a tool on a softer surface are unique to that tool.

**Statistics, Primer Shear, Striation Pattern** 

### A206 Forensic Latent Fingerprint Decisions: How Accurate Are They?

Bradford Ulery, MS\*, and R. Austin Hicklin, MS\*, Noblis, 3150 Fairview Park, Falls Church, VA 22042; JoAnn Buscaglia, PhD\*, FBI Laboratory, CFSRU, FBI Academy, Building 12, Quantico, VA 22135; and Toni Roberts, MS\*, Federal Bureau of Investigation, 2501 Investigation Parkway, Quantico, VA 22135

The goal of this presentation is to inform attendees of the results of this large-scale study of latent print examiner accuracy and to demonstrate the feasibility of the "black box" model for objectively assessing the accuracy and effectiveness of forensic examiners.

This presentation will impact the forensic science community by serving as a preliminary step in demonstrating potential areas of strength and weakness within the latent fingerprint discipline as well as offer some objective measures to support admissibility requirements.

Despite over one hundred years of the forensic use of fingerprints, the accuracy of decisions made by latent fingerprint examiners has not previously been ascertained in a large-scale study.<sup>1</sup> Recently, there has been increased scrutiny of the discipline resulting from publicized errors and a series of court admissibility challenges to the scientific basis of fingerprint evidence.<sup>2-5</sup> In response to the misidentification of a latent print in the 2004 Madrid bombing, a Federal Bureau of Investigation (FBI) Laboratory review committee evaluated the scientific basis of friction ridge examination.<sup>6</sup> That committee recommended research, including the study described in this report: a test of the performance of latent print examiners.<sup>7</sup> The need for evaluations of the accuracy of

fingerprint examination decisions has also been underscored in critiques of the forensic sciences by the National Academy of Sciences (NAS) and others.<sup>8-11</sup> This study is based on a black box approach, evaluating the examiners' accuracy and consensus in making decisions rather than attempting to determine or dictate how those decisions are made.

This study evaluated examiners on key decision points during fingerprint analysis, comparison, and evaluation. One-hundred and sixtynine latent fingerprint examiners were tested, each of whom compared approximately 100 pairs of latent and exemplar fingerprints randomly drawn from a pool of 744 pairs. The pool was constructed to be representative of difficult comparisons from searches of an automated fingerprint identification system (AFIS) containing more than 58 million subjects. The fingerprints were selected to include a range of attributes and quality encountered in forensic casework. Latents of low quality were included in the study to evaluate the consensus among examiners in making value decisions about difficult latents. Image pairs were selected to be challenging: mated pairs were randomly selected from the multiple latents and exemplars available for each finger position; non-mated pairs were based on difficult comparisons resulting from searches of AFIS.

Examiners frequently differed on whether fingerprints were suitable for reaching a conclusion. More than 99.8% of individualization decisions were correct, and 86.6% of exclusion decisions were correct. Procedures used operationally to reduce the possibility of error would have improved these rates, such as examination of original evidence or paper fingerprint cards, review of multiple exemplars from a subject, consultation with other examiners, revisiting difficult comparisons, verification by another examiner, and quality assurance review.

A follow-up study of the repeatability of latent print examiners' decisions (intra-examiner variability) was also conducted, in which examiners were presented with image pairs that they had assessed weeks or months previously as part of this research effort.

The results of these studies, as well as the applicability of the black box test model for assessing examiner performance in other forensic disciplines, will be discussed.

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Latent Fingerprint, Error Rates, Examiner Accuracy

### A207 Dimensional Stability Concerning Exposures of Polymers to Solvents

David S. Pierce\*, Sarnia Police Service, 555 North Christina Street, Sarnia, ON N7T 7X6, CANADA

After attending this presentation, attendees will be introduced to a new research study exploring dimensional stability issues that can affect impression evidence comparison and testimony. This research provides insight into the behavior of polymers under a range of situations ranging from casual exposures to extreme workplace conditions.

This presentation will impact the forensic science community by acknowledging that exposure to solvents is known to cause swelling or shrinkage of exposed materials; and by discussing how the immediate impact of this research is limited to those conditions where exposure to solvents are known or suspected, where there is a size difference between a suspect specimen and either of the crime scene markings or a new specimen. This extends to cases where the occupation or activities of a suspect need to be considered or eliminated. While it is not possible to make definitive assertions regarding a particular specimen, this research does provide some basis from which to consider size differences and unusual conditions with an affected specimen.

Exposure to solvents is known to cause swelling or shrinkage of exposed material. This is a topic that can provide necessary background information for anyone giving evidence that pertains to polymers. Dimensional stability has long been investigated by engineers concerned with achieving improvement of specific properties such as resistance to oils or acids.

Industrial interest regarding dimensional stability issues is concerned with the quality control of specific materials and products. Commercially motivated engineering studies are frequently conducted at elevated temperatures that are designed to provide a quick analysis of material designs. This presentation examines results conducted to more closely resemble either casual exposures or actual working conditions.

Dimensional stability issues are not currently included in the literature or methodology of forensic comparison. The effects can be minute, but as with most forensic concerns, small details are no less important than larger counterparts. Current analysis of footwear or tires is conducted almost entirely based on pattern transfer. Dimensional stability (except in extreme cases) cannot be expected to have the same effect with large pattern areas as it does in the comparison of much finer details within a pattern.

The scope of the research presented includes exposure of outsole and tire specimens for varying periods of time, and by different methods, to some common solvents including fuels and water. The methods and conditions of experimentation are noted. Efforts were made to record, limit, or eliminate extraneous influences such as temperature and exposure to UV radiation by the use of selective conditions and control samples.

This particular research was inspired by previous studies which began by examining the stability of specimens immersed in particular solvents including lard, motor oil, and diesel fuel. The addition of casual exposures and controlled comparisons has yielded some interesting results that also suggest the nature of how specimens can behave once the exposures have been removed.

Results of the study will be presented with a caution that in each case, where dimensional stability issues could play a role, specific proof will be required to support findings and opinions. This research builds on previous studies conducted by the author with the intent of providing a starting point for examination of the topic and its relevance to the forensic science community.

**Dimensional Stability, Polymers, Solvents** 

#### A208 Design of a Prototype Mid-IR Imaging System for Visualizing Blood at Crime Scenes

Stephen L. Morgan, PhD\*; Michael L. Myrick, PhD; Heather Brooke, BS; Megan R. Baranowski, BS; and Jessica N. McCutcheon, BS, University of South Carolina, Department of Chemistry & Biochemistry, 631 Sumter Street, Columbia, SC 29208

After attending this presentation attendees will learn about advances in imaging that may enable visualization of blood at crime scenes via noninvasive remote infrared imaging with adequate sensitivity and with designed selectivity against interferences.

This presentation will impact the forensic science community by exploring how a common thread in the recent discussion of forensic techniques is the need for continuing research to establish limits and measures of performance for forensic analyses: to clarify the applicability and reliability of techniques for various purposes. The present work constitutes the first steps in evaluation of the performance of infrared imaging for crime scene investigations.

Current visualization methods for blood and semen are not specific, require dark conditions, and often are not very sensitive. Further, high discriminating power is important at crime scenes so that time and resources of forensic investigators are not wasted on the collection and analysis of false positive samples. A prototype portable camera using mid-infrared (IR) spectroscopy with a thermal imaging detector has been designed that has a spectral response tuned by filters of polymer films. Lock-in amplifier techniques are used to construct the contrast image of the scene, on a pixel-by-pixel basis in real-time, using techniques designed to enhance visualization of blood. An infrared source (e.g., a small heating plate, glow-bar, or space heater) is employed to illuminate a scene with IR light. Light reflected from the scene is employed to achieve imaging by chopping the source and digitally processing each pixel by a lock-in amplifier approach, producing an output that is proportional to contrast between stain/no-stain regions.

The infrared camera response is also sensitized to spectral regions where blood components (e.g., proteins) show absorbance using a combinatorial simulation-driven design process that selects chemical filters to maximize discrimination between blood-stained and unstained surfaces. There are many factors involved in optimizing discrimination by using optical filtering aids, including, but not limited to, the detector response, optical throughput of the system, optical properties of the samples, and optical properties of the materials for sensitizing films/filters. There are nearly infinite possible setups for the system, which means it is neither cost- nor time-efficient to physically test each one. In lieu of this, simulations of camera output were used, per pixel, beginning with measured spectra of calibration samples or standards, using an objective function or figure of merit (FOM) to measure simulated performance. Combinatorial simulations are performed to select from the large number of different filtering possibilities and the combinations of filter parameters that best discriminate between neat fabrics and one stained with blood were used.

Further data processing methods develop and display scene images, with regions indicative of the target analyte (latent blood) showing contrast from background. This approach has produced acceptably high signal-to-noise ratios and enabled visualization of blood well below  $100 \times d$ ilutions with visible contrast, while providing discrimination against some substances reported to give false-positive responses with other techniques. Besides being rapid, IR imaging for bloodstain detection offers advantages: examiners are not exposed to chemicals, the technique can be used indoors or outdoors under ambient light, patterns are not smeared, and stains are not diluted or altered by chemical reagents.

Fundamental studies were concurrently conducted to advance the scientific basis and the understanding of infrared imaging for crime scene

visualization. Additional instrument development and validation research is necessary for realization of the ultimate forensic goals of the present research. However, this research has opened up novel and intriguing applications of imaging, based on diffuse reflectance in the mid-infrared region of the spectrum that may have valuable forensic applications.

Imaging, Blood, Detection

## A209 Combination of Evidence in Complex Casework Using Bayesian Networks

Gerard J.Q. van der Peijl, PhD\*, Netherlands Forensic Institute of the Netherlands Ministry of Justice, PO Box 24044, The Hague, 2490 AA, NETHERLANDS; and Marjan J. Sjerps, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, The Hague, 2497GB

After attending this presentation, attendees will understand the pros and cons of using Bayesian networks for combining evidence in a complex case.

This presentation will impact the forensic science community by evaluating a combination of evidence from a wide range of expertise areas in a transparant and logical way. This will assist forensic experts and, in the future, also law and court officers in correctly weighing the combined evidence in criminal investigations and ensuing court procedures.

In forensic casework, often multiple investigations are performed. Reporting the evidential value of the combined results within a single casework investigation is desirable in many respects. Bayesian networks have been proposed in the literature as a useful tool for combining evidence; however, many challenges are encountered when putting theory into practice in complex casework investigations. Nonetheless, it is concluded that this is the way forward. This presentation illustrates the pros and cons of using Bayesian networks in complex casework investigations using a real case, involving many questions and many types of forensic evidence.

Late in a summer night, two incidents occurred in a rural Dutch area. A motorcade of a car with trailer, an excavator, and a private car are observed by the police. This is a combination that is often used for a hit on an ATM. The excavator is used to break into the ATM, the last private car is used to hinder pursuers (e.g., by throwing spikes on the road). The police pursue and encounter the motorcade. On a side road, the car advances and hits the police car in the flank. A police officer standing outside the police car feels threatened and shoots five times at the vehicle. Subsequently, an injured male is encountered on the driver's seat in this car. An excavator is found abandoned a little further down the road.

A few days afterwards, in the woods close by, the body of a man is found. His head and back are covered with maggots of different sizes and a large part of the soft tissue has gone. On his skull, an injury (impression) is observed. This male is identified shortly afterwards. His DNA matches blood and tissue samples taken from the vehicle and a guardrail next to it. The main question: Was this man (mortally) injured by the police officer during the shooting incident?

Many forensic investigations were made including comparisons of bullets to the police officer's gun, DNA-investigations on many samples, fiber comparison of materials found on the bullets to garments and balaclavas, comparison of paint and glass particles found on the bullets to the car paint and (broken) window glasses. Especially the micro traces (human tissues, glass, paint, fiber) on two bullets found in the vehicle appear to link both the deceased and the injured male to the incident and to each other. GSR investigations on clothing around damages (holes) were made to verify if these damages could be bullet holes. The FT-IR spectrum of a white substance found on one bullet matched the spectrum of bone material. An investigation of the tool marks on the head injury did not provide a definitive answer whether this injury was caused by a bullet. The autopsy and subsequent pathology and toxicology investigations could not determine a cause of death for the deceased due to the advanced decomposition of the body and the lack of soft tissue. Through hair and fiber investigations a link with the excavator was investigated. In total, 11 expert areas were involved.

The use of Bayesian networks was explored in this case. This presentation shows a network that was made for answering a part of the main question: the manner of death. Some progress was made to combine DNA, glass, paint, fiber, (white) material, bullet trajectory, toolmarks, and pathology evidence. The assumptions made in the structure of the model and the probability tables behind it shall be discussed, as well as the various advantages (explicit derivation of the combined evidential value, transparancy of reasoning and assumptions, sensitivity analysis, information analysis) and disadvantages (many assumptions required, possibly misleading suggestion of exactness, sensitivity to small changes in formulation). This presentation concerns work in progress. No claim is made that the present approach provides the final answer although there appears to be no viable alternative to the Bayesian network modeling and look forward to a fruitful discussion to advance the evaluation of a combination of forensic evidence on a scientific basis.

**Evidence Combination, Micro Traces, Bayesian Networks** 



## DIGITAL & MULTIMEDIA SCIENCES



#### B1 The Digital Forensics Certification Board (2009-2010): Activities and Certifications

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After attending this presentation, attendees will be persuaded to review the process and will be ready to go for the Phase II with the automated system.

This presentation will impact the forensic science community by promoting trust and confidence in the digital forensics profession by providing an objective and validated certification process which will help the maturation of digital forensics as a science.

The Digital Forensics Certification Board (DFCB) will have a great impact due to its launching at the right time, being weeks after the initial National Academy of Sciences report in 2009. Certification for digital practitioners is imperative. The DFCB is a professional certification requiring a clean background review.

The Digital Forensic Certification Board was created to develop a professional certification process for digital evidence. After numerous meetings with digital evidence practitioners and various agencies, digital certification has finally come to pass. The process was begun at <u>www.dfcb.org</u> with the first application taken on February 9, 2009 and ended in September 16, 2009. Sam Guttman, retired as the Assistant Inspector General for Investigations for Postal OIG, volunteered to be President of the DFCB.

DFCB developed a "Founders" process for those who have had over five years of experience in the field of digital forensics. Accreditation of our certification process was planned via the Forensic Specialty Accreditation Board (FSAB). The founders were given a registration that could substantiate their experience, education, training, publications, positions held, testimonies given, their background and other relevant activities. Each founder must have scored 100 points on the application form to qualify. They wrote 15 questions in the domains of their expertise each with four incorrect answers and one correct answer. These questions became part of the collection of questions that have been sorted and verified. There were 600 viable questions that resulted from the founders group that will be validated when the next round of applicants begin the electronic version of the application process. There were 130 founders that successfully completed the certification process.

The DFCB started Certification Phase II from June 14<sup>th</sup> thru July 31<sup>st</sup>, 2010 and developed an automated application online. The application for Phase II had the following criteria:

- Score a minimum number of points on the Assessment Scoring
- Sheet and provide supporting documentation
- Meet and continue to comply with the DFCB Code of Ethics and Standards
- Pass a background review
- Develop 15 usable test questions with multiple choice answers. Applicants shall author questions and answers following the instructions posted on the DFCB website
- Take a non-score test sampling from questions developed by the founders. Instructions for developing multiple choice questions are available on the website.

The next phase will focus on the development, validation, and delivery of DFCB Certification tests.

This project was supported by NIJ Grant. Digital Evidence, Certification, Compliance

### B2 Communicating Results: The Digital Forensic Report

Mark Pollitt, MS\*, Daytona State College, 1200 West International Speedway Boulevard, Daytona Beach, FL 32114

After attending this presentation, attendees will have an understanding of how digital forensic reports can be organized and written in a manner that will assist the reader to better understand the examiner's results while aiding the examiner in structuring their testimony.

This presentation will impact the forensic science community by improving the quality and content of digital forensic examinations.

One of the criticisms articulated in the National Academy of Sciences report, *Strengthening Forensic Science in the United States: A Path Forward*, concerned the content and effectiveness of forensic reports. The structure, content, and detail found in forensic reports vary widely in the profession and often are written for the examiner's benefit, not the reader. This presentation will look at the requirements for forensic reports, based upon the Federal Rules of Evidence, and how those translate into a set of requirement. An example will be offered as to how these might translate into a report format. Utilizing genre theory, the report format will then be examined to determine the most effective. At the conclusion of this presentation attendees will have an understanding of how digital forensic reports can be organized and written in a manner that will assist the reader to better understand the examiner's results while aiding the examiner in structuring their testimony effectively.

Forensic Reports, Exert Testimony, Technical Writing

B3 Development of an Offender Classification Based Investigative Protocol for Use With Online Consumers of Child Pornography Cases: An Information, Technology, and Behavioral Sciences Approach

Kathryn C. Seigfried-Spellar, MA, 401 North Grant Street, Knoy Hall of Technology, West Lafayette, IN 47907; and Marcus Rogers, PhD\*, 401 North Grant Street, West Lafayette, IN 47907

The goals of this presentation are to provide an introduction to a developing investigative model, introduce an offender classification scheme for use with online consumers of child pornography, and assist in the establishment of scientifically derived protocols for digital evidence investigations.

The presentation will impact the forensic science community by providing crucial insight into using investigative models for dealing with online child pornography investigations. The proposed model assists in the identification and collection of digital evidence from computer systems using established offender classifications.

There has been a lack of meaningful discussion and research related to effective and efficient investigative models and protocols for investigating consumers of online child pornography. The few studies done to date have focused primarily on clinical populations and physical/environmental factors that might affect the investigation. As more resources are being devoted to child pornography related investigations it is paramount that more efficient methods are developed for deriving pertinent data that can be used as evidence to either prosecute or exonerate those people charged with this type of criminal offense. Anecdotal and research based evidence indicates that law enforcement spends an increasing amount of their limited resources (both time and personnel) dealing with cases involving online child pornography. It has also been speculated that technology innovations such as the Internet, webcams, and social networks have greatly assisted child pornographers with their criminal tradecraft.

Whether the rate of child pornography is increasing or not has been the topic of very heated debate. What is not debatable is the technical difficulty that arises when investigating this type of criminal activity. This difficulty has been correlated with the increased size of available storage devices and the falling cost to consumers. The days of simply looking at every sector on a storage device for possible evidence is no longer practical and in some cases not technically feasible within a reasonable time frame. What is required are more tactical and focused approaches to investigating large amounts of data. These approaches or process models need to be informed by research that studies the personality and motivational characteristics of the offenders in question.

The presentation will introduce an offender characteristics based investigative protocol that assists investigators looking for digital evidence. The protocol combines advances in the behavioral analysis of online consumers of child pornography with common locations of digital evidence found on computing system. Previous behavioral characteristics based models, such as Lanning (2001) and Krone (2004) were modified to take into account the security precautions commonly implemented by these offenders. The Rogers-Seigfried model identifies common types and locations of digital evidence available to investigators based on the classification of the offender. The process model advocates treating the computing system as a digital crime scene analogous to the physical crime scene where context and evidence proximity (both physical/virtual and temporal) are important considerations.

The model also leverages the constraints and default behaviors built into the various operating systems, file systems and applications based on usability and human computer interaction standards. These constraints limit the potential virtual space that must be examined by investigators looking to identify and understand the context of any digital evidence.

The proposed model has been used successfully in several investigations involving online child pornography. The presentation will present a brief case study to illustrate the concepts introduced. Limitations of the model and suggestions for future research will also be discussed.

**Evidence, Pedophilia, Investigations** 

### B4 Exploring the Progression of Nondeviant and Deviant Pornography Use By Age of Onset and Sex

Marcus Rogers, PhD, 401 North Grant Street, West Lafayette, IN 47907; and Kathryn C. Seigfried-Spellar, MA\*, 401 North Grant Street, Knoy Hall of Technology, West Lafayette, IN 47907

After attending this presentation, attendees will be presented with the results of an empirical study assessing the difference in age of onset for engaging in various forms of nondeviant and deviant pornography and the progression of pornography use between men and women. In addition, attendees will learn whether there is a progression of increased risk for engaging in nondeviant to deviant forms of pornography based on age of onset.

This presentation will impact the forensic science community by adding to the body of knowledge examining the age of onset for consuming various forms of nondeviant and deviant pornography. Although seemingly counterintuitive, research indicates the collections of child pornography users not only contain sexualized images of children, but other genres of pornography both deviant and socially acceptable in nature (c.f., Quayle & Taylor, 2002; Quayle & Taylor, 2003).<sup>1,2</sup> In fact, interviews with child pornography users have suggested that some offenders move "through a variety of pornographies, each time accessing more extreme material" (Quayle & Taylor, 2002, p. 343)<sup>1</sup> as a result of desensitization or appetite satiation, which lead to collecting and discovering other forms of deviant pornography (Quayle & Taylor, 2003).<sup>2</sup> Also, some consumers stated they downloaded the images simply because they were available and accessible, making the behaviors primarily a result of compulsivity rather than a specific sexual interest in children (Basbaum, 2010).<sup>3</sup>

Some child pornography consumers exhibit a complex array of sexual interests, which may be representative of a more general level of paraphilic tendencies rather than a specific sexual interest in children. In a study conducted by Endrass et al. (2009),<sup>4</sup> the collection of images from 231 men charged with child pornography use also revealed other types of deviant pornography. Specifically, nearly 60% of the sample collected child pornography and at least one other type of deviant pornography, such as bestiality, excrement, or sadism, with at least one out of three offenders collecting three or more types of deviant pornography (Endrass et al., 2009).<sup>4</sup> This research suggests the majority of Internet child pornography users are collecting a wider range of deviant pornography, which may reflect a general level of sexual deviance rather than a specific paraphilia, such as pedophilia. In other words, some child pornography consumers may be dissidents within the normal population who exhibit a wider range of sexual interests.

Although case studies exist, few empirical research studies have assessed the question of whether individuals who use nondeviant forms of pornography (e.g., adult pornography) are at a greater risk for consuming deviant forms of pornography (e.g., animal and child pornography). The current study adds to this body of knowledge by examining the age of onset for consuming various forms of nondeviant and deviant pornography. Specifically, the present project will explore at what age individuals first knowingly searched for, downloaded, and exchanged/shared the following pornography genres: adult-only, animal (bestiality), and child pornography. By examining the interrelations among the self-reported age and pornography use variables, the goal is to provide a better understanding of how nondeviant pornography use either facilitates or accelerates the probability of engaging in more deviant forms of pornography.

The first goal of this study is to determine whether or not the *age of onset* is a risk factor for engaging in deviant pornography. In other words, are individuals who engage in nondeviant pornography use at an earlier age more likely to engage in deviant forms of pornography use compared to late onset users? The second goal of this study is to determine whether or not the age of onset for nondeviant and deviant pornography differs by sex (male, female). Finally, the third goal of this study will explore the frequency of pornography use by collapsing the respondents into pornography categories: none, adult-only, animal-only, child-only, adult-animal, adult-child, animal-child, and adult-child-animal. This will assess whether self-reported child pornography users are more likely to self-report adult and animal pornography behaviors compared to the other categories of users.

Results and future implications of the study's findings will be discussed.

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Deviant Pornography, Adult Pornography, Age of Onset

#### B5 An Approach for the Detection of the Illicit Use of Legitimate Network Access Credentials by an Intruder

#### Christopher W. Day, BS\*, 2 South Biscayne Boulevard, Suite 2800, Miami, FL 33131

After attending this presentation, attendees will understand a typical Persistent External Targeted Threat (PETT) attack and one of the most common follow-on modes of persistence utilizing stolen, but valid, network access credentials. Previously, PETTs were most commonly observed in government agencies but are now becoming more prevalent in the private sector as demonstrated by the so-called "Aurora" attacks against Google in January of 2010. A common result of a PETT attack is the compromise of valid user and system administrator credentials that are then subsequently used by the adversary to continue to access the victim network via VPN, RDP, Enterprise Portals, or other remote access platforms. Due to the fact that the credentials used by the intruder are valid access credentials, the intruder is simply "logging in" and no longer required to utilize an exploit or some other form of "hacking" to continue to intrude into the network. This form of intrusion is difficult to differentiate from legitimate user access and hence challenging to detect.

This presentation will impact the forensic science community by providing practitioners with awareness of this form of illicit activity as well as network and system observables to detect it in networks of interest. Given that one of the primary results of a PETT attack is sensitive data exfiltration, it is important for investigators to know how to identify the patterns and observables that the malicious misuse of valid network access credentials leave behind in the forensic record. Finally, a new free, extensible, open-source tool will be introduced to help investigators process many common log file types to highlight the observable patterns of the above-mentioned malicious misuse of valid network credentials.

**Intrusion, Detection, Credentials** 

#### B6 Preventing a Rush to Judgment: Application of Computer Forensics in Data Breach Cases

Jason M. Paroff, JD\*, Kroll Ontrack, 1 Harmon Meadow Boulevard, Suite 225, Secaucus, NJ 07094; Stephen D. Baird, MS, Kroll Ontrack, 1166 Avenue of the Americas, 23rd Floor, New York, NY 10036; and Alan E. Brill\*, MBA, Kroll Ontrack, 1 Harmon Meadow Boulevard, Suite 225, Secaucus, NJ 07094

After attending this presentation, attendees will understand how the pressures on corporate and governmental managers who believe that their organization has been the target of a successful breach of sensitive personal or health-care data can lead to jumping to conclusions that, in fact, a suspected breach is a real one. This can result in the notification of thousands of people, informing them that they have been victims of an incident that, in fact, never occurred. The application of computer forensics often provides the best way of determining whether an actual

incident occurred and whether that incident meets the varied criteria for victim notification under the 47 United States State laws, plus applicable United States and international federal laws and regulations.

This presentation will impact the forensic science community by demonstrating that through the use of digital forensics, companies can avoid needless large outlays for notification and remediation cost if it can be shown with forensic accuracy that an incident did not occur (or that it is different in scope than assumed) and avoid the creation of unnecessary anxiety on the part of persons who would be concerned about identity theft when, in fact, their data was not at risk.

When an organization has reason to believe it has suffered a breach of sensitive personal or health-care information, there are literally dozens of state and federal laws and regulations that may, depending on the nature of the data compromised, and the home jurisdiction of the individuals involved, require specific notification of affected individuals as well as governmental entities. These notifications are often tied to tight timelines in the law, but it has been found that with proper project control and forensic discipline, an investigation can be carried out within the allotted time frames that can provide management (and usually counsel) with the best information available to support their decision making process. Recent surveys indicate that the cost to an organization of a data breach can exceed \$20 per victim simply for notification and basic remediation assistance, so breaches of as little as 50,000 records can quickly result in a million dollar unplanned expense. This is, of course, in addition to what can be substantial costs related to reputational damage, and the potential costs of litigation, or added regulatory oversight that can result from reported cases of data loss - even where it is later found that the event did not actually occur.

The forensic work has the added benefit, in many cases, of providing valuable insights into exactly what happened, the vector through which an incident originated, and sometimes information about the perpetrators. It is not unusual to be able to provide some assurance that an incident has been stopped and that there is not a continuing leakage of sensitive data.

A series of case studies based on the team's work that will demonstrate actual situations in which computer forensics proved that an incident did not occur, or that it was less severe than had been assumed will be provided.

Data Breach, Personal Data, Data Compromise

### **B7** Finding Light After Dark: Low Light Photography for Forensic Investigators

Francesca M. Daher, BA\*, Harris County Institute of Forensic Sciences, Imaging Department, 1885 Old Spanish Trail, Houston, TX 77027; Dustin C. Hatfield, BA, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054; and Desmond R. Bostick, and Andre Santos, BA, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will learn techniques used by night shift scene investigators in a medical examiner setting to photographically document decedents and places of death in low light situations.

This presentation will impact the forensic science community by providing information on photography training initiatives used in a medical examiner setting for advanced photography techniques to enhance scene investigations.

While the burden of photographic documentation may fall on a professional forensic photographer for some fortunate agencies, many non-photographers in the forensic community find themselves struggling to take high-quality photographs, especially in low light situations. Since many forensic scenes occur at night or in low light situations, the use of these techniques can prove to be very useful to the forensic community as a whole.

Untrained or inexperienced photographers often face several challenges when it comes to low light photography. These challenges are most commonly associated with the limitations of a mounted flash unit. By removing or disabling the flash unit and manipulating the camera settings, an apparent daylight photograph can be achieved. These types of photographs can add valuable information to an investigation or serve as a permanent visual record of the scene prior to evidence being removed for processing.

While inexpensive point and shoot cameras appear to be a simple solution for investigators lacking photography experience, these cameras often fail to produce the desired results. These cameras often have severe limitations and pre-fixed settings which can prevent the photographer from achieving the desired exposure. For these reasons, SLR cameras are recommended for most typical forensic photography situations and required for low light situations. This presentation is designed to show how previously untrained investigators are now using these SLR cameras and manual camera settings to perform the advanced photographic techniques used to document scenes in low light situations.

Once a basic understanding of camera operation is established, investigators should be entrusted with SLR cameras capable of long exposures and tripod mounting. Through training the investigative staff is allowed to experiment with manual camera settings, tripods, and SLR cameras under the direct supervision and instruction of a trained forensic photographer. By learning how shutter speed and aperture affect the exposure, investigators can begin to use the minimal available light to illuminate the scene beyond what is visible with the naked eye. The investigators begin to understand the limitations of a traditional flash and how street lamps, headlights, and even flashlights can provide the needed light to capture the entire scene.

With proper equipment, training, and practice, scene investigators have achieved outstanding results with time exposures and "painting with light." These valuable tools provide the quality of photographs needed for proper scene documentation and, therefore, the transfer of all available and potentially crucial scene information onto the forensic pathologist or other investigative agencies.

The result of these training initiatives is demonstrated in the photographs themselves by comparison of traditional methods to these more advanced techniques. The discussion will encourage agencies to enhance their use of photographic documentation and training across multiple disciplines as well as provide examples of how this has been accomplished in our agency.

Photography, Low Light, Investigations

#### B8 Imaging the Unseen with Digital UV/IR Technology: Preliminary Bruising and Tattoo Studies

Stephanie L. Hoffman, MSFS\*, 27811 Northbrook Avenue, Canyon Country, CA 91351; and Wilson T. Sullivan, MPA, Chaminade University of Honolulu, 3140 Waialae Avenue, Honolulu, HI 96813-1578

After attending this presentation, attendees will learn about the electromagnetic spectrum and its relevance to visible, ultraviolet, and infrared digital photography. This presentation will also cover the documentation of visible, ultraviolet, and infrared radiation and their affects on different items of evidence as they transmit (remove), reflect (lighten), and/or absorb (darken) with each type of radiation, with digital single lens reflex (SLR) camera systems. Camera/lighting settings and equipment will also be suggested for specific types of evidence. In particular, the preliminary research results on revealing/documenting both fresh and healed bruising and removing/darkening tattoos with ultraviolet (UV) and infrared (IR) radiation will be discussed. These preliminary research findings aim to highlight the possible evidentiary results that can be obtained through the use of these techniques, and how

easily they can be accomplished with a UV/IR modified digital SLR camera. Successful results were obtained with each tattoo and bruise tested; of particular note is a case where the bruise was revealed seven months after it had disappeared from human sight. It is clear that both of the referenced techniques have yielded results proving they could be of great use at investigative stages with identifications and abuse cases.

This presentation and the research proposed within it will impact the forensic science community by demonstrating: (1) the need for the everyday use of both visible and ultraviolet/infrared (UV/IR) photography in forensic cases; (2) the ease with which these techniques can be accomplished via digital photography; and, (3) the impacts possible at various stages in the criminal justice process which can be achieved by revealing and documenting evidence that often goes unnoticed.

The potential of ultraviolet/infrared (UV/IR) photography to aid in investigations has yet to be fully realized and utilized by the forensic community. Past film based methodologies for this type of photography were difficult, unpredictable, inconsistent and/or expensive, resulting in its limited use. However, the once popular film based camera systems are being replaced by digital equivalents, and as such, it is time these old film methodologies were reevaluated as potential tools with the new digital single lens reflex (SLR) camera systems. UV/IR photography no longer needs to be thought of as difficult, unpredictable, inconsistent, and/or expensive since digital camera systems offer a framework to make these techniques and applications a more feasible option. This research demonstrates the need for the standard use of both visible and UV/IR digital photography in forensic cases by identifying the possible unique evidentiary items these techniques can now provide for identifications and abuse cases; as well as the ease with which these techniques can now be accomplished through digital means. The speed with which such results can now be obtained and distributed has the potential to greatly impact and expedite cases at both the investigative and prosecutorial stages.

Infrared, Ultraviolet, Photography

## **B9** Camera-to-Subject Distance and Facial Comparison Examinations

Richard W. Vorder Bruegge, PhD\*, Federal Bureau of Investigation, OTD-DES, Building 27958A, Pod E, Quantico, VA 22135

After attending this presentation, attendees will learn how to calculate the subject-to-camera distance necessary to achieve an orthographic projection for the face over the region extending from the nose to the ears, as well as the implications of this result for individuals and agencies involved in the capture and analysis of facial images, particularly when using measurements of facial features for comparison analysis.

This presentation will impact the forensic science community by informing it of a technical issue relating to forensic facial comparison that could lead to multiple false exclusions of subjects.

Forensic facial comparison examinations often incorporate a combination of morphological and anthropometric analyses.<sup>1</sup> Recent efforts have begun to rigorously assess the utility of anthropological landmarks<sup>2,3</sup> and morphological features<sup>4</sup> as objective, measurable characteristics of faces that can be used for comparison analysis. It is anticipated that forensic facial comparison analyses in the future will incorporate a more explicit determination of the size and shape, relative or otherwise, of specific features of the face and head than has been done in the past. Such features include the eyes, nose, mouth, and ears.

While many key facial features used in forensic comparison (as well as in facial recognition applications) are practically co-planar (e.g., eyes and mouth), the nose and ears are distinctly out of the facial plane. As a result, they are prone to perspective distortion and the relative size of these features will vary with camera position relative to the subject.

The degree to which this effect impacts the accuracy of automated facial recognition (FR) algorithms and systems has not been reported. Since the ears are not considered by most FR algorithms, any distortions to them can be expected to have a negligible effect on the automated portion of the systems. However, practical experience has shown that human reviewers of FR system output frequently focus on the ears and nose to quickly sort through a candidate list. As a result, distortions in ear and nose can negatively impact the overall effectiveness of a human-computer FR system.

The effect of perspective on facial images is well established in the photographic and forensic communities.<sup>5,6,7</sup> An analysis of anthropological landmarks on several subjects included in the MAGNA database demonstrated the degree of measurement errors that ensue for different camera-to-subject distances.<sup>8</sup> In particular, measurements of features associated with the ears were shown to be especially prone to perspective error. This result was not unexpected, given the fact that the ears are the most remote part of the face and head that is visible in a frontal image.

Under ideal conditions, 2-dimensional images of the face and head (photographs or video images) would be acquired in a way that perspective distortions were removed, and measurements taken from the photograph would accurately reflect the true physical measurements – so long as those measurements were taken in a plane parallel to the plane of the camera sensor. Images which depict three-dimensional objects with no perspective distortion, such as architectural drawings, are referred to as being in orthographic projection<sup>9</sup> and they reflect a situation in which all rays of light reflected off a subject enter the camera lens parallel to one another.

Existing guidance for the acquisition of frontal photographs (e.g., ANSI/NIST-ITL 1-2007)<sup>10</sup> describes a typical camera to subject distance of 1.5- to 2.5-meters. In this paper, it will be demonstrated that this distance is insufficient to generate an orthographic projection of the face and ears when a frontal photograph is acquired to meet resolution requirements for SAP Levels 40 and above. Instead, a distance of approximately 70-meters would be necessary to achieve orthographic projection for a facial image that incorporates the entire region from the nose to the ears at SAP Level 40 (approximately 200-pixels between the pupils), while a distance of approximately 125-meters is necessary for SAP Level 50 (approximately 600-pixels between the pupils). Such distances are impractical in virtually every controlled capture (e.g., enrollment) scenario. As a result, facial comparison practitioners must actively incorporate anticipated perspective effects into their analyses. Likewise, any other forensic or biometric application that incorporates the nose and ear as components must take this effect into account. This particularly applies to anyone who would use photo-anthropometry alone as a basis for inclusion or exclusion of a subject.

It is important to note that human beings are not accustomed to viewing each other in orthographic projection, but in perspective projection. Under such conditions, a photograph depicting a subject in an orthographic projection could lead to an improper exclusion by a screener. As a result, the requirements of manual screening and forensic analysis may be somewhat at odds with one another.

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Facial Comparison, Image Analysis, Orthographic Projection

#### B10 A Review On Automatic Footwear Retrieva Systems From Crime Scene Shoe Marks

Federico Cervelli, PhD\*, Via Valerio 10, Trieste, 34100, ITALY; Francesca Dardi, BS, and Sergio Carrato, PhD, University of Trieste, Via Valerio 10, Trieste, 34100, ITALY

After attending this presentation, attendees will have a clear understanding of the state of the art systems for the automatic retrieval of the footwear that left the shoe mark found at the crime scene and will gain a basic understanding of automatic "one to one" comparison techniques between the shoe mark and the suspect's footwear.

This presentation will impact the forensic science community by providing knowledge of state of art automatic footwear retrieval systems, developed to help forensic scientists in finding the shoe that left the mark at the crime scene.

The activities performed during the crime scene analysis are of paramount importance for the investigation. The crime scene expert is in charge of the detailed documentation of the crime scene status, as well as the search for fingerprints, shoe prints, biological fluids, chemicals, firearms ammunition, and the collection of the items pertaining the crime for a later and deeper analysis in the laboratory.

In particular, shoe marks play a key role to understand the crime and can help investigators gain precious information: when there is no suspect or few elements are available, knowing the make and model of the shoe sole that left the shoe mark on the scene can point a path, while, on the other hand, if there is a known suspect, his or her shoes can be compared to the shoe mark found on the crime scene to evaluate his or her involvement in the criminal act.

Two different approaches can be followed in order to find the make and the model of the shoe which produced the shoe mark on the crime scene: (1) a forensic shoe print expert analyzes the shoe mark and looks for the corresponding shoe on electronic and paper catalogs; and (2) a footwear retrieval system is queried with the crime scene shoe mark, and the results of the query are then analyzed by the expert.

Some semi-automatic systems have been proposed and face the problem: shoe prints are classified by a human expert which describes them with a series of geometric patterns. This work will guide the audience through the systems proposed in literature, starting from the very first work to current algorithms. Fourier based systems, fractals based systems, invariant moments systems, and feature based systems will be shown and their working principles will be explained in all relevant details to guarantee their understanding. A comparison among the different approaches will be made showing the advantages and disadvantages of each method under a forensic point of view.

Results of the performance of some of the most successful methods will be presented, both on synthetic and on real shoe marks, showing the difference between ideal and practical performance from the perspective of the forensic expert in the need to choose among one of the available approach.

The presentation will end with a brief overview on automatic method to compare the shoe mark with the suspect's footwear.

The audience will be aware of the advantages and disadvantages of the current state of the art on automatic footwear retrieval systems. Shoe Mark, Automatic Retrieval System, Footwear

#### B11 Preliminary Assessment of Discrimination of Twins in Photographs Based on Facial Blemishes

Nisha Srinivas, MS\*, Matthew Pruitt, BA, Gaurav Aggarwal, PhD, and Patrick J. Flynn, PhD, University of Notre Dame, Department of Computer Science and Engineering, South Bend, IN 46556; and Richard W. Vorder Bruegge, PhD, Federal Bureau of Investigations, OTD-DES, Building 27958A, Pod E, Quantico, VA 22135

After attending this presentation, attendees will be aware of efforts to utilize facial blemishes as a potential means of identification in photographs.

This presentation will impact the forensic science community by exposing them to current research efforts to establish a statistical basis to support the Digital and Multimedia Sciences (DMS) discipline of forensic facial comparisons – a critical aspect of all forensic disciplines in light of the National Academy of Science report. Attendees should recognize a need to develop a deeper understanding of this and other emerging forensic disciplines within DMS and consider their applicability to their forensic laboratory.

Digital cameras capable of recording both still images and videos are ubiquitous in our society. Likewise, video surveillance through the use of closed-circuit television systems is becoming more prevalent. Frequently, law enforcement or intelligence agencies have a requirement to identify subjects depicted in those photographs and videos. The growing interest in both facial recognition and facial identification led the Federal Bureau of Investigations to create the Facial Identification Scientific Working Group (FISWG) in 2009. The mission of FISWG is to develop consensus standards, guidelines, and best practices for the discipline of image-based comparisons of human features, primarily face, as well as to provide recommendations for research and development activities necessary to advance the state of the science in this field.<sup>1</sup>

As background, FISWG defines facial recognition as "[t]he automated searching of a facial image in a biometric database (one-tomany), typically resulting in a group of facial images ranked by computer-evaluated similarity," while facial identification is defined as "[t]he manual examination of the differences and similarities between two facial images or a live subject and a facial image (one-to-one) for the purpose of determining if they represent the same person."<sup>2</sup> In a practical sense, (automated) facial recognition systems generate candidate lists which must then be evaluated by a human reviewer performing facial identification.

While there has been extensive research conducted in facial recognition and, to a lesser extent, facial identification within both academic and industrial settings, this research has not yet converged to a single consensus set of standards of practice comparable to fields such as DNA analysis or fingerprint analysis, nor has a statistical basis for identification or exclusion been established. As a result, fully automated face recognition has not yet achieved a level of reliability and repeatability that make it suitable for use as a means of identification in the court room. Likewise, while the manual comparison process of facial identification has been accepted for expert testimony in multiple United States federal and state courts, it is lacking in a statistical basis from which conclusions may be drawn.

One attempt to address the statistical basis for human identification from facial images is the Magna Study.<sup>3,4</sup> In this large scale study involving over 3,000 subjects, it was determined that a meta-analysis of anthropometric measures of landmarks common to all human faces is not sufficient to discriminate between individuals. Put another way, this study found that the geometric distribution of a large set of landmarks common to all human faces (e.g., corners of the eyes and mouth) is not sufficiently unique to allow one to individualize a subject. Additional work remains to be performed on the Magna data to determine to what degree individuals might be segmented into individual classes based on the distribution of these landmarks. However, the Magna result indicates that facial features other than common landmarks will be necessary to support classification to groups smaller than 1% of the population. Facial blemishes, such as freckles or moles, are currently considered to be the best candidates for these features, as they are presumed to be comparable to friction ridge minutiae.

Incorporation of facial blemishes as features has already been shown to improve automated face recognition software.<sup>5</sup> The current effort to be described in this presentation would extend the utilization of such features for automatic face recognition, but will also have applicability to the forensic use of facial identification. More specifically, the current effort is intended to assess the ability to discriminate between identical and fraternal twins based solely on the distribution of facial blemishes. The hypothesis to be tested is that the distribution of blemishes on one twin's face will differ from the distribution of blemishes on the other's face. A secondary hypothesis to be tested is that the distribution of blemishes on any given face is random.

Data supporting this effort has already been collected for approximately 100 pairs of twins in 2009 at the Twins Days convention, held annually in August in the town of Twinsburg, Ohio. An additional collection is planned for 2010. For this analysis, each facial image will be processed using a face detection algorithm based on the work of Viola and Jones<sup>6</sup> that returns the bounds of a candidate face.

An Active Shape Model, first developed by Cootes et al.,<sup>7</sup> is fit to the face's bounding region, yielding a set of face feature locations that correspond to anthropometrically or photometrically significant points. Combinations of these points define one or more face-centered coordinate systems, providing a normalized basis for feature location. After normalization, blemishes will be identified using an automated extraction technique based on an approach first developed by Park and Jain.<sup>5</sup> Once blemishes have been marked, their distribution will then be determined for each subject and comparisons performed between subjects – not just within twin pairs, but across the entire population of subjects.

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Facial Identification, Photographic Comparison, Facial Blemishes

#### B12 The Reduction of False Negative Errors in Fuzzy Hashing

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After attending this presentation, attendees will understand: (1) the algorithms behind fuzzy hashing; (2) be presented the inadequacy of the false negative error rate of the original algorithm; (3) receive the statistical evidence to support the changes made; and, (4) see that the new changes do not greatly effect performance.

This presentation will impact the forensic science community by spurring discussion on error rates in digital forensics and introducing people to an improved implementation of an underused algorithm in digital forensics.

Fuzzy hashing is a hashing technique that uses a context triggered piecewise hash to compare files. The engine continuously sums bytes until the sum modulus of the block size is equal to one less than the block size. This process repeats with different block sizes until a block size is found that creates a target hash of 33-64 characters. Originally, while this is occurring, another hash is being created that is appended to the original to allow for comparisons of files with block sizes that were either 50% or 200% of the original.

This brought about a relatively serious false negative rate as the block sizes changed for specific files that were just on the edge between two different block sizes. For files of this type, fuzzy hashing is unable to match files that were  $\sim$ 50% of the size of the original or greater than 200% of the size of the original for certain cases. In testing, this proved to be troublesome as the file was still very similar to the original, but due to the intolerance of differences in block sizes there would be no match.

This problem would occur a relatively significant amount of the time as the length of a fuzzy hash is between 33 and 64 characters, but the distribution is uniform, meaning that these tail conditions are just as likely to occur as the more generally tolerant conditions of a hash around 48 characters which can be appended to either way and still achieve relatively good matching distances.

During testing, it was observed that the match scores reported back were generally linearly dropping to a range around 40-50 and then suddenly falling to zero. After some investigation, it was found that this had to do with the block sizes being incompatible. This generally seemed counterintuitive as a large portion of the file was still a perfect match and one would expect the match score to drop to a number where an examiner would probably need to look at the file to determine whether or not it was actually a derivative of another file.

The solution to this problem was to expand the tolerance of the hashes to block sizes. This was done by keeping a third counter which would set off to create a hash character when the rolling sum modulus four times the original block size was equal to one less than that number.

This could lead to some questioning as to how one could be sure that this modulus would be hit as the odds of a larger block size triggering a hash are considerably worse than those of a smaller block size. The principle that allows this to occur is that bytes can be treated as something close to independent uniform distributions from 0-255. The PDF of a sum of bytes is the convolution of the two uniform distributions, which in statistical terms forms a Gaussian curve about twice the average. Some simple math can show that discrete Gaussian modulus of the block size creates a uniform distribution from 0-(block size-1), meaning that it is simply a negative binomial distribution that repeats itself each time it ends.

Knowing that the statistics approach the expected value the longer they continue, it was safe to assume that in almost all cases (for non trivial files) that the third hash would be between 8 and 16 characters which is enough information to reliably trigger a match. Producing a fourth hash though, would max out at eight characters, and since the algorithm requires more than seven consecutive characters to be the same for any match to be declared, as well as the tendency for the values that are effected in the hash to change with relatively little data added, only an exact match of the file could be found, and only if that file happened to be one of the 20% of files that happened to have an 8 byte hash.

When using this third hash appended to the first two, false error rates were on average halved, but they became much more useful in the sense of appearing more like what you would expect a human to be able to detect. It is easy for the human brain to determine visually that a file is 40% identical to another and he/she will then wonder why the hash fails to accurately classify this homology. Using the old method, files would (on average) fall off when there was a 37.5% match, but now this level of accuracy has been doubled allowing files to match down to an 18.75% match. This level of matching allows for the limitation to become the actual algorithm to match files rather than the generation of hashes.

The tradeoff for this would be an expected increase in processing time as the program is now computing three hashes instead of two. There is a very slight increase in processing time due to the fact that the extra hashes only provide new code when they are invoking their own encoding of data which is one-sixth as often as the original two pieces of the hash are invoked. This combined with the fact that there is only one extra operation per cycle which does not get invoked this allows for the increase in execution time to be only 10%.

**Digital Forensics, Fuzzy Hashing, Error Rate** 

#### B13 Computer Forensic Bitmaps and Visualization for Data Identification

Shane A. Macaulay\*, Security Objectives Corporation, 1100 Dexter Avenue North, Suite 100, Seattle, WA 33487

After attending this presentation, attendees will understand some of the principles of visualization techniques which can be applied to data identification.

This presentation will impact the forensic science community by discussing the components used to generate bitmaps from opaque data regions. Attendees will be presented visualized examples from several public data sources and understand how these can be applied toward forensic methods and tools.

These images are generated fundamentally by using variable block hashing combined with advanced search techniques (akin to an overlay network of a distributed hash table) and guaranteed binary clone detection.

Using established cryptographic algorithms, or "digital fingerprints", with scaled variable size, applied to an opaque data region, illustrates a domain of knowledge when extrapolated against what is currently known in an existing database. Database seed material would typically be data from sources such as National Institute of Standards and Technologies (NIST) National Software Reference Library (NSRL), or other similar collections.

Bitmap generation applications can be used for coverage analysis, isolation of unknown or new artifacts and also data recovery. Data

interpretation, comprehension, recovery, and analysis of many forms may not need to be visualized but do benefit from bitmaps without coloring for determining probability or even partial matches.

Results of some recent colorized (visual) bitmaps are of a gigapixel class. Measuring the overall process of generation in some tens of minutes is an encouraging sign for future terapixel scale images. **Forensic Visualization, Variable Block Hashing, Data Search** 

#### B14 Facial Comparison Using 3D Techniques

Arnout C. Ruifrok, PhD\*, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS; Chris van Dam, MSc, and Robin T.A. van Rootseler, MSc, University of Twente, Hallenweg 15, Enschede, 7522 NH, NETHERLANDS

After attending this presentation, attendees will understand the possibilities and limitations of the current biometric facial comparison techniques, facial comparison techniques using uncalibrated surveillance video footage, and some basic principles of 3D multi-frame approaches to facial comparison.

This presentation will impact the forensic science community by providing insight into current and new techniques for facial comparison and on how to optimize the use of image information in surveillance video for person identification based on facial images.

Although biometric techniques exist for facial comparison using image material, these techniques only show acceptable performance when using full frontal images of good quality in a controlled environment. As soon as the quality of the image material is gets below ISO 19794 requirements, the performance of the current biometric systems quickly deteriorates. As it is extremely rare that surveillance video footage material complies with the ISO 19794 requirements, automated biometric systems in practice are useless for forensic casework.

Current forensic facial comparison techniques using un-calibrated CCTV footage are based on visual comparison by human operators using 2D images. Forensic experts take only one or a few frames from a video on which they base a facial comparison with images or reconstructed video footage of a suspect. During this process, potentially valuable information from unused sub-optimal frames is discarded.

To improve the use of the available image information and to develop techniques to determine the evidential value of the surveillance video, the Netherlands Forensic Institute started a collaborative project with the University of Twente, The Netherlands, titled "Person Verification 3D."

Using a multi-frame approach, more of the available information can be used. In contrast to 2D image comparison, 3D model comparison is more reliable, because it doesn't suffer from changes in lighting conditions and/or pose. It also offers the possibility to combine the information in the multiple frames.

The main three techniques to reconstruct 3D facial models from 2D video footage are Structure from Motion, Shape from Silhouettes, and the use of Morphable Models. Each of these techniques has its own advantages and disadvantages. Shape from Silhouettes is robust against pose and lighting conditions resulting in easy and quick binary comparison for fitting but a PCA shape model is needed for accurate reconstruction. Structure from Motion is robust against lighting conditions but results in a relative sparse model. Morphable Models generate a dense 3D model based on shape and texture but are computationally expensive.

In evaluating existing techniques for building 3D face models from uncalibrated multi-frame video material this collaborative project aims to combine the complementary techniques to: determine the within and between variability of reconstructed 3D models; determine the influence of frame rates, resolution and amount of movement of a person on the accuracy of a 3D model; compare the constructed 3D models with 2D and 3D reference material; and to quantify the evidential value of the available footage in terms of a Bayesian likelihood framework.

An overview of existing biometric techniques, visual comparison of CCTV footage, the techniques used to construct 3D models, the progress of the project, and initial results using CCTV footage of varying quality will be presented.

Facial Comparison, Surveillance Video, 3D Models

# B15 Removing JPEG Artifacts in Skin Images for Forensic Analysis

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After attending this presentation, attendees will understand a new method which removes blocking artifacts in JPEG-compressed skin images for forensic analysis.

This presentation will impact the forensic science community by providing a useful method to remove blocking artifacts in JPEGcompressed skin images. With this technique, biometric traits in evidence images can be used for criminal and victim identification reliably.

Recent technological advances have allowed for a proliferation of digital media. This media can be used as evidence in legal cases and hints for investigation. Increasing capability of processing this media for criminal and victim identification is becoming an important task. In some cases (e.g., child pornography and masked gunman), faces of criminals or victims cannot be seen, because they are covered or obstructed. Biometric traits on/in the skin (e.g., skin marks and veins) become important features for identification. Dr. Craft and Dr. Kong were recruited by the United States Department of Justice as expert witnesses for a legal case, United States v. Michael Joseph Pepe (2008), which involved sexual acts with seven pre-teen girls in Cambodia. Dr. Craft was required to identify skin marks in digital images (evidence images) collected from a crime scene and skin marks of the suspect, Mr. Pepe, for verification, because the face of the criminal in the evidence images could not be observed. Unfortunately, Dr. Craft's expert opinion was challenged, partially because of blocking artifacts in the evidence images.

Using biometric traits in/on the skin for criminal and victim identification highly depends on quality of evidence images because of the size of these traits. Evidence images, taken by consumer cameras, are always compressed by the JPEG algorithm. Blocking artifacts are a well known problem caused by this algorithm. As a result, vein patterns can be broken and skin marks can be blurred, or even totally removed, especially under high compression ratios. Although many methods have been proposed to remove blocking artifacts, none are developed specifically for skin images. In fact, they make the situation even worse, because they generally smooth images, including the traits, to alleviate blocking artifacts.

This presentation introduces a new algorithm to remove blocking artifacts in skin images. This algorithm formulates skin image deblocking as an estimation problem and embeds statistical information of skin images into a maximum a posteriori (MAP) model to perform the estimation. A statistical analysis is carried out on a skin image database to obtain a cumulative distribution function (CDF) of difference between
two neighboring pixel values. Because intensity values usually do not change abruptly in a skin image, the CDF of the database and that of a testing image are quite similar. However, the JPEG algorithm removes high frequency information and changes the original CDF. This phenomenon is more serious in the U and V components, because of the down-sampling process and because of the even larger quantization parameters in the JPEG algorithm. The proposed algorithm uses the CDF of the database as a target to modify a compressed image. A potential function is designed based on the difference between the CDF of the database and that of the compressed image. In this way it connects the MAP model and the statistical information from the skin image database. The gradient descent method is utilized to minimize the potential function.

The proposed algorithm has two novel characteristics: first, it exploits the prior knowledge of skin images extensively; and, secondly, it guarantees that the resultant and compressed images have the same quantized Discrete Cosine Transform (DCT) coefficients, which cannot be achieved by most other methods. The performance of the algorithm was evaluated on 762 skin images with different compression ratios, and compared with four other deblocking methods. Both subjective and objective evaluations demonstrated that the results from the proposed algorithm were more close to the original images. It not only removed blocking artifacts, but also recovered skin features. The out-performance was more obvious when the compression ratio increased. From the resultant images, vein patterns and skin marks can be extracted efficiently for forensic analysis.

Child Pornography, Vein Pattern, Skin Mark

## B16 Conversion of AVI "txts" Stream Data to Adobe® Premiere® Pro Title Files

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After attending this presentation, attendees will understand how to identify and extract text or caption data embedded in an Audio Video Interleave (AVI) multimedia container format file. Once extracted, this information can then be converted into Adobe® Premiere® Pro title files, which can then be used in conjunction with the imported video/audio information.

This presentation will impact the forensic science community by allowing video examiners to more comprehensively review a video/audio recording contained in an AVI file. The embedded text information, which may be overlooked, can prove to be vitally important in establishing an accurate timeline of events.

AVI files may contain video, audio, and/or text data, multiplexed and indexed as separate data streams ("vids," "auds," and "txts," respectively) within a single container file. Multimedia software such as Windows Media Player allows for playback of both the recorded video and audio information and, through an optional setting, enables the user to display the embedded text or caption data, if present. Typically, this text data is displayed by the software outside of the video playback area, and not as an overlay of the recorded visual information.

With AVI files produced by digital video recorders (DVRs), embedded text information typically consists of corresponding date and time information for the captured images/audio, an assigned name for the particular camera view, and/or other identifying information. Date and time information may also be "burned" into the visual data, but the embedded information sometimes provides additional detail. For example, a video frame may have a "burned" time of "09:55:44," and an embedded time of "09:55:44.197," thereby providing timing information out to the millisecond. Unfortunately, when AVI files with embedded text data are imported into non-linear video editing software such as Adobe® Premiere® Pro for more detailed review, the embedded text information is no longer accessible and is unable to be displayed.

Through detailed analysis of an AVI file's structure and index, the locations of the embedded "txts" stream data entries can be determined, and accordingly, they can be extracted into a separate data file. By creation of a template Adobe® Premiere® Pro title file, separate title screens can then be produced for each extracted text entry. These title screens can then be imported into Adobe® Premiere® Pro and overlaid on their respective video frames, allowing for a more detailed analysis of the recording. Batch processes can be used to expedite the location, extraction, and conversion steps.

Two case examples in which they produced automated scripts for the location, extraction, and conversion of embedded "txts" stream data into Adobe® Premiere® Pro title files will be presented. These title files were then used to establish accurate timelines of the depicted events, to find discontinuities in the original recording processes, and to provide additional information which was not readily apparent from analysis of only the video and audio data.

While this presentation concentrates on the use of Adobe® Premiere® Pro, the methodology and steps which will be discussed may be applicable to other non-linear video editing programs, which feature title screens or a similar ability to overlay text information on a video file. **Digital Video, Embedded Text, Metadata** 

#### B17 Recovering Multimedia From File Fragments

Michael Piper, BA\*, United States Secret Service, 950 H Street, Northwest, Suite 4200 - FSD, Washington, DC 20223

After attending this presentation, attendees will gain an understanding of how multimedia files, commonly used in cell phones, are structured and how that structure can be exploited to recover the audio and video contents of fragmented files.

This presentation will impact the forensic science community by bridging the gap between the computer forensics and multimedia forensics disciplines by demonstrating a methodology for recovering the multimedia payload within damaged or partially recovered files.

Cell phone forensic examinations are performed everyday to recover existing and deleted data. Reviewing file fragments can be problematic depending on the type of data contained within the file and the scheme used to encode it. ASCII text characters have a direct representation in digital data and can be interpreted easily. Multimedia information (audio and video) is more complex. Audio and video encoders exploit human perception to reduce data redundancy. This results in algorithms that are highly complex and have many variable options. Knowing the state of these variables can distinguish streaming multimedia from gibberish.

In this case study, fragments of two deleted files recovered from separate cell phones will be examined – a 3GP file and an MP4 file. In each case, an exemplar file was provided from the phone. Attempts to play these fragments directly were not successful, but suggested that information was present. Successful decoding of the multimedia payload required understanding the file specifications of the data involved, exploiting the exemplar files to form assumptions that reduced the unknown variables, and exploiting existing metadata to calculate the missing metadata. Once sufficient metadata was reconstructed, a standard multimedia viewer could be used to play the recordings.

Multimedia, File Fragments, Recovery

#### B18 Quantifying Phase Changes in Audio Authenticity Examinations

David J. Snyder, BS\*, William L. Hinton, BS\*, Andrew Galotti, BS\*, and Kenneth Marr, MS\*, Federal Bureau of Investigation, Engineering Research Facility, Building 27958A, Quantico, VA 22135

The goal of this presentation is to present testing results of manual and automatic methods for detecting phase changes in forensic audio recordings when conducting audio authenticity examinations.

This presentation will impact the digital and multimedia forensic science community by defining the limitations of using phase change as an analysis tool in audio authenticity examinations. Phase changes by themselves may not provide insight as to whether an event is an alteration or not, but shows promise as a method to correlate multiple events. If the accuracy of detecting phase changes is unreliable, this will also impact the value of it being used as potential method for forensic audio authentication.

**Hypothesis:** To determine whether a phase change in a forensic audio recording can accurately be identified as an alteration or edit and develop criteria or parameters classifying the event as such.

**Synopsis:** A series of test recordings that include various digital and analog formats, which are both altered and unaltered with discrete reference tones recorded at various amplitudes will be used to test manual and automatic methods for a change in phase. Testing results provide answers to the question: Is a phase change in an audio file synonymous with alterations?

The use of changes in phase is not a new analysis method for forensic audio authenticity examinations. Interest in expanding its role to detecting edits in digital recordings has increased in the last several years. Several concerns accompany this increased interest. Automatic detection of phase shifts can help speed a cumbersome time-consuming process of manually locating events, but accuracy and the thresholds of such detection methods are not widely known. Even if events are detected accurately there is not any specific criteria to determine whether the phase shift is the result of an alteration or naturally occurring event during the time of recording.

Test recordings will be produced to include reference tones at various amplitudes to determine if changing the amplitude of the reference tone will directly impact the accuracy of automatic phase detection systems. The test recordings will also contain a range of naturally occurring events and files with various alterations. The use of several common digital formats that represent the type of audio being received for examination and the method in which they are recorded may show that some formats may or may not maintain phase. An automatic detection system may falsely identify phase changes that may be inherent to the recording process, format, or recording environment and not necessarily the result of an edit or alteration. It may also identify phase changes where they do not exist or not identify them at all. Being able to accurately account for the number of phase changes detected or not detected is important.

The second part of this presentation will attempt to clarify what a phase change means to the authenticity of an audio recording. Even if the detection of phase changes is accurate, what correlation does a phase change have to an alteration or edit? Examination of known events may help correlate phase changes caused by edits or naturally occurring ones. Criteria for establishing which phase changes are the result of an alteration and which ones are not is lacking. This problem is similar to identifying events as pause or voice-activated for analog recorders. Both of these events are caused by stopping the transport of the recorder without disengaging the record and erase heads. A pause event is generally associated with a potential alteration, where as a voiceactivated event is generally not. Often the events are identified through testing the operation of the analog recorder, these methods do not translate to digital recorders. **Statement:** Phase changes by themselves may not provide insight as to whether an event is an alteration or not, but shows promise as a method to correlate multiple events. If the accuracy of detecting phase changes is unreliable, this will also impact the value of it being used as potential method for forensic audio authentication.

Audio, Authenticity, Data Analysis

#### B19 Camera Identification in Large Databases of Images

Zeno J. Geradts, PhD\*, Netherlands Forensic Institute, Ministry of Justice, Laan van Ypenburg 6, Den Haag, SH 2497 GB, NETHERLANDS; and Wiger Van Houten, MS, Netherlands Forensic Institute, Laan van Ypenburg 6, Den haag, NETHERLANDS

After attending this presentation, attendees will be informed on different methods for camera identification, ranging from pixel defects to PRNU in digital cameras. Limitations and posibilities are discussed.

This presentation will impact the forensic science community by demonstrating the possibilities for extracting evidence from larger databases of images, such as child pornography and allow examiners to link cases to each other.

A digital camera consists of many electronic components. After the image has been formed on the image sensor, the image information will pass through all of the components before the final data file is written to flash memory. Each step in this process may add random noise to the image. Even during the image formation process itself, a noise-like pattern from the sensor may be introduced in the image. This noise-like pattern is a small but measurable systematic contribution to the signal, and is called the Photo Response Non Uniformity (PRNU) pattern. The visibility of this signal is limited and may be a small difference depending on the intensity of the signal. In practice, this means that well illuminated images will result in a better extraction of this signal compared to when the image is dark. Extraction of these patterns is done with complex filters, such as wavelet filtering.

The PRNU pattern itself can be determined from the image and it preferably is done with images with no discernible textures (flat field image, for example from a grey surface). In the past, the influence of strong compression was examined, and it appeared as though it was still possible to extract the PRNU pattern; however, it initially turned out to be more complicated than once thought. The examining of the PRNU pattern for forensic use is well researched by Jessica Fridrich and others.

There is a standard working procedure for the examination of PRNU in casework. The examiner will compare the retrieved pattern with one or more images. It will also be determined if the pattern is specific for the sensor (i.e., device characteristic) and determine the influence of possible class characteristic signals in this signal (i.e., brand or model characteristic). For this reason, at least three, and preferably ten cameras of the same make and model were used to validate the method for PRNU comparison.

In practice, it is not always possible to have the camera for casework; however, it is possible to determine if a set of images have been made with the same camera or different cameras based on the PRNU pattern. By comparing the pattern from a questioned image with the pattern from a set of reference images made with a suspect camera, it can be determined whether the questioned image was produced with the suspect camera or not. This works when the image is authentic, but fails when the image underwent any spatial transformations (e.g., rotation, shearing, resizing) because the "fingerprint" is desynchronized, unless the same transformations are applied to the reference material. It is also possible to alter the image such that the PRNU pattern is filtered out, although this is complicated and time consuming.

Other techniques for camera identification also exist, mainly based on statistical features. However, these approaches often involve time consuming feature extraction and SVM training and only work on the classification level.

With this research, the influence of aging of cameras was also examined. This research also showed that matching based on a PRNU pattern within a two-year time frame for different models and brands of cameras was still successful. Also heating the cameras to 50°C, and freezing to -20°C, did not significantly alter the PRNU pattern within the tested cameras.

The use of large image databases, such as child pornography and other databases of relevant images will require significant processing power. For these, it is important to have a better pre-selection, based on other information, such as quantization tables of images which can be extracted instantly. The results of the examination based on images that were downloaded from online photo websites are demonstrated, and groups of cameras between the different images are found. For a good comparison, it is important to have the most original images, or to know exactly what kind of operations have been conducted on the image. Since in casework the ground truth may not be known, it is important to draw conclusions based on a Bayesian framework.

PRNU, Camera Identification, Image Comparison

#### B20 Macintosh® Forensics: A Crash Course

Gavin W. Manes, PhD\*, Avansic: E-Discovery and Digital Forensics, 401 South Boston Avenue, Suite 1701, Tulsa, OK 74103

The goals of this presentation are to discuss: (1) data preservation and forensic collection from a Macintosh<sup>®</sup> system; (2) forensics investigation of a Macintosh<sup>®</sup> system; (3) differences between Windows<sup>TM</sup> and Macintosh<sup>®</sup> systems related to forensic collection and investigation; and, (4) additional challenges of the Macintosh<sup>®</sup> based on file system structure, file formats, and caches.

This presentation will impact the forensic science community by providing the basic tools and techniques to forensically acquire Macintosh<sup>®</sup> computers. Participants will also be aware of the complexities and difficulties of Mac computers vs. Windows<sup>TM</sup>.

Most investigators spend their time an energy learning and keeping up with the latest tools and techniques for Windows<sup>™</sup> investigators. However, the steadily increasing market share of Mac OSX means that most investigators will find themselves in a position to examine these systems. This presentation will give forensic investigator with little knowledge of the Mac OSX platform the basic knowledge necessary to acquire and analyze one of these systems.

The first step in any digital forensics investigation is to imaging evidence drives. However, Apple<sup>®</sup> has made it very difficult to work with hardware components, meaning that this seemingly simple task can become a complicated operation. A brief overview will be given of how to "crack the cases" for some of these machines and retrieve hard drives for imaging. Alternative means of acquiring images will be discussed, particularly when hardware, time, or other circumstances require that clever techniques be employed. Solutions presented will include the well-known "Firewire Target Mode" workaround, as well as instructions in using common Live CDs for imaging Apple<sup>®</sup> computers.

Once the basics of acquisition have been covered, A basic overview of the of the Mac OSX file system will be provided. The basic challenges to investigators will be described, the tools used to address those challenges, and the differences in the ways that each of those tools approaches the problems. Interpretation of HFS+ MAC times and unusual file types that have significant evidentiary value will be discussed. Other important evidence caches include information related to such user activity as web browsing, file usage, iPod and iPhone usage.

Working with Macintosh<sup>®</sup> computers is an increasingly necessary tool in the forensic investigator's arsenal. But the Macinstosh operating system has proved a very unique beast with its own set of challenges, requiring a body of specialized knowledge to tackle it effectively. The goal of this presentation is to familiarize the investigator with many of these basic problems and arm them with the some of the tools needed to level the playing field.

**Digital Forensics, Data Preservation, Investigation** 

#### B21 Macintosh<sup>®</sup> PList Files: Hidden Information for Digital Forensics Investigators

Gavin W. Manes, PhD\*, Avansic - Digital Forensics, 401 South Boston Avenue, Suite 1701, Tulsa, OK 74103

The goals of this presentation are to: (1) educate the audience about forensically relevant information contained within Macintosh<sup>®</sup> Plist files; (2) discuss the basic structure, functions, and location of Plist files; and (3) provide the audience with tools and information to appropriately interpret Plist files (particularly those who have digital forensics experience).

This presentation will impact the forensic science community by arming attendees with techniques and information in order to retrieve forensically relevant information from Macintosh<sup>®</sup> Plist files.

Although extremely common in system running Mac OSX and devices such as the Apple<sup>®</sup> iPhone and iPad, the structures of Apple<sup>®</sup> plist files remain mysterious to many forensic investigators. This creates an obstacle for many investigators tackling OSX systems, since plist files contain valuable information relevant to forensic investigations. Further complicating the issue, graphical interfaces for handling with these files are confusing and primitive at best.

An in-depth overview of the Apple<sup>®</sup> plist format in all of its incarnations, including the "binary," "XML," and "ASCII," or "oldfashioned" formats will be given. Furthermore, information will be provided to help decode other formats an investigator may consider "unusual" that are sometimes seen stored in plist files relevant to forensic investigations. These include formats such as the "alias records," which can be found storing potentially useful information about files that may exist in different locations across a system.

Finally, tips to efficiently process and analyze these files when encountered in the field using several readily available tools will be provided. The structure of commonly examined plist files will be discussed, along with important practical examples for handling some of the more complex structures.

This will give investigators inexperienced with this aspect of Mac OSX investigations a deep and informative look at what challenges might be normally encounter in the field.

**Digital Forensics, Investigation, Computers** 



## **ENGINEERING SCIENCES**



#### C1 Understanding and Applying "Daubert's Error Rate" - Part One

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After attending this presentation, attendees will learn one important form that an error rate can take and how to develop an uncertainty interval, derived from the Chebychev inequality, which can serve as an error rate for a measurement process. This presentation will impact the forensic science community by providing attendees a better position to qualify or cross-examine an expert with respect to this area.

Since the *Frye* decision was superseded by Rule 702 of the Federal Rules of Evidence, as interpreted by the *Daubert, General Electric*, and *Kuhmo Tire* decisions (The *Daubert* Trilogy), it is no longer enough to show that basis for the admission of expert evidence in a judicial proceeding is that it is generally accepted in a relevant field of study. These Federal Rules of Evidence require that the evidence itself be reliable. In 2009, the National Academy of Sciences issued a report, entitled *Strengthening Forensic Science in the United States: A Path Forward* (the NAS Report). It looked at the state of the art of forensic science and found that, in many respects, forensic science disciplines were wanting.

One of the criteria that the court set forth in the *Daubert* Trilogy and discussed in the NAS Report is the (known or potential) error rate of the hypotheses or techniques being proffered. The concept of error rate causes consternation and confusion to both forensic practitioners and to the legal community. This presentation will impact the forensic science community by abating some of that confusion by: (a) defining in practical terms what an error rate is or can be; and (b) offering guidance to practitioners how to develop an error rate for techniques that they utilize. Thus, the impact to will be for practitioners to improve their own practice of forensic science and, by instilling this knowledge in the legal community, members of which can then ask appropriate questions, to make sure that forensic science practitioners do indeed treat error rate in a meaningful manner.

The trial judge that must act as a "gatekeeper" to determine admissibility of expert testimony to the trier of fact on the basis of both: (a) the ability to assist the jury; and, (b) whether or not the information the expert intends to offer is indeed "scientific knowledge." *Daubert* sets forth a non-inclusive list of criteria to be considered, among them, whether there is a "known or potential rate of error", a concept from metrology: the science of measurement.

*Error rate* is an appropriate term when one deals with, for example, a dichotomous classification, e.g., whether or not there is or is not a match between a suspect and a piece of evidence.<sup>1</sup> Unfortunately, "error rate" is problematic when it comes to *measuring* things of forensic import, because it conflates *error* with *measurement uncertainty*, which is not "error" at all but, rather, a concept in metrology that reflects the fact that measurements are never without at least some variability.<sup>2</sup>

While expert testimony must be grounded in science (for example, mechanisms of friction). Once the science is established then the admissibility of expert testimony revolves around things more quotidian (the actual measuring of the friction between a reference material and a given floor surface). In place of hypothesis testing and its associated errors, there is accuracy, standardization, and calibration issues. One tool that has been used for this purpose is a *Confidence Interval* (CI), which depends upon measures of the central tendency (typically, the Arithmetic Mean) and the dispersion (typically, the Standard Deviation). A CI is expressed as a range of values subject to a level of confidence in that range of values. For example, one can state that a tribometric test sequence determined that the friction between the floor and a reference surface was between 0.37 and 0.47 with 95% probability:  $P(0.37 \le \mu \le 0.46) = 95\%$ . The production of a CI requires the collection of data in a contextually-appropriate manner, the calculation of sample statistics, typically the mean and standard deviation, and from that, a calculation of the CI using straightforward and well-known formulae. CIs for single or small samples require that the underlying probability distribution follow a Normal (Gaussian) Distribution.<sup>3</sup> Unfortunately, many forensically interesting phenomena are not Normally distributed.<sup>4</sup>

Parts one and two of this paper look at alternative ways of developing uncertainty estimates:

- 1. The Chebychev inequality (discussed in this part of the paper).
- 2. The sign-test derived confidence interval on the median (discussed in part 2).
- 3. The uncertainty interval based upon the cumulative function (discussed in part 2).

1-**The** Chebychev Inequality: The Chebychev inequality, which requires no information about the underlying probability distribution other than its mean  $(\mu)$  and a standard deviation (s), states that the probability of being farther than k standard deviations from the population mean is less than  $1/k^2$ :



$$P(|x-\mu| \ge k\sigma) \le \frac{1}{k^2}$$

For example, the probability of being greater than two standard deviations from the mean is less than 25%:

$$P(|x - \mu| \ge 2\sigma) \le \frac{1}{2^2} = \frac{1}{4} = 25\%$$

This result is weak compared to the narrower confidence interval that can be arrived at given information about the underlying probability distribution. If the underlying probability distribution was normal, for example, the probability of being greater than two standard deviations from the mean is about 5%, one-fifth of the Chebychev-determined value. (See the graph above.)

#### **Example 1: The Chebychev Uncertainty Interval**

Col. (1)	Col. (2)	Col. (1)	Col. (2)	Col. (1)	Col. (2)	Col.(1)	Col. (2)
0	0	7	1	14	6	21	0
1	0	8	2	15	3	22	1
2	2	9	4	16	1	23	0
3	0	10	3	17	1	24	0
4	1	11	7	18	1	25	1
5	0	12	11	19	0	26	0
6	0	13	8	20	2	27	0

The following data represents the time, in milliseconds, that it takes a laser rangefinder to stabilize its reading. Column one represents the stabilization time, rounded to the nearest millisecond, that the rangefinder takes to settle; column two represents the number of times that that particular reading is seen out of 55 readings. For example, the device took 2 ms to settle on two occasions and on eight occasions, it took 13 ms to settle. The mean and standard deviation can easily be calculated in Excel to be 12.31 and 4.09 ms respectively.<sup>5</sup>

Thus, 25% of the readings are within  $12.31 \pm 2x4.09 = 12.31 \pm 8.18 = (4.13, 20.49).$ 

If one is working with single samples or very small samples, as is often the case with forensic samples, and if there is historical instrument data from which to build a standard deviation, the Chebychev Inequality will enable one to develop an Uncertainty Interval (UI), albeit a rather wide one.

**Discussion:** An instrument or process, absent an externally imposed rule or standard, cannot "pass" or "fail" an UI or a CI. Rather, and this is important, the utility or lack of utility is situational. A hypothetical floor-friction example illustrates this. In civil litigation, a plaintiff must prove that a floor is "slippery." If the tribometer gave us a 95% CI or UI of the available friction as  $(0.35 \le \mu_a \le 0.43)$  on the true coefficient of friction, i.e., it is 95% certain that the true value of floor friction is between 0.37 and 0.43, that in and of itself cannot determine whether the floor is or is not slippery. There must also be an external reference, a threshold: if the pedestrian-required friction is, say,  $\mu_r$ =0.45, with values at or above 0.45 being slip resistant, and values below that slippery. If the high side of the CI or UI is, say, 0.43, the floor would be proven, within reasonable certainty, to be slippery.

One might argue that a one-sided CI is more appropriate because we are only interested in one side of the error rate CI (or UI). In this frictiontesting example, where the movant wants to prove that the friction is, within reasonable certainty, below slipperiness threshold, one could argue that a one-sided, "high side" CI, better suits the task (if the high side of a 2-sided CI-or a 1-sided, high-side CI-is below the friction threshold, this will prove the slipperiness. Conversely, if the high side of the available-friction CI is above friction required by the pedestrian, then the floor cannot be proven to be slippery). A one-sided, upper CI would give a lower high side, making it easier to show that the floor was slippery. The more conventional two-sided CI is preferable because (a) the two-sided interval gives a larger interval on the side of interest (whether that's the low or the high end), reflecting a larger uncertainty, making it harder for the movant to prove the slipperiness hypothesis, and is thus more conservative and, (b) the uncertainty of most processes is in fact better reflected by a two-sided UI.

#### **References:**

- <sup>1</sup> Dichotomous classifications, like this 'match' example, actually have two error rates: the probability of a false positive (that a match is mistaken) and the probability of a false negative (that a match will be missed). *Daubert* concerns itself with the former.
- <sup>2.</sup> Measurement Uncertainty is the parameter, associated with the result of a measurement (the measurand) that characterizes the dispersion of the values that could reasonably be attributed to that measured value. (Guide to the Expression of Uncertainty in Measurement, ISO, Geneva, 1993.)
- <sup>3.</sup> For the sample mean (the arithmetic average), the Central Limit Theorem states that as the sample size increases, the distribution of that mean rapidly converges to the Normal Distribution. Practically, if the underlying distribution of the values has a unimodal (one-hump) shape, the average of a sample of 10 or more will converge to a normal distribution; Regardless of the distribution's shape, the average of a sample of 30 or more will onverge to a Normally-distributed statistic. Unfortunately, many forensic analyses use single or very small samples, making the Central Limit Theorem inapplicable.

- <sup>4</sup> The distribution of Perception-Reaction Time is log-normal; the distribution of the probability of an indicated slip on a Brungraber Mark II walkway friction tester is binomial, and the CF can be determined using Logistic Regression. ("The Relationship Between the Measured Friction Coefficient and the Safety of a Walkway Surface," 2010 Proceedings of the American Academy of Forensic Sciences, Vol. 16, p. 159-160, Seattle, WA, and part 2 of this paper).
- <sup>5</sup> In Excel, the formulae are =average(range) and =stdev(range) respectively, where range is the selection of the individual values.

Daubert Error Rate, Forensic Science, Uncertainty Interval

C2 Overbilling of Governmental Contracts on Engineering Services of Construction – Case of Black-Box Operation 2009: Brazilian Federal Police

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The goal of this presentation is to demonstrate to attendees how Brazilian expert engineers are using techniques of cost engineering to assist police investigations of fraud in government contracts for engineering services for construction.

This presentation will impact the forensic science community by demonstrating how successful investigations by the Brazilian Federal Police are leading to requests to use forensic engineering cost analysis in other types of criminal probes, including undercover operations, courtordered electronic surveillance, and use of informants. Police investigations of fraud in contracts for airport infrastructure work confirmed that it is possible to identify fraudulent overbilling using forensic engineering cost analysis techniques to study public works budgets. Overpricing and shorting of services actually delivered are identified as a form of corruption in the procurement process.

Authorities responsible for investigating possible corruption in public works projects are the primary users of civil engineering forensic specialties provided by the Brazilian Federal Police. One such investigation, code-named Black Box, looked at twelve airport infrastructure projects, estimated fraud, and other misuse of public funds to total about US \$500,000,000, a huge sum equivalent to about 57.8% of the total projected costs of the projects. Various overbilling schemes were involved. It is estimated that billions of dollars are siphoned from government contracts for construction engineering services each year. In cases of suspected overbilling, forensic engineers must estimate how much public money has been diverted elsewhere. Cost analyses are complex and time-consuming. Over the past ten years, technical standards for use by forensic engineers studying public works fraud cases were written up by the Unit for Forensic Engineering Examinations within SEPEMA, part of the National Institute for Criminalistics (INC). This institute is in reality the central unit of forensic science for the Federal Police. These standards include methodologies for locating different kinds of financial fraud, define nomenclature, and provide ways to link activities to specific criminal organizations. There are multiple forms of overbilling and shorting of services and these must be analyzed with specific techniques. Once identified, this fraud must be linked back to individuals and their criminal schemes.

Overbilling and shorting of services were found to take various forms, identified below:

- a. Billing for materials and services that were not actually provided at the level listed on the invoice.
- b. billing at rates that represented major over-pricing relative to market or to negotiated rates.

- c. Substituting lower quality materials, or providing poor and shoddy services, while invoicing for high quality products and services.
- d. Padding contracts by changing product, quantities, and level of services mid-stream to create substantial overruns.
- e. Manipulation of time schedules, pricing, and contract terms to increase overall contract cost.
- f. Deliberate over-estimation of project cost for materials, equipment, and services at the point of inception, above and beyond cost standards and market rates.

Successful techniques to identify, investigate, and prosecute criminal overbilling and shorting are described within the Brazilian Federal Police experience. This information may be useful to other nations and jurisdictions coping with the same problems and the same huge misuse of scarce resources.

Engineering, Overbilling, Corruption

# C3 Environmental Forensics: A Repository for Junk Science

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After attending this presentation, attendees will be able to identify the indicators of junk science in environmental forensic science cases.

This presentation will impact the forensic science community in learning how to deal with junk science in environmental forensic litigation.

The inconvenient truth about environmental forensics is that the U.S. Supreme Court rulings of Daubert,<sup>1</sup> Joiner,<sup>2</sup> and Khumho Tire<sup>3</sup> backfired. The groundwork laid in these cases was intended to provide a series of tests for a judge, as the "gatekeeper", to discern and allow appropriate science to be heard by the trier of fact, and to keep "junk science" out of the courtroom. Instead, it has led to the promotion of junk science. The U.S. Supreme Court's rulings are being used to advance the specific junk science needed to aid the "expert's" client and not the good science needed to inform the trier of fact. This so-called expert publishes the pertinent method and case studies in a peerreviewed journal, which immediately addresses and satisfies Daubert's peer-reviewed test and implies complete acceptance by the scientific community.

This is wrong because: (1) they are purportedly publishing a scientific method (one they know little about in many instances) when in fact, they are using the article as a vehicle to solidify or validate their position in a particular litigation case; (2) the "science" and its application that they espouse is seriously flawed and not worthy of publication; and, (3) publication of information from an ongoing litigation matter is professionally and ethically wrong and potentially prejudicial when offered to the court as evidence of the validity of their approach. These published papers have several attributes in common that should caution the reader and a judge about the objectivity of the interpretation of the environmental forensics used to form the so-called expert's conclusions

The author or authors have little or no formal training concerning some of the disciplines in the article or case. For instance, in *Cornell-Dubilier Electronics, Inc. vs. Home Insurance Company*,<sup>4</sup> in which the scientific disciplines were chemistry and hydrogeology, an expert was accepted by the court as an environmental scientist even though this "expert" testified that he had no expertise in either chemistry or hydrogeology.

A case study is used that is in litigation. The litigants are not given but the data maps and case description are included in the article. The fact that the author's interpretation of the data has been accepted for publication has made it at least ironclad, most likely gold-plated, for use in the trial. The publication will contain numerous general references. For instance, a ten-page article might have 50 references, which gives the article the illusion that the article is of a very scholarly work.

The interpretation of the data in the article is rarely accompanied by any actual data. There are tables of general or average results, with little, if any, backup sampling and analytical information.

Age-dating releases are a favorite topic in these publications. In each article there are many variables given, which are basically "fudge factors," used to determine the date of a release of a substance. A favorite tactic is the use of numerous methodologies that provide the same result. These methodologies are accepted as good science, with lots of general references and very little data and quality control. Numerous avenues filled with fog give the impression of a clear and definitive picture.

The development of the *Daubert* hearing for experts has the inconvenient consequence of promoting peer-reviewed junk science publications. What should have created rigorous hurdles that good science could easily clear has, instead, become a pole vault. No wonder the "gate keepers" are in Never Never Land.

**References:** 

- <sup>1.</sup> Daubert v. Merrell Dow Pharmaceuticals, 509 U.S. 579 (1993).
- <sup>2.</sup> General Electric Co. v. Joiner. 522 U.S. 136 (1997).
- <sup>3.</sup> Kumho Tire Co. v. Carmichael, 526 U.S. 137 (1999).
- <sup>4</sup> Home Insurance Comapany v. Cornell-Dubilier Electronics, Inc. Superior Court of New Jersey Law Division: Mercer County, Civil Action No. MER-L-5192-96.

**Environmental Forensics, Junk Science, Age-Dating** 

## C4 Understanding and Applying "Daubert's Error Rate" - Part Two

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After attending this presentation, attendees will be in a better position to qualify or cross-examine an expert with respect to this area. Error Rate is used as an indicator of the reliability of a scientifichypothesis, technical test, procedure, or process, and is often required for admissibility of expert testimony. Technical attendees will learn two forms that an error rate can take and how to develop uncertainty intervals, derived from the Cumulative Function and from a confidence interval derived from a sign test on the median, which can serve to characterize the error rate for a measurement process.

This presentation will impact the forensic science community by providing practitioners with the resources to improve their own practice of forensic science and, for the legal community, to give insight into how an uncertainty interval is developed.

Rule 702 of the Federal Rules of Evidence require that the evidence in a forensic proceeding be reliable. One of the criteria that the court set forth in interpreting Rule 702 is the error rate of techniques being proffered. This paper will impact the forensic science community by offering two methods of determining an error rate.

One classic metric of measurement uncertainty is the confidence interval on the mean (the arithmetic average), a function of that mean and the sample standard deviation, which connects the sample and the population using a *Student's-t* probability distribution.

This paper (and Part 1) together, looks at alternative ways of developing uncertainty estimates, which vary in their simplicity and their efficiency:

- 1. The Chebychev inequality (discussed in part 1);
- 2. The sign-test derived confidence interval on the median (discussed in this paper).

3. The uncertainty interval based upon the cumulative function (discussed in this paper).

**Example 2: A confidence interval based upon the sign test on the median:** This method requires only that the samples be independently drawn from the same continuous distribution; it makes no assumption concerning the form of that distribution. The sample values are placed in order from smallest to largest. For the laser-rangefinder-settling-time data used in example 1, rearranged in ordinal, rather than tabular form, the results obtained are:

Without going into theory, the confidence interval rests upon the assumption that the median lies either (a) before the first point (4) or after the last point (25), (b) between the first and second data points (4 & 7) or between the next-to-last and last data points (22 & 25), (c) between the second and third data points (7 & 8) or the third-from-last and-second from last data points (20 & 22) or (d) between the third and fourth

The widest possible confidence interval, (4, 25) fails to capture the median only if the true median is less than four or greater than 25; the next widest confidence interval (7, 22) fails to capture the true median only if it is not within the data range (except for the end points). The probability calculation turns put to be binomial with  $p(success) = \frac{1}{2}$ . Thus, the confidence level for a confidence interval that extends k points from the extreme values of the data set is:

1 -  $(2 \ge b(k; n, \frac{1}{2}))$ , where

 $b(k; n, \frac{1}{2})$  is the binomial distribution probability of k successes in n trials with a probability of success at each trial of  $\frac{1}{2}$ .<sup>1</sup> The upper limit results only show:

Upper	Distance	from	Confidence
Limit	(either) end		Level
25	0		100.0%
22	1		100.0%
20	2		100.0%
20	3		100.0%
18	4		100.0%
17	5		100.0%
16	6		100.0%
15	7		100.0%
15	8		100.0%
15	9		100.0%
14	10		100.0%
14	11		100.0%
14	12		100.0%
14	13		100.0%
14	14		100.0%
14	15		99.9%
13	16		99.8%
13	17		99.6%
13	18		99.2%
13	19		98.4%
13	20		97.2%
13	21		95.3%
13	22		92.8%
13	23		89.6%
12	24		86.2%
12	25		82.9%
12	26		80.2%
Median	27		

**Discussion of the sign-test-based confidence Interval:** One thing is clear: one cannot in general specify a specific confidence level, e.g., 95%, because the confidence interval is based upon discrete data points. (Here, you can get *near* that 95% value: The 95.3% confidence interval is between 13 and 21 milliseconds; the 92.8% confidence interval is

between 13 and 22 milliseconds.) This discreteness is a function of the discrete nature of the underlying (binomial) distribution, which has non-zero probability only at the integers. That said, (a) this confidence interval is exact and not subject to any limitations on the underlying distribution; and, (b) only tradition lies behind using 95% confidence limits, or any other specific confidence level.

#### Example 3a: An Uncertainty Interval From Raw Data

Col.	Col.	Cal	(2)	Cal (4)
(1)	(4)	0	(0)	0.00
0		U	0	0.00
1		U	U	0.00
2		2	2	0.04
3		0	2	0.04
4		1	3	0.05
5		0	3	0.05
6		0	3	0.05
7		1	4	0.07
8		2	6	0.11
9		4	10	0.18
10		3	13	0.24
11		7	20	0.36
12		11	31	0.56
13		8	39	0.71
14		6	45	0.82
15		3	48	0.87
18		1	49	0.89
17		1	50	0.91
18		1	51	0.93
19		n	51	0.93
20		2	53	0.96
21		ñ	53	0.96
22		ĭ	54	0.98
23		'n	54	0.00
24		n	54	0.00
25		1	55	1.00

The data here relates to the same 55 observations<sup>2</sup> of the time (in milliseconds) to stabilize a laser rangefinder. The first column is the millisecond value; the second column is the number of readings (of the 55) in each millisecond "bin": the third column is the cumulative number of occurrences, and fourth column is the cumulative frequency. More specifically, Col. (1) represents time in milliseconds, and is graphed on the x-axis. The x-values, here, represent how many milliseconds it takes to stabilize the rangefinder, rounded to the nearest second. The values in column one are from a continuous distribution, e.g., a value of four seconds is recorded for any actual value:  $3.5 \le t \le 4.5$  (the shaded row is an example within an example, with x = 4.) Col. (2) represents the number of occurrences: How many observations, out of the 55, does the settling time take on the value x, e.g., for x = four seconds, one value out of the 55 values is recorded. Col. (3) represents the cumulative number of occurrences: How many times in the experiment was a value of x or *fewer* seconds recorded. On the x = 4 line, the four-or-fewer seconds occurred three times (none for 0 seconds, none for one second, twice for two seconds, none for three seconds, and once for four seconds. The shaded portion of col. (2) contains these values.) Col. (4) represents the Cumulative frequency: and is graphed on the y-axis. Col. 4 is but Col. 3 divided by 55, the sample size, expressing the cumulative results in column three, as a decimal. For the shaded row, representing four or

fewer seconds (actually a time of 4.5 seconds or less), there are three or fewer occurrences, giving a cumulative frequency of 5%.

Graphing the first column as the independent variable on the x-axis and the fourth column as the dependent variable on the y-axis, and drawing lines horizontally from the 5% and 95% ordinate values to the CF, and then down to the x-axis, the 90% CI is seen for the stabilization time for the laser rangefinder ranges from 4 to 19.6 milliseconds. (This can, of course, also be seen on the chart, just above.)



**Example 3b: The confidence interval from a regressiondeveloped cumulative function:** The following diagram illustrates the uncertainty interval from the cumulative function by depicting two logistic regressions, developed from real data collected using a novel tribometric device:



Here, two datasets are compared by comparing the two curves, again drawing horizontals at 5% and 95% probabilities. Where each of the two horizontal lines cross the CFs, verticals have been drawn down to the x-axis. For the leftmost, shallower-sloping curve, the P(slip) = 5% is 2.25° away from the 5.4°, the median value. The P(Slip) = 95% is 2.38° from the median. Thus, the 90% CI can be expressed as (2.15°, 7.78°). Similarly, the rightmost logistics regression curve yields the CI:  $8.5^{\circ}\pm 0.7^{\circ}$ . Obviously the rightmost curve represents an over three times more accurate instrument-and-process than the leftmost curve.

**Discussion of the Cumulative Funtion methodology:** Given the analysis, above, it appears that measurement uncertainty-the metrological equivalent of "error rate" in categorical classification-can be reasonably characterized by the methodology above. Moreover, by drawing the horizontals across at different ordinal values, we can obtain other CIs. For example, drawing horizontals at 25% and 75%, we obtain a 50% CI; by drawing across at  $2\frac{1}{2}$ % and  $97\frac{1}{2}$ %, we obtain the commonly-used 95% CI.

This is *not* a a true confidence interval, meeting the definition:  $P(I \in \text{parameter} \in I) = (1 - \alpha)$ , where

$$P(\underline{L} \leq parameter \leq L) = (1 - \alpha)$$

 $\underline{L}$  and  $\underline{L}$  are lower and upper bounds, respectively, and  $(1-\alpha)$  is the level of confidence, i.e., the probability that the *true parameter* lies between the lower and upper limits. There is no 'true parameter' coming into the analysis. This is not really a legal handicap, as none of the error rate

explications require a confidence interval. Rather, what is described here is an uncertainty interval.

**Discussion—General:** The techniques developed in this paper provide tools to meet the error-rate criterion of *Daubert*.

An appropriate experimental design under which data is collected is essential to prevent the parameter under analysis from being confounded by some unanticipated factor. Given an appropriate experimental design, more data provides more reliable results than less.

The methodologies described above should allow forensic practitioners to estimate their own processes' or tests' uncertainty *via* calculation of the error rate expressed as a CI or UI. This is one valuable component of meeting the requirements of Federal Rule of Evidence 702 as explicated by the *Daubert* Trilogy. Accomplishing a data-specific error rate will make your tests and analyses better able to withstand scrutiny. Familiarity with the general principles described above will assist those in the legal community in proffering competent (or rejecting problematic) technical evidence.

#### **References:**

2

The formula for calculating the binomial probability of x successes in n trials with  $p(success) = \frac{1}{2}$  is =binomdist(x, n,  $\frac{1}{2}$ ) The mathematical formula is:

1.

$$b(x,n,\frac{1}{2}) = \frac{n!}{x!(n-x)!} (\frac{1}{2})^x (\frac{1}{2})^{(n-x)!}$$

.While the appropriate sample size always depends upon the characteristics of that which is being characterized, a sample size of 55, is in our opinion, on the small side of reasonable for developing *Daubert* –purposed Confidence Intervals.

Daubert Error Rate, Forensic Science, Uncertainty Interval

#### C5 Forensic Examination of an Unwanted Seat Belt Release in a Rollover Collision With Occupant Ejection

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After attending this presentation, attendees will have been introduced to a case where a seat belt restrained occupant in a large SUV was ejected and severely injured in a multiple-impact rollover collision. The goals of this presentation are to describe in detail the physical evidence and to clearly explain the mechanism of unwanted seat belt release.

This presentation will impact the forensic science community by illustrating how a detachable anchor design can be susceptible to release.

**Collision Overview:** A 1999 Ford Expedition with six occupants was traveling in lane #3 of a 3-lane highway when it was struck from the rear by an errant Ford Explorer. The rear impact caused the Expedition to spin out of control, impact the concrete median barrier, and roll over onto its right side. The Expedition was subsequently struck by two vehicles. The latter more severe impact caused structural deformation to the Expedition with the unwanted release of the third row, right outboard occupant's seat belt. The occupant was ejected and sustained a severe closed head injury. This presentation discusses the reconstruction, injury, buckle design, and mechanism of the unwanted belt release.

**Materials Reviewed:** The traffic collision report provides details of the type and location of physical evidence, and a scaled, hand-drawn diagram. Color digital photographs (29) taken at the scene by police were reviewed. The subject vehicle was inspected. Medical records detailed the occupant injuries. **Collision Reconstruction:** In this multi-vehicle collision, there were eight distinct impacts.

- Impact #1: Right front corner of the Explorer rear-ends the left rear corner of the Expedition: The Expedition was traveling approximately 65 mph when the right front corner of the Explorer struck its left rear corner, evidenced by paint transfers on the Expedition matching the color of the Explorer.
- **Impact #2:** Explorer rolled on embankment: The Explorer careened off the right side of the highway and overturned on a landscaped embankment.
- **Impact #3:** Explorer struck a palm tree: The Explorer descended the steep slope, struck a palm tree, and continued to its point of rest (POR) on its wheels.
- Impact #4: Right front corner of the Expedition strikes the concrete median barrier: The Expedition was propelled 174 feet, yawed counterclockwise about 125° (when viewed from above) to an angle of about 18° relative to the concrete median barrier, and struck the barrier at 45 to 47 mph, evidenced by tire friction marks.
- **Impact #5:** Right rear corner of the Expedition strikes the concrete median barrier: The Expedition continued to yaw counterclockwise for about 27 feet on its wheels until, at 42 to 44 mph with a principle direction of force of 160° to 180°, its right rear corner collided with the barrier, evidenced by the tire friction marks on the roadway and horizontal scrapes on the Expedition.
- **Impact #6:** Expedition tripped and rolled in lane #1: The Expedition continued to yaw counterclockwise, slowed to 34 to 37 mph, tripped, and rolled <sup>3</sup>/<sub>4</sub>-turn driver-side leading. Then, the Expedition slid on its right side in lane #1 for about 92 feet, evidenced by metal scrapes to the vehicle exterior.
- Impact #7: Corolla vs. Expedition: The Toyota Corolla, traveling 65 mph in lane #1, skid into the concrete median barrier, causing left front wheel and suspension damage. Then, the Corolla was redirected, continued skidding for 148 feet, and, traveling approximately 22 mph, struck the front undercarriage of the Expedition, which was stationary on its right side. The Expedition rotated clockwise (when viewed from above) and became aligned within lane #1 with its rear end exposed to approaching traffic.
- Impact #8: RAM vs. Expedition: The Dodge Ram, traveling 65 mph in lane #1, skid for 46 to 57 feet, slowed to 50 to 53 mph, and hit the right rear corner of the Expedition, which was stationary on its right side, evidenced by a tread imprint from the Ram's left front tire on the Expedition's exhaust pipe and paint chips matching the Ram embedded in the Expedition spare tire bead. The Expedition's velocity change was 28 mph. The Expedition, on its right side, rotated counterclockwise for more than 360° to its POR, ejecting the third row, right outboard passenger.

**Expedition POR:** At its POR, the Expedition faced east in the middle of the #1 lane.

**Injury Description:** The 14 year-old, 135-pound female, 3rd row, right outboard belted passenger sustained severe head trauma (right basal and right frontotemporal skull fractures with right frontal epidural or subdural hematoma, diffuse subarachnoid hemorrhage, and pons diffuse axonal injury with residual right 6<sup>th</sup> nerve palsy), with blunt right orbital injury, severe frontal laceration, occipital bleeding, nose fracture, and chipped teeth.

**Injury Causation:** Inspection of the Expedition's interior rear occupant compartment, roof rail and headliner revealed no blood spatter or physical evidence consistent with a forceful head strike. At their POR's, the ejected third row, right outboard occupant was lying on the

roadway adjacent to the concrete median barrier approximately five feet from the underside of the Expedition. Therefore, the severe injuries sustained by the victim were likely the result of a forceful impact with the concrete barrier after ejection.

#### Seat Belt Assembly Inspection:

Third Row Outboard Seat Belt Design: The 3rd row, outboard seat belt assemblies feature continuous loop webbing, a free-sliding latch plate, fixed D-ring and an emergency-locking retractor. The seat belt assemblies contain a seat-fixed, end-release buckle and a lower outboard detachable anchor. These design features facilitate the complete removal of the third row seat assembly to expand cargo volume. The 1998 through 2002 model year Ford Expeditions and Lincoln Navigators contain the same third row detachable anchor design. The detachable anchor resembles a side-release seat belt buckle encased in a plastic boot (photo 01 detachable anchor). The other side of the detachable anchor is called the anchor tongue, and resembles a chrome-plated latch plate tongue that is secured to the vehicle inside a recessed floor pocket. The Expedition Owner's Guide explains how to disengage the lap-shoulder belt from the floor: Insert a key or small screwdriver through a hole in the boot, press on the release button, thereby separating the detachable anchor from the anchor tongue (figure 01 illustration). During the inspection of the subject vehicle, the detachable anchor of the right third row outboard seat belt assembly was found detached from the anchor tongue.



**Belt Usage:** Physical evidence on the right 3<sup>rd</sup> row outboard seat belt assembly was conclusive for use at the time of collision. Conspicuous striations to the polymer over mold of the latch plate and D-ring confirmed occupant loading to the seat belt system.

Third Row Outboard Seat Belt Release Mechanism: The impact by the Ram caused the Expedition's right rear floor structure to deform upward toward the headliner (photo 02 rear corner damage). Deformation to the area surrounding the detachable anchor was significant enough to allow the detachable anchor to contact the outboard seat back recline lever. In the undeformed condition, the hole in the boot is approximately four inches from the lower edge of the recline lever when the seat belt is fastened. Scratches consistent with interaction with the recline lever were visible on the side of the boot that conceals the release button facing the lever (photo 03 boot evidence). The boot also showed evidence of being compressed in the form of discoloration or lightening of the plastic. The physical evidence indicated that it was most likely interaction between the seat back release lever and the plastic boot that led to an unintended release of the detachable anchor.





Third Row Outboard Seat Belt Detachable Anchor Compression Testing: Compression tests were conducted on the bootencased detachable anchor assemblies sectioned from two exemplar seat belt assemblies salvaged from 1999 and 2000 model year Expeditions. The 2000 specimen exhibited more oxidation to the metal plating of the detachable anchor, likely a result of exposure to the elements during storage.

First, a force gauge was used to quantify the average minimum force needed to depress the release button and disengage the anchor tongue. Ten tests were conducted on each specimen.

- The 1999 specimen needed an average 4.1 pounds and approximately 1/8 inch depression to release the anchor tongue, whereas
- The 2000 model needed an average of 5.3 pounds to release the anchor tongue.

Next, a test fixture was designed to hold the detachable anchor in a horizontal orientation (photo 04 fixture). A 1.5-inch diameter ball was used as an impactor centered on the hole in the boot, with a preload of about 20 pounds. The impactor was attached to an Instron model 1123 tension-compression machine with a crosshead speed of 0.5 inches/min. A 5.0-pound weight was secured to the anchor tongue to aid removal from the detachable anchor once sufficient compression force was attained. Data was collected with a 5,000-pound load cell at 20Hz.

- For the 1999 specimen, a compression force of approximately 760 pounds and a displacement of about 0.13 inches was necessary to eject the anchor tongue.
- For the 2000 model specimen, a compression force of about 1,110 pounds and a displacement of about 0.17 inches was necessary to eject the anchor tongue.

The difference in force and displacement values between the specimens may have been the weathered condition of the 2000 specimen, or a result of the eccentricity of the impactor position relative to the release button underneath the plastic boot.



**Conclusions:** A large SUV was involved in a multiple-impact rollover collision from which a third row, right outboard seat belt restrained occupant was ejected and seriously injured.

Physical evidence on the third row, right outboard seat belt detachable anchor suggested that interaction with the seat back recline lever led to unwanted release of the seat belt.

Compression testing on exemplar detachable anchor specimens showed that latch release can be obtained with 760 to 1,110 pounds compression and as little as 0.13 inches of release mechanism depression.

The detachable anchor design feature of a removable third row seat has been found to be prone to unwanted release, permitting occupant ejection with the potential for serious, life-threatening, or fatal injury. Unwanted Seat Belt Release, Detachable Anchor, Rollover Occupant Ejection

#### C6 The Importance of Proof Marks in Vehicle Accident Reconstruction

## Michael L. Shirley, BS\*, Innovators Research, Inc., 2290 CR 23, Waterloo, IN 46793-9415

After attending this presentation, attendees will gain an understanding of the physical architecture and function of the steering system components, including the steering axle, as commonly used in heavy duty commercial vehicles, e.g., trucks, intended for use on paved and unpaved roads. Scientifically accurate reconstruction of vehicle accidents may require detailed examination of the internal mechanisms of components. Attendees will learn how impact forces that are generated by tire-to-road contact can create internal witness marks in other interconnected components.



This presentation will impact the forensic science community by emphasizing the necessity to consider multiple pieces of evidence from the roadway and vehicle(s) along with detailed evidence that is not visible in a macro examination.

A rural highway frontal collision occurred between a tractor-trailer combination (semi) and a pickup truck, resulting in significant physical damage to both vehicles and personal injury to the two occupants of the pickup truck. On-scene photographs depicted the vehicles in their post collision, at-rest positions. Investigators–including engineers, generally concluded that the driver of the (semi) had failed to control his vehicle while crossing a dual set of angled railroad tracks resulting in his (semi) then crossing the highway centerline and subsequently colliding with the approaching pickup truck, whose driver had already begun taking evasive action. Visual examination and photographs of the (semi tractor) revealed a component of the (semi's) steering linkage (the pitman arm) was fractured into two pieces and no longer provided an integral mechanical connection between the driver's steering wheel and the road wheels.

The collision between the two vehicles was slightly oblique, with the driver side frontal area of the (semi) contacting the frontal area of the pickup truck. The driver's side front-end of the (semi), being the initial contact area on the (semi), seemed to support a conclusion that the steering gear pitman arm was fractured during the collision sequence. Subsequent reports by investigators and a consulting engineering firm, so-stated this theory, and their conclusion and opinion was that the steering (pitman arm) fractured upon impact with the pickup truck. A pictorial representation of a typical heavy duty commercial vehicle steering system is presented in Fig. 1 for reference.

A further, detailed examination of the on-scene photographs and details related to the highway conditions at the railroad crossing, presented an additional option for further scientific investigation. An engineering report that had previously been prepared by a professional metallurgical engineer regarding the fracture of the steering gear pitman arm and was included in the litigation discovery documents was also important in the ensuing, additional analysis.

The attendees will be guided through a photographic inspection and analysis of the inner workings of the (semi's) steering gear and steering linkage. The results of this new portion of the investigation will be combined with other previously established evidence, and a resulting and different conclusion will be presented.

Steering Gear, Proof Marks, Impact Force

C7 Analysis of Cutaneous/Cortical Head, Extremity, and Thoracic Trauma Associated With Glass Impact in Automotive, Industrial, and Residential/Commercial Building Construction Applications Utilizing the Forensic Engineering Method

Laura L. Liptai, PhD\*, BioMedical Forensics, 1660 School Street, #103, Moraga, CA 94556

After attending this presentation, attendees will understand how to utilize the Forensic Engineering Method to teach the forensic engineering analysis of the signature of physical evidence remaining on soft tissue and cortical trauma as a function of the varied material properties of glass.

This presentation will impact the forensic science community by providing new insight into incident causation, trauma biomechanics and prevention involving glass-like material property impacts.

Glass is utilized in various applications as a retention material. In building construction to prevent falls from height and in vehicles as a retention mechanism to prevent occupant ejection (Willke).<sup>1</sup> Over time, glass has been designed to help reduce occupant glass trauma upon impact. Tempered glass and high penetration resistant (HPR) laminated glass are commonly utilized due to their preferential tensile properties and the mechanisms by which they shatter. Tempered glass is treated with either heat or chemicals to increase its strength, and is designed to have outer compression and inner tension allowing it to divaricate throughout the circumscription of the plate boundaries, thereby creating diminutive fragments (Lawn).<sup>2</sup> HPR laminated glass is composed of two layers of glass interpolating a polymer interlayer. The design of this glass allows it to withstand higher breakage and penetration forces, and causes the glass to fracture into small fragments while the component itself remains largely intact (Rieser).<sup>3</sup>

In order to analyze the injury trauma associated with glasses possessing different material properties, a wide variety of glass related trauma incidents were examined. Occupant retention requirements varied in vehicular, industrial, residential, and commercial building applications. Given these different retaining surface properties and human dynamics, data were gathered relating to the glass composite responses both with, and without, visible fracture patterns; these data were analyzed and the results used to study the inter-relationship between retaining surface properties, human dynamics, and trauma sustained by humans.

Data collection methods included observation, research, experimentation and/or calculation. The forensic engineering methodology utilized, and outlined in Figure 1, illustrates the ultimate objective of uncompromised data collection that results in systematically considered, iteratively derived, and objectively balanced findings. Test designs consisting of a drop test mechanism were utilized to generate a range of impact velocities. A glass suspension and release mechanism was designed that demonstrated a dramatic improvement in experimental repeatability. The glass suspension mechanism also enabled experiments that included a pre-failed condition of microscopic, as well as linear and spider web glass fracture patterns, to be performed. Instrumentation protocol included use of a anthropometric dummy to simulate the subject. Triaxial accelerometer data were collected at a frequency significantly exceeding Nyquist, conditioned to boost the signal; converted from an analog to a digital signal, adjusted via a dac card to format compatibility, and passed through a Butterworth filter.

Data analytics for a variety of configurations are presented, including alternatives. Ranges of acceleration required to achieve glass fracture modalities were measured and associated with specific trauma sustained. The results provide new insight into incident causation, trauma biomechanics, and prevention involving human impact with glass.



Figure 1: Forensic Engineering Method **References:** 

- <sup>1</sup> Willke, D., Summers, S., Wang, J., Lee, J., Partyka, S., Duffy, S. "Ejection Mitigation Using Advanced Glazing: Status Report II." National Highway Traffic Safety Administration, Transportation Research Center, Inc., August 1999. Collins, J. et al. "Accident Reconstruction." Charles C. Thomas, Publisher. Springfield, IL, 1979.
- <sup>2</sup> Lawn, B.R., Marshall, D.B. "Contact fracture resistance of physically and chemically tempered glass plates: a theoretical model." Physics and Chemistry of Classes, Vol. 18, No. 1, February 1997.
- <sup>3</sup> Rieser, R.G., Chabal, J. "Safety Performance of Laminated Glass Configurations." #670912, February 1967.

Cutaneous/Cortical Head, Thoracic, Trauma

#### C8 Human Injuries Accompanying Acute Angle Automobile Collisions With Wood Plank Fences

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Automobile or light truck collisions at acute angles with wood plank fences can result in human injury due to post-dislodgement and horizontal wood plank penetration of the passenger compartment. This hazard is largely unappreciated due to its sporadic publication in isolated case reports. The goal of this study is to show how such collisions, even those recorded during a limited time and area, are more prevalent than suspected and that the associated human injuries appear "binary" in severity.

Study of vehicle collisions with roadway signs and guard rails have led to new designs and materials that have reduced motorist injuries, but wood plank fences have escaped comparable attention. The present study offers new information regarding the incidence of motor vehicle – wood plank fence collisions as well as the type and severity of human injuries that can occur. This presentation will impact the forensic science community by increasing awareness of the hazards posed by wooden plank fences aligned parallel to the highway and to motivate additional studies and engineering efforts that will result in fence redesign, altered placement, or use of new materials to mitigate injury risk to motorists who collide with fences at an acute angle.

Wooden plank fences are commonly used to delimit real estate or constrain the movement of large animals. These fences are typically constructed of three or four horizontally placed oak or hickory boards (~2" thick, 4" – 6" wide, 6' – 16' long) nailed to intermittently spaced (6'-16') vertical round wooden posts (~6" diameter, ~8' long, ~4' of which are underground). Isolated sporadic reports exist of injuries to errant motorists who strike these fences at an acute angle, but this hazard is largely unrecognized. Similarly, the frequency and type of injuries suffered by these motorists are also not well known. The purpose of this study was to quantify the frequency and severity of injuries to motorists due to this mechanism that were observed during more than a decade at a single location.

The databases of a major University Level I Trauma Center and the County Coroner were retrospectively analyzed over the period 1995-2007. This study was IRB approved. Hospital charts, operative reports, and charges were abstracted retrospectively to confirm fence contact, injury data, subject demographics, and hospital costs. Motorcycle collisions were excluded. Mean values were compared by using Student's t-test; correlation was analyzed by using regression techniques.

One hundred and twenty eight subjects were involved in 127 acuteangle collisions of automobiles or light trucks with wooden plank fences during this period. Of these 128 subjects, 123 were evaluated at this Trauma Center and of these, 35 (27%) had a documented wood fence plank-patient interaction (PPI). Mean subject age was 32.8 years. Males (30 of the 35) were more frequently (86%) represented and 91% of these 35 subjects were in the driver's seat at the time of injury. Fourteen of the 35 (40%) died from injuries related to PPI. Blunt injury predominated over penetrating injury; only one subject had a mortal penetrating head injury from PPI. Survivors of PPI had a lower (p=0.05) Injury Severity Score (14.5 vs. 27) than non-survivors. Restraint data were available for 87 of the 128; 48.5% were restrained. No correlation was detected between restraint status and level of injury or mortality.

Two-thirds (64%) of the impacts occurred on the subject's right side. The most common body region of plank contact was the head

(13/14, 93%) and as expected, brain injury was the most common cause of death in that group. The upper torso (chest and shoulder) was the next most common region of injury; PPI was associated with significant soft tissue, bone and vascular injuries as well as tissue loss. PPI involving the upper extremity was also associated with neurovascular compromise and these injuries required extensive operative intervention for salvage or repair. Near complete amputation of the involved extremity after plank contact was not uncommon. Neck injuries were uncommon but when present, they were associated with significant vascular and soft tissue injury. A single penetrating abdominal injury (fatal) occurred in this group of 35 subjects.

Total mean hospital incurred costs for 13 of the 35 PPI related injuries were 50,530 for those requiring surgery (n=6) and 34,256 for those not requiring surgery (n=7). The latter value was skewed because four of the seven subjects expired shortly after arrival at the hospital.

This study adds new data underscoring the frequency of this injury mechanism and suggests that injuries to motorists who collide with wooden plank fences at acute angles are binary in severity; either none/minor or major/fatal. This conclusion is based upon a limited time and region sample; the national extent of this problem is unknown due to the lack of standardized databases linking this specific mechanism. Engineering initiatives to mitigate injuries associated with PPI are complicated by the absence of wood plank fence construction standards and the lack of information regarding the mechanism by which wooden planks enter the passenger compartment of roadway errant automobiles and light trucks.

Additional studies are needed to quantify the extent of the problem nationally, understand the mechanism of vehicle penetration by wood planks, increase public awareness of the hazards attending collisions with these fences at acute angles, and develop injury-mitigating fence construction strategies or new frangible material alternatives.

Human Injury, Automobile Accident, Wood Fence

### C9 So You Think You Can Testify? Cross-Examination for Expert Witnesses

Betty Layne DesPortes, JD\*, and Steven D. Benjamin, JD\*, Benjamin & DesPortes, PC, PO Box 2464, Richmond, VA 23218-2464

The goal of this presentation is to assist experts in understanding the purposes and goals of cross-examination and will illustrate topics about which they are likely to be questioned. For attorneys, this presentation will outline the issues they should include in their research, trial preparation, and cross-examination in order to better elicit the facts relevant to determining reliability.

The presentation will impact the forensic science community by explaining the purpose and nature of cross-examination and will assist the forensic science community to present reliable results with credible testimony in criminal cases.

Expert witnesses-particularly in the criminal justice system-often complain that attorneys fail to "focus on the science" or ignore reliable results in favor of personal attacks. Attorneys, on the other hand, complain that experts wrap questionable opinions in an undeserved aura of science, but fail to confront experts in a meaningful and probative way.

In the wake of the United States Supreme Court decision in *Melendez-Diaz v. Massacuhsetts*, 129 S.Ct. 2527 (2009), more forensic scientists and other expert witnesses are being called to testify in criminal cases. Expert witnesses can no longer rely on their written reports being admitted into evidence without their testimony and attorneys now must confront live witnesses instead of documents at trial. The purpose of testimony is to present reliable evidence to the trier of fact (judge or jury) for consideration in reaching judgment. The specific purpose of cross-examination is to ensure the reliability of the evidence presented by full disclosure of all relevant facts–a purpose so integral to

our system of justice that "the very integrity of the judicial system and public confidence in the system" depend on the right to cross-examination. *United States v. Nixon*, 418 U.S. 683, 709 (1974).

The reliability of scientific evidence not only depends upon the forensic science method or technology used, but also upon the expert. The trier of fact cannot determine the reliability of the evidence presented without assessing the reliability of the method or technology and the reliability of the expert who determined the results. The reliability of the science and the reliability of the expert witness are both areas that must be explored on cross-examination.

To assess the reliability of the science, relevant areas for inquiry include: (1) the extent to which a particular discipline is founded on reliable scientific methodology that gives it the capacity to accurately analyze evidence and report findings, and, (2) the extent to which practitioners in a particular discipline rely on human interpretation that could be tainted by error, bias, or the absence of sound operational procedures and robust performance standards. The case-specific areas of inquiry include the capabilities or limitations of the specific laboratory facilities, evidence handlers, and technicians, including the potential for contamination.

To assess the reliability of the expert, relevant areas include education, training, professional associations, certifications of competence, proficiency testing, unexpected result incidents, and, as with all witnesses, potential bias or motivation.

The assessment of reliability, while necessarily personal because the expert's own work is at issue, should not be conducted or viewed as a personal attack. The reliability of evidence, including the reliability of the science and the expert witness, is a fundamental issue in our system of justice. The purpose of cross-examination is not to discredit a witness; it is to separate truth from fiction and exaggeration from accuracy. Cross-examination seeks the full disclosure of all facts necessary for the judge or jury to be able to assess the reliability of the evidence and to determine at the truth of the case.

Expert Witness, Cross-Examination, Reliability

#### C10 Misleading Expert Testimony: Examples, Causes, and Reforms

Paul C. Giannelli, JD, LLM\*, Case Western Reserve University Law School, 11075 East Boulevard, Cleveland, OH 44106

After attending this presentation, experts and attorneys should be able to recognize a variety of testimony that may mislead a jury and how to avoid such testimony.

This presentation will impact the forensic science community by alerting experts and attorneys to problematic testimony and ways to avoid it.

This presentation will provide examples of misleading testimony from actual cases. Expert testimony may be misleading for several different reasons. Obviously, false testimony is misleading. More commonly, testimony may mislead because it includes overstatements or because statistics are improperly used. It can also be misleading because it is incomplete. Even technically accurate statements may mislead the jury. Sometimes the cause of misleading testimony is the expert and sometimes it is the fault of the attorneys.

Misleading Expert Testimony, Expert Testimony, Misleading Testimony

#### C11 Incompatible Codes of Ethics and Inherent Conflicts Between Lawyers and Their Expert Witnesses

Thomas L. Bohan, PhD, JD\*, MTC Forensics, 54 Pleasant Avenue, Peaks Island, ME 04108

After attending this presentation, attendees will receive observations about attorney/expert incompatibilities from an attorneyphysicist who has been noting them for 35 years. The objective is to sort out conflicts between lawyers and experts when they work together.

The presentation will impact the forensic science community by making practitioners aware of those incompatibilities which are resolvable so that they can resolve them and those that are not so that they can be aware of them.

This presentation highlights the conflicts between the two types of practitioners, identifies those that appear insurmountable, and suggests ways to ameliorate the rest.

Aside from personality conflicts, most problems between forensic experts and their lawyer-clients are traceable to their radically different codes of ethics. Lawyers are obliged to provide the best representation possible for their clients consistent with the law. They are not permitted to suborn perjury; however, short of that and with the exception of prosecutors, they have no obligation to the truth. It is not a violation of legal ethics for a lawyer to shop for experts, a specialist who will say the "right thing," regardless of the lawyer's suspicion that the "right specialist" may be incompetent. Once a case is underway, the lawyer is not seeking the truth but rather trying to establish his or her client's theory of the truth. Indeed, for a lawyer to reveal too much truth at trial can be a violation of legal ethics. The reasons for these ethics rules are fundamental to our system of justice; in following them, lawyers are acting perfectly honorably.

Scientists are ethically obliged to tell the truth. They not only must not tell lies, they must avoid making deliberately misleading statements. Most scientists also have an urge to clarify, to explain, and to ensure that the other person understands. They therefore have a difficult time complying with the standard instruction lawyers give their witnesses before trial or deposition: Your interrogator is neither your friend nor your student; just give the simplest, briefest answer to the questions and do not answer questions not asked. President Clinton, in his Paula Jones deposition, showed how not to comply with this instruction, and suffered as a result. Asked, "Is there a... relationship between you and Ms. Lewinski?" he tried to help out his hostile interrogator, responding with his famous "It depends on what the meaning of "is' is." The correct response would have been "No," leaving it up to the interrogator to sharpen up his questions. Some scientists-as well as probably most laypersons-believe that this approach is lying by omission and therefore unethical. Moreover, they assert that it is a deliberate attempt not to tell the whole truth and therefore a violation of the witness's oath. Those scientists generally do not serve as expert witnesses.

It is difficult for scientists first encountering the legal system to accept the fact that certain phrases have taken on secondary meanings. The oath to tell "the truth, the whole truth, and nothing but the truth," if interpreted in the manner that most not familiar with the system do, would lead to the conclusion that the majority of witnesses testifying under oath are committing perjury. As a practical matter, only the first and third parts of this ritualized recital mean anything in court. There is another ritual phrase, one pertaining just to expert witnesses, that is even more removed from the meaning assigned it by the non-legal world. The experienced expert witness knows that he or she must answer affirmatively to questions of the form: "Is this your conclusion to a reasonable (medical, engineering, scientific...) certainty?" Unless one's conclusion relates directly to a fundamental law of science or the like, the most one can say about it is that it is far more probable than not. "Certainty" is a foreign concept with respect to what most specialists spend their lives doing, no matter how successfully.

A scientist documents what he or she is doing while carrying out a professional investigation by recording all the facts that have apparent relevance to the investigation, by describing the actions taken by the investigator in the course of the investigation, and by memorializing his or her tentative conclusions. When confronted by an edict to put nothing in writing, the honest professional usually is at a loss. While it is understandable that the lawyer hiring the expert wants to avoid paying to create material detrimental to the underlying case, this particular edict hamstrings the specialist. Moreover, it can leave the expert in an untenable position months or years later at trial trying to support his or her conclusion without any documentation to support it. Indeed, not have documented an investigation is so unprofessional and inimical to its quality that acceding to this edict would appear to be unethical on the part of the scientist.

Although few trial lawyers go so far as to direct their experts to make no fields notes, many try to restrict the experts' reports, even sometimes demanding that there be no written report. Field notes and an oral conclusion are all that is wanted. As litigation strategy, this may make sense, and it certainly is not unethical for either the lawyer to request no written report, or for the scientist to accede to the request. However, the experts generally do not like it, and for good reason that should convince lawyers as well.

The "no written report" demand may appear to arise from, at best, lawyerly superstition and, at worst, a wish to submerge the truth. While both motivations do appear, there is another, one that is the result of the adversarial legal system. It is in the nature of this system that one's legal adversary will seize on anything that appears to undercut one's case, something that regrettably leads to a great deal of demagoguery in litigation. Anything in an expert witness's writing that has the slightest appearance of contradicting the expert's conclusions may be, and often is, used to attack the expert and through the expert, the lawyer's case. An expert who is prepared can fend off these attacks. However, from an economic standpoint has (both in terms of time and in terms of expense) dictates that such discussions be avoided completely if possible–a special case of the rule that far better than successfully defending a lawsuit is not having to deal with it in the first place.

Although from the perspective of legal *ethics* the "no written report" rule has nothing wrong with it, from the legal *strategy* perspective, it has two very serious flaws. The less important is occasioned by the delays that drag out litigation. Not having prepared a detailed written report places the expert in the position of either being vulnerable at trial or of having to carry out the investigation all over again, and under less propitious conditions than existed the first time around. An added benefit of having a detailed expert report early in litigation is that through being deposed on it, the expert is in a better position to defend the report's reasoning at trial. These benefits should be clear to everyone, regardless of whether they have a scientific education or bent.

The more important reason for permitting, encouraging, the expert to prepare a formal report has to do with how scientists work. For all but a few, it is during the writing of a formal report that scientists do the final testing of their ideas. It is when they realize which of the elements of their theory are not adequately supported, giving them the opportunity to seek better support or, if necessary, discard them. How much better for this to happen months or years before trial than when the expert is under cross-examination on the witness stand!

The "no written report" trial lawyers appear to be getting few in number, and may disappear completely if and when the anticipated reforms in forensic practice take place. Nevertheless, probably a majority of them are afflicted by what may be called magic-wordsphobia. Most scientists, and lawyers, too, when they are writing internal memos, feel compelled to present the full picture, the full array of facts underlying an incident in litigation. Invariably, some facts will exist that appear to undercut the scientist's theory, whether he or she is analyzing a murder case or seeking to explain an electron paramagnetic resonance spectrum. To simply ignore those facts is to stop short of completing the job. They need to be stated and, if possible, an explanation provided as to why they do not torpedo the theory being presented in the conclusions section. Yet lawyers repeatedly will ask the consultant to not even mention the inconvenient facts, regardless of how small a fraction of the total picture they represent or, and this is the crux of the scientist's annoyance, no matter how well known those facts are to everyone concerned with the case. Refusing to mention inconvenient facts known to all is a form of superstition, or at the very least, the reasoning of a child: "If I don't mention that it is sleeting, maybe we will go on the picnic after all." This reluctance to mention inconvenient facts is traceable to the adversary nature of the system, and is understandable in that context. The scientist; however, feels put in the position of trying to slip one over when these facts are passed over without mention.

Another source of experts' annoyance with the lawyers they consort with is the failure of the latter to believe them. Although trial lawyers (a category which, contrary to the propagandized image, includes civil defense lawyers as well as plaintiffs' lawyers) are an unusually intelligent bright, ethical group, a pleasure for scientists to work with, there remain a few for whom superstition or perhaps pessimism trumps analysis. They are the lawyers who demand that their experts not carry out tests that the expert has explained will demonstrate the ridiculousness of the adverse expert's theory: "What if it turns out that they are right?"

Finally, with respect to lawyers' flaws, mentioning those people who do not understand their own case well enough to rehabilitate their expert after he or she has had to endure a series of "yes or no" questions that on their face make the expert witness appear ridiculous. A lawyer is not ready for trial until he or she is ready to do expert rehabilitation following a series of bogus "yes or no" questions.

Looking in the other direction, lawyers appreciate experts who understand the basics of the American trial system. Hard as it is to fathom, there are university professors and others who, after decades of testifying in court, are still unsure in a civil case as to which side is the plaintiff and which the defendant. Then there is the seriously incompetent expert witness who, rattled on cross, and responds to an apparently reasonable request for technical information: "I have absolutely no idea."

**Ethical Conflict, Experts, Lawyers** 

### C12 Differing Perspectives on the Use of Experts in an Adversarial Litigation System: Are Experts Misunderstood & Misused? Are Court Appointed Experts the Ultimate Answer?

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After attending this presentation, attendees will gain knowledge of techniques that may be utilized to understand an expert's perspective of the litigation process, maximize expert performance, and minimize the risk of bad feeling at the end of a project. The improper use of experts will be discussed from the perspective of an attorney, and case studies will be employed to illustrate techniques employed to mischaracterize expert opinions to the advantage of the opposing side. The undesirable effects of the adversarial process upon the relevancy, reliability, and validity of scientific evidence, and its ultimate value to the finder of fact, will be discussed. Additionally, the pros and cons of utilizing a court appointed expert will be discussed from the perspective of an expert who has been retained in that function.

This presentation will impact the legal and forensic science communities by drawing attention to how easily forensic experts can be misused, and impress that all stakeholders (experts, attorneys, plaintiffs, defendants, and the courts) must share responsibility to ensure that experts are properly utilized, with the goal of presenting relevant, reliable, and valid science in the courtroom. If adopted, the techniques described in this presentation will impact the way that attorneys and courts interact with experts, help them maximize expert usefulness, and improve post project harmony, thereby improving the litigation process for all participants.

Many attorneys view scientific experts as quirky, twitchy, socially inept geeks with personality disorders. In many instances they are correct. Attorneys work their way through a process of expert/consultant administration. This begins with research, and then moves through selection, engagement, casework, deposition, and trial. There are many human interactions along the way, and these are primarily between the expert, the retaining attorney, and his law firm. However, the importance of interactions with other parties should not be overlooked, and it is common for experts to interact with the retaining attorney's client, other experts, the opposing attorney, opposing attorney's client, and the court.

Many of these human interactions relate to issues of scheduling, estimates, milestones, report content, deposition & trial preparation, travel & accommodation, payment, and general administration of the project. An attorney is a project manager, and as such needs finely honed personnel management skills.

These complex multiple party interactions, and multiple activities, are a recipe for a strained relationship between expert and retaining attorney. This potential is amplified by the inherent differences in personality traits between attorneys and experts. While stereotypes are not 100% applicable, it is reasonable to assume that most attorneys are objective driven organizers with outgoing personalities, whereas most scientific experts tend more towards the absent minded, somewhat disorganized, introverted type of personality.

It is vitally important to realize that just because there is no open animosity or adverse feedback from an expert, this does not mean that he or she is happy. That introverted personality and an inherent dislike of conflict, means that even thoroughly disgruntled experts may not communicate their dissatisfaction to the retaining attorney. With the correct approach an attorney can, to a large extent, avoid expert dissatisfaction, and simultaneously create an atmosphere of trust whereby an expert will feel comfortable discussing potential problems before they develop into case busting nightmares. The advantages for all parties involved are significant.

Irrespective of personality traits, adherence to a few simple guidelines, and an understanding of the expert's mindset and project perspective, will greatly assist the attorney in achieving his objectives, minimizing stress on the expert, and building and sustaining a relationship that will benefit future projects.

All aspects of the litigation process will be discussed, as viewed through the expert's eyes. From initial contact and interview, through provision of information, analysis, liaison with other parties, reporting, deposition and trial preparation, and trial testimony and cross examination. Checklists of what to do, and what not to do, when dealing with an expert, will be presented.

An Attorney's View on Expert Testimony - Keeping It Relevant, Reliable, and Valid: Virtually every homicide case that results in a trial includes testimony from a forensic pathologist about the autopsy performed, the detailed findings, and the conclusions drawn as to cause and manner of death. Sometimes an inadmissible opinion testimony will be elicited and admitted, even when there is proper objection by opposing counsel. In one jurisdiction, the medical examiner who performed the autopsy in a recent case was asked by the prosecutor, and was allowed by the trial court, to opine as to whether the decedent's injuries were inflicted by the perpetrator with intent to kill, even though case law in that jurisdiction for the last fifteen years or more held an inference of intent to kill a matter for the jury, not an expert, to draw.

In the same jurisdiction, a young man was recently indicted by a grand jury for first degree murder in the death of his infant son, who was chronologically three months old at time of death, but biologically only

\* Presenting Author

five weeks old, having been born at 32-weeks gestation. The child was unresponsive when the parents brought him to the hospital, he never recovered and died within four days of his collapse, after support efforts were discontinued. Both mother and father reported that the child appeared fine the day of his collapse, while both parents were home with him. While the mother left the home for about 45-minutes to pick up food for dinner, the child was in the care of the father and went into arrest. Upon the mother's return, the child was taken to hospital. The mechanism of death was an hypoxic-ischemic encephalopathy due to cardiopulmonary arrest. The child had established subdural bleeding and cortical vein thrombosis at the time of his arrest. The child had no skull fractures and little evidence of impact trauma (a tiny area of intra-scalp bleeding above the left ear). The medical examiner performing the autopsy opined that the cause of death was traumatic head injury and the manner of death as homicide. The grand jury heard from, inter alia, the child's paediatrician, who testified that it is not medically possible for the child to have sustained injury prior to the mother leaving the home on the day of his arrest, and from the medical examiner, who testified that "the history we have, normal infant up until a certain point would suggest that the injury occurred at that point." In rendering this opinion, the medical examiner discounted the significance of the histological evidence (iron stains of the SDH and the CVT were markedly iron positive). The medical examiner mischaracterized the findings and opinions of the forensic pathologist retained by the defense, whose written report concluding that the manner of death was undetermined was introduced as an exhibit by the prosecuting attorney; and he misrepresented medical intervention procedures utilized during the child's last hospitalization (testifying that a "shunt \*\*\* was placed to remove hemorrhage or blood from the head"; when in fact it, was a camino bolt, which was used to insert a pressure monitor into the brain), which, whether or not intentionally done, was done in the context of downplaying the significance of the markedly iron positive SDH and CVT.

The case studies in this presentation, although illustrating just a couple of examples of how the expert can be misused, should inspire forensic scientists and jurists to strive for the presentation of relevant, reliable, and valid science in the courtroom.

**The Court Appointed Expert:** The adversarial litigation system employed in the US, and many other countries, pits opposing experts against one another in an often unpleasant battle that results in personal attacks, and ultimately tarnishes both individuals, and their profession. Courts have the authority to appoint an independent expert of their own - a third expert to mediate between the two experts employed by the opposing parties in the litigation. The pros and cons of court appointed experts will be discussed from the perspective of an expert who has acted in that capacity.

Expert-Attorney Relationships, Expert Misuse, Court Appointed Experts

#### C13 Court Appointed Expert Selection Methodology: Federal Rule 706

#### Helmut G. Brosz, PEng, BASc\*, Brosz & Associates, 64 Bullock Drive, Markham, ON L3P 3P2, CANADA

The goals of this presentation are to give an example of methodology used for the selection of a court appointed expert, highlight some of the important attributes a court appointed expert needs to possess, and propose the use of a multi-dimensional matrix system to determine the most appropriate expert witness.

This presentation will impact the forensic science community by addressing the important attributes needed by court appointed expert witnesses.

Court appointed experts simplify and make easier for the courts to understand highly technical and complex cases, particularly electrical matters. Court appointed experts, agreed to by both the plaintiff and defendant, play an important role in giving judges an easier understanding of complex and technical cases. Cases have settled where court appointed experts were used in the interest of expediting justice and reducing costs.

Rule 706 of the Federal Rules of Evidence states that a 'court may appoint any expert witness agreed upon by the parties, and may appoint expert witnesses of its own selection.'

It is suggested that the key attributes are education, training, experience, reputation and track record. In addition, the court may require specific experience that is relevant to the particular technical engineering issues facing the court. If this attribute is missing, the court may not recognize the individual as qualified to testify as an expert. The matrix for evaluating potential expert witnesses can be appraised based on the following categories: possible conflict of interest, education, practical experience, publications, forensic experience, design experience, available support and professional affiliations. Typically, plaintiffs and defendants, each, nominate potential experts for consideration who are then interviewed via conference call and scored according to the multi-dimensional matrix proposed by the author. Each party pays the pro-rata expert fees while the court is exempt from paying.

**Conclusion:** The matrix system provides a logical method to overcome the difficulty faced by opposing parties and experts on being able to agree on an independent court appointed expert. This methodology worked well in the subject product liability case involving a double fatality by immolation of two utility linemen resulting from an exploding pole top oil filled utility transformer that had a defective gasket. The two experts for the two deceased, one the expert for the utility, and the expert for the transformer manufacturer ultimately agreed on one court appointed expert.

The court expects the expert to review opposing reports and obtain information, and then interpret its meaning. The court appointed expert must prepare a protocol for questioning information and then obtain it, using the power of the court. The protocol would describe documents needed, testing needed, and experiments and inspections necessary. In this way, the use of a court appointed expert is efficient, decisive and cost effective.

Appointment of an expert will undoubtedly remain an unusual event, suited only to the most demanding cases and judges. Regardless, Rule 706 remains an important alternative source of authority to deal with some of the more scientifically demanding evidentiary issues that arise in courts.

Expert Witness, Rule 706, Selection

## C14 Litigation and Attempts to Pierce the Peer-Review Process

Robert O. Andres, PhD\*, Ergonomic Engineering, Inc., 20 Gulf Road, Pelham, MA 01002

The goal of this presentation is to discuss the problems encountered when a manuscript is involved in the peer-review process and attorneys attempt to interfere with its publication. The responses of the respective parties, including the plaintiff and defense attorneys and the journal editorial board and legal team, are described.

This presentation will impact the forensic science community by serving as a cautionary tale for experts who also find themselves involved in original research. It will also make the argument for establishing the peer-review process as work product in the legal sense, at least from a scientist's perspective.

An original study, replicating an unpublished investigation done by another scientist for a litigation matter was undertaken and results were reported at a scientific conference and published in the proceedings. When the results were used in reports written for plaintiffs, railroad defense attorneys focused on the fact that the proceedings publication was not the same level of peer-review as a refereed journal article. They also subpoenaed the raw data, which was turned over in response to a court order at the urging of the plaintiff's attorney, who did not want to lose his expert in the case. When the principal investigator finally found time, the results were put in manuscript form and submitted to a refereed journal.

The ensuing legal maneuvers went far beyond what most scientists experience during the publication process. Subpoenas were served on the Associate Editor of the journal demanding the original manuscript and the confidential reviews. The Associate Editor had to consult his legal department, who then referred the matter to the corporate legal people outside the United States. The journal turned over both the original manuscript and the confidential reviews to the defense attorneys without ever informing the principal investigator (who learned about it when he was being deposed). The ensuing back-and-forth will be described in lay terms, and the potential chilling effect on those agreeing to review manuscripts on an ad hoc basis will be discussed.

After revisions, the article was finally published in the refereed journal and a second more extensive study on the same general topic was proposed to all of the Class 1 railroads, the railroad trade association (Association of American Railroads, AAR) and the Federal Railroad Administration (FRA). The FRA eventually funded the study, but not before railroad defense attorneys contacted the general counsel at the FRA and attempted to prevent the study from being funded. The AAR then tried to insert adversarial experts of its choosing into any peerreview done of the study by the FRA, at which point the FRA and investigators decided to go directly to a refereed journal with any manuscripts instead of providing a report to the funding agency. The second study was completed and the first manuscript was submitted to a refereed journal. Railroad defense attorneys issued more than 30 subpoenas to co-authors and their institutions to obtain the original manuscript and reviews. However, lessons learned the first time around prevented them from interfering with the peer-review process again. The second article is now in press.

Peer-Review, Litigation, Expert

### C15 Examining the Sources of Expert Opinions

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After attending this presentation, attendees will gain a better understanding of how journal articles, textbooks, studies, and experiments may be used to substantiate or attack an expert opinion in all phases of court proceedings.

This presentation will impact the forensic science and legal community by distinguishing how and when forensic experts may rely upon or be confronted with written materials that substantiate or contradict their professional opinions.

Most forensic disciplines have reduced their general principles and methods to writings in textbooks and journal articles. The Federal Rules of Civil Procedure at Rule 26(b) and several state codes of procedure or evidence mandate that an expert written opinion shall contain, amongst other items:

- 1. A complete statement of all opinions rendered in the case before the court;
- 2. The basis and reasons for the opinion;
- 3. The data and information that form the basis of the opinion;
- 4. Any exhibits to include appended texts from treatises, journal articles and studies; and,
- 5. The expert's *curriculum vitae* and list of publications in the past ten years, etc.

Ideally, an expert witness report should cite to relevant journal articles or texts to substantiate an expert opinion. These reference materials may be reviewed by opposing attorneys and later, judges who are called upon to review an expert's opinion that is challenged pre-trial in a motion to suppress an opinion or dismiss a case, or at a *Daubert* or *Frye* hearing (or motion submitted on papers) to limit or preclude an expert opinion. In these pre-trial proceedings, the proponent of the expert opinion must lay bare the underlying rationale for the opinion.

Since the discovery process is more liberal than trial rules, inquiries as to the underlying basis for experts' opinions and their sources of such knowledge will invariably be made by trial attorneys. The foundation for the expert's source of knowledge will invariably be disclosed and experts should therefore be prepared to handle such inquiries when generating an opinion. While such disclosure may leave the judge with contradictory studies and journal articles, it is the role of the forensic expert to educate and convince the attorney and judge that the expert's opinion is reliable and therefore, admissible at trial. Whether the opinion is reliably based upon applying sound methodology and procedures that are generally accepted within the expert's field may be substantiated by the prior publication of a journal article or text that is peer reviewed. Whether the content of journal articles, textbooks or studies is ever disclosed to a jury is a different legal determination.

The Federal Rules of Evidence in Rules 703 and 705, and their corresponding state rules of evidence provide that while the expert may have reasonably relied upon some facts or data (found in written materials), those (hearsay/out of court) facts or data that are generally not admissible at trial may be disclosed to the jury if the judge rules that the probative value in assisting the jury to evaluate the expert's opinion substantially outweighs the prejudicial effect. However, on cross-examination, the expert must testify about the underlying facts or data if asked to disclose it.

Federal Rule of Evidence 803(18), known as the Learned Treatise exception to the Hearsay Rule (the rule that generally precludes the introduction of out of court written materials at trial), allows the use of a treatise (text, journal article, or study) either on direct examination of an expert to substantiate the basis for the expert's opinion, or on cross examination to impeach the expert's opinion.

In some states such as New York, written materials such as treatises, textbooks, journal articles or studies may not be used on direct examination, but may only be used on cross examination if the expert acknowledges that the written material is "authoritative." This gives expert witnesses the ability to stifle cross examination by merely stating that they do not recognize the written materials as authoritative. However, some clever techniques may be used to overcome this resistance to disclosure of the experts' basis of knowledge for their opinions.

Distinguishing between the use of written materials at trial and in a pre-trial setting, such as in a written expert report, a deposition, or an affidavit in support of or in opposition to a motion to suppress or dismiss, is a distinction forensic experts and attorneys must be aware of and prepared to handle.

Journal Articles, Pre-Trial/Trial Proceedings, Evidentiary Rules

## C16 Establishing Causation in Environmental Toxin Cases – Optimal Interaction Between Lawyers and Experts

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After attending this presentation, attendees will have an overview of the methodology for establishing general and specific causation in cases of individuals exposed to environmental carcinogens. These cases are unique in their requirement for a team of experts with widely differing areas of expertise each of whom must rely on the one before him. If one of the expert's reasoning is found to be flawed by the court, it can result in a *Daubert* challenge against all the subsequent experts who relied upon that one expert. This presentation will impact the forensic science community by providing an understanding of the newlyevolving scientific and legal requirements for these cases which will allow a better understanding of the interpretation of the results, how much the case will cost and it will allow the experts to evaluate the validity of each other's testimony.

The tools available to establish general causation are largely in the form of animal studies performed by regulatory agencies such as the Centers for Disease Control (CDC) or compiled and discussed by agencies such as the Institute of Medicine (IOM), together with human studies which are essential to support general causation. Except for a few chemicals such as the dioxins which have been followed in exposed populations prospectively, proper controlled studies are not available since it is not ethical to expose humans to carcinogens. In the absence of controlled studies on carcinogen exposure, published exposure studies are primarily at the workplace. The rate of cancer in workplace studies is always underestimated because of the methodology by which the studies are conducted. Judges have adopted the rule that a chemical cannot be legally found to cause a cancer unless there is a report showing a relative risk of 2.0. In addition, if the confidence interval crosses 1.0, then the study can be excluded from evidence as not being statistically significant. The usual result of this is that the plaintiff presents a barrage of appropriate studies, the defendant counters with another onslaught of epidemiologic studies that do not meet the statistical requirements, and then the expertise, preparation, and quick wittedness of the expert comes to the fore in his/her ability to point out the flaws in the studies. These flaws are predictable and will be discussed.

Probably the greatest challenge in establishing general causation is identifying the chemical and, therefore, the disease(s) in which it could be implicated. The safest way to find a good case is probably to follow on the studies performed by the regulatory agencies that are mandated to identify and quantify the environmental chemical, usually carcinogens, once a superfund site is identified. In these studies, the raw data is usually accurate although the scientific interpretation may be politically dictated.

*Specific causation* is very much a matter of precise, careful histories to exclude other causes of the target illness, and optimally to identify a mechanism of action by which the targeted chemical caused the disease.

This is routine medical practice, and a competent physician who is experienced in the range of chemicals which may cause the disease should handle it well. However the devil is in the details, and in the case of specific causation, the devil is in the dose.

The EPA holds that a dose which increases risk of cancer by  $>1/10^5$ in a population can be considered significant. Although workplace exposure is helpful, most environmental toxic exposures are domestic. A large number of methods must be utilized to determine what the dose of a suspect chemical was at the time that critical exposure occurred, made more difficult by the long latency period of most cancers. Most physicians will agree that there is a range of carcinogen exposure which can cause a significant increased risk of cancer (usually considered to be  $>1/10^5/year$ ) in a population. Because of the enormous biological variability in exposed humans, many will refuse to opine on what was a cancer dose for a given individual. As judges and defendants have come to realize that the difficulty in establishing causation they have crafted a series of questions which can quickly reduce the expert who refuses to play the game to idiot status. Those questions will be discussed, as will the many ways that a dose can be established.

**Environmental Toxins, Domestic Exposure, Dose** 

#### C17 Downed Power Line Electrocution Case: Legal and Engineering Issues

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After attending this presentation, attendees will understand the importance for lawyers, judges, and engineers to recognize how the use of the forensic sciences affects the behavioral, legal and causal issues of a litigated case. This stresses the importance that admissible evidence, codes and standards, operating procedures, videos, photos, eyewitness accounts, and autopsy reports all fit the puzzle pertaining to downed power line electrocution matters.

This presentation will impact the forensic science community by expanding the knowledge of factors affecting downed power line electrocution litigation so the competence and performance of lawyers and engineers, vis-a-vis the court, will be enhanced.

**Legal Summary & Description:** Timothy Cutler was 32 and resided in California. He was an interesting and exciting young man who was looking forward to his wedding on January 24, 2004. Early in the morning of January 23, 2004, a 7,200 volt power line, owned and maintained by the local utility, fell. The power line caused a fire in the front yard of Cutler's house.

No one saw what happened to Tim Cutler, but he was severely burned by the electricity, arcing, and fire, and three days later, succumbed to those injuries.

The utility managed the news story by showing a picture of a dead pigeon in Cutler's driveway and suggesting Tim had picked up the live conductor, thereby causing his own fatal injuries. Being a single man, his parents were his only heirs, and hired the a law firm to investigate the circumstances of their son's death.

Forensic experts were retained to examine the scene, evaluate the damaged conductor, inspect the infrastructure and substation, and analyze the injury to Cutler's hands and face.

The biomechanical expert concluded Cutler didn't grasp the conductor, but rather used his hand to deflect it when it whipped off the ground.

The forensic electrical engineering experts concluded the splice, not the pigeon, was to blame for the line separating and that the utility violated numerous standards and operating procedures.

Forensic electrical engineers were enlisted to tie all the other forensic facts together to hypothesize how Tim Cutler got out of his house after the power went out and the fire started in his front yard, where he innocently encountered the live conductor.

The forensic work and conclusions caused the utility to settle this case before a lawsuit was filed for \$1.5 million.

**Chronology:** An automatic splice on a #6 solid copper wire let go on a 200 foot span between poles – Energized wire (at 7200 volts) fell on sidewalk and grass boulevard – Arcing to ground ensued – 100 amp fuse protecting the #6 copper wire lateral does not open - Substation recloser set at 149 amp ground fault pick up trips and recloses - Arcing stops and resumes – Recloser trips a second time and locks out - Victim approaches downed wire in driveway – Utility log shows 3389 customers out of power – Utility operator closes recloser switch via remote control – Reenergized wire bounces up and contacts victims hand, finger burnt off -Victim is electrocuted and engulfed in flame

**Violations/Errors/Issues:** Manufacturing and installation errors -Lateral fuse size protecting line too large - Utility operator violated utility written operating instruction. - "...if the location of the trouble (fault) is <u>not</u> known, sectionalizing and testing should begin immediately."

Pathology Facts: consisted of various injuries and burns References:

Institute of Forensic Electro-Pathology Database - California General Order 95 - Transmission & Distribution handbook - Distribution construction reference manuals (various utilities) – National Electrical Safety Code – Overhead distribution standards – REA – Sectionalizing guide

**Electrocution, Downed Power Line, Splice** 

## C18 The Difficult Task of an Independent Expert in an Environmental Justice Project

James Millette, PhD\*, MVA Scientific Consultants, 3300 Breckinridge Boulevard, Suite 400, Duluth, GA 30096

After attending this presentation, attendees will understand how difficult it can be when acting as an independent technical advisor in an environmental justice situation.

This presentation will impact the forensic science community by increasing the general knowledge of how the expert technical advisory process works in environmental justice cases.

Hunters Point in San Francisco, California was formerly a U.S. Naval Shipyard from 1941 to 1974. The major activities included ship building and repair and research on exposure to radioactive fallout. In the mid 1950s the shipyard employed 8,500 civilians. The Navy deactivated the shipyard in 1974. In 1989, following the Navy's environmental investigations, the U.S. Environmental Protection Agency (EPA) placed the shipyard on its National Priorities List, thus, designating it a federal "Superfund" site. The shipyard was divided into six parcels with Parcel A containing the housing structures on 88 acres of hillside overlooking the Bay. The Parcel was ultimately transferred to the City of San Francisco for development. The City contracted with Lennar to oversee the development. Immediately adjacent to the Hunters Point redevelopment property are homes in the community known as Bayview/Hunters Point. The community consists of almost 90 percent minority populations. According to the Agency for Toxic Substances and Disease Registry (ATSDR), the Bayview/Hunters Point community is similar to many urban, industrial, minority communities across the United States and has higher than the national average rates of asthma, respiratory disease, breast cancer, and diabetes. Therefore, they are considered a vulnerable population and may be more sensitive to the effects of exposure to hazardous substances. It was determined that the rocks in the redevelopment property had natural occurrences of asbestos fibers. A number of areas in the State of California have natural occurrences of asbestos and special precautions are required to prevent raising high levels of asbestos-containing dusts during any work that disturbs the rock. For the Hunters Point project, a Dust Mitigation Plan including community air monitoring stations was prepared and approved.

Because of environmental exposure concerns over the redevelopment of the property that is being done in several phases, community residents formed 'SLAM' - Stop Lennar Action Movement. Under an EPA program that provides the services of technical experts to community groups in Environmental Justice situations, a contractor to EPA was tasked to "Identify a technical expert to assist SLAM with the results of EPA's asbestos-related updates, including improvements to the monitoring plan, mitigation measures, and EPA's conclusions with regard to risk." This task was to provide independent technical assistance to SLAM and eventually other community members. In addition to providing general technical assistance in asbestos-related matters, there was a specific request to review one particular EPA draft document: Draft Technical Summary of EPA's Analysis of Hunters Point Air Monitoring Filters for Asbestos, December 22, 2009. The EPA technical summary and the documents that formed its basis were reviewed. These included the Dust Mitigation Plan, reports by the US EPA, the Department of Health of San Francisco, and the Department of Health of the State of California. The results of approximately 5,000 ambient air samples collected in and around the redevelopment property that had been analyzed for asbestos were also reviewed. A site visit was made to the neighborhood around the site and members of SLAM and other residents were interviewed. The findings at a public meeting held in San Francisco were presented. The meeting was attended by about 100 people: residents, activists and local clergy. It was a difficult setting to attempt to present technical conclusions. Although there was evidence that the local residents had not been treated fairly at all times by Lennar, the redevelopment contractor, my review of the data concerning asbestos exposure did not show evidence of increased risk of asbestos-related disease from the redevelopment activities. This paper reviews the project and highlights the difficulties in presenting scientific environmental forensic information to a largely emotional audience concerned about health risks to themselves and their children.

Hunters Point, Asbestos, Community Exposure

#### C19 Scatter Enhanced X-Ray Imaging – A New Tool in the Fight for Aviation Security

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After attending this presentation, attendees will gain an appreciation for the problems faced by airports to identify concealed items such as knives, explosives, and narcotics before their placement onboard an aircraft. The attendees will be made aware of the current state of the art technology before being introduced to a relatively immature screening technique that could aid in combating the problem.

This presentation will impact the forensic science community by revealing the current limitations in the threat detection performance of security screening systems. Attendees will be introduced to a new technique at an early stage in its development that one day could be routinely used as a diagnostic tool in the forensic community. Although marketed here primarily toward its use as a screening system, it should be noted that this new technique could conceivably have applications in numerous forensic fields where the ability to rapidly visualise volumes in three dimensions as well as chemically identify their contents would be advantageous.

A single approach that can effectively and non-destructively screen volumes for illicit materials is yet to be adopted by the security screening industry. In the wake of the 9/11 attacks, items of most concern include weapons (such as knives and scissors) that could be used for the implementation of a hi-jacking. However, the dangers of other materials such as concealed explosives, and in the long term the illegal trafficking of narcotics (both domestically and internationally) cannot be overlooked. To combat this issue we introduce a new diagnostic technique brought about by the amalgamation of two existing technologies. The 3D power of Kinetic Depth Effect X-ray (KDEX) developed by collaborators at Nottingham Trent University is united with the materials discriminating ability of angular dispersive X-ray diffraction (ADXRD).

KDEX utilizes multiple X-ray transmission images captured at differing perspectives to the inspection volume. Displaying the images in sequence provides depth perception to the screener via motion parallax. This technology has been shown to increase threat detection performance of familiar threat shapes in cluttered environments. Angular dispersive X-ray diffraction can provide materials identification of any material of a crystalline nature (which includes the majority of explosives and narcotics). The amalgamation of these techniques provides unprecedented specificity. This is because KDEX increases the likelihood of identifying threat shapes where as X-ray diffraction can identify chemical threats, which may have an innocuous shape.

Although these techniques are generated from a similar source, they are fundamentally different. To bridge the gap an extensive body of work has already been conducted by this research group to combine them. Contrastingly to KDEX, angular dispersive X-ray diffraction typically describes a single known point in space. To combine these techniques the research group has already investigated novel scatter sensor geometries that work to pinpoint the locations of threat scatter signatures within the inspection volume. These geometries simultaneously decipher the source to sample distance and the angle of the materials scattered radiation (indicative of the material and usually referred to as  $2\theta$ ). In principle diffraction patterns are obtained from a series of linear detectors arranged normal to the primary X-ray beam but at differing positions along it. The tangent of the gradient formed from the change in peak position from one detector to the next provides the actual  $2\theta$  values. The location of the diffracting material is also derived as part of this process. The sample position is the point on the primary beam that would be intersected by a straight line originating from the peak position on the CCD (at a given detector position) traveling with respect to its measured 20. This latest body of work involves a quantitative review of the KDEX images to compute object disparity (where by the relative movement of object gives an indication of its location in depth. This provides an alternate measurement of the locations of objects inside the inspection volume which is paramount for the correct interpretation of the scatter signatures. It is hoped that should a successful corroborative relationship be built between these techniques then the forensic community will have a new diagnostic tool in their fight against crime.

Security Screening, Narcotics, Explosives

#### C20 Forces Required for Stabbing Through a Variety of Clothing Types

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The goal of this presentation is to show how single and multiple layers of fabric influence the force required for stabbing. The presentation will show that for a variety of blunt and sharp weapons, the force required for penetration can be changed significantly depending upon the items of clothing worn. The educational message is that the clothing should be given consideration when estimating the force required for a stabbing incident.

This paper will influence the way in which forensic evidence related to knife crime is interpreted. This presentation will impact the forensic science community by demonstrating how often, the effect of clothing is disregarded but depending on the type and number of layers worn, the forces required for knife penetration can change significantly.

In the United Kingdom, murder by stabbing is the most common form of homicide. Previously, the forces required for stabbing with knives have been considered and shown that the tip radius is important for defining the sharpness of the stabbing implement. Further, recent work has shown that for blunter instruments such as screwdrivers, the cross-sectional area is important for determining the forces required for penetrating a silicone rubber/foam skin analogue. A related factor for understanding the forces required for stabbing is to consider how much the victims clothing influences the results. Thus, as part of a program of work to determine the forces required for stabbing with sharp and blunt implements, the effect of clothing in changing the forces required for penetration with a weapon were considered. Often, the effect of clothing is considered to have a minimal effect on the forces required for penetration. However, the forces required can be significantly altered, particularly when multiple layers of clothing are worn. A series of experiments have been conducted using a silicone rubber/foam skin simulant and a Materials Testing System. The knife is pressed into a skin simulant block and the load and displacement curve recorded. A load drop is seen when penetration of the skin simulant occurs. This has allowed the maximum force for penetration with single and multiple layers of clothing to be established. A range of clothing materials including t-shirts, cotton shirts, jeans, jumpers, and leather jackets have been tested both alone and also with layering as is typical of the clothing worn by victims in knife attacks. As an example, figure 1a shows an example of a load-displacement curve for a sharp knife into a skin simulant test block. The point at which the knife penetrates the silicone skin is arrowed and occurs at 15N. Figure 1b shows a similar loaddisplacement curve for a test with a three layer clothing system, a plain cotton T-shirt with two sweatshirt layers on top. The penetration force is again arrowed and it can be seen that the force required for penetration of the clothing system has increased to over 40N for the same knife.

The results show that the victims clothing should be considered in trying to answer the question as to degree of force involved in a particular stabbing incident.



Fiure 1a: Load-displacement curve for a sharp knife penetrating a skin simulant test block.



Figure 1b: Load-displacement for knife penetrating through three layers of clothing into a skin simulant test block. Knife, Stabbing, Penetration Force

#### C21 Assessing the Mechanical Properties of Skin by Indentation Methods

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The goal of this presentation is to discuss the complexities of the mechanical properties of skin relevant to penetration by sharp and blunt weapons. Skin is a complex material that has time dependent properties and the goal of this presentation is to discuss the relevance of these properties to the ability of a weapon to tear and puncture skin.

This presentation will impact the forensic science community by demonstrating to those interested in how weapons penetrate skin, particularly forensic pathologists, but also forensic scientists and engineers that are asked to provide expert opinions on the force required for penetration by weapons.

Stabbing in the United Kingdom with sharp weapons such as knives, glass shards and bottles is a major problem with high numbers of deaths and injuries caused by such crimes. One of the difficulties in understanding the forces required for stabbing is in deconvoluting the effect of the large number of different variables involved (such as weapon geometry, type of stab, type of clothing etc.), for which there is very little data in the scientific literature. Figure 1, for example, shows a glass bottle penetrating a silicone rubber skin simulant. The large elastic deflection of the surface is apparent. The bottle makes contact at a number of points and the large elastic deflection, rigidity of the supporting under layer and viscoelastic response of the rubber are allimportant for the penetration force required in this case. The influence of the mechanical properties of skin itself in determining the force required for penetration is discussed. Skin has a complex constitutive behavior and the aim of the work in this paper is to discuss how the mechanical properties of skin vary by using indentation techniques to study the mechanical response. The advantage of modern instrumented indentation techniques is that they allow the load-displacement and time

of an indenter into a material to be continuously monitored and thus the mechanical response of materials can be determined with precision. Additionally, compared to traditional mechanical testing techniques where the specimen preparation and instrument set-up can be complex, instrumented indentation allows a larger number of experiments to be performed and therefore the scatter in the mechanical response to be better established. Skin as a material is viscoelastic, i.e; the properties are time dependent. Also, the mechanical properties of skin can vary with age, location on the body, and levels of hydration. This paper reports on a number of indentation experiments on porcine flesh at different times from death. The experimental methodology has been standardized to ensure repeatability. Additionally, flesh has been tested with differing levels of hydration. The results are compared to the mechanical properties of silicone rubber/foam skin analogues. A number of indenters with both sharp and blunt tip radii have also been tested and the wounds imaged using microscopy techniques to establish whether or not the wounds are of the form of incised wounds or tears.



Figure 1: Complex fracture geometry of a broken glass bottle showing the bottle just out of contact and just in contact with silicone rubber/foam skin simulant. The large elastic deflection of the skin simulant is readily apparent.

Skin, Mechanical Properties, Stabbing

#### C22 Legal Issues in a Metallurgical Failure Resulting in Death

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After attending this presentation, attendees will understand the close relationship between metallurgical analysis and litigation. The attendees will also learn the legal issues involved in presenting a case based primarily on metallurgical evidence. This presentation will impact the forensic science community by illustrating an example of the relationship between legal and scientific activities in a death case.

Effective metallurgical investigation can be important in developing an understanding of the sequence of events associated with an accident.

Harry was a World War II hero and a diabetic. He lived alone in a very hilly section of San Francisco. Because of his limited mobility, Harry decided to buy an electric power scooter from a national manufacturer. The local dealer came to Harry's house to assess his needs and sold Harry an option for the "durable heavy duty transmission" because of the steep hills that the scooter would be traveling. Harry decided to do some shopping and upon returning, he turned down a street with a six percent slope. It appeared that he quickly picked up speed, the scooter flipped, and Harry hit the pavement head first. He was pronounced dead at the scene by paramedics.

The manufacturer's initial legal position was that there were no manufacturing or design defects in the equipment. Their explanation was that the old man simply was inattentive and recklessly drove the cart down the hill, causing it to overturn resulting in his death. There was even mention of the unopened six-pack of beer that he had just purchased at the grocery store. To change the focus of the case, counsel pled a "survival cause of action" which allows punitive damages to be recoverable. Decedent's heirs in a wrongful death claim cannot recover punitive damages. This legal position made the defendant's manufacturing methods relevant, and the speed of the free wheeling cart relevant to the lawsuit. Hence, recreation of and calculations of the speed of the out-of-control cart were required, as well as a thorough metallurgical analysis of the failed metal components.

Examination of the scooter showed two failures: (1) that the steering mechanism had broken free from the left front steering arm, allowing the wheel to be free to swivel. The result of the front wheel swiveling could explain the scooter flipping. A metallurgical examination of the aluminum steering mechanism showed a "Swisscheese" array of shrinkage holes in the aluminum casting that severely compromised its strength. The fracture surfaces were examined by Scanning Electron Microscope (SEM) and indicated fractures consistent with the steering mechanism failure preceding and causing the accident, rather than occurring as the result of the accident; and, (2) the metallurgical analysis indicated that the transmission components had also failed before the scooter flipped. This particular electric scooter had a regenerative brake attached to the motor, and the motor was in turn attached to a differential, and then the differential was attached to the rear axle. In the event of a failure anywhere between the motor and the rear axle, the scooter had no capability for braking. The analysis showed that the transmission components were defective and unsuitable for their intended use.

The accident sequence was determined to be that the scooter transmission failed first, causing braking system failure and rapid increase in speed. This was followed by the failure of the steering, causing the scooter to flip.

The legal issues in building the case based on the metallurgical findings, and the ultimate satisfactory resolution are presented.

Metallurgical Failure, Legal, Fatality

#### C23 Absorption Refrigerator Fires

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After attending this presentation, attendees will learn the characteristics and modes of failure that cause fires in absorption refrigerators used in the recreational vehicle industry.

This presentation will impact the forensic science community by aiding forensic engineers and fire investigators in the determination whether an absorption refrigerator failed and caused a fire.

Standard refrigerators use a basic process of thermodynamics whereby the cooling process is based on Charles' Law. Absorption refrigerators provide the cooling through evaporation so that the heat is carried away from one material to another that absorb the hot molecules. Absorption refrigerators send the refrigerant, commonly an ammonia product, into a hydrogen gas. As the ammonia evaporates in the presence of the hydrogen gas, the cooling effect is produced. This hydrogen ammonia gas passes through a water containment vessel, which absorbs the ammonia. The water ammonia solution is then heated, which boils the ammonia out of solution. The ammonia gas then condenses back into a liquid so that the liquid ammonia is then sent back through the hydrogen gas on the reduce. At room temperature, ammonia is generally a gas so that it needs to be pressurized in order to turn it into a liquid at room temperature. The typical pressure of the system of an absorption refrigerator is approximately 300 psi.

Sodium chromate is introduced into the closed system to serve as a corrosion inhibitor for the steel tubing and components of the system. Absorption refrigerators are popular in the recreational vehicle industry because the refrigeration cycle can be run through the introduction of electrical or gas heating to boil the ammonia out of solution. This feature allows the refrigerator to be used when electrical shore power is not available at a camp site or while the vehicle is in motion without the use of an electric generator to provide power to the refrigerator. This feature is especially advantageous at camp sites where the use of a generator is prohibited because of the noise and exhaust pollution that would be produced by a generator. The basic components of the absorption refrigerator include an absorber vessel, a liquid temperature exchanger, a boiler, a water separator, a condenser, an evaporator, a gas temperature exchanger, an absorber, and the requisite tubing to connect the components together in a closed cycle.

Attendees will gain experience in the determination of the common mode of failure of these refrigerators that produce fires. Additionally, a simple mode of testing will be discussed in order to determine whether the refrigerator failed and caused the fire.

Absorption Refrigerator, Sodium Chromate, Fire Dynamics

### C24 Failure Mode Analysis of Resistance Exercise Product Failure Resulting in Eye Injury

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The goal of this presentation is to illustrate how packaging constraints can cause permanent sets in latex tubing thereby shortening the life span of the product, leading to catastrophic failure.

This presentation will impact the forensic science community by illustrating how packaging constraints will have catastrophic effects on the life span and failure mode of some resistance fitness products.

**Resistance Band Overview:** Resistance bands and tubing are commonly used fitness products on the consumer market. Fitness routines and specific body movements are crafted to focus the workout of certain muscle groups and to enhance overall body strength for specific sporting activities. Resistance exercisers are commonly used for injury prevention and rehabilitation. The benefits of using resistance exercisers for strength training are compelling; they transport easily, take up far less space than a free-weight room, and are affordable compared to monthly gym memberships. The current study closely examines the failure of a resistance exerciser in which a teenager suffered a serious eye injury.

**Case Description:** The incident occurred at a family fitness center where stretch tubing fitness products were available to its members. A teenager was a member in the studio during a supervised exercise class when the injury occurred. The class instructor demonstrated the use of the resistance exerciser intended for the particular exercise before instructing the class to perform the exercise. The student was performing a leg extension exercise, holding the handle of one end of the exerciser while the student's foot was placed in the loop on the other end, and the exerciser was stretched to provide resistance on leg extension. During one of the repetitions the latex tubing broke and the broken end of the tubing struck the student's eye. The impact caused multiple injuries to the eye, including corneal abrasion, retinal detachment, and a torn retina. As a result of the injury, the student required four surgeries over two years. For unknown reasons, the evidence was discarded by the fitness center personnel.

The resistance exercise products used in the studio were of three distinct designs: a circular shape, or loop, with two foam handles, one opposing the other; a larger loop in a figure-eight configuration with foam handles at either end and third foam cylindrical retainer in the center; and a straight tube with handles at each end (photo 01 figure-eight). All three product designs are constructed of latex tubing, and are offered in five different colors. The tubing color denotes wall thickness, and resistance increases with wall thickness. As the user's muscle

strength improves, upgrading to a thicker tubing (different color) will correspondingly increase the resistance of the workout, much like adding more weight to a curling bar.



**Joint Description/ Failure Mode Hypothesis:** The loop and figure eight exercisers feature a joint. The joint does not use adhesive, but rather each end of the tubing is stretched over opposite ends of a solid rubber plug in the shape of a barrel (photo 02 tubing joint). First, one end of the tube is stretched over the plug, and then the other. The overlapping ends of tubing are secured only by friction between the layers. Since the evidence in this case was unavailable to ascertain the failure mode, failure of the joint was proposed as an initial hypothesis. To test this hypothesis, tension tests were conducted on new products to quantify joint design strength.



**Tension Testing:** Two new, figure-eight exercisers with the same tubing color (0.0625 inch wall thickness) as the one used by the victim were purchased directly from the product manufacturer. A long section of tubing containing the joint and another without the joint were sectioned from each sample. A total of four specimens were tested; two with the joint and two without. An Instron model 1123 tension-compression machine was used. The cut ends of the tubing were held using two inch diameter split-drum grips to reduce the chances of fixture induced stresses (photo 03 joint testing fixture). The crosshead speed (grip separation rate) was ten inches per minute, and data was sampled at 20 Hz.



The tubing specimens that did not contain a rubber plug joint were found to break at an average force of about 97 pounds with an average maximum elongation of about 19.0 inches (780%). The tubing specimens that included a joint were found to break at an average force of about 88 pounds with an average elongation of about 19.6 inches (803%). The test results revealed that the rubber plug joint was robust with regard to the test protocol and the tubing failure occurred near the grips. The test failure mode appeared to be induced surface tears resulting from friction where the tubing stretched around the grip or was allowed to contact the tubing secured in the grip's slot. With increased tension, these surface tears grew, causing complete transection of the tubing. The test protocol did not yield insight into the cause of failure of the incident product, because the test failure mode did not replicate the hypothesized failure mode at the joint.

**Inventory Inspection:** The fitness center resistance product inventory was categorized by design and color, and their storage methods were inspected. Loop, figure-eight and straight exercisers hung from rubber-coated hooks on a wall organizer inside the dojo. Following the inspection of the products actively used by gym members, an inspection of the discarded inventory was also undertaken.

Latex-based resistance exercisers degrade over time. At the time of inspection (2.5 years after the injury), much of the inventory had been taken out of use and maintained by the gym for inspection purposes. Inspection of the discarded inventory identified two areas of latex tubing degradation in the form of surface tears. First, tears were observed adjacent to the joint where the tubing ends were stretched over the rubber plug. The tears were observed to be pulled open by the inherent surface tension caused by the joint design. Second, tears were observed midlength in the tubing and with higher frequency than those found near the joint (photo 04 tubing tears). These mid-length tears were found to correspond to a permanent set. A permanent set in latex tubing is a defect that is permanent in nature and consists of a kink or fold in the tubing wall caused by bending the tubing below its designed bend radius and maintaining that bend long enough for the tubing to acquire a permanent set. It was hypothesized that these permanent sets causes the propagation of tears, and this was more likely the reason the exerciser broke when used by the victim.



The gym's resistance exerciser inventory was inspected again, this time focusing only on those products hanging on the wall organizer. Remarkably, permanent sets and associated micro-tearing was observed on numerous samples. Even the new products with manufacturing residue on the tubing, not yet removed by use, revealed permanent sets and signs of localized tearing.

**Product Packaging:** The packaging of the new exercise products purchased was evaluated. The exercisers are placed into compact clear plastic heat-sealed bags with dimensions that guaranteed folding or rolling the product in order to fit. The compact product packing caused permanent sets in the tubing. In fact, permanent sets were observed in the new exercisers inside unopened packages (photo 05 product packaging).



**Endurance Testing:** Endurance testing was designed to determine if micro-tears associated with permanent sets in the latex tubing would lead to failure of the resistance exerciser. Prior to testing, two specimens were aged in accordance with American Society for Testing and Materials (ASTM) D753-04, Standard Test Methods for Rubber - Deterioration in an air oven. The specimens, maintained in their original sealed bag with air holes, were independently aged at 70°F for 63 and 72 hours, respectively. After aging, the two specimens were inspected and no changes in the tubing were observed.

Each test specimen was then placed in a test device whereby the foam center was placed over a cylinder between two dynamic arms while the two handles were independently secured to fixed arms (photo 06 endurance testing fixture). The dynamic arm would rise approximately 15 inches and then return to the start position where the tubing would rest, thereby completing one cycle. The test and cycle was not intended to simulate any single particular exercise. Rather, the test stretched the exercise product over a modest distance.

In fact, the test did not stretch the product beyond what one would anticipate when adults use the figure-eight exerciser.



Without any tubing surface artifact, the figure-eight would be expected to eventually break after sufficient repetitive cycles of use, because each cycle will naturally cause a minute amount of degradation of tubing material.

Endurance testing was performed at a constant 0.1Hz, and continued until product failure. The first test specimen broke after 1,118 cycles, with complete tubing transection at the location of the deepest permanent set. Testing of the second test specimen was stopped after 2,498 cycles when a deep tear was observed to be forming at one of the permanent sets.

**Conclusion:** A resistance exercise product failed during use under foreseeable tension forces. Although the product that actually failed was not available for inspection, investigation supported the hypothesis that it failed due to weakness and tearing at a permanent set in the tubing.

The fitness center personnel were remiss in their inspection of the resistance bands products used by gym members.

A permanent set in the tubing is likely to cause incipient tearing that is not perceptible to the user who doesn't know what to look for

(including gym personnel or physical therapists). If a permanent set exists, the tubing will become progressively weaker at this location. The incipient micro-tears will after some period of use suddenly expand and lead to tubing failure.

It is critical to inspect tubing for permanent sets (under adequate light, using fingers to feel the tubing to detect small tears) before each use. Appropriate warning and instruction should be given to users. Because the risk of sudden failure may not be perceptible, these exercisers should be discarded before incipient tears are permitted to enlarge.

Different packaging is required. Aside from material uniformity (which may vary according to different product processes used by various latex tubing manufacturers), inadequate packaging is a cause of permanent sets in latex tubing. In addition, permanent sets create the risk of failure and injury.

Latex Tubing, Permanent Sets, Resistance Exercise Products

# **GENERAL**

**CHICAGO 2011** 



#### D1 Development of a Science Based Fingerprint Curriculum

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After attending this presentation, attendees will be introduced to a project that is developing a science-based curriculum addressing the recommendations set forth in the National Academy of Science (NAS) Report.

This presentation will impact the forensic science community by demonstrating the process of a first-principles approach to forensic fingerprint curriculum.

In order to fully understand and appreciate the study of friction ridge skin and its resultant impressions, it is necessary to have a basic understanding of the fundamental principles upon which this discipline rests. Forensic science can be traced back to the comparative methods proposed by Aristotle. The later writings of Georges Cuvier and Thomas Huxley further supported the utility of comparative methods in science. Ernst Mayr stated in his publication *The Growth of Biological Thought* that "the branches of science that depend on the comparative method are not inferior" to experimental methods (p. 32); however, he also stressed that scientific progress is made "with the introduction of new concepts or improved old ones" (p. 23). Friction skin formation has its foundations in anatomy, physiology, anthropology, and embryology; and through research has evolved to a source of personal identification using the comparative method.

The NAS Report suggested the need for an understanding of the "principles, practices, and contexts of science" (8-1) in conjunction with hands-on training that closely mimics the experiences of forensic practice. It is through "formal education, training, and the proper conduct of research" that the "scientific knowledge, principles, and practices of forensic science disciplines must be based" (8-1). Academic curricula guided by the requirements set forth by organizations such as SWGFAST and ASCLD-LAB are needed.

The goal of the fingerprint curriculum is to be comprehensive, covering core and discipline specific elements, and to include with each module teaching information, learning materials, practical exercises, and assessments. As the curriculum progresses, it will reference the first principles outlined in beginning sections and published materials to insure mastery and include frequent hands-on and interactive lessons. Module topics and lesson objectives will be available to the attendee for review. Once complete, the comprehensive curriculum will be available publicly at no cost for use by practitioners, educators, students, and trainees. The modules are designed to be used independently, in whole or in part, based on the instructors' goals.

The goal is to provide a course to be included in a forensic science program whose goal it is to produce forensic scientists well versed in science, the law, quality assurance procedures, research, and discipline specific information and techniques. Research has been conducted to gather information regarding curricula in other scientific disciplines, current fingerprint curricula, history of the comparative method, and fingerprint specific history, practice, and ongoing research. The following module topics will be included in the curriculum:

Module 1:	Science and First Principles
Module 2:	What is a Fingerprint: Anatomical, Physiological,
	Anthropological, and Embryological Considerations
Module 3:	History of Identification
Module 4:	Classification and Taxonomy
Module 5:	Forensic Science and Fingerprints
Module 6:	Fingerprint Detection, Visualization, and Preservation
Module 7:	Comparisons and Conclusions
Module 8:	Fingerprints and the Law
Module 9:	Research and Quality Assurance

Traditionally, fingerprint training has been based on historical or experiential curricula and materials (top-down) rather than from a fundamental starting point and building to actual practice (bottom-up). This new approach will first create an understanding of the science supporting the discipline followed by an incorporation of the practice. Education, Fingerprint, Curriculum

## D2 Incorporating Forensic Nursing Education Into Undergraduate Nursing Programs: A Simulation Approach

#### Stacy A. Drake, MSN, MPH\*, 6510 Acorn Court, Pearland, TX 77584

After attending this presentation, attendees will have a blueprint for integrating forensic knowledge in nursing education or other health care curriculums. The presentation embraces the premise that forensic nursing should be included in the Bachelor of Science in nursing curriculum because of the potential impact on the forensic community and the public health care system.

This presentation will impact the forensic science community and public health care system by serving as an example of how forensic nursing content can be included within undergraduate nursing curriculums. The examples provided include knowledge of forensic nursing as well as permitting the student to synthesize this information via simulation of forensic cases.

Because of the national nursing shortage, nursing education nationwide is pared to the essentials required by the state boards of nursing and accreditation agencies; programs are accelerated leaving no room for alternate tracks or electives. However, given that nursing students in their clinical rotations will inevitably be exposed to wound care, family dynamics, pediatric and elder patients suffering from abuse, and like situations, scenarios that deal with forensic nursing principles can be built into existing courses. This permits students and new graduates to expand their nursing competencies. With this added information, students can assist in recognizing and documenting injury patterns, identifying and preserving gunshot residue, or implementing policies to preserve biological specimens in case of suspected toxicological death.

As a growing number of nursing programs and hospital orientation programs employ simulations in training, scenarios can focus on the role of the nurse in a forensic application. In this era of cost consciousness in the hospital setting, the nurse is often the most logical person or the first line of opportunity for documenting and preserving forensic evidence. In fact, the nurse may be the only person available for this documentation. This type of role responsibility is portrayed in the simulation. Thus the student nurses learn practical implementation of forensic science in an intra-professional setting as they also learn wound care and other medical-surgical procedures. These simulations are distributed throughout the three to four semesters of nursing education, beginning with physical assessment courses and continuing through senior level courses where students learn to deal with death and dying and patient care in hospital emergency and other high acuity settings. In the last semester of training, seminars focus on forensic knowledge that permit attendees to learn about recognizing, collecting, preserving, and documenting evidence; supporting sexual abuse survivors; and working with forensic pathologists; medicolegal death investigators; and law enforcement personnel. When the simulation scenarios and simulations are pulled together in a package, they also provide continuing education units to Registered Nurses (RN). In addition, this focus on forensic nursing through simulation provides basic training for RNs needing certification that will enable them to actively participate in a forensic nursing team. The presentation will include our initial experiences with this curriculum and some early outcome data.

Forensic Science, Forensic Nursing, Undergraduate Nursing Curriculum

#### D3 The Need for Mandatory Continuing Education for Forensic Science Professionals

Lindsey E. Crass, BS\*, Forensic Science Initiative, 1600 University Avenue, 208 Ogelbay Hall, Box 6217, Morgantown, WV 26506

After attending this presentation, attendees will understand the need for mandatory continuing education requirements for any person practicing in the field of forensic science.

This presentation will impact the forensic science community by highlighting the importance of continuing education for all forensic science practitioners.

Other professions such as doctors and lawyers have continuing education requirements that are mandatory in order to continue practicing in the field. The information presented will show that continuing education should be mandatory in the forensic field like other professions because the level of knowledge across the different disciplines is ranging due to the lack of continuing education available. Some continuing education for forensic professionals is currently offered, yet the cost and availability may cause some professionals to not be able to attend

Based on data gathered from over 1,000 forensic practitioners at four training programs over three years, the continuing professional education needs of the forensic industry are going unmet. The forensic science community is interested in gaining more knowledge and training, but opportunities for this education vary greatly. For example, some professionals were able to attend five different training programs in one year while others were only able to attend one; this does not account, of course, for those who were not able to attend any. Also, professionals had to use personal time off or personal funds to attend continuing education opportunities. Gathering data from professionals employed by agencies with differing training resources was important because the data reflects the range of continuing education courses being received by forensic professionals and how attendance was funded for these and other programs.

In the National Academy of Science report, *Strengthening Forensic Science in the United States: A Path Forward*, the need for forensic science continuing education was emphatic. No mandatory standardized continuing education requirements exist in the forensic industry. Although, individual certifying or accrediting bodies may require continuing education, these are not universally accepted, even within specific disciplines. If all forensic scientists were required to gain a certification or license in order to practice their profession, mandatory continuing education should be part of the certification or license renewal process.

Mandatory continuing education for professionals is achievable; doctors, teachers, and lawyers are required to obtain continuing education to retain their licenses or certification. Continuing education requirements vary by state but some are national. The same could hold true for forensic professionals. As a science-based industry, the continuing development and attainment of knowledge and skills is critical to assure forensic work is being performed correctly and accurately using validated techniques and standards. By requiring all forensic scientists to gain a set number of hours of continuing education every year, for example, the entire field of forensic science would be strengthened.

After compiling the data from four continuing education programs, reviewing other professional groups' continuing education requirements and reviewing *Strengthening Forensic Science in the United States: A Path Forward*, the need for mandatory forensic science continuing education is clear. By pursuing mandatory continuing education, professionals need to agree on what requirements are most important to the field. With industry consensus, policies by local and national agencies could follow the guidelines and funding agencies could then assist with sponsorship and resources.

**Continuing Education, Training, Professional Development** 

## D4 Determination of Animal Law Enforcement's Capability to Conduct and Manage a Dog-Fighting Investigation and Recommendations for Identified Deficiencies

Dena M. Mangiamele, DVM\*, Animal Legal & Veterinary Medical Consulting Service, Inc., 10725 Atrium Drive, San Diego, CA 92131

After attending this presentation, attendees will learn about the origins of the blood sport termed dog fighting, learn that successful prosecution of these cases is important because commission of this felony crime has been proven to be a precursor to more serious crimes, understand the current status of animal law enforcement's capability to conduct and manage a dog fighting investigation, and be provided with creative recommendations for identified deficiencies in investigative practice and management of dog fighting cases that can be useful to animal law enforcement, animal care staff, and prosecutors.

This presentation will impact the forensic science community by emphasizing the importance of successful investigative practices and case presentation of the felony crime of dog fighting (animal cruelty). It is crucial that all law enforcement and animal sheltering agencies investigating these cases are knowledgeable and well trained in up-todate forensic techniques in order to successfully prosecute these cases which could potentially prevent future progressive adult violence.

This paper answers the question, "How prepared is animal law enforcement to conduct and manage a dog-fighting investigation?" A Knowledge/Attitudes/Practices survey was developed and administered to national dog-fighting experts who have past experience working with animal law enforcement in the capacity of field investigators, specialty shelter veterinarians, and local prosecutors and ranked animal law enforcement's capability to conduct and manage a dog-fighting investigation. The survey questions explored three main areas: (1) preparation prior to crime scene investigation; (2) techniques of crime scene investigation; and, (3) post-investigation case management. Each area focused on specific practices and skill sets. Experts categorized these skill sets in a 1-5 ranking, with 1 indicating total competency in that area. The analysis of all expert responses revealed that animal law enforcement agencies have deficiencies in each of the three phases of the investigative process.

Research was conducted on the availability of advanced training and continuing education opportunities for animal law enforcement in dog fighting investigative skills and case management. It was discovered that there were few opportunities for officers in this specialty. As a result of these findings, the most effective recommendation to improve investigative techniques performed by animal law enforcement in dog-fighting cases would be to develop and distribute a Dog-fighting Investigation Manual for Animal Law Enforcement as the training tool of choice. Supplemental to the manual, instructional videos should be created and used in training conferences that could be organized and scheduled throughout the country.

Survey findings also indicated that collaboration among agencies is a key factor to successful prosecution. With this in mind, the recommendation of a written manual and training conferences becomes even more effective when it is structured to integrate local law enforcement, animal care staff, veterinarians, and prosecutors.

At the end of the presentation, examples of the current progress of implementation of the recommendations will be shared with the attendees. Instructional videos in dog fighting investigation will be viewed in correlation with the investigative manual manuscript that is currently being reviewed for publication.

Dog, Fighting, Investigation

#### D5 Characterization and Testing of Canine Training Aids for Forensic Victim Recovery Investigations

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After attending this presentation, attendees will have a better understanding of the challenges facing forensic canine specialists in today's world. There are several key challenges that need to be addressed, particularly the consistent or appropriate training of victim recovery canines across the community. If canines can be trained using a common training aid, the overall consistency of results should improve.

This presentation will impact the forensic science community by expanding the core group of people aware of these issues, which in turn may lead to faster and/or better research and development to address these key issues. Of equal importance is the benefit of having well trained, efficient canines that are consistently able to locate clandestine burials (i.e., human remains) and provide case related information, as well as some measure of closure for the families of these victims.

The use of canines in law enforcement and military applications is well-known. Canines are used to screen for drugs and explosives, to locate missing persons, to associate crime scene evidence with a suspect, and to locate victims of violent crimes. To do this, these canines require extensive training and conditioning. This training includes many facets, one of the most important of which is the use of training aids. In most of the situations listed above, standard training aids have been developed that can be used by the canine community and these training aids have been proven very successful for training canines. Of the group listed above, training aids for locating clandestine burials is of keen interest. The community of so-called victim recovery canines is quite diverse and not very well organized and training aids can vary widely. Some handlers acquire human tissue, human bone, human blood, "other"

For a training aid to be effective, it must very closely simulate the actual material that the canine is attempting to locate. In the case of human remains, this is not a simple task. There is some research that describes what volatile compounds have been found emanating from human remains. However, there are a number of factors that influence what compounds might be detected at the site of human remains: time since death, disposition of the body (surface, buried, or submerged), environmental factors, influence of scavengers, etc. As such, developing a training aid that can account for all of these variables is quite challenging. It is also possible that one training aid will not be sufficient to address all of these variables and canine handlers will have to use training aids that target the general conditions in which they find themselves at the time of their search. This presentation describes the characterization of commercially available training aids and the initial attempts at developing a training aid based upon current research activities in our laboratory.

Several different analytical approaches have been utilized to characterize the commercially available training aid formulations studied here. The basic approach involves collecting air samples from the training aids and analyzing those samples using gas chromatography with mass spectrometric detection (GC/MS). Air samples are collected primarily because they are the most relevant to canines. In this research, air samples have been collected and analyzed using thermal desorption methods, cryogenic methods, and solid phase microextraction (SPME). Preliminary results show how commercial training aids contain very few (i.e., <3) of the chemical signatures previously found to be associated with buried human remains. For the development of a new training aid, data from past research was utilized that identified 33 headspace components found above the sites of buried human remains. Mixtures of these compounds were then prepared and tested using canines to determine if positive responses could be achieved.

Canine, Clandestine, Burial

## D6 Blunt Force Trauma Patterns in Suspected Animal Abuse Cases

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After attending this presentation, attendees will gain a better understanding of the value of forensic anthropological assessments in investigations of animal abuse. The goals of this presentation are to highlight blunt force trauma fracture patterns among canid skeletons and to discuss the value of detailed trauma assessment in animal abuse investigations.

This presentation will impact the forensic science community by providing awareness of perimortem trauma patterns from suspected animal abuse cases, and will highlight novel areas where forensic anthropologists can contribute to medico-legal investigations.

In October of 2009, the California State University, Chico Human Identification Laboratory (CSUC-HIL) was consulted by law enforcement to search the side yard of a private residence in northern California. The property contained the remains of several pets suspected to have died under suspicious circumstances. The recovery team located three shallow gravesites and excavated the remains of four domestic canids (*Canis familiaris*). The decomposed remains were transported to the CSUC-HIL for inventory and analysis. Investigators provided

antemortem documentation to assist in individuating each canid, which was confirmed through assessment of sex (e.g., presence/absence of a baculum), age (epiphyseal union, dental development, dental attrition), bone size, craniofacial morphology, and fur coloration pattern.

The remains were photo-documented prior to the removal of adhering soft tissue and fur through maceration. Each skeleton was laid out in anatomical position and analyzed with the assistance of two zoo archaeologists and a veterinary pathologist. The presence/absence of a baculum identified two of the canids as male and two as female. Three of the canids were skeletally mature and one was juvenile based on dental eruption and epiphyseal union sequences. These findings are consistent with antemortem records and witness statements provided to law enforcement. A detailed trauma assessment indentified perimortem blunt force trauma (BFT) on all four canids.

Canid #1 shows evidence of BFT on the craniofacial skeleton, thorax, vertebral column, and metacarpals. On the skull, there is a circular depressed fracture along the midline at the intersection of the parietals and the occipital. A radiating fracture propagates from this impact site along the sagittal suture, and then terminates on the left side of the frontal. The left hemi-mandible shows evidence of a "butterfly" fracture on the medial aspect. Peri-mortem fractures were observed on several ribs, a spinous process of a vertebra, and on left metacarpals III-V. Well-healed fracture calluses were also observed on five left ribs.

Canid #2 shows evidence of BFT on the craniofacial skeleton, the thorax, the vertebral column, and the pelvis. Both zygomatics exhibit peri-mortem fractures. BFT of the nasopalatal region is associated with a linear radiating fracture that propagates into the lateral squama of the left parietal, terminating at the temporal line (Impact #1). In addition, there is a fracture to the left side of the cranium, which displaced bone endocranially (Impact #2). There are additional peri-mortem fractures of multiple ribs, one vertebral spinous process, and the left publis.

Canid #3 shows evidence of BFT of the pelvis. The innominate is fractured along the midline (at the pubic symphysis), and a fracture propagates into the dorsal aspect of the left iliopubic ramus. No other evidence of trauma was observed.

Canid #4 shows BFT of the left thorax, which involved three ribs. No other evidence of trauma was observed.

Although all four canids exhibited BFT, the pattern of involvement varied substantially. Canids #1 and #2 showed extensive trauma to the head, including depressed cranial vault fractures, as well as numerous rib fractures. Appendicular fractures were only observed on the left metacarpals of Canid #1. Canid #3 and #4 showed less traumatic involvement, represented by pelvic and rib fractures, respectively. In summary, the distribution of BFT is consistent with reported cases of animal abuse. Injuries to canids commonly involve the craniofacial region and the thorax and pelvic area, and less commonly occur on the appendicular skeleton.

Forensic anthropologists are uniquely suited to assist with animal abuse investigations because of their advanced knowledge of skeletal anatomy and ability to analyze traumata on the skeleton. However, trauma assessments of non-human animal skeletons provide unique challenges, such as morphological differences in anatomical features, inter-specific variation in areas of buttressing, and differences in bone density. A multidisciplinary approach involving expertise from veterinary pathology and zooarchaeology is essential for accurate reconstruction of trauma to the nonhuman skeleton. These case studies highlight new areas where forensic anthropologists can contribute to medico-legal investigations.

Animal Abuse, Blunt Force Trauma, Forensic Anthropology

## D7 Advancing Fire Debris Analysis through Chemometrics: An Overview of Research at the National Center for Forensic Science

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The goal of this presentation is to describe the chemometric methods currently being studied at the National Center for Forensic Science for the analysis of fire debris data and to show how the methods can assist the analyst in their casework.

This presentation will impact the forensic science community by raising the awareness of research efforts to introduce new mathematical approaches to the analysis of fire debris which facilitate the detection of ignitable liquids in the presence of interfering pyrolysis products, allow for rapid searches of ignitable liquid and pyrolysis product libraries, and support the use of probabilistic classifiers.

Current practice in fire debris analysis often relies on ASTM-E 1618-06, which defines a standard method for ignitable liquid residues analysis and classification in extracts from fire debris samples using gas chromatography-mass spectrometry. Identification of the presence of ignitable liquid residues is accomplished by the analysts' recognition of chromatographic profile patterns and target analytes. Laboratory-to-laboratory variations in chromatographic conditions can complicate the automated search of large databases and libraries that are shared between laboratories. Covariance maps of the chromatographic data and the total ion spectrum (TIS) are chromatography-invariant for a given set of analytes and therefore form the basis of robust pattern recognition and classification methods which allow spectral libraries to be searched for the identification of an unknown.

Ignitable liquid identification challenges resulting from sample contamination with pyrolysis products can be overcome by target factor analysis of a set of spectra, each containing differing contributions from ignitable liquid residues and pyrolysis products. Target factor analysis reduces the dimensionality of the dataset, producing a set of latent variables that do not necessarily resemble the spectrum of a single component in the fire debris. The abstract solutions can be tested one at a time to determine if an oblique rotation (to an angle other than 90° with respect to the latent variables) can lead to a vector that correlates strongly with a known spectrum obtained from a library. When this procedure is applied to a set of spectra of known ASTM classification comprising a library, the resulting correlations allow for the use of several probabilistic classification techniques to identify classes of ignitable liquids potentially present in the samples.

Analysis of a large set of total ion spectra from ignitable liquids (greater than 450 samples) and a set of spectra from pyrolysis products (greater than 50 common building materials) has demonstrated that the data from each set can be independently modeled and new samples correctly classified with a high degree of accuracy as either resulting from an ignitable liquid or a substrate. Further classification of ignitable liquid data to the correct ASTM class is less accurate and the correct classification rate is significantly reduced when the miscellaneous and oxygenate classes are included. The use of other chemometric methods for the implementation of hard and soft classifiers will also be discussed

The chemometric methods are applied to datasets resulting from laboratory burns, large scale burns of furnished 20'x8'x8' containers designed to replicate structure fires, and from libraries of hundreds of commercially available products that are highly flammable. This presentation will provide detailed information about ongoing research conducted at the National Center for Forensic Science. Large scale burns were performed in collaboration with the Bureau of Fire Standards and Training, Ocala, FL, and the Bureau of Forensic Fire and Explosives Analysis, Tallahassee, FL.

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 U.S. Government and no official endorsement should be inferred.

Chemometrics, Fire Debris, Factor Analysis

#### D8 Dating Spores With the Carbon-14 Bomb Pulse

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After attending this presentation, attendees will understand how biological materials produced in the past 55 years can be dated using the carbon-14 ( $^{14}$ C) bomb-pulse. Attendees will learn how the carbon-14 ( $^{14}$ C) content of biomolecules serves as a chronometer of synthesis between 1955 and the present.

The presentation will impact the forensic science community by showing how a recently produced bioagent can be distinguished from one drawn from a historical archive.

Investigators of bioagent incidents or interdicted materials need validated, independent analytical methods that will allow them to distinguish a recently made bioagent sample from material drawn from the archives of a historical program. Accelerator mass spectrometry (AMS) precisely measures <sup>14</sup>C/C concentrations in biological materials and has been used to date the synthesis of biomaterials over the bomb pulse era (1955 to present), fulfilling the law enforcement need to place bioagents in a chronological context.

Atmospheric testing of nuclear weapons during the 1950s and early 1960s doubled the concentration of <sup>14</sup>C in the atmosphere. After cessation of atmospheric tests in 1963, the level of <sup>14</sup>CO<sub>2</sub> has decreased with a mean life of about 16 years, not due to radioactive decay, but due to mixing with large marine and terrestrial carbon reservoirs. The temporal variations of artificially high levels of atmospheric <sup>14</sup>C have been captured in organic material world-wide and thus offer an opportunity to determine a date of synthesis for biomolecules. Since <sup>14</sup>C is incorporated into all living things, this bomb-pulse is an isotopic chronometer of the past 55 years. The enhanced level of <sup>14</sup>C worked its way up the food chain from CO<sub>2</sub> so that all living things were labeled with the pulse.

The concentration of  ${}^{14}$ C/C was measured in a variety of media, *bacillus* spores, and separated proteins from *bacillus* spores. Bacteria convert the carbon in their food sources into the biomolecules they need, just like plants and animals. The  ${}^{14}$ C concentration of *Bacillus* spores reflects the radiocarbon content of the media in which they were grown. The incorporation of the food source isotopic signature occurs if the media is primarily carbohydrate (e.g., high glucose), primarily protein derived (excess nitrogen), or a blend. In a survey of commercial media we found that the  ${}^{14}$ C concentration indicated that carbon sources for the media were alive within about a year of the date of manufacture and of terrestrial origin. Hence, bacteria and their products can be dated using their  ${}^{14}$ C signature.

*Bacillus* spore samples (BSL1, biosafety level 1) were obtained from the LLNL archive as well as generated on site. The standards were

*B. thuringiensis kenyae* spores (Bt ken, control spores) generated onsite with defined media and carbon free purification; The test samples include *B. thuringiensis israelensis* (Bti), *B. globigii* (Bg), and *B. thuringiensis kurstaki* (Btk) from the LLNL archive. The archive spores were produced and purified by means unknown to the researcher performing the extraction, in order to mimic real world specimens. The archived spores were contaminated with petroleum-derived carbon from solvents and detergents used during processing. Using a mechanical lyser and a variety of washes with carbon free KOH, HCl, and HOOH, contaminant carbon was removed from soluble proteins. Samples were dried and combusted to  $CO_2$ . The evolved  $CO_2$  was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors. Graphite targets were measured for <sup>14</sup>C content by accelerator mass spectrometry.

Soluble proteins were purified sufficiently for accurate <sup>14</sup>C bombpulse dating. The insoluble fractions could not be cleaned using our procedures. Since media is contemporary, <sup>14</sup>C bomb-pulse dating of isolated soluble proteins can be used to distinguish between historical archives of bioagents and those recently produced.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory. Bomb Pulse Dating, *Bacillus* Spores, Protein

#### D9 The Topic of Anchoring When Determining Likelihood Ratios in Fingerprint Comparison

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After attending this presentation, attendees will understand the importance of correct conditioning when determining likelihood ratios in fingerprint comparison.

This presentation will impact the forensic science community by illustrating the importance of correct conditioning when determining likelihood ratios in fingerprint comparison.

Following recent challenges in court on fingerprint evidence evaluation, statistical research on this topic has become more and more important. The question is how to evaluate the strength of evidence for the similarity of high-quality, rolled fingerprints, and low-quality fingermarks, which may be distorted, partial, or smudged. The similarity is based on the comparison of discrete characteristics (such as general pattern of prints and marks) and continuous characteristics (such as the minutiae, level II characteristics of the finger denoting locations and orientations of ridge endings and bifurcations). The Likelihood Ratio, or LR, is a statistical measure of the strength of the similarity, defined as:

 $LR = Pr(E \mid H_p, I)/Pr(E \mid H_d, I).$ 

Here Pr(.|.) indicates the conditional probability of an event, *E* the evidence (some expression of the similarity between marks and prints),  $H_p$  the hypothesis that the mark and print have a common origin,  $H_d$  the hypothesis that this is not the case, and *I* all relevant background information (such as tactical information). Evaluation by LRs yields an objective measure of the strength of evidence, as opposed to assessment by expert opinion (which is basically subjective). In the current study, the focus is on the impact of different ways of conditioning numerator and denominator of the LR on the numerical result.

In two papers by Neumann et al (J.For.Sci., 2006, p.1255-66, 2007, p.54-64), an analysis is given for LR computations based on the general pattern of print and mark, the number of minutiae on the mark, and the similarity of the minutiae configurations. A Euclidian distance is used to quantify similarity of the minutiae configurations. In Egli et al

(For.Sci.Int., 2007, p.189-95), the similarity between marks and prints is determined by an automated fingerprint identification system (AFIS), and LRs are evaluated based on prints made by one fixed thumb. Moreover, a simulated case study is performed with a fingermark containing 10 minutiae, leading to an LR of 85.6 million. This finding indicates that high LR values can be attained for fingermarks that in most countries would be unusable in court since the number of minutiae is too low.

For forensic application, it is important to know whether the reported LRs are accurate and robust. It turns out that within-source variability of similarity scores may differ for different fingers, even from the same donor. Next to this, the distribution of between-source similarity scores may depend on the fact whether a fixed mark is compared to a non-matching database of prints, whether several marks of the same finger are compared to one fixed fingerprint, or to several marks of different fingers, etcetera. The above is closely related to the topic of "anchoring" of evidence (which can be suspect anchored, crime scene anchored, or not anchored at all). In Neumann et al, it is unclear what conditioning is used to obtain distribution for the within - and between - source variability. In Egli et al, both in the numerator and the denominator the LR is conditioned on the number of minutiae of the mark. However, the conditioning is asymmetric in the sense that for the within-source variability comparisons are used of multiple marks with multiple prints of a fixed finger, whereas for the between-source variability comparisons are used of a fixed mark with multiple prints of databases of non-matching prints.

The results of the current study, which uses similarity scores generated by an AFIS system, show that there is a considerable effect of both the particular finger, and of any particular reference print used on the distribution of within-source similarity scores. This illustrates that there is a choice to condition either on a particular reference print of the suspect, or on several reference prints. Whatever the choice is though, it has to be used both for the numerator and denominator of the LR formula. The resulting LRs may be quite different from the ones obtained when using non-symmetric conditioning in numerator and denominator of the LR formula.

Bayesian Approach, Anchoring, Fingerprint Comparison

### D10 Blended Learning – An Effective Approach to Training for Forensic Science Disciplines

Kevin Lothridge, MSM, Eileen M. Fynan, BA\*, and Jane Smith, BS, National Forensic Science Technology Center (NFSTC), 7881 114 Avenue North, Largo, FL 33773

After attending this presentation, attendees will understand the blended learning approach to forensic training and the benefits of this model, such as reducing costs and reaching more practitioners with state-of-the-art training.

This presentation will impact the forensic science community by describing how the blended learning model is a highly effective approach to forensic science training. The online environment provides many benefits to stakeholders, including best value for training delivery, collaborative learning environments, consistent content, secure access and randomized testing capabilities. In addition, robust reporting features provide comprehensive reports on performance metrics. When combined with the hands-on, scenario-based activities in the classroom, this approach to training can be a highly cost effective alternative that promotes peer interaction and produces well-trained, highly proficient forensic practitioners.

Reduction in training budgets requires agencies to carefully consider options that maximize training opportunities while minimizing cost. This presentation will provide information on the blended learning approach to forensic science training and the benefits of this model, such as reducing costs and reaching more practitioners with state-of-the-art training.

From training members of the U.S. military to preparing forensic practitioners who work in publicly funded crime laboratories and law enforcement agencies, the National Forensic Science Technology Center (NFSTC) educates professionals on the front lines of ensuring public safety. To broaden the availability and reduce the cost of training, the NFSTC often uses a blended learning model that combines web-based distance learning with onsite instruction and hands-on activities. The distance learning component maximizes class preparation, assessment activities and learning results, while the complementary classroombased training uses realistic scenarios that allow trainees to practice their skills in real-world situations.

The NFSTC maintains a web-based Online Learning System (NOLS) that supports blended learning by serving as a virtual learning community that includes program information, course content, resources, discussion forums, communication tools, surveys and autograded testing. Before and after each classroom session, participants complete online work through NOLS to master basic subject area knowledge or to reinforce and apply skills learned through classroom instruction, discussions, demonstrations and hands-on activities.

NFSTC's delivery of two *Latent Print (LP) Examiner Training* programs provides an example of forensic science training that was developed using the blended learning model. Offered at no cost to entry-level examiners, the selection process includes an online interactive visual acuity test. The program combines classroom training, online distance learning and practical exercises and is designed to help prepare trainees to successfully meet the challenges of certification examinations provided by the International Association for Identification (IAI).

This comprehensive, 11-course *Latent Print Examiner Training* program provides each trainee with more than 380 hours of classroombased training over an 8-month timeframe. An additional 6 to 10 hours of online pre- and post-coursework is assigned for each of the 11 courses, providing an average of 88 hours of web- and practical-based instruction. Five 2-week classroom-based sessions are held at the NFSTC facility in Largo, FL. Between sessions, trainees complete online reading assignments, exercises and assessments in preparation for the next classroom-based training session. During the two years that these programs have been offered, the NFSTC has trained a total of 33 practitioners in the latent print examination discipline.

The most recent graduating class achieved average grades of 97.25% on individual course assessments and 93.78% on the comprehensive program assessment. In a three-part mock certification exam, grades averaged 98.4 %, 86.4% and 76.8% for each individual component. Trainees also participated in a video-captured moot courtroom testimony experience.

The blended learning model is a highly effective approach to forensic science training. The online environment provides many benefits to stakeholders, including best value for training delivery, collaborative learning environments, consistent content, secure access and randomized testing capabilities. In addition, robust reporting features provide comprehensive reports on performance metrics. When combined with the hands-on, scenario-based activities in the classroom, this approach to training can be a highly cost effective alternative that promotes peer interaction and produces well-trained, highly proficient forensic practitioners.

Distance Learning, Forensic Training, Blended Learning

D11 A Forum on Forensic Science Education: Do University Forensic Science Education Programs Meet the Needs of Forensic Laboratories and How Can Forensic Laboratories and Universities Work Together to Improve Forensic Science Education and Practice?

Scott Stoeffler, MSFC\*, McCrone Associates, 850 Pasquinelli Drive, Westmont, IL 60559; and Todd Zdorkowski, MS, Midwest Forensics Resource Center, United States Department of Energy Ames Laboratory, Ames, IA 50011

After attending this presentation, attendees will be informed of the issues discussed during a recent forum on the collaboration between crime laboratories and university forensic science education programs.

This presentation will impact the forensic science community by expanding the awareness of forensic science educators and crime laboratory managers and bench workers of different ways in which academia and the working professional community can interact and collaborate to improve the education of forensic scientists and the quality of work done in the forensic science field.

The Midwest Association of Forensic Scientists and the Midwest Forensics Resource Center held a forum on forensic science education, June 15-17, 2010 in Indianapolis, Indiana. It asked: "Are university forensic science education programs meeting the needs of forensic science laboratories?" and "How can forensic laboratories and universities work together to improve forensic science education and practice?"

The Forum's discussion-leaders included:

- Forensic laboratory directors.
- Recent graduates of university forensic science programs working in forensic laboratories.
- Forensic scientists employed in crime laboratories who also instruct in university programs.
- University administrators, instructors and researchers.

Nineteen of the forty participants were from municipal, county, state, and federal forensic laboratories and twenty-one were from university forensic science programs.

Forum discussion was frank. Afterwards, participating educators reported making curriculum and instruction changes on the basis of forum discussion, and participating forensic laboratory administrators reported new collaborations with university forensic education programs. The dominant suggestion in the participant-evaluations was to establish a continuing forum.

The forum consisted of sessions during which three-member panels (each panel representing a participant group) addressed the topicquestion from experience. Each 45-minute stimulus-session was followed by a 45-minute interactive discussion. Panels and discussions on the final morning addressed the potential for collaboration in curriculum improvement and forensic science research. For example:

Participating forensic laboratory directors expressed concern that college and university forensic science education credentials do not help when make hiring decisions.

One laboratory director participant asked the forum to consider the situation of a forensic manager seeking to hire a recent university graduate. He or she wants a candidate with several general characteristics:

- Good writing and speaking skills.
- Logic and reasoning skills.
- Ethics and morals.
- Inquisitiveness.

• A bachelor's degree (indicating significant laboratory skill and experience, a grasp of science and scientific method, and topical knowledge in chemistry or another lab science)

The participant reported investing significant time and effort to assess points 1 through 4 directly (because university credentials do not/cannot document these effectively). It was also reported the investment of significant time and effort in assessment of university degrees.

This participant stated that the proliferation of forensic science programs complicates the hiring process with its plethora of "qualifications" which was illustrated with excerpts from applications that documented over 60 different forms of university qualification, including: forensic science workshops, training, certificates, institutional awards, associate's degrees, bachelor's degrees, master's degrees and doctorates.

Forensic scientists who also instruct in university education programs, expressed concern that college and university forensic science education programs ignore critical components of forensic practice.

One panelist noted that universities seem more-oriented toward forensic programs that correspond to their already-existing university departments (like DNA and forensic chemistry) than to other forensic disciplines, like trace evidence, firearms and tool marks, impression evidence, questioned documents, photography, and/or digital evidence.

This presenter suggested that university programs and their graduates could benefit from a Courtroom-testimony-cum-public speaking course positioned early in the curriculum. He also proposed a class addressing the scientific method in forensic science.

A second panelist noted that some university programs fail to meet routine ethical standards. The panelist documented cases in which a university forensic science program was reluctant to fail students on tests and from classes in which they were caught cheating. Several more-serious ethical lapses were also documented.

Recent graduates of university forensic science programs now working in forensic laboratories said that university forensic science education programs failed to address knowledge critical to their work.

One participant had graduated from an ASC-accredited BS chemistry program with a forensic science emphasis. He described his academic program as heavy on theory and basic science. He noted that his daily on-the-job challenge was to apply science to specific cases and said that he had not practiced the application of science at the university.

- He also felt his university education had not adequately addressed: • Scientific quality assurance
- Forensic ethics
- Reproducibility
- Writing skills
- Verbal communication skills

This speaker was being trained as a firearms examiner, and further noted that firearms, fingerprints, footwear, tire tracks, pattern matching, and trace evidence were largely absent from his university and undergraduate program.

Administrators and staff from forensic science education programs described their programs and their response to university demands in the development and administration of them.

Several speakers described how they have structured programs in response to interactions with crime laboratory directors and staff, and the work they do to systematically implant forensic content into existing university classes and programs. However most said that laboratory concerns were secondary to traditional university curriculum development issues.

In response to laboratory requests for assistance in protocol development and validation studies, several mentioned that these forms of research do not really contribute to faculty promotion or tenure, and are not considered sufficient for master's degree projects. In response to requests for fundamental research into forensic science, one noted that these are not commonly funded; that they may take many years to complete; that the academic audience for such studies is restricted, and that few upper tier journals are oriented to the publication of such studies.

Education, Laboratory, Collaboration

### D12 Genetic Influences on Count and Distribution of Forks and Ridge Endings in Fingerprints

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The goal of this presentation is to investigate whether fingerprint minutia, a type of detail used in fingerprint identification, is influenced by heredity.

This presentation will impact the forensic science community by contributing to the understanding of fingerprint uniqueness.

This research is accomplished by comparing the number and distribution of forks and ridge endings (the two most fundamental types of minutia) in the fingerprints of 151 biologically related British adults from eight different families to those of 304 unrelated British adults.

Several prior studies have suggested a strong genetic component to the number of minutiae in fingerprints, as evidenced by strong correlation coefficients between family members and monozygotic twins. The relationship between heredity and minutiae distribution has been largely unexplored.

In this study, fingerprints were collected either by rolling, inking, and printing on high quality white printer paper, or by using black powder and white adhesive labels affixed to a plastic transparent sheet. The prints were then scanned into digital format at 12,000 dots per inch resolution.

The portion of the fingerprint examined consisted of a square grid positioned over the center, or core, of the fingerprint pattern, which was further subdivided into four quadrants. First, the core of the print was marked. Next, a gridline distanced 10 transecting ridges from the core was placed. That distance was then used as the basis for a square grid placed over the fingerprint to define the sample area. The number of ridge endings and of forks occurring in each quadrant of the grid was recorded for each fingerprint.

Chi-square tests comparing the variation in total minutiae for the 8 family groups were statistically significant (p<0.001) (expected values were calculated from the control sample). Analysis of variance tests comparing the number of minutia occurring in each of the four quadrants in the eight families to the quadrant data for subsets of the control sample was not statistically significant, either when the minutiae counts were considered as a whole, or when forks and ridge endings were considered separately. No individual in the study presented less than one fork, less than one ridge ending or fewer than four total minutiae in the central portion of their fingerprints.

The results of this study indicate a genetic influence on the number of forks and ridge endings in fingerprints, though not necessarily on their distribution. There is more similarity in the number of minutiae occurring in the fingerprints of individuals from the same family than would be expected if compared to an unrelated individual. This study found no familial correlation in the distribution of minutiae, though distribution was examined only narrowly in this study, in terms of quadrants of the fingerprint, but not in terms of proximity to the core or to other minutiae for example. Also of note, is the finding that the fingerprints of adult individuals display a minimum number of forks and ridge endings.

The validity and reliability of fingerprint identification in the United States, when carried out by a qualified latent print examiner is largely unquestioned, though it has occasionally been challenged under the *Daubert* standard. Despite the wide acceptance of identification from fingerprints, the ability to quantify fingerprint uniqueness would still be useful, for example in calculating a minimum number of corresponding points necessary for identification from fingerprints by matching minutiae, from both a legal and scientific standpoint.

Fingerprints, Minutiae, Heredity

#### D14 Detection and Prevention of Elder Abuse: A Pilot Study in Italy

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The goal of this presentation is to inform attendees about an emerging phenomenon of human dominance, the so called "elder abuse."

This presentation will impact the forensic science community by demonstrating an inadequate awareness of detecting and preventing violence against elderly people, primarily among health professionals, in Italy.

It is estimated that people over the age of 80 will be the fastestgrowing group in Europe over the next decades, rising from 4% today to 11% in 2050 (V. Turkulov et al., 2007). Even though elder abuse in institutional and domestic settings is increasingly being recognized as a major social problem, it is still underestimated in Italy.

The abuse against elderly people includes several types of damages and situations. The most typical victims are those carrying particular risk factors such as poor general health, disabilities, dependence on others. Abuse may take many different forms: physical, psychological/emotional, neglect, financial, legal, or sexual. Elder abuse is often the result of a lack of adequate knowledge, overburdening, and stress on the part of professional and family caregivers alike.

The supposed lack of appropriate measures to protect elderly people particularly vulnerable to abuse led to a project with the goal of giving a better estimation of the phenomenon and consequently acting in order to prevent it.

Purposes of this study were to assess the significance of this social problem in Emilia Romagna and Liguria (the "oldest" Italian Region) and to understand the level awareness of juridical or medico-legal measures to be adopted in the case of elder abuse or neglect. This has been performed by distributing an anonymous questionnaire to health operators and other professionals dealing with elderly patients (geriatrists, E.R. doctors, general practitioners, nurses, physiotherapists, social service operators, professional caregivers).

The results of the study conducted in these two Italian regions showed the little emphasis given to this significant problem in Italian society. For improving legal and medical protection, a more specific medico-legal and clinical definition of the elder and a higher awareness and alertness of medical personnel involved in old people care are mandatory. A preventive action for the subjects with high risk factors could be carried out to stop repeated violence and chronic abuse.

The first step to prevent violence against elder people should be fostering the ability of recognizing forensic markers of elder abuse such as bruises, deficient nutritional status, dehydration, bad hygienic conditions, decubitus ulcers, broken bones, sharp wounds, and skin tears. These markers, right now, are too often ignored or underestimated. In this sense, the purpose of the study is to overcome the low level of knowledge through new multidisciplinary screening tools as well as uniform and validated guidelines. Such an approach could be extremely helpful not only for doctors, but also for all professional care workers and generally for people in contact with elderly people.

Moreover, the evaluation and comparison of other legal systems that provide a definition and a specific protection of the elderly in different European countries are fundamental.

In conclusion, elder abuse is a widespread serious problem. It is recommended that both forensic and health operators become familiar with this phenomenon, especially those who are involved in old people care. A widespread training of health professionals, justice and social sectors is the only mean to increase the awareness of elder abuse and to create multidisciplinary teams, developing research in this field.

Elder Abuse, Multidisciplinary Approach, Forensic Markers

#### D15 An Evaluation of Digital Radiography and Multi Detector Computed Tomography (MDCT) in Gunshot Wound Trauma

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After attending this presentation, attendees will recognize the principle differences between Digital Radiography and Multi-Detector CT Scanning (MDCT). Attendees will understand the relative advantages and disadvantages of each method for the documentation and evaluation of soft and hard tissue trauma resulting from ballistic injury. A case study demonstrating the use of both methods in the investigation of ballistic trauma will be presented.

This presentation will impact the forensic science community by increasing awareness of the potential offered by modern medical imaging techniques and afford a greater understanding of their application in the investigation of ballistic trauma.

Although radiography has long been the primary method used to evaluate ballistic trauma, interest is increasing in the use of Multi-Detector Computed Tomography (MDCT). Recent studies have demonstrated significant advantages of this method over traditional film-based radiography. However, advances in detector and computing technology used in digital radiography now offer an alternative to traditional radiographic methods. The portability and lower capital cost of such units make this an attractive alternative imaging method in situations where MDCT is not possible for logistical or financial reasons.

A case study in which three experimental subjects (pigs, humanely killed) were subjected to postmortem gunshot trauma via a series of controlled ballistic discharges is presented. All subjects were examined both prior to and following shooting using MDCT and digital radiography. Following postmortem imaging, the subjects were examined using a conventional necropsy. Postmortem and antemortem image data from both modalities was evaluated by a team of Consultant Radiologists and compared to the necropsy findings.

Many studies have demonstrated the advantages of MDCT for evaluation of postmortem pathology due to its high resolution digital acquisition permitting both sectional and 3D reconstructions. In this study, MDCT proved very effective at demonstrating entry & exit wounds, projectile pathway and the extent of both temporary and permanent cavity. However, in order to demonstrate and evaluate this information, a complex and time-consuming computer post-processing sequence is necessary, requiring specific specialist skills. Equipment is both large and expensive and may be outside the budget of many jurisdictions. However, in certain situations the additional information acquired may significantly reduce the time taken for autopsy, thus providing a cost-effective solution in busy jurisdictions.

Digital Radiography (DR) enabled both hard *and* soft tissue trauma to be recorded, documented and evaluated, offering significant advantages over its film-based predecessor. Radiographs were rapidly acquired to determine the presence or absence of underlying fractures and to establish whether any ballistic material remained within the soft tissue. Despite these advantages over conventional radiography, DR is not a 3D imaging technique and proved less effective at evaluating projectile pathway or bullet fragmentation than MDCT. It is also subject to errors of magnification and distortion and complicated superimposition. It is however, a more cost effective and simpler technique offering the user greater operational freedom and improved workflow, decreasing the overall postmortem examination time when compared to film radiography. It may be particularly useful in field applications due to its portability.

Both MDCT and DR are effective methods of evaluating ballistic trauma. While MDCT offers significant advantages in providing a 3D demonstration of soft tissue damage from entry to exit, it is a complex and time-consuming process. DR offers a rapid and effective primary tool for such investigations which is significantly superior to its film-based predecessor and less complex than MDCT. It may prove to be a more versatile option in many circumstances.

Multi-Detector Computed Tomography, Digital Radiography, Forensic Imaging

### D16 Tracking Bullet Trajectories: A Comparison of Multi-Detector Computed Tomography and Magnetic Resonance Imaging

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After attending this presentation, attendees will understand that although radiography, either using film or a digital recording format, is currently the primary means for the examination of gunshot wound victims, interest has increased in employing advanced imaging modalities such as Multi-Detector Computed Tomography (MDCT) and Magnetic Resonance Imaging (MRI). Although vendors suggest the equipment is easily operated, a skilled technologist is required to manipulate the unit in order to obtain the optimal images. This presentation will consider optimizing protocols which will maximize image quality and, in addition, compare the advantages and disadvantages of MDCT and MR.

This presentation will impact the forensic science community by demonstrating how when operated by a skilled technologist, MR and

MDCT can be employed to produce complementary images of gunshot wounds. MR will demonstrate the soft tissue response to the forces created by the projectile. MDCT will more clearly visualize lead fragments from the projectile and resulting damage to bony structures.

**Hypothesis:** Both MDCT and MR provide excellent sectional images of anatomic structures. However, with each of the advanced imaging modalities, skilled equipment operators are necessary in order to manipulate the protocols necessary to generate the best obtainable images.

**Synopsis of Content (Materials & Results):** As part of a study to determine the effects of gunshot wounds on soft tissue and bone, three pigs were shot with two different caliber handguns and subsequently imaged using various imaging modalities including computed radiography (CR) and plane (conventional) radiography, Multi-Detector Computed Tomography (MDCT), and Magnetic Resonance Imaging (MRI). Prior to being shot, the animals were stunned using a "capture bolt" and then exsanguinated by via a throat slash. Because of the length of time necessary to acquire the MRI images, only one pig was imaged with all four imaging modalities including MRI and MDCT.

**Conclusion:** Compared to MDCT, MRI produces superior sectional images of soft tissue structures; however, specific parameters regarding slice orientation must be programmed prior to the initiation of the examination. Conversely, MDCT best visualizes bone and more dense materials that are not clearly demonstrated with MR. In addition, MDCT initially collects a volume of tissue that can later be reconstructed in virtually any plane and can also generate three-dimensional images with varying degrees of transparency.

Magnetic Resonance Imaging (MRI), Multi-Detector Computed Tomography (MDCT), Gunshot Wound Imaging

## D13 Forensic Polygraph Testing: A Response to the Call for Strengthening the Forensic Sciences

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After attending this presentation, attendees will have a better understanding of how polygraph testing fits in the general forensic sciences. In addition, attendees will learn how practitioners of this forensic technique of polygraph testing, like a number of other techniques, struggle with the demand for better "science" and improvements in the credentials and training of practitioners.

This presentation will impact the forensic science community by presenting two points of information: (1) the polygraph testing community, as will be discussed in the presentation, has a strong historical connection to the forensic sciences, even though in many applications today may not be apparent to uninformed observers; and, (2) the recent reviews by the National Research Council (NRC) of the National Academy of Sciences, of polygraph testing and, then, of the forensic sciences, have promoted a strong and sustained interest in leaders in the polygraph testing community to attend to the call for a strengthening of the field in line with the NRC's recommendations. Attendees will learn how these recommendations are being addressed and what remains to be done.

The National Research Council (NRC) of the National Academy of Sciences (NAS) released a public report on polygraph testing in 2003 which was based on an extensive review of the available empirical research. The report was widely publicized and was generally considered critical of polygraph testing. The NRC's initial charge was to consider the use of polygraph testing in the context of personnel security screening, e.g. police and intelligence agency applicant testing and government employee security clearances. However, after a review of the literature, it was determined that there was a significant lack of empirical evidence regarding the use of polygraph testing in screening applications. The NRC subsequently expanded their efforts to include "specific-incident testing," which involves the use of polygraph testing for forensic purposes, such as in criminal investigations. The NRC concluded that "specific incident polygraph tests can discriminate lying from truth telling at rates well above chance, though well below perfection." Additionally, the NRC provided commentary and recommendations regarding a number of additional issues in polygraph testing that needed attention. This included special emphasis on the need for more and better research, careful attention to the development of theory and theory directed research, and a strengthening of the "standardization" of the testing process. Though the 2003 NRC report had considerable influence on guiding some changes in the polygraph community, many of its recommendations were viewed with considerable skepticism, which likely occurred as a result of several issues noted by the polygraph community. One of the most important of these was that the NRC committee which was established to review the research evidence was comprised only of persons who had no interest or involvement in the polygraph testing community, whether as researchers, practitioners, or scholars who focused on the topic. In short, the committee lacked representative spokespersons with personal and professional experience in the field who could have balanced the strong, abstract considerations the committee focused on with specific concerns that arise in real-world application. Such input may have led to a report that was more influential than was actually the case in this instance. Unlike the NRC report on polygraph testing, the now widely known and highly influential 2009 Report on the forensic sciences by the National Academy of Sciences included a number of multi-discipline scientists and multiple persons with real-life experience in a number of forensic practices. This, has led to a report with far greater influence than the 2003 NRC report and, has led to a response to the more recent NRC report by leaders in the polygraph community that has a greater sense of direction and urgency, than was the case previously. The recommendations of the 2009 NAS Report will be discussed and consider them in relation to disparities among practitioners in the polygraph community. Concerns in the polygraph testing community about how to deal with enhanced research activities, accreditation of training facilities, certification of practitioners, quality control and other oversight mechanisms will be highlighted. Further, this paper will outline the significant changes that have already occurred in the field and the changes which are planned for implementation in the near future.

From this presentation attendees will have a better understanding of forensic polygraph testing and why, even though such testing may differ in nature from other forensic techniques, the difficulties and disparities in the polygraph examiner community are similar to those in other areas that were considered in the 2009 NAS Report. Furthermore, this presentation will provide attendees with information regarding the current state of the polygraph testing field and how its leaders intend to strengthen its role as a forensic science.

Forensic Polygraph, Polygraph Examiners, National Academy of Sciences

#### D17 A Case Study of a Murder Staged to Look Like a Suicidal Hanging: Focusing on the Small Details That Led to the Successful Resolution of the Investigation

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After attending this presentation, attendees will be familiar with the key investigative steps needed to successfully detect a staged crime scene. The benefits of not making a hasty judgment while conducting a death scene examination and preliminary investigation will be discussed. Keeping the focus of the investigation broadly to verify or rule out all possibilities will be espoused. A multidisciplinary approach to investigations is the key in obtaining outstanding results in the pursuit of the truth.

This presentation will impact the forensic science community by exposing participants to various key steps in solving some of the most difficult death investigations.

In late October 1992, a quiet military housing area was stunned by the apparent suicide of a 21-year-old army wife and mother of two small children. Military members represent all spectrums of our society. Military families often face extreme challenges that are unique that frequently cause stressors to build to the point of tragedy. Murder and suicide are sometimes the unfortunate outcome. The United States Army Criminal Investigation Division is chartered to investigate all unattended deaths on U.S. Army installations worldwide.

CID Special Agents responded to the scene and began documenting and processing the crime scene, processing the body, and interviewing friends, neighbors, and family members. The 23-year-old soldier reported his wife went upstairs to take a shower. After hearing the water run for an unusually long time he went to check and found her hanging in the nude from an electrical cord attached to the shower head. The soldier additionally reported his wife had made prior suicide attempts following the recent death of her mother and had a family history of depression. The body was taken to the North Carolina Medical Examiner's Office, Chapel Hill, NC, where an autopsy supported the asphyxial death of the wife. A review of her medical records supported the Soldier's allegation of the prior suicide attempt.

The soldier was allowed to take his wife to Texas to be buried and his children taken to their grandparents for long term care. Upon his return to duty nearly a month later, CID Special Agents conducted a thorough interview in which the soldier was confronted with inconsistencies between his statements, other interviews conducted and the evidence from the death scene. He subsequently confessed to getting into a fight with his wife during which she knocked herself unconscious. At that time he formed the plan to hang her from the shower to make it look like a suicide. He placed the electrical cord on the shower head and then hung his wife.

Murder, Hanging, Suicide

### D18 If I Had a Hammer 2: Another Example of Improvised Firearm Construction and Use

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After attending this presentation, attendees will have a better understanding of improvised firearms. This presentation will illustrate the construction and firing of an improvised firearm from a suicide. This presentation will impact the forensic science community by illustrating the use of commonly found items to construct a plausible and effective weapon.

Crime scene investigators work scenes that become almost too familiar to them in that there are similar facets that often repeat themselves depending on the scenario. However, occasionally scenes have something different that sticks out and attaches itself to that case from which it will forever be known to those involved.

Although there have been ways to manufacture weapons for many years, it is not often seen in practice. It is often thought by law enforcement that the removal of weapons will prolong the life of the individual expressing suicidal thoughts, thereby giving him or her time to be evaluated, receive treatment, and be released to friends or family that will take care of them. There are circumstances where law enforcement is asked to remove weapons under court order for fear the individual will do themselves or others harm.

Removal of weapons from the possession of someone with questionable intentions should increase the difficulty in actually harming themselves. Firearm "building" is a relatively simple yet illegal alternative to acquiring another weapon. The use of such improvisation demonstrates, in the case of a suicide, sheer determination on the part of the victim to end his or her life.

One such case involved pieces of metal piping, a live shotgun round, a nail, and a hammer. The shotgun round was held inside a piece of pipe using a metal coupling and fitting with a hole drilled in to the end the same size as the width of a nail. The hammer was used to strike the nail, thereby mimicking the action of a firing pin and discharging the round through the pipe which was held against the victim's chest. Because there was not an alternative route for the gases to escape, the result was exceedingly devastating to the upper left chest cavity.

The device was disabled by the hazardous device team using a percussive disruption device. In order to demonstrate the weapon construction, PVC pipe was used to build a scale model. The model was made two times larger than the original improvised firearm for three reasons: constructing a firearm is a federal offense, even if it is used for education purposes. There would not be ammunition available for this size on a civilian level. The PVC would not contain the "explosion" cause by the striking of the round, further proven by the emergency room injuries from the commonly constructed "potato gun."

This presentation will describe the circumstances of the scene, the evaluation by the crime scene investigator and the hazardous device team, and the reconstruction of the improvised firearm using materials that were legal and safe.

Crime Scene, Improvised Firearm, Suicide

### D19 Towards Standards in Forensic Archaeology: Examining the Impact of Method on Interpretation

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The goal of this presentation is to demonstrate that different methods of excavation and recording systems applied to the same archaeological features result in different reported interpretations, and therefore reconstruction of events at crime scenes. The results may impact on how field archaeologists worldwide undertake excavations, apply methods and interpret their work.

This presentation will impact the forensic science community by demonstrating the assessment of the different methods used to excavate archaeologically justifies the need to question and test methods used to collect evidence for forensic investigations. The level of confidence we can place in different methods is questioned.

Forensic science and the standards within its' various disciplines are under the spotlight and forensic archaeology is no exception. The National Academy of Sciences (NAS) Report 2009 highlighted the need for review, and the work of the Forensic Regulator of the Home Office in the United Kingdom developing standards within forensic science show the demand and active movement towards standards determination for forensic science disciplines.

Examination of standards in forensic archaeology, quantitative determination of accuracy of contrasted methods and critical assessment of the suitability of methods for forensic and legal cases has not been undertaken systematically. Excavation method is one of these and publication of research in this area began at Bournemouth University by Hanson in 2004. This has continued and accelerated, with an increase in contributors and data collected; Cheetham 2005; Wright, Hanson and Sterenberg 2005; Hanson 2007; Cox et al 2009; Cheetham and Hanson 2009; Wright and Hanson 2009; Hanson et al 2009, critically assessing excavation, management and practice and highlighting limitations in these areas.

Archaeologists and excavators have for too long described standard practice without an assessment of what this means and whether the methods used maximize data identification and recovery. The luxury of doing without such an assessment cannot be delayed when courts and legal cases have begun examining archaeological practice and standards are being set. Now is a critical time to conduct research that can contribute data to advise the working groups and regulatory bodies as to what standards should be set for archaeological excavation methods, recording and interpretation for forensic and legal purposes.

This paper examines archaeological and forensic case studies where interpretations have been questions and the "unrepeatable experiment" of excavation has been repeated with differing results. Experiments to test methods in a controlled manner have determined levels of archaeological and evidence identification and recovery differ depending on the methods used. Excavation of simulated and identical archaeological features was undertaken by a series of participants using two different excavation methods, which were compared: (1)stratigraphic excavation, as described in detail by Harris (1989) and Harris et al (1991); and, (2) arbitrary excavation described in detail and critically compared with the former method by Praetzellis (1991). The results showed that stratigraphic excavation provides a higher level of accuracy in evidence location and identification of archaeological contexts. They also suggest that levels of accuracy are dependent on practitioner experience and speed of excavation. Arbitrary excavation methods produce a common level of accuracy independent of experience, but this level is not accurate enough to provide confidence in this method for use forensic cases, other than in specific circumstances. This variation in results dependent on excavation method not only affects confidence in the nature and context of evidence recorded but also in the interpretations given and reported upon.

Forensic Archaeology, Excavation Methods, Standards

#### D20 Retaliation Against a Police Officer, or Not? A Case Study

#### Claire E. Shepard, MS\*, 501 Varsity Road, Griffin, GA 30223

After attending this presentation, attendees will learn how to recognize signs of police retaliation at a crime scene and recognize clues that indicate staging of a crime scene. In addition, attendees will learn the importance of being aware of workplace events and how they may tie into future investigations.

This presentation will impact the forensic science community by showing the importance of recognizing clues at crime scenes that may or may not point toward the original hypothesis regarding what occurred. Knowledge of the characteristics often seen at crimes scenes where there was police retaliation and crime scenes that were staged will be gained.

On a Sunday morning around 6:00 a.m., police were called to check an apartment where neighbors noticed there was an open door. Upon arrival, the police determined a burglary had occurred and crime scene investigators were called to process the scene. The burglary was located in a third floor apartment in a gated complex. When the CSI arrived at the scene, specific messages to the victim and a gang sign were observed written on the walls of the apartment. One message was obviously directed toward the police and was initially thought to be a message to responding officers. However, it was later determined that the victim of the burglary was a police officer who was on-duty at the time of the incident.

Because of the specific messages written on the walls, the condition of the scene and the time frame during which the burglary occurred, investigators initially hypothesized the burglary and vandalism was a result of retaliation against the police officer who occupied the apartment. Steps to ensure the safety of the police officer victim were immediately begun by the internal affairs and homeland security units. Detectives on scene canvassed the apartment complex and the victim officer's superiors were notified. In the meantime the roommate of the officer arrived on scene and was also interviewed by the detectives.

Throughout the scene investigation evidence that was inconsistent with the initial hypothesis of police officer retaliation was noticed. For example, several neighbors reported hearing three gunshots during the night, but no evidence of gunshots was found at the location. Additionally, while the incident had all the makings of a burglary, nothing appeared to be missing from the apartment. The officer's uniforms were intact and accounted for and there was not severe damage to any personal items (including weapons) located inside the apartment. Other hypotheses were suggested including a random occurrence of the burglar happening upon the officer's apartment and that juveniles living in the apartment complex committed the burglary. However, neither of those hypotheses fit the characteristics observed at the scene.

The crime scene was documented according to procedure and the investigation continued by detectives. Throughout the investigation more information regarding the history of the roommate's professional and personal life was gained and ultimately provided the clues needed to determine if the burglary was conducted by an offender retaliating against a police officer or by someone else. The keys to solving the investigation and reconstructing the crime scene were prior experience working retaliatory scenes and the knowledge of current events at the workplace regarding the police officer and the roommate. While a scene may initially appear to fit one hypothesis careful documentation and analysis of the evidence, along with a little workplace gossip, can turn the investigation toward a different conclusion.

Staged Crime Scene, Police Officer Retaliation, Details

#### D21 Drowning in Fuel: A Case Report

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After attending this presentation, attendees will learn about drowning in fluids other than water.

This presentation will impact the forensic science community by presenting a case report of a man who drowned in fuel.

Drowning in liquid, other than water is a non-frequent occurrence. From the analysis of *forensic* literature it is deduced, in fact, that a
systematic study doesn't exist on this matter. The drowning in fluid different from the water usually happens in liquid not mixable with the blood as the fused fat, the oil and its by-products or in substances like beer, wine and them by-products, where the alcoholic vapors compete in the reduction of the consciousness, favoring the drowning. The nature of the event is usually accidental although some of these deaths can cause safety problems in the working places.

Personal observations of a drowning case in gasoline is described. Two young people of black race, one male and one female, were found deceased on board of a craft used by clandestine immigrants for the crossing of the Channel of Sicily. The short distance between the southern coast of Sicily and the northern coast of Africa favors the crossing of the Channel of Sicily from precarious and overloaded boats of men that seek their fortune through the clandestine immigration in Europe. During the judicial inspection (conducted by another expert) the corpses were found inside two cans of metallic material. At inspection, the corpse of the female showed all the characteristics of the drowning in water of sea (as then confirmed by autopsy examination). The male corpse had maceration of the skin and strong smell of gasoline.

The autopsy examination, ordered by the Judicial authority, showed the presence of the characteristic signs of the asphyxia (conjuntival petechiae, blood fluidity, blackish-colored blood) and atypical signs of a drowning in water, as the oily surface in the blood, on the pulmonary surface and pulmonary squeezing, as well as the presence of abundant oily material inside the stomach. Such liquid, exposed to the action of a flame, got burned. The withdrawn material, preserved in special containers of plastic material, determined the corrosion of the same subsequently confirming the hypothesis of the drowning in gasoline.

Histological investigations, conducted through the use of colorations of base and colorations contemplated for the specific diagnostic question, confirmed the drowning in gasoline, on the base of the comparison of pulmonary parenchima homogeneously turned necrotic with destruction of the cellular structures, excluding the presence of any other pathology able to autonomously determine the death of the young male.

Despite the indication of drowning in gasoline, there were no signs of battering were found on the victim, which excluded the cause of death being a homicide. It is reasonable to suppose that the death of the youth was accidental, although the judicial investigations have not confirmed the real nature of the event.

Drowning, Fuel, Asphyxia

# D22 Use of Proposed Standardized Geophysical and Archaeological Forensic Techniques to Supplement Crime Scene Investigations: Concepts and Examples of Applications

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After attending this presentation, attendees will have an enhanced understanding for standardization of methods used in combined geophysical and archaeological activities in the field of crime scene investigation.

This presentation will impact the forensic science community by comparison of successful and unsuccessful geophysical deployment strategies as well as argue strongly for use of combined interdisciplinary geophysics and archaeology to establish basic methodologies for discovery and recovery of burials.

Here, some of the major issues encountered during the brief history of the North Carolina Program for Forensic Sciences will be discussed.

This presentation will better motivate the forensic science community by providing an understanding of how standardized field techniques used in combined geophysical and archaeological forensic investigations can be beneficial to the success archaeological forensic techniques by improving the chances for successful criminal burial site recognition.

This project reviewed successful and unsuccessful combined geophysical and archaeological crime scene investigations and identified key components that were lacking in unsuccessful programs that were present in successful investigations. After a review of the findings, recommendations are presented for the establishment of standardized field techniques the geophysical and archaeological forensic specialist. The goal of this discussion is to provide additional initiatives that will foster communication within the geophysical/archaeological scientific communities.

Geophysical and archaeological field sampling techniques require the establishment of anticipated results, and a clear identification of physical parameters that need to be collected to satisfy the anticipated results. This includes: identification of target (i.e., weapons made of steel permit a restricted number of geophysical techniques for evaluation), or other parameters, such as potential depth of burial, size of object, and overall site conditions may further restrict the selection of available geophysical sampling solutions. This selection process is part of what we call "mental mobilization" and is key to selecting the appropriate search methodology. These simple principles have been widely adapted by other professionals in non-criminal applications (i.e., location of underground pipe, tanks, and buried waste). Although the crime scene presents a unique set of issues during the investigation, the scientific principles remain the same.

This presentation will provide examples of geophysical/archaeological combined investigations, and how by following the basic principles of an organized search the chances of success have been improved. Simple guides (tables) will be provided for the selection of investigation methodology using the current knowledge base of geophysical/archaeological investigatory techniques including but not limited to:

- Single and multi-frequency electromagnetic detectors (for large to small area surveys where rapid assessment is desired);
- Total and Gradient Magnetometers (for large and small area survey are desired, and potential targets are composed of iron/nickel compounds);
- Ground-penetrating radar for medium to small areas (where soil and ground conditions permit use); and,
- Other methods such as remote sensing using aerial photography, mapping using GPS, and reflectance and thermal infrared imaging will also be considered.

Results of geophysical/archaeological evaluations provide a valuable and constructive feed-back mechanism to facilitate discovery and recovery of human remains and/or associated crime scene evidence. These data may also be used to provide important information for the inclusion and exclusion of potential areas for evaluation.

Geophysics, Archaeology, Standardization

# D23 Geographical Spatial Analysis of Homicide Offenders' Residences in Baton Rouge, Louisiana: An Example of How to Use GIS in Forensic Investigations

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After attending this presentation, attendees will gain knowledge of how a GIS (Geographic Information System) can be created to aid in forensic investigations. Also, attendees will learn how GIS can provide investigators with statistical results in map-form. The resulting maps provide insight into how features in the landscape affect human behavior.

This presentation will impact the forensic science community by showing attendees the process of identifying central points and hot spots associated with where homicide offenders once lived in Baton Rouge, Louisiana. From the generated maps identifying the highest concentration of offender residences, researchers and members of law enforcement will gain a necessary understanding for applying Central Crime Theory to homicides occurring outside of Baton Rouge (Chainey & Ratcliffe, 2005).<sup>1</sup> Also, attendees will learn how statistical spatial analysis can be used to determine if offenders' residences are clustered or dispersed.

Homicides involve a minimum of two individuals-the victim and the offender-yet a majority of the geographical discussions on homicides only reference the location where the victim's body was found. Rather than focusing on the geographical areas associated with homicide victims, this presentation focuses on the geographical areas associated with offenders. Often criminals will operate from an anchor point that he or she feels comfortable with. The criminal will leave the anchor point to commit the crime and then return to the anchor point because it is the offender's "safe haven." Often the anchor point is the offender's residence, so comparing the geographic distributions of homicide offenders' residences within a certain area can offer a new approach to learning about the criminal activity within the area as well.

The Baton Rouge Sherriff's Office provided the addresses for the residences of offenders committing homicides in Baton Rouge, Louisiana, between 1991 and 1997. The addresses were geocoded and assigned X and Y geographic coordinates, which resulted in 304 point locations. A GIS was created using these points, and CrimeStat 3.1 and ArcMap 9.3 were used to analyze the geographical distributions of homicide offenders' residences. The results were obtained using various techniques associated with three different types of statistical spatial analysis: Descriptive spatial statistics, nearest neighbor analysis, and spatial cluster analysis.

The results from this research indicate that homicide offenders in Baton Rouge tend to live in one generalized location in the northern point of the city. The spatial analysis from this research also indicates that the presence of major features in the landscape (e.g., Mississippi River, Interstate 10, shopping malls, etcetera) influenced the overall spatial distribution of where homicide offenders reside. Awareness of how features in the landscape can influence human behavior is an important component to any criminal investigation, and the use of GIS can aid in the identification of these influences.

**References:** 

<sup>1.</sup> Chainey, S. and Ratcliffe, J. 2005. GIS and Crime Mapping. Cichester, West Sussex, England: John Wiley & Sons.

GIS, Spatial Analysis, Homicide Offender

# D24 Analysis and Classification of .22 Caliber Firing Pin Impressions

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After attending this presentation, attendees will be able to describe the .22 caliber firing pin classification system and its application to forensic investigations. The objectives of this presentation are to explain a .22 caliber firing pin classification system, describe how to examine and compare morphological variations and measurements in cartridges, and to describe how to code and retrieve cartridge cases.

This presentation will impact the forensic science community by providing an innovative firing pin classification system that can be used to associate cartridge cases found at crime scenes to a firearm based on the firing pin morphology.

The classification system is an alternative to storing images with ballistic scanning software. Using this system, examiner's can code, file and retrieve .22 caliber cartridge cases for comparisons. The firing pin impressions from unidentified .22 caliber cartridge cases found at crime scenes can be associated with similar .22 caliber firing pin impressions on file using the classification system.

Twenty-five different types of .22 caliber firearms, ten rifles and fifteen handguns were test fired to collect a variety of firing pin impressions for analysis. The fifteen handguns included seven pistols and eight revolvers. The pistols tested included: Beretta Model 21A-22LR, Cobra Derringer, Davis Derringer, Ruger Mark II, Sig Sauer Mosquito, Thompson Center, and a Walter P22. The revolvers included: FIE Model 15, FIE Model TEX 22, Harrington & Richardson Model 922, High Standard, Iver Johnson, RG-10, RG-24, and Smith & Wesson Model 617. The rifles included seven semi-automatics and three single shot bolt actions. The semi-automatics included: Browning, Marlin Model 60, Marlin Model 75, Ruger 10/22, Savage Model 64G, Winchester Model 190, and Winchester Model 290. The single shot rifles included: Mossberg Model 26B, Remington Model 510, and Remington Model 547. All firearms used in this study were pre-owned and obtained from gun shops for test firing.

The bullet and propellant were removed from the cartridges and the priming mixture was neutralized before collecting the firing pin impressions. The priming mixture was soaked in isopropyl alcohol for 12 hours followed by another 12 hours in water and dried at 20°C (68°F). This process neutralized the priming mixture and prevented detonation while test firing to obtain an impression. A stereoscopic boom microscope equipped with a digital camera and measuring software was used to record the measurements of each cartridge impression.

The firing pin impressions were divided into groups based on geometric shapes and measurements. Group I firing pin impressions were square to rectangular in shape, Group II impressions were circular or semi-circular, and Group III impressions were angular or wedge shaped.

Group I impressions were first separated into small (S), medium (M), and large (L) based on the amount of surface area made by the firing impression. The (S) range for surface area was  $0.60 - 0.89 \text{ mm}^2$ , (M)  $0.90 - 1.79 \text{ mm}^2$  and (L)  $1.80 - 2.00 + \text{mm}^2$ . Next, they were separated into (S), (M), and (L) based on the width of the firing pin impression. The width range for (S) was 400-599  $\mu$ m, (M) 600 - 799  $\mu$ m, and (L) was 800 – 899 +  $\mu$ m. Finally, they were separated into (S), (M), and (L) based on the height of the firing pin impression. The height range for (S) was  $800 - 1199 \mu m$ , (M)  $1200 - 1599 + \mu m$ . Using these measurement ranges, the firing pin impressions could be classified into one of 27 divisions. In the firing pin classification system developed, the first letter in the three-letter arrangement represents the amount of surface area made by the firing pin impression, the second letter represents the width of the firing pin impression and the third letter represents the height of the firing pin impression. The classification

system was ordered as follows: SSS, SSM, SSL, SMS, SMM, SML, SLS, SLM, SLL, MSS, MSM, MSL, MMS, MMM, MML, MLS, MLM, MLL, LSS, LSM, LSL, LMS, LMM, LML, LLS, LLM, or LLL.

There was only one category for Group II impressions and that category was based on the radius of the firing pin impression. It was divided into (S), (M) and (L) divisions based on the radius of the firing pin impression. The (S) range for the radius was 200- 499  $\mu$ m, (M) 500 – 599  $\mu$ m, and (L) 600 - 699 +  $\mu$ m. There were no wedge shaped firing pins in the firearms sampled; however, they would be separated by surface area using the same range as used for Group I impressions.

When Group I firing pin impressions were classified based on the square surface area of the impression, 28% were (S). Within this (S) group, 6% were classified as SSM, 6% SSL and 6% SLS based on the firing pin impression height and width respectively. Seventeen percent of the impressions were (M) and within this group 11% were MMM. Only 1% of the firing pin impressions were in the (L) group.

Consequently, using this classification system, 20 out of 25 firearms could be eliminated in the SSM classification, 24 could be eliminated in the SSL, 24 eliminated in the SSM, and 24 in the SLS classification. For firing pin impressions with "M," out of the 25 impressions, 23 could be eliminated in MMM, 22 in MML, 22 in MLM and 24 in LLL.

In conclusion, this system associates firing pin impressions from .22 caliber cartridge cases found at crime scenes to similar .22 caliber firing pin impressions filed in this classification system. As a result, cartridge cases can be examined to determine association to a firearm based on the firing pin morphology.

Firing Pin Impression, Cartridge Case, Firearm

# D25 The Effects of Gunshot Trauma on the Rate of Colonization by Flesh Eating Insects Using Pig Carcasses

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After attending this presentation, attendees will have a better understanding of the effects that gunshot trauma have on the insect's role in the decomposition of swine carcasses.

This presentation will impact the forensic science community by helping to overcome the obstacle of PMI being compromised when gunshot trauma is involved and allow forensic entomologists more accurate assessments.

It was hypothesized that swine carcasses inflicted with gunshot trauma would significantly vary from carcasses with no gunshot trauma. There has been little research done on gunshot trauma and the effect it has on decomposition. The focus of this project is how gunshot wounds effect blow fly colonization. When gunshot wounds lead to death, the arrival of insect fauna is inevitable. There were different weapons used in this project as follows: 12ga. shotgun loaded with 2.75" shell containing 7.5" bird shot; .40 caliber pistol using 150 grain hard ball ammunition; 9MM parabellum pistol firing hard ball ammunition; and .22 caliber LR pistol firing hard ball ammunition. Throughout the course of this project 100 pig carcasses were used. Three research runs were conducted; summer 2009, fall 2009, and spring 2010. Each of the four calibers had five replicates as well as five controls for a total of 25 pigs per research trial run. The location of the research area was an enclosed compound measuring 50'X50'X5' high. The area also had a concrete footer that kept scavengers out as well as a random pulsating electrified fence along the top to keep scavengers out. Locations of pig carcasses were randomly assigned using a random number generator. The carcasses were monitored and photographed twice daily and notes were taken to document blow fly activity and beetle activity. Wound

diameter was measured daily to track wound decomposition. The major stages of blow fly activity were noted: adult flies, fly eggs, fly larvae, migrating fly larvae. The presence of beetles and the end of maggot migration (characterized by the absence of observable larvae on the body) was also noted. This can allow researchers to document differences in development time as well as the initial onset of blow fly life stages. Blow fly adults and larvae were collected in accordance to the standard operating procedures outlined in Haskell and Williams (2008). This was done each day to document any differences in species composition or development among the different treatments. Adult flies were collected and preserved in 70% EtOH and collected fly larvae were killed in KAA (composed of 95% ethanol (77%), acetic acid (15%) and kerosene (8%)) and transferred to 70% EtOH for preservation.

Forensic entomologists are often asked by law enforcement agencies to provide an estimation of the PMI using insects. If wounds such as gunshot trauma are present and this has an effect on the blow fly activity, then the estimation of the PMI is therefore compromised. The data obtained from this research will impact the forensic science community by helping to overcome this obstacle when gunshot trauma is involved and allow forensic entomologists more accurate assessments. **Forensic Entomology, PMI, Gunshot Trauma** 

# D26 Application of Micro Digital Measurement in Fingerprint and Firearm Comparisons: A New Method for a Reliable and Valid Approach

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After attending this presentation, attendees will receive sufficient information of the micro digital measurement, the new device, the operating steps, and the unique features of the new technology, as well as, observe a live examination by the presenter.

This presentation will impact the forensic science community by introducing a new technique for fingerprint and firearm examinations: micro digital measurement using a palm-sized digital viewer. The device and the technique will impact the forensic science community by providing statistical and geometrical measurements of the evidence compared, and thus increasing greatly the levels of reliability and validity.

Current fingerprint (Integrated Automated Fingerprint Identification System – IAFIS) and firearm (National Integrated Ballistic Information Network – NIBIN) database systems are able to use digital technology for a comparison via a probability analysis. Yet to a large extent the two systems are solely based on the pattern and minutia characteristics between the known and the unknown samples without any statistical and geometrical measurements. Therefore, an examiner often has to rely heavily on his or her experience to make a decision and faces routine accusations of using "subjective factors" from the defense in court. This is exactly the focal point in the recent debate and accusation of fingerprint and firearm examination as non-science disciplines from the influential report which was issued in February of 2009.

To address the issue of lacking digital statistics and geometry that other advanced forensic examinations are using, the two fingerprint images will be used from the Madrid bombing case and illustrate the advantages of this new device. First, the equipment is a palm-sized device and can be connected to a laptop via a USB, thus being portable for a crime scene examination. Second, the device takes digital pictures of fingerprints and bullets/casings for comparisons, which makes it easier for online communications and evidence storage. Next, the device can be connected with a projector for a live comparison and analysis at any locations, such as the police department, the district attorney's office or in court during an expert testimony. Further, the device has multiple light sources with black/white, UV, infrared, and polarized lights, each with a magnification range from 1 to 250x. Most importantly, the new technique can provide micro digital measurements, which is a very practical comparison technique for fingerprint and firearms examinations. The measurement unit can be set at inch, mil (0.001 inch), um, or mm. the micro digital measurement is able to calibrate nine types of digital statistical and geometrical measurements simultaneously. The nine formats are: (1) line; (2) continuous line; (3) polygon; (4) radius circle; (5) diameter circle; (6) three points circle; (7) three points arch; (8) three points angle; and, (9) four points angel. With the nine formats, the author is trying to explore comparisons on partial fingerprints and heavily distorted bullets/casings.

With the three unique features of being portable, digital, and practical, this new device should be considered as a great tool in teaching forensic science in classrooms, conducting a preliminary examination at the scene, or even performing a supplemental or even verification examination in the lab. Finally, this pale-size device can provide an effective live demonstration in court with straightforward statistical and geometric digital images (measurements) of the evidence between the known and the unknown samples to the jury that no other device can display a similar function.

The forensic science community now has come to a crossroad that both qualitative and quantitative measurements are critical in courtroom battles. It is suggested that this new technique may provide a new direction for the fingerprint and the firearms examinations and promote them to be more reliable and valid disciplines.

Micro Digital Measurement, Fingerprints, Firearms

## D27 Observed Microscopic Changes of Bullets Fired From Barrels After Cleaning With Bore Brushes

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After attending this presentation, attendees will gain an appreciation for the effect that metal bore brushes commonly marketed and sold in retail firearm stores for cleaning have on the striations in a bullet's land impression and the need for care when analyzing firearms that are subject to frequent cleaning.

This presentation will impact the forensics science community by demonstrating the susceptibility of changing land impressions that are frequently cleaned with bronze or steel bore brushes over the long term. It will also encourage firearms examiners to be cautious when comparing firearms likely to have been cleaned after a shooting incident, either through an extended lapse of time or through recovery of such cleaning brushes along with evidence firearms.

Although it is considered common knowledge that using steel bore brushes to clean firearm barrels may have potential to change the pattern individual characteristics, no documentation of the effect is to be found in the literature. In an effort to confirm this effect, several 9mm semiautomatic pistols were field-stripped and their barrels subjected to a simulation of long-term use of various bore brushes. Using Hoppe's No. 9 Solvent as a cleaning solution, bore cleaning brushes of various compositions (nylon, bronze, or steel) were passed through the barrels 1,000 times using a standard cleaning technique. Test groups of three bullets each were fired before cleaning began and at varying intervals during the process, and the bullets of each test group were compared to each other and earlier test groups. The results demonstrate that metal bore brushes have an ability to affect the land impressions. In most instances this appears to be through erosion, as fine striations were broadened and lowered by smoothing or obliterated entirely. Coarse marks were less likely to be affected. While steel brushes were most effective, the effect was also observed after cleaning with bronze brushes.

While all final test fires could still be matched to the initial test fires, fewer areas of good correspondence were observed, and often an easy match became a difficult one with only one or two lands having sufficient striations remaining. In addition, the effect appeared somewhat random, with unequal results on particular land impressions within the same barrel. It is conceivable that with some firearms, this change in the individual\_characteristics could render the entire bullet unmatchable to an earlier-fired bullet.

Firearms, Land Impressions, Bore Brushes

#### D28 The Use of Infrared Imaging to Facilitate Fired Cartridge Case and Bullet Comparisons

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After attending this presentation, the attendees will have an overview of different image acquisition techniques for cartridge cases and bullets. A near-infrared (IR) camera connected to a stereomicroscope that is capable of acquiring IR images of fired evidence will be demonstrated in this presentation.

This presentation will impact the forensic science community by exploring the microscopic capabilities of IR research as applied to the comparison of fired bullets and cartridge cases. IR has been extensively used for night-vision, search-and-rescue operations, navigation, astronomy, and medical body scans. Current forensic use of near-IR involves detecting gunshot residue and biological stains. This research explores the microscopic capabilities of near-IR research as applied to the comparison of fired bullets and cartridge cases. Advanced machine learning technology can also be easily implemented with the IR images. Time saved will enable examiners to reduce backlog by efficiently and effectively comparing firearms related evidence.

Lighting is a common problem faced by firearms examiners when comparing cartridge cases and bullets. Visible light interacts with the surface texture of these items resulting in the production of shadows. These shadows are key for comparing and identifying striations and impressions on evidence fired from the same firearm. However, if the lighting is not exactly the same for the two items being compared, differences in the shadows may be created, which could make an evaluation of the items more difficult. Other problems related to the use of visible light for cartridge case and bullet imaging include reflected light issues, glare, and the incident angle of oblique lighting. Image acquisition techniques such as 2D and 3D laser imaging, scanning electron microscopy, and thermal infrared microscopy will be reviewed and compared.

In this work, a digital SLR camera with IR capabilities will be fitted with an IR filter, mounted to a stereomicroscope, and used to capture images of cartridge cases and bullets. Different light sources will be compared for use with the camera. Test fired cartridge cases and bullets from multiple makes and models of firearms will be photographed using both near-IR and visible light. Both faint and exaggerated striations and impressions will be photographed in order to determine the sensitivity of using near-IR for imaging this type of evidence. The difference between this work and other related research is that near-IR imaging will be used to facilitate manual comparison of firearms-related evidence by examiners. IR images produced by near-IR light are expected to reveal more detail than visible light microscopy, resulting in a detailed image that is suitable to assist forensic firearms examiners in their evaluation of fired cartridge cases and bullets. It will be shown that the connection of a near-IR camera and a stereomicroscope is an affordable, efficient, and useful adjunct to visible light microscopy for crime labs.

This research will evaluate the use of near-IR light to examine fired bullets and cartridge cases using a comparison microscope. Forensic firearms examiners will compare bullet and cartridge case images captured with near-IR light and visible light. The benefit of using near-IR light will be determined. It is anticipated that this new way of microscopic image acquisition will facilitate forensic firearms examinations.

Infrared, Firearms, Identification

#### D29 Features of Gunshot Wounds to the Head on Postmortem Computed Tomography

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After attending this presentation, attendees will be able to identify the characteristic features of gunshot wounds to the head, as seen on Postmortem Computed Tomography (PMCT).

This presentation will impact the forensic science community by providing pictorial information of characteristic features of fatal gunshot injuries to the head on PMCT. With the increasing use of postmortem imaging methods, forensic pathologists should be aware of special imaging characteristics of gunshot wounds to the head, such as entry and exit wounds, projectile paths and projectile localization. After the presentation, the audience will improve knowledge on typical patterns of bullet wounds to the head and learn about the limitations and advantages of PMCT concerning this topic.

**Purpose:** Fatal head injuries due to gunshots can be either penetrating or perforating and projectiles may or may not be found inside the cranial cavity. Describing the exact path of the projectile, beveling, and resulting injuries, as well as determining the entry and exit wounds, are of great importance in forensics. There are special macroscopic characteristics, which a forensic pathologist uses to distinguish between entry and exit wounds during autopsy. Reconstruction of events is feasible based on these findings. The goal of the present retrospective case study was to determine if PMCT examination in cases of gunshot fatalities to the head could answer relevant forensic queries. Similar studies in the past, with fewer cases, have already stated the importance of PMCT when investigating such fatalities.

**Material and Methods:** The Forensic Institute database was retrospectively evaluated for lethal gunshot wounds to the head. In a five-year period (March 2005 to March 2010) 46 cases were identified. The postmortem interval (time interval between death and the CT imaging), the manner of death (suicide, homicide, accident) as well as the age and sex distribution of the fatalities were evaluated. Axial, sagittal and coronal multiplanar reformations, as well as volume rendered models, were constructed. Primary image review and 3D reconstructions were carried out on a CT workstation and on the local picture archiving and communication system (PACS).

**Results:** Image findings were analyzed for projectile-type, penetrating or perforating injuries and specific location of entry wound. Typical imaging characteristics for each group were determined. The presence of gas, metal parts, projectiles, or osseous fragments in the cranial cavity as well as parenchymal brain injuries and intracranial hemorrhages were evaluated.

Discussion: PMCT is a reliable technique to depict fatal gunshot injuries to the head. Identification of beveling, reconstructions of a bullet path and identification of gunshot entry or exit wounds are feasible. Moreover, PMCT as a cross-sectional imaging modality allows for precise anatomic localization of bullets in penetrating trauma, clearly superior to two-dimensional plain radiography. Identification of millimeter-sized foreign objects (e.g. metal or osseous fragments) is feasible on PMCT whereas such findings might be missed during manual dissection or not even be dissected (e.g. face, cranio-cervical junction, vertebral column). Image reconstructions of PMCT are easy understandable for lay-people and at court, besides the advantage of permanent documentation and the possibility of re-evaluation. Findings are displayed in a time saving manner without any obligatory destruction during classical autopsy. Limitations of PMCT imaging, such as depiction of soot residues, skin patterns and vascular lesions are discussed. Besides external inspection, these limitations can (partially) be overcome by complementary surface scanning, photogrammetry and PMCT-angiography/magnetic resonance imaging.

**Conclusions:** PMCT is an excellent tool for displaying perforating and penetrating gunshot wounds to the head and allows for reconstruction of events. In order to improve quality in the field of forensic pathology, PMCT should be applied in routine investigation of legal cases, especially in cases with fatal gunshot wounds to the head.

Virtopsy, Gunshot Wound, Postmortem CT

## D30 Heating Up "Cold Cases": Research and Service for Unsolved Cases of Human Identification

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After attending this presentation, attendees will be presented with examples of cold cases undergoing reinvestigation utilizing methods that were not readily available during the initial investigation. The objective of this presentation is to establish a systematic and comprehensive set of methods applicable to unsolved cases involving unidentified human remains, as well as, to present a comprehensive model that includes the latest tools and technologies for investigating unsolved cases of human identification. Attendees will be presented with examples of field, laboratory, and morgue methods for ongoing investigations.

This presentation will impact the forensic science community by providing an example of a collaborative, multidisciplinary approach to research and casework involving unidentified human remains, and by also providing examples of collaborative, multidisciplinary research and casework involving unidentified human remains. Specifically a protocol is presented for cold case investigations with particular attention to the challenges that may arise when new methods are presented in court.

Over the past year, a service oriented research initiative among the Forensic Anthropology Laboratory at the University of South Florida and the Hillsborough, Pinellas, Pasco, and Hernando County Medical Examiner's Offices in Florida was undertaken in collaboration with local law enforcement agencies throughout the Tampa Bay region to apply a range of methods to unsolved cases dating back to 1969. The goals for this project were to: (1) assist local medical examiners with unsolved identification issues; (2) systematically apply a comprehensive set of methods to all unsolved cases in the region; and, (3) document osteological, chemical, burial and postmortem factors about each case to develop a baseline of data relevant to the local population and natural environment in the area. Through the course of this project, a wide variety of field, morgue, and laboratory methods were applied and the preliminary findings are discussed in this presentation.

In addition to analyzing unidentified skeletal remains retained by the medical examiner's offices, this project exhumed graves of unknown individuals buried in numerous cemeteries as "John" or "Jane Does." Ground penetrating radar was used to accurately document each exhumation case and to establish the parameters for grave excavation. A full osteological analysis was completed for each case to re-evaluate the initial parameters of identity, such as age, sex and ancestry. Some of the earliest cases had not previously been analyzed by an anthropologist. Two programs were used to evaluate the biological parameters for each individual. Additionally, bone and tooth samples for DNA and chemical isotope analysis for each case was completed. In cases where an original sketch or photograph from the autopsy was not available, 2D facial composites were created. To date, more than 35 cases have been included in this project.

Previously published research on unidentified remains in Florida showed that many individuals come from immigrant, migrant, or at-risk groups. In this investigation, several trends emerge among males and females in terms of the demographic profile, the type of original burial site, cause and manner of death, and the rates of decomposition given the burial context. Initial analysis shows that males (n=13) and females (n=13) representing African-American (n=7), European-American (n=5), Mesoamerican (n=4), South American (n=6), and Circumcaribbean (n=1) ancestry. Currently, craniometric analyses indicate there are more African-American and South American individuals found in the Tampa Bay region with distinct trends among males and females.

The methods and results of this project are presented here. Additionally, legal issues that have challenged anthropological methods in court in these districts are outlined. The ways in which regionally specific data and case studies can offer demonstrative examples in court are addressed. Finally, this project resulted in new course development at the University of South Florida for graduate students in Anthropology. The new course is a service learning course in which students work with community partners throughout the medicolegal community on the problems of missing and unidentified persons. The ability of anthropologists to use their research and casework for education is also critical for practioners who work in university settings. This project reflects forensic anthropology today and the changing role of anthropologists in death investigations.

Identification, Cold Cases, 3D-ID

## D31 Autoerotic Deaths: A 25-Year Retrospective Epidemiological Study

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After attending this presentation, attendees will have a better understanding of the epidemiology of autoerotic deaths.

This presentation will impact the forensic science community by providing new insight into the incidence of autoerotic deaths.

**Introduction:** Autoerotic deaths have been defined as accidental deaths occurring during individual, usually solitary, sexual activity in which a device, apparatus, or prop employed to enhance the sexual stimulation of the deceased in some way caused unintentional death. It

is written in almost all papers on autoerotic deaths that autoerotic fatalities account for about 500 to 1,000 deaths per year in the United States. This highly cited number is now of general acceptance, and was generalized to estimate the number of cases in Canada as well. However, a closer look at the original reference reveals that contrary to the general belief, this incidence does not originate from an epidemiological study in United States, but from an estimation from unpublished data available from England and Canada. However, a Canadian study challenged this number in 2008 and demonstrated that the incidence was significantly lower in the province of Quebec. Presented here is a 25-year epidemiological study of autoerotic deaths in the province of Alberta, Canada.

**Material and Methods:** The Province of Alberta (Canada) is divided in two Offices of the Chief Medical Examiner, one in Edmonton and one in Calgary. The database of both offices was searched for the following keywords: sexual, autoerotic, sex, naked, penis, semen, breast, vagina, porn, pornography. These keywords were not only searched for in the cause of death but in all other parts of the file as well (for example, in the investigators summary and investigators notes). All non-suicidal hanging and asphyxial deaths were also reviewed.

**Results:** From 1985 to 2009, 38 cases of autoerotic deaths were found (incidence of 0.56 per million inhabitants per year). The number of cases per year varied from none to four (average  $1.52 \pm 1.08$ ). Victims were all males, aged from 16 to 74 years (average  $33 \pm 12$ ). Most victims were single. The vast majority of deaths were related to typical methods (36 cases, 95%). The most common method was hanging (28 cases, 74%). Atypical methods were encountered in 5% of cases: one case of electrocution combined with hanging, and one case of atypical asphyxia method by inverted suspension. The most common location the bodies were found was basement (34%), followed by bedroom (24%), and bathroom (13%). The majority of victims were not under the influence of ethanol or drugs when the accidental death occurred. In 23% of the victims, an acute ethanol intoxication above 80 mg/100 ml was found. Cannabis, methamphetamine, or cocaine was found in 13% of the victims. In 23 cases, the investigation established if the event occurred in the morning, afternoon, evening, or night. There was no clear evidence of a preferential time of day for these deaths. However, it seems that autoerotic deaths might be slightly more common during summer (37%). The geographic distribution of autoerotic deaths reveals a preferential distribution in big cities compared to rural areas: the incidence in Calgary was 0.76 per million inhabitants per year, compared to 0.57 in Edmonton, and only 0.44 in the rest of Alberta.

**Discussion:** The widely cited incidence of 500 to 1,000 autoerotic deaths per year in the United States is based on data from 1983. Considering that the population of United States in 1983 was of 226.5 millions, this incidence corresponds to 2.2 to 4.4 cases per million inhabitants per year. In 2010, the population of United States has increased to 309 million. Therefore, the incidence of 500 to 1,000 autoerotic deaths per year in the United States should be changed to 700 to 1,400 deaths per year considering the population increase. However, these numbers are largely overestimating the reality in Canada, and are probably overestimating the reality in United States as well. Epidemiological studies are needed to better assess this estimate in United States. Further studies are needed to better assess the incidence of autoerotic deaths in different geographical and socio-economical areas.

Autoerotic Death, Autoerotic Asphyxia, Incidence

#### D32 Death Investigations in Rural Meigs County, Ohio Under the Coroner's Inquest System

#### Larry D. Marshall, MFS\*, Meigs County General Health District, 112 East Memorial Drive, Suite A, Pomeroy, OH 45769

After attending this presentation, attendees will understand the coroner investigation system in Ohio, the challenges faced by rural counties investigating deaths, and how regional forensic systems in Ohio, such as the Montgomery County Coroner's Office, assists with rural county investigations.

The presentation will impact the forensic science community by providing a view that a coroner system need not be arcane and antiquated; rather, if properly regulated by state law, the coroner investigation system such as exists in Ohio can be as effective a tool for death investigations as the medical examiner system.

Ohio is a coroner state. Each of the 88 counties elects a physician who serves for four years in that capacity. Ohio law requires the coroner to be a licensed physician who has been in practice a minimum of two years and is mandated 16 hours of professional development per year. Larger cities such as Columbus, Cincinnati, Cleveland, and Dayton have forensic centers that serve as forensic resource centers for counties that do not have the fiscal resources to support such centers.

Meigs County, Ohio, with a population of about 23,500 persons is situated in the Appalachian plateau region of southeast Ohio. The county is economically challenged with a systemic unemployment rate of over 15%. Medicolegal investigation in this fiscal environment is a challenge. The current annual budget for the coroner's office is \$29,096.00. The office employs an elected coroner, the incumbent of which is a family practice medical doctor and has been the coroner since 1988, as well as an investigator who is also the County Health Commissioner. The office receives an average of 50 cases calls per year of which about 24 are sent to the Montgomery County Coroner's office for autopsy. The cost for an autopsy, including transportation of remains, is about \$1,800.00. These costs are paid out of the County General Fund and are not part of the coroner's budget. The budgets of the sheriff's office and local police jurisdictions for investigations as well as training are likewise restricted and as a result, the Ohio Bureau of Criminal Identification is often called upon to assist in death investigations.

Because of fiscal constraints, not all cases reported to the coroner's office are scene investigated beyond the telephone call. Natural manners involving, hospice, nursing homes, and cases with a documented medical history, especially the elderly, do not normally receive a response. Sudden and unexpected death involving adults and children are investigated but not all are sent for autopsy depending on investigative circumstances. Homicides and undetermined are autopsied. Most suicides not involving the elderly with a medical history and most accidental deaths are sent for autopsy.

It is believed that the Ohio coroner's system serves medicolegal purposes satisfactorily. Cases are investigated on a case by case basis with resources directed to those cases that most impact the public safety and health of the community.

**Coroner, Medical Examiner, Death Investigations** 

# D33 The Society of Medicolegal Death Investigators is Now Operational

Mary Fran Ernst, BLS\*, Saint Louis University School of Medicine, Division Forensic Pathology & Education, 1402 South Grand Boulevard, Saint Louis, MO 63104

After attending this presentation, attendees will be informed of the Society of Medicolegal Death Investigators, a newly created educational organization to promote medicolegal death investigators and their profession.

This presentation will impact the forensic science community by introducing attendees to The Society of Medicolegal Death Investigators (SOMDI), the newly created professional organization for medicolegal death investigators.

Medicolegal death investigators are employees of medical examiner or coroner offices who investigate violent, suspicious, sudden and unexpected deaths. Following office standard office procedures, they are the first line of defense of the jurisdiction's death investigation system. Their decisions at the time of the initial death report can mean the difference between a homicide being overlooked or recognized or a dead person never being identified, misidentified or scientifically identified.

Lay investigators began working for medical examiner systems in the mid-1960s. In the early 1990s, a group of veteran, experienced death investigators from throughout the United States collaborated and developed a set of voluntary guidelines for their profession. The National Institutes of Justice then published these in December 1997. The term "medicolegal death investigator" was selected to differentiate these individual investigating deaths for coroner and medical examiner offices from investigators working for law enforcement agencies.

Since 1998, the American Board of Medicolegal Death Investigators (ABMDI) has been professionally certifying medicolegal death investigators. As of July 31, 2010 there are more than 1,550 medicolegal death investigators certified in North America and four foreign countries. It is estimated that there are more than 8,000 medicolegal death investigators working in North America.

In February 2009, the National Academy of Sciences released their report, *Strengthening Forensic Science in the United States: A Path Forward.* The report noted that the forensic science community is plagued by poorly funded systems and inconsistent practices in federal, state and local crime laboratories and medicolegal offices. The Report emphasized the future needs of forensic science practitioners to include:

- mandatory professional certification for all practitioners;
- development of discipline-specific practice standards; and,
- high-quality education, training and continuing education opportunities should be available to all those working in the forensic sciences

The United States has two types of medicolegal death investigation systems: the coroner and medical examiner systems. The coroner is an elected official that is responsible for death investigations and administrative duties of his/her office. A medical examiner is often an appointed official who is an ABP board-certified forensic pathologist who oversees the entire death investigation process, handles administrative duties and performs autopsies for his/her office. Both groups currently have their own professional organizations that representing them – the International Association of Coroners and Medical Examiners (IACME) and the National Association of Medical Examiners (NAME). Medicolegal death investigators may join both organizations but they are not the primary focus of either organization.

That is why The Society of Medicolegal Death Investigators has been created – to represent the medicolegal death investigator profession, its needs, and concerns.

The Society of Medicolegal Death Investigators (SOMDI) will be operational January 1, 2011. This will be a voluntary professional

membership organization specifically dedicated to medicolegal death investigators and the work they do investigating deaths for medicolegal offices. It will be an organization inclusive of all individuals currently employed as medicolegal death investigators in either medical examiner offices, coroner offices, equivalent military authority offices whose responsibility includes conducting death scene investigations and ABMDI certified individuals.

Organizational details will be presented including membership criteria, application information, organizational structure, contact and website information, training and educational opportunities, and annual meeting details, etc.

Society of Medicolegal Death Investigators, SOMDI, Medicolegal Death Investigators

#### D34 Caffeine Related Deaths in Young Adults

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After attending this presentation, attendees will understand the possible detrimental effects of periodic fluctuations of blood pressure, caused by the intake of caffeinated drinks, substances containing caffeine, or caffeine supplements, can have on the heart, potentially leading to death.

This presentation will impact the forensic science community by provoking thoughts and questions as to the relationship between death and the intake of caffeine, regardless of the presence of postmortem toxic levels. It will also discuss caffeine's effects on otherwise minor, undiagnosed conditions, in young adults.

This presentation will address the levels of caffeine found in coffee, tea, caffeine drinks, caffeine supplements, and some over-the-counter medications containing caffeine. These levels will then help the audience understand the effects caffeine has on the normal physiology; specifically caffeine's effect on the cardiovascular system and blood pressure.

The different cultural and psychological influences in which caffeine is incorporated into the lives of young adults will also be discussed. For instance, some manufacturers of caffeine products use cars, sporting events, athletes and music events to promote their product. Some exploit the alcohol use of young adults by combining their caffeine drinks with alcoholic beverages. Others align their products with the images of nature and the environment.

The motivation to consume products containing caffeine and the timing of consumption will also be addressed. Some caffeinated products promote their use prior to physical activity. This is believed to improve physical endurance or athletic ability. For examples, some of these products specifically target the drinks to those who participate in extreme sports; resulting in the combination of high levels of stress to the body and high levels of caffeine. Others market their products to maintain mental sharpness. For example, some of these products specifically target the drinks to 'gamers'; resulting in the combination of no physical stress to the body and high levels of caffeine.

Young adults may not be aware their diagnosed or yet to be diagnosed medical conditions may be exacerbated by the intake of caffeine. Others willingly accept the potential complications or deny the personal applicability. Diabetes mellitus, stomach ulcers, kidney disease and seizures are conditions that may be exasperated by the ingestion of caffeine.

The amount of caffeine in the body at the time of death may be at non-toxic levels but the periodic use of substances containing high levels of caffeine may harm the cardiovascular system or other organ systems. Deaths potentially attributable to caffeine may be similar to deaths involving alcohol. Deaths from the effects of long-term alcohol use are seen frequently but an overdose of alcohol is less frequent.

Education of young adults as to the effects the caffeinated products is imperative, especially when taking into account the increased numbers of caffeinated products being marketed and the decrease of physical activity and fitness in youth.

Four case studies will be presented, of the deaths of young adults who were known to have ingested caffeinated drinks, substances containing caffeine or caffeine supplements for a period of time prior to death.

Caffeine, Death, Heart

#### D35 The Killer Economy

Bethany L. Bless, MS\*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will understand the relationship between the economic downfall and the suicide rate as it pertains to Harris County, Texas. Attendees will also have an increased awareness of the underlying stressors that result in an individual being vulnerable to suicide. Analysis of statistics in Harris County, Texas will be completed during the years 2006-2009.

This presentation will impact the forensic science community by serving as an educational tool for the impact of the poor economy as a possible risk factor for suicide. A full understanding of the risks and factors involved in suicide will help elucidate the interaction between economic cycles, unemployment, and suicide. Improved understanding may help organizations provide services to at risk individuals, possibly preventing some of these deaths in the future.

Recent economic turmoil, increased unemployment and record foreclosure rates have spurred inquiries about whether these changes have led to an increase in suicides. The suicide rates in Harris County, Texas for the period of years 2006-2009 were used to test the general hypothesis that the suicide rate is affected by economic variables and, in particular, to explore the relationship between the economy and the suicide rate. This review shows that a possible relationship exists between unemployment, the economy, and suicide. Although it is difficult to determine the exact stressors that were involved with an individual who commits suicide, a suicide note or comments made to friends or family will give clues into what stressors were involved. This study used investigator reports for Harris County, Texas, and suicide notes left on scene to determine the stressors involved. These statistics were compiled and showed a steady increase in the number of suicides and the number of suicides where the economic downfall, unemployment, or financial strain was specifically mentioned as a primary stress leading to the individuals decision to commit suicide. Statistics showed a steady increase over the years of 2006-2009 in the number of suicides involving financial stress as a factor. The year 2006 was used as a comparison basis for this study as the economic crisis was noted to begin in 2007. There were 363 suicides in 2006, 27 of which revealed suicide notes or comments from family that suggested some financial stress. This is equal to 7.4% of overall cases for the year. In 2007, the number of suicides where notes or family comments confirmed economic stress, increased to 47 cases of the total number of suicides numbering 436 or 10.8%. The trend continued through the 2008 year with 55 of 459 cases or 12.0%. The peak of suicides has been in the year 2009 with 76 of 488 cases or 15.6% of the suicides being directly related to the failing economy. Not all reports contained information valuable in ascertaining whether the economy was a stressor involved. In addition, not all cases involved a suicide note, therefore; many cases where the economy may have been a stressor were unable to be applied with these statistics.

A common "chain of adversity" can begin with job loss and move toward depression through financial strain and loss of personal control. With a better understanding of the risks of suicide, leaders and their organizations can take steps to lessen the impact of the economic downturn. Organizations in the public and private sectors should help make key services more accessible, especially high-quality, comprehensive transition services for the unemployed and assistance for homeowners threatened by foreclosure. Individuals in distress can take action to reduce their own levels of distress. Individuals can engage in activities that relieve anxiety and emotional distress and focus on managing areas in their lives where they still have some control.

Suicides, Economy, Unemployment

# D36 Managerial Responsibilities in the Homicide Investigation Process: Making a Case for Periodic Reviews of All Ongoing Death Investigations

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After attending this presentation, attendees will learn about the frequency of homicides in the United States and the clearance rates associated with them; will learn about some of the reasons wrongful convictions have occurred; will learn about other investigative failures that have occurred; and, will be provided with a sample of managerial tasks, that if properly instituted could increase solvability (clearance) rates for homicides.

This presentation will impact the forensic science community by detailing investigative failures in death cases that have contributed to their "cold case" status of being unresolved and will impact the community by outlining a proposal of instituting certain managerial tasks into the investigative framework to increase the number of solved cases.

Over the past five decades the clearance rate of homicides in the United States has dropped from 92% to 62%. In 1993 the highest number of homicides occurred with 24,530 murders that carried with it a clearance rate of 67%. During the next 15-18 years the number of murders dropped to 16,000 while the clearance rate remained in the low sixty percentile.

With the emergence of DNA the innocence projects found a significant number of people wrongfully convicted and contributed those convictions to incorrect identifications of suspects, false confessions, faulty forensic science and scientists, and jail hHouse snitches or informants. With the exception of faulty forensic science and scientists, the majority of reasons cited for wrongful convictions are directly related to the investigative process.

Furthermore, over the years, during the evaluation of numerous cold cases of unresolved homicides it became evident there are a significant number of investigative failures found within these case files that probably contributed to the fact these investigations are still unresolved. A common thread found throughout these evaluations was that there were no supervisors conducting periodic reviews of these investigations to ensure the detectives remained on track.

Detectives, as a whole, are given complete freedom and latitude to conduct their investigations as they see fit. While most of the time this is probably alright, tunnel vision, for example, has a way of surfacing and the investigation goes awry, wasting time and money. However, the inserting of a periodic review process of these cases by a supervisor would serve to identify problem areas and issues early on in the investigation hopefully preventing the investigation from becoming an unresolved cold case.

With this concept in mind two models of a review process will be addressed. One is subjective while the other is objective in nature. The

subjective review process is basically where the supervisor/reviewer conducts regularly scheduled reviews and in most situations uses his/her experience and knowledge to identify and address problem areas. It is subjective in the sense that this process always opens the door to bias and prejudices from the reviewer who is limited to his/her level of expertise. And, in the experience of the author, the criticism from these reviews sometimes becomes more negative than positive that can create an unhealthy atmosphere and even resentment within the investigative unit.

The second approach is objective and comes to us from a police detective in Great Britain who was attempting to address a way to curb the wrongful convictions. He subsequently designed a "structured and guided approach" to conducting a review of ongoing murder investigations "as opposed to the reviewing officer using their own knowledge, experience and skill," as previously described. The objective review tool he designed, while considered to be somewhat labor intensive, has 31 categories that comprehensively cover the aspects of any murder investigation. As these categories are outlined, one will clearly see how objective this approach to reviewing murder investigations is structured.

In light of what this presentation has uncovered it behooves us to search for other avenues to correct the mistakes found in all investigations, not just death cases. Because of the magnitude of the crime of murder these get more attention and our efforts should be limitless. The author would proffer that periodic reviews of either type, subjective or objective, would move us in the right direction while increasing the solvability rate. But that the structured and objective approach would better serve the criminal justice system and its victims. **Death, Homicide, Clearance Rates** 

#### D37 Postmortem MR and CT in Fire Fatalities

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After attending this presentation, attendees will be able to identify the typical Postmortem Computed Tomography (PMCT) and Postmortem Magnetic Resonance (PMMR) imaging findings in fire fatalities, identify the burn related findings that are seen better on imaging than at autopsy, and describe the fire-related findings that are not well seen by imaging and those not seen by either imaging or autopsy. In sum, attendees will have a thorough understanding of forensic findings in fire fatalities across the spectrum of the major forensic tests that are currently available.

This presentation will impact the forensic science community by demonstrating that imaging methods provide excellent depiction of charred bodies and the majority of forensically important findings that can be identified in such cases. This presentation will provide the forensic investigator with additional tools and means to reach forensic conclusions as well is to display important findings to non-medical personnel such as family members, the police, members of the legal profession, and jurors.

This presentation will describe the evaluation of burn victims using advanced forensic imaging (including PMCT and PMMR), detail the typical imaging findings on both modalities, and finally compare the imaging results with results from traditional autopsy and other forms of forensic investigation to highlight the strengths and weaknesses of the various techniques in fire death investigation.

A retrospective review was performed of burn fatalities who were evaluated at our institution by both forensic imaging (either PMCT, PMMR, or both) and traditional autopsy. The fire-related imaging findings were evaluated and the frequency of injury type and location where recorded. A description of the typical imaging findings is provided in addition to a detailed pictorial review. Finally, the autopsy related findings are compared to the results of imaging. These comparisons are discussed, as is a comparison with results of other published studies on forensic findings in fire related deaths.

Nearly all typical autopsy findings in charred bodies are visible in PMCT and PMMR, including: thermal tissue loss, large areas of skin splitting, heat epidurals, separation of the inner and out tables of the skull, contractures, fire related and non-fire related fractures, organ protrusion, and puppet organs. Imaging by PMCT better demonstrates internal gas collections. PMCT also better demonstrates global findings such as the distribution of debris that may be present and the distribution of fractures in the skeleton as a whole. PMMR best display the rarification of bone marrow that can occur in bones exposed directly to flame, a finding that is difficult to dissect and therefore not often seen at autopsy. Autopsy better demonstrates airway soot deposition, minute surface changes (for example crow's feet), coloration changes in the surface of burned bone, and the fine details of fracture margins. Neither autopsy nor imaging can identify carbon monoxide, although both can be used to collect toxicology samples.

In conclusion, forensic imaging by PMCT and PMMR is well suited for evaluation of fire fatalities. The combination of advanced imaging with the traditional external forensic examination can identify the majority of known and forensically important fire-related findings. PMCT and PMMR can be a useful adjunct or, in selected cases, possibly a replacement for traditional autopsy in the investigation of fire-related deaths.

Radiology, Fire, Virtopsy

# D38 Women: Invisible Territory of Violence in Colombia

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After attending this presentation, attendees will understand the reality of female victims of violence, specifically of domestic violence and spousal abuse in Colombia. The figures according to the 2010 report published by the Reference and Forensic Information Division of the National Institute of Legal Medicine, are a matter of concern. Two cases of female victims of domestic violence and spousal abuse will be presented.

This presentation will impact the forensic science community by providing figures that prove how violence against women is a serious problem in Colombia. Two cases of female victims of spousal abuse who arrived at the coroner's office will be presented. Attendees will conclude that action is required to address these cases in an integral manner. The problem requires involvement from both criminal justice officials and health care professionals, who must create awareness and educate judicial officials who deal with domestic violence victims.

When there is spousal abuse, aggressions against women are seldom isolated cases. On the contrary, these actions are systematic attacks that escalate over time in terms of frequency and intensity and may cause serious injuries to the victims. The likelihood of the victim's death as a result of these systematic aggressions is high.

The first case involves a young woman who was frequently battered by her partner. According to her account, after her spouse choked her mechanically, she lost consciousness and sphincter control; forensic examinations revealed that she had dysphonia, multiple small petechial hemorrhages, bilateral punctiform retinal lesions in both eyes, and several bite marks, among others. The patient was medically diagnosed unable to work for ten days. The forensic examiner's report warned the authorities about the victim's high risk of death. In Colombia, cases where the patient is medically diagnosed as unable to work for less than 30 days are considered misdemeanors and must be settled by the parties. However, the importance of this type of report is that it should be covered by domestic violence laws. In theory, domestic violence laws have different connotations from the legal standpoint. When this woman submitted the medical examiner's report to law enforcement, she was informed that the claim could not be taken and that she could not file a complaint against her abuser because, based on the time of medical disability, it should be settled by the parties, despite the offender's repeated death threats against the woman and her child.

Case two involves a young woman whose fragmented body was found inside several garbage bags in a neighborhood in Bogotá. Seventeen fragments were found. The chest fragment became relevant because it had evidence of a tattoo and multiple sharp force wounds. The body was identified a few days later on the basis of the signs that matched those of a mother of two daughters who had been reported missing by her husband. According to the husband's account, the missing woman had left home after a domestic fight. Investigators suspected that the woman was a victim of spousal abuse and domestic violence by her husband, who was jealous because "his" woman was working. The offender dismembered the victim's body after physically attacking her, which caused her death.

Although significant efforts have been made in Colombia to give women a dignified position in society, legal statutes are still unable to prevent violence against women and establish integral intervention programs aimed at breaking the psychological, emotional, and financial chains that keep women tied to their abusers. Therefore, women are highly vulnerable. Their private spaces, i.e., their homes, can become terrifying places for them and for their children.

Violence Against Women, Domestic Violence, Female Victims

# D39 The Relevance of Scientific Evidences in Criminal Investigations of Mafia's Crime: The Experience of "Capaci's Bloodshed" and the Murder of the Judge Giovanni Falcone

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After attending this presentation, attendees will learn the importance of using a strictly objective scientific method to collect evidence to solve complex judiciary cases concerning Mafia's crime.

This presentation will impact the forensic science community by presenting scientific data and images about the brutal murder of the Judge Giovanni Falcone during the so called "Capaci's Bloodshed."

For over thirty years, the Institute of Legal Medicine of Palermo has worked with the judiciary bench in order to obtain close to certain judgments about the causes of death and the instruments that have caused it, even and especially in range of many different crimes that the mafia committed. In regard of scientific and didactic function of forensic medicine, the Institute of Legal Medicine of Palermo finds it essential to highlight the importance of scientific evidence in any investigation relating to heinous Mafia's crimes. The goal of this research was to gather scientific data, not only forensic, but also those related to other sciences (ballistics, forensic genetics, judicial inspection), from forensics police and any other specialists in the most important killings by mafia during the past few decades.

On May 23, 1992 at 5:58 p.m., while the judge was returning from Palermo's airport, driving one of three armored cars that made up his

escort, he was killed by a violent explosion of TNT along with his wife Francesca Morvillo and three other men in his escort. Only after a thorough judicial investigation which included rebuilding the exact dynamics of events, the investigators were able to trace the names of the murderers who carried out the explosion. In particular, through careful analysis and thorough inspections of the sites, it was established that the car driven by Judge Falcone was placed in the middle in vertical line between the two escort cars; the group's first car with three bodyguards, a brown "Fiat Croma" was fully engulfed in the explosion, and remains were thrown over the opposite traffic lane. The second car, the white "Fiat Croma" driven by the Judge crashed against the concrete wall; Giovanni Falcone and his wife Francesca Morvillo were thrown violently against the windshield and both died during transport to hospital due to serious internal hemorrhaging, while the driver, who took the back seat, survived with no injuries. The bodyguards in the third car, a blue "Fiat Croma," escaped miraculously, as the people that at the time of the attack were passed with their cars in the highway.

Only after time, surveys suggest that the explosion was caused by a charge of five pounds of dynamite placed in a tunnel under the highway and that it was triggered remotely by Giovanni Brusca, an assassin commissioned by Mafia boss Toto Riina. Brusca was located on the top of the hill facing the highway. This location was discovered through a botanical survey carried out on some broken branches and through forensic tests carried out on cigarette butts found in large quantities there.

This work would show how often the resolution of complex legal and forensic issues is only possible thanks to scientific evidence that is relevant and conform to the rules of its scientific field of reference, accompanied by appropriate guidance to clarify the reading and interpretation of scientific data (e.g., specificity and sensitivity of a given test). In the research of the evidence, therefore, it can not be left aside a strict objective method so that, therefore, only a multidisciplinary approach (legal, forensic, and scientific), with the final purpose to collect scientific evidence, delivers the resolution of complex cases.

Scientific Evidence, Mafia's Crime, Giovanni Falcone's Homicide

# D40 Experience of Assisted Suicide in Switzerland

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After attending this presentation, attendees will know about the Swiss experience of assisted suicide, especially laws, associations performing assisted suicide, and the position of medical doctors and of the population.

This presentation will impact the forensic science community by giving information on assisted suicide, which is allowed only in some countries, like Switzerland and a few states in the United States.

Access to effective palliative care offers a dignified end of life to most patients. However, this option doesn't always correspond to patient expectations. Some patients would ask for euthanasia, which is, in most countries, not allowed. Some other patients prefer to end their lives by asking for assisted suicide from their doctor.

Assisted suicide is authorized in Belgium, Holland, Luxemburg (since 2008), Switzerland and several states in the United States – Oregon, Washington (since 2009), and Montana (since 2009).

In Switzerland, some associations offer assisted suicide, under certain conditions, to sick people wanting to end their lives. These associations are, essentially, for the French part of Switzerland, "EXIT-ADMD" (association for the right to die with dignity), as well as "EXIT Deutsche Schweiz" and "Dignitas" (in the german part of the country).

When patients fulfil certain conditions or criteria, EXIT-ADMD provides to the patient sodium pentobarbital which causes rapid loss of

consciousness and death usually within 30 minutes without suffering. The five required criteria are: discernment capacity, incurable disease, serious and repeated asking, severe physical and/or psychological suffering, fatal prognosis or severe invalidity. Most patients asking for assisted suicides suffer from cancer, neurological (like Parkinson disease, multiple sclerosis or lateral amyotrophic sclerosis), cardiovascular, or respiratory diseases.

The rate of assisted suicide in Switzerland has increased during the last fifteen years, but appears to be stabilizing. Probably the number of cases didn't increase so much, but more of them were officially announced, as doctors know better about their rights concerning assisted suicide. This kind of death represents about 25% of suicides in Geneva, but only a minor part of all deaths (about 0,4%).

The practice of assisted suicide has prompted numerous debates among legal, medical and ethical professionals. It is sometimes mistaken with euthanasia, which is quite different because the act is then performed directly by the doctor. The problem of patients who are, for physical reasons, not able to perform assisted suicide (for example not able to swallow or completely paralysed) is still not resolved.

In 2009, 166 members of EXIT-ADMD asked for assisted suicide and 69 assisted suicides were performed. Other have been refused, some of these patients finally died from natural death or are still alive.

Doctors have been asked about their position on assisted suicide. Some of them (2/3) had already been questioned by their patients. Most of these doctors are favourable even if they're not sure to be willing to practice assisted suicide themselves. Two third of them said that assisted suicide should be allowed in nursing homes and hospitals. The problem in nursing homes is that the patients live there and should have the same rights as people living by their own.

Recently, a sample from the Swiss population was questioned and 75% were favorable to assisted suicide for patients with incurable diseases which cause physical and/or psychological suffering, or for very old people. Moreover 63% of the population say assisted suicide should be authorized by nursing homes.

Assisted Suicide, EXIT Association, Switzerland

#### D41 The Effectiveness of Interdisciplinary Forensic Science Education for Multiple Audiences

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After attending this presentation, attendees will understand the importance of educational initiatives in forensic science at all levels and audiences.

This presentation will impact the forensic science community by illustrating the critical need for integrated forensic education on all levels - middle school, high school, and college students and their teachers, as well as law enforcement and judicial practitioners. A discourse between these often disparate groups within the forensic science community is essential to elevate the perception of forensic science as a valid and scientific field.

Recently, Fradella et al. (2007) described the forensic science field as "plagued with poor education and training" while the National Institute of Justice Special Report on Education and Training in Forensic Science (NIJ 2004) assessed the educational training needs of the forensic science community as "immense." Holland et al. (2006:30) acknowledge that all too often, individuals working in the realm of forensic science may have limited knowledge of crime scene evidence collection, processing, analysis, and presentation of this evidence in a court of law. They go on to state: "continuing this trend is unacceptable, as forensic science is the essential link between the crime scene, the forensic laboratory, and the legal system." It is also clear that many of the recent criticisms of the scientific credibility of the field as well as the response to these criticisms (as evidenced by this year's AAFS theme of "Reliable, Relevant and Valid Forensic Science") revolve around issues of forensic science education.

This presentation explores forensic science educational initiatives aimed at different levels and audiences: law enforcement officers working in the field, high and middle school teachers offering instruction in forensic science, and high school and college students interested in forensic science. These initiatives have taken place over a period of several years at Radford University through the RU Forensic Science Institute. Results of these different initiatives are compared and discussed in relation to nation-wide trends in forensic science education.

In 2009, through funding from the National Institute of Justice, the goal was to deliver at no cost to law enforcement officers a series of four 2.5 day workshops focused on "Innovations in Forensic Science"-two of which have already taken place, serving a total of 120 attendees. The target audience was the mid-Atlantic and mid-Appalachian region, an area with numerous small, rural-based law enforcement agencies. Topics of instruction include digital forensics, forensic biology and chemistry, the medical examiner's role in death investigation, forensic entomology, mass fatality incidents, and forensic anthropology and forensic archaeology. Presenters included a mix of forensic scientists (chemists, medical examiners, criminalists), academicians with many years teaching experience in forensic science, as well as law enforcement specialists working in various forensic science fields. Instruction was based on a combination of lecture, laboratory, and field exercises as well as roundtable discussions. Pre-workshop surveys indicated that digital forensics and forensic anthropology and archaeology were among the areas of greatest interest to attendees. Although law enforcement officers demonstrated a wide range of variation in their knowledge about different areas of expertise in the forensic sciences (based on their education and years of experience), nearly half of the attendees (47.5%) indicated that they had received no training or a minimum of two weeks or less prior training in any of the forensic science topics covered. On a scale of 1-5 (1 being not knowledgeable, 5 being proficient), a mean score of 2.19 on the major topics covered indicated that most attendees felt only slightly knowledgeable about these topics before the workshops. Most attendees felt that the major topics covered were relevant to their jobs. In postworkshop surveys, a mean score of 3.3 indicated a 22.4% increase in knowledge by participants. However, at least 18% of participants indicated little interest in learning about the topics discussed and saw little relevance of these topics to their jobs. These individuals apparently attended solely to obtain the 20 hours of in-service training credit offered for participating in the workshop. In addition, overall attendance of these fully funded workshops was low-statistics indicate that rural police agencies and sheriff's offices with small staffs have little financial opportunity to send their officers to forensic science institutions for training.

The results of these educational initiatives in forensic science aimed at law enforcement officers are compared in this poster to similar ones for high school and middle school teachers and students. Significant differences are found in terms of interest, participation, and application of materials taught. It is concluded that encouraging a collaborative discourse between the often disparate communities of criminal justice and forensic science teachers, students, and practitioners is greatly needed.

Forensic Science, Education, Interdisciplinary Training

## D42 A Study of Fingerprint Literature as a Basis for Educational Curricula

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After attending this presentation, attendees will leave with an understanding of how content analysis may be used to create a basis for academic curricula in professions where a significant amount of academic literature exists, but no standard curricula have yet been created.

This presentation will impact the forensic science community by emphasizing the value of curriculum development in forensic science.

Forensic science, being partially born from the practice of law enforcement and the needs therein, does not have standard academic curricula available for many of its subdisciplines, such as firearms, tool marks, and questioned documents. As a result, there was a historical shift in some disciplines of forensic science from a science based on research to a science based on application. This shift may potentially prevent a science from growing and progressing as it should.

It is up to the profession to extract its own curriculum with its own sources, including past and existing procedures and peer-reviewed publications. Developing a curriculum would be useful in outlining the scientific foundations from which the science grew, and perhaps more importantly, to promote that continued scientific growth, as well as providing forensic science educators and trainers a basis to develop their own coursework.

Working groups, such as the Technical Working Group on Education and Training (TWGED), have previously recognized the value of curricula. While these projects do develop a consensus curriculum, they can be lengthy and consumptive of resources. This study seeks to use another means to develop a type of consensus curriculum: published content analysis. Content analysis is a method of analyzing communication in order to produce an objective and quantitative assessment of the content within. It can do this by various methods, including word counts, frequencies, or spatial and time analysis. Content analysis has been used to compare literature in cases of disputed authorship, to compare writing style and technique, and to quantify the effect the communication had on its audience. The benefits of using content analysis for curriculum development are that the literature used is provided to the public for review and it also consists of a diversity literature that spans over long periods of time.

In this study, a content analysis was performed of nine well-known fingerprint books as a way to develop a curriculum for the fingerprinting and friction ridge profession. Specifically, this study uses the table of contents of each written work to compare the chronological and relative order of topics. The order that is deduced from the analysis will provide an "informational hierarchy" for the subject matter. For example, topics appearing closer to the beginning of tables of content may be more basic, more fundamental, or both. Therefore, when taught, the subject should be treated in a similar fashion.

**Content Analysis, Curricula, Education** 

#### D43 Field-Purposing Technologies: Placing Forensic Tools Into the Hands of Field Practitioners for Timely Intelligence

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After attending this presentation, attendees will be provided with insight into this leading-edge area of forensic science, including overview of how Department of Defense (DoD) units analyze evidence in the field to develop forensic intelligence, insight into the training requirements of field personnel, overview of the development of field protocols and procedures, examples of portable forensics technologies already deployed, benefits and limitations of on-site analysis, deployable forensics laboratories and reach back support, and a description of the Forensic Technologies CoE technology evaluation process.

This presentation will impact the forensic science community by explaining how these efforts hold the potential of producing dramatic gains in public safety. This combination of technology, quality assurance and support to practitioners at the point of need provides field personnel with the ability to conduct examinations and quickly develop actionable intelligence. In addition, by utilizing proper protocols, practitioners can run tests while maintaining the integrity of the evidence for follow-up laboratory analysis as needed. By deploying our nation's forensic technologies and knowledge into the field, agencies can increase their capability to predict and prevent events rather than react to them.

As the military, law enforcement and homeland security communities are called to meet challenges such as narco-terrorism, border incursions, and terrorist threats; the need for rapid analysis of forensic evidence becomes paramount. To provide field personnel with the forensic intelligence to conduct investigations and aid missions, agencies are equipping first responders and military service members with portable forensic analysis tools. These technologies allow complex analyses to be conducted outside of the conventional laboratory environment. This capability serves to not only expedite the rapid development of intelligence to lead the investigation, but also to dramatically reduce unnecessary processing by already backlogged laboratories.

However, providing practitioners with deployable technologies is only part of the solution. Ensuring practitioners also have the knowledge, skills and support to properly apply these tools to analyze compounds and gather vital forensic data is just as important.

This presentation will provide an overview of how the National Forensic Science Technology Center (NFSTC) has assisted the Department of Defense (DoD) in this effort by developing programs of instruction, providing reach back assistance, and establishing field techniques and protocols. In addition, an overview of the technology evaluation activities conducted by the Forensic Technologies Center of Excellence (FTCoE) will be presented. Through the FTCoE, forensic scientists evaluate emerging technologies by furnishing unbiased information regarding the performance and usability of new tools. These evaluation reports provide agencies with impartial data to assist them in making the selection of the most appropriate technologies for meeting their operational objectives.

These efforts hold the potential of producing dramatic gains in public safety. This combination of technology, quality assurance, and support to practitioners at the point of need provides field personnel with the ability to conduct examinations and quickly develop actionable intelligence. In addition, by utilizing proper protocols, practitioners can run tests while maintaining the integrity of the evidence for follow-up laboratory analysis as needed. By deploying our nation's forensic technologies and knowledge into the field, agencies can increase their capability to predict and prevent events rather than react to them.

Forensic Intelligence, Portable Technologies, Technology Evaluation

#### D44 From the Bed to the Bench: Defining the Vaginal and Cervical Environment for Post-Coital DNA Recovery

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After attending this presentation, attendees will understand the cyclic variability in the vaginal and cervical environment across the lifespan that may influence the recovery of seminal DNA following rape.

This presentation will impact the forensic science community by helping attendees understand that there may be reasons for absence/presence of DNA beyond the expected time-frame commonly accepted by the forensic community.

The forensic laboratory community has developed a number of advanced technical methods for DNA recovery. Recently, two important studies have: (1) evaluated recovery of DNA past the historical 72 hours promoted as the outside limits for a rape evaluation evidence recovery; and, (2) compared recovery of DNA evidence from the vaginal and cervical sample collected from rape victims. The impetus for both studies came from scholarly presentations and discussions with advanced practice forensic nurses, physicians, laboratory directors, and forensic nurses. The results from the pilot study with three couples looked at the timing of recovery in the post-coital couples. The results from the small sample was that DNA was found routinely at 3-4 days after coitus, but also at 5-6 days post-coitus, and also 7 days post-coitus using enhanced methods for DNA detection. These results question the prevailing practice of limiting evaluation post-rape to a 72 hour period. In the second study, vaginal and cervical samples were compared for the presence of DNA. The results of this study were that cervical samples produced the positive DNA samples when vaginal samples failed to produce recoverable DNA. Both studies challenge the prevailing wisdom, practice and protocols that direct investigation to limit evaluations to 72 hours and collections sites to the vagina for DNA recovery. Specifically, these studies challenge: (1) the limitation of rape evaluation and evidence collection to 72 hours for recovery of DNA; and, (2) the optimum location for the recovery of DNA in the post-coital sample.

The literature review reveals that the medical literature reports a variety of physical and environmental changes in the genitourinary structures impact fertility. In addition, it is known that the normal appearance of genital and urinary structures change throughout the monthly cycle, are predictable, and documented over the lifespan of the female. Tools have been developed to help classify the changing appearance. However, the research has not quantified the changes in females that includes cyclic variances, e.g., the monthly cycle of a reproductive female, or the expected changes across the life span, e.g., infant, pre-pubertal, pubertal, reproductive aged, peri-menopausal, menopausal, to late menopausal changes.

To complicate the interpretation, the addition of ejaculate to the genitourinary environment, the nature of the semen, sperm and the influence of the cyclic vaginal environment on seminal properties in and out of the environment have not been considered by the forensic community. In addition, the vulvovaginal environment across the life span is not studied in the context of the forensic sciences or recovery of post-coital DNA.

This presentation will lay the foundation for understanding embryology and physiology of the vaginal and cervical environment, and the maturation of the vulvovaginal and cervical structures across the life span. The presentation covers the estrogenic changes and the impact of mixing ejaculate in the environment in the context of the post coital environment with the purpose of laying a foundation for future research questions and explanations for why a forensic sample produced (or did not produce) recoverable DNA.

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DNA, Rape, Post-Coital Environment

#### D45 Elder Abuse: Keep Your Family Close and Your Wallet Closer

#### Amy Y. Carney, PhD\*, 210 Ivory Gull Way, San Marcos, CA 92078

After attending this presentation, attendees will understand the dynamics of elder abuse, identify three major forms of elder abuse in San Diego County, and identify an at-risk population for financial abuse.

This presentation will impact the forensic science community by raising the awareness of financial abuse in the elder community, showing perpetrator and elder relationships, and highlighting the need for further forensic investigation and research in financial abuse.

Elder abuse is a recognized social problem in the United States. It was first labeled as "granny bashing" in British medical journals in the 1960's but this problem remained poorly addressed until one of the earliest studies in America surfaced in 1979. In 1976 the Subcommittee

on Housing and Consumer Interests noted that no group of American citizens suffered more painful losses at the hands of criminal predators than the elderly. The Administration on Aging had stressed the importance of investigation into elder mistreatment in 1978. Originally studied under the umbrella of family violence, maltreatment of the elderly has received more funding and research attention in recent years. The National Center on Elder Abuse has defined domestic elder abuse as "any of several forms of maltreatment of an older person by someone who has a special relationship with an elder, such as a spouse, sibling, child, friend, or caregiver. The American Medical Association also provided a definition of elder abuse that stated "abuse shall mean an act or omission which results in harm or threatened harm to the health or welfare of an elderly person. Abuse includes intentional infliction of physical or mental injury; sexual abuse; or withholding of necessary food, clothing, and medical care to meet the physical and mental needs of an elderly person by one having the care, custody, or responsibility of an elderly person." Multiple aspects of elder abuse have been examined in the literature including measures for detection, assessment, and documentation. Sexual abuse of the elderly has been documented in several studies, often including victims with functional or cognitive impairment. Sexual homicide is also well documented in the literature. Financial exploitation is the inappropriate use of an elderly person's resources for personal gain, and has become much more prevalent in recent years. Telemarketing fraud, extortion, theft, and credit card fraud has left older adults unable to pay for food, medication, and medical care. Coercion to change a will, signing over deeds, and transfer of personal belongings or giving of material goods without consent all constitute abuse. Financial abuse is expected to increase in the coming years as the population ages. Frequently seen as a valuable and vulnerable target the elderly often have assets that are desirable such as property or good credit. Financial, physical, and sexual abuse are often seen in combination. Although studies have examined the circumstances surrounding abuse as well as theories of causation and characteristics of the abuser and abused, studies of the relationship between the abuser and abused are less well documented. This study examined the abuser relationship, family or non-family, and types of abuse (financial, physical, sexual) in the elderly through evaluation of prosecuted cases of elder abuse by the San Diego County District Attorney's Office. 155 cases of elder abuse were identified in Court records evaluated for the years 1996 through 2009. Incidence of types of abuse are presented as well as findings for statistical significance for abuser-abused relationship, type of abuse, and demographic predictors for financial and physical abuse, as well as future directions for forensic study in elder abuse.

**Elder Abuse, Financial, Perpetrator Dynamics** 

#### D46 An Interdisciplinary Approach to Child Sexual Abuse Investigation in Colombia

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After attending this presentation, attendees will have a general knowledge about the importance of a multidisciplinary approach in order to do a proper forensic analysis as well as social, legal, and criminal in cases related with sexual child abuse, at the same time attendees also will understand the Colombian forensic work in the reported cases.

This presentation will impact the forensic science community by showing how hundreds of people in Colombia had been found guilty of child sexual abuse without a scientific interpretation of forensic evidence and/or a proper criminal investigation.

Since 1999, when Luis Alfredo Garavito, one of the worst serial killers and child abusers of Latin-America was arrested, the number of accusations of child sexual abuse in Colombia have increased year by year and in 2009 the Colombian Institute of Family Welfare reported

17,000 cases in which most of the victims are children under 14-yearsold of which 75% are girls. But it is possible that an important number of these cases are just false reports

Within the Colombian criminal code, child sexual abuse is represented by two kinds of crimes, sexual touching and sexual assault. In the first case there are not any physical evidences in victim's body; while in the second the victim has been penetrated. In accordance with statistics children less than 12 years old are in most of the cases, victims of sexual touching while children up to 13-years-old are victims of sexual assault.

Beginning January 1, 2005, with the implementation of the adversarial system in Colombia, hundreds of people had been found guilty of charges in cases related with child sexual touching and child sexual assault without any forensic evidence or a proper criminal investigation.

Child sexual touching cases around the world as well as in Colombia can be very difficult to prove largely because cases where definitive, objective evidence exists are the exception rather than the rule. When child sexual abuse occurs the child victim sometimes becomes the only witness and the child's statements are usually the only or most important evidence. In such cases, the central issue becomes whether the child's statements can be trusted but neither prosecution nor defense are doing an appropriate legal and/or forensic interview to find the true.

In spite of the high number of accusations and the Colombian State's efforts for protection and prevention, the criminal and forensic investigation of child sexual abuse cases in Colombia is still far away from international levels due to several factors. Within these are an inadequate criminal investigators' training on these kinds of crimes, the analysis, reports, and testimonies of non-forensic experts in the court, manipulation of victims' minds, the use of neither scientific nor technical resources by psychologist and a bad praxis from some medical doctors, especially in rural areas.

Together with this scientific issue, there are other difficulties. On one hand, judges, attorneys, and prosecutors are not trained sufficiently to use and understand physical and forensic evidence but on the other, the mass media and social prejudgment about these cases are taking an important impact in judges 'minds.

Since 2007 with the new children's protection code, people who are condemned by child sexual abuse in Colombia have no legal benefits even if they confessed to their crimes. This is the only crime that has no benefits for offenders in this country and unfortunately some people are using it for personal revenge or economical interests.

Through a multidisciplinary analysis from the legal context, forensic sciences (psychology and medicine), criminal investigation, and social anthropology, an interdisciplinary team working in some cases for the defense and in others for the victims, will bring real cases, their physical and testimonial evidences, criminal processes and sentences.

Child Sexual Abuse, Forensic Interdisciplinary Work, Crime Scene Investigation

# D47 From this Day Forward: To Have and to Hold – Spousal Rape of Asian Immigrant Wives

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After attending this presentation, attendees will better understand salient elements germane to select cases of immigrant spousal rape, and understand gaps and deficiencies in care, evaluation, and guidance for marginalized victims of sexual trauma. This presentation will also heighten awareness and promote collaboration among medical, legal, and other professionals who may encounter immigrant victims of spousal sexual trauma.

This presentation will impact the forensic science community by contributing to the understanding of sexual violence in marginalized groups of women, e.g., Asian immigrant victims of spousal rape, improving collaboration among all professionals who intersect victims of immigrant spousal rape, and providing a framework for the forensic evaluation of victims of immigrant spousal rape.

During the 1970s, concomitant with the rape crisis movement, society finally admitted the possibility of rape within marriage. It was 1993 before marital rape became a crime in all 50 states. Differences exist in how states implement the laws (NCVC: 2010). In 1993, California's law was amended. The reporting period was expanded to 1 year (vs. 3 years for non-spousal rape). A comprehensive body of research exists on marital rape. What can be said about men who target females that they believe embody mythical stereotypes of Asian women?

Asian Mail-Order Brides –The Circuit of Culture, explored the historically-prized Asian mail order bride industry. Both mass-produced paper catalogues and countless web pages continue to depict a seemingly infinite supply of eager young Asian prospects for marriage. They are, according to Ms. Ho, "submissive, obedient, loyal, soft-spoken, and meek." Other images that come to mind are the "geisha girls, China dolls, Miss Saigons, and the Comfort women that fill the media, books, and popular culture." (Christine Ho, USAsians.net: 2003).

Regardless of their real-life personality traits, a myriad of factors can increase the vulnerability of these women to fall prey to those with the motivation to control and victimize. While mail-order brides are targeted products that advertise cultural stereotypes, these stereotypes are not exclusive to the mail-order bride business.

Barriers exist for all victims of marital rape. For groups that are marginalized, e.g., immigrant women controlled by abusive spouses, the web is a tightly woven noose. Along with expected stressors of sexual trauma, they face fear of deportation, possibility having to leave their children or new, loving relationships.

From the lens of altered cultural perspectives, marginalization, and marital rape, two young Asian women who were likely perceived not as *mail-order*, but perhaps *made-to-order*, by their assailants, legal American citizens will be discussed. Both cases occurred in Northern California; both sexual assault victims faced deportation back to their country of origin. The discussion includes steps taken by the FCNS by a San Francisco Bay Area legal advocacy caucus. The Criminal Investigative Profiler provides a conceptual framework about the assailants to understand the dynamics of the assailants.

**Materials and Methods:** <u>Case 1</u>: 33-year-old Asian female; an aunt introduced her to her future husband in East Asia. She was approaching age 30 and under family pressure to marry. After dating in her country for a year, they married in the United States. The spousal assaults began shortly afterwards. The duration was 15 months and often included daily episodes of painful anal penetration. The FCNS evaluation occurred 1.75 years after the last episode of sexual assault.

<u>Case 2</u>: 33-year-old Asian female. Three years earlier, an aunt introduced her via email to an American male. She later visited her aunt in California and met the man in person. Forced sexual acts, including sodomy, commenced shortly after the marriage, a few months later. She later learned that her marriage was not legal. Fifteen months after the last sexual assault, she was evaluated by the FCNS.

**Discussion:** *Cracks, Gaps, Barriers, or a Need to Change?* These cases cross more than national and cultural boundaries. Superimposed upon the myriad sequellae of sexual assault trauma are a host of ubiquitous factors such as language barriers, lack of medical insurance, isolation, lack of funds, and cultural disparity. Most tragically poignant may be the disbelief that even in America, marital obligation is synonymous with endurance, even with criminal activity. The words of one Asian woman perhaps best summarize the pathos:

"Life felt like a daily torture but I did not know what to do about it. I never imagined that my married life would be like this, but thought that I had to endure the pain because it was my duty as his wife. "

Numerous roadblocks were encountered in efforts to evaluate the first case; lessons learned sped the evaluation of the 2<sup>nd</sup> case. They also confirmed the glaring gaps in our provision of care and services to these women.

These cases are discussed in an effort to clarify gaps and missed opportunities by the medical-legal system. All women, regardless of their background, language, culture, or circumstances, deserve assistance, and support after such intense levels of prolonged intimate sexual violence.

Immigrant Spousal Rape, Forensic Clinical Nurse Specialist, Criminal Investigative Profiler

## D48 Suspect Examinations for Evidence in the Investigation of a Sexual Assault

Diana K. Faugno, MSN\*, Forensic Registered Nurse Consultants, 1351 Heritage Court, Escondido, CA 92027; and Patricia M. Speck, DNSc, 1740 Overton Park Avenue, Memphis, TN 38112

After attending this presentation, attendees will be able to list several barriers as to why suspect examinations are not considered, as well as, be able to give examples of when a suspect examination should be considered.

This presentation will impact the forensic science community by expanding knowledge and will review common misconceptions about examination collection and potential interpretation as well as the information that can be obtained during the examination process.

All too often suspect examinations are often overlooked in a sexual assault investigation. Most law enforcement agencies as well as nurse examiner programs have failed to establish appropriate policies and procedures for obtaining comprehensive forensic examinations for sexual assault suspects. The purpose of this presentation is to make the case for the importance of suspect examinations, for the collection of evidence from both the suspect's body and clothing, to explore some of the reasons and barriers as to why they often are not done, and to provide concrete recommendations for overcoming these barriers and using suspect examinations effectively in your community. Any evidence that provides corroboration of the victim's account and documents force or injury is absolutely critical for the investigation of sexual assault. The forensic examination is arguably the most critical component in the aftermath of a sexual assault. The exam has two main goals: (1) to treat the survivor of the assault for any medical injuries that may have resulted from the assault; and, (2) to collect precious evidence that may eventually lead to the arrest, prosecution, and conviction of the offender. At the completion of the examination, the medical forensic report is generated. The suspect examination form will have information that can be impact the investigation of sexual assault. Common misconceptions will be reviewed about examination interpretation as well as the information that can be obtained during the examination process. When evaluating potential sources of evidence, law enforcement professionals often focus on anything that might have transferred from the suspect to the victim; thus, forensic examinations of the victim are seen as critically important. However, keep in mind that any evidence that could potentially be transferred from the suspect to the victim may also be transferred from the victim to the suspect. Therefore, depending on the type of contact involved in a sexual assault offense, the suspect's body may actually be a better source of probative evidence than the victim's. For example if the suspect forced his penis into the victim's mouth during the sexual assault, his penis maybe a richer source of evidence than the victim's mouth. Clearly, any evidence from the suspect's body that establishes the identity

of the victim will be important in the investigation and prosecution of sexual assault. It is therefore surprising that so few law enforcement agencies routinely collect forensic evidence from the body of the suspect. It's Not Just About DNA Identification.<sup>1</sup>

Yet the importance of the suspect examination is not solely based on the potential for documenting the victim's DNA for identification purposes. Depending on *where* the victim's DNA is found on the suspect's body, it may provide a better idea of the specific acts that were involved in the sexual assault (e.g., penile-vaginal penetration, digital penetration, oral copulation). This type of evidence may be particularly helpful with very young victims or with victims who are under the influence of drugs or alcohol, because they may not recall or may not be able to articulate exactly what happened to them. Evidence of the victim's DNA on the suspect's body can also be important in cases involving multiple perpetrators, where the victim knows that a suspect participated in the assault but is not sure if he penetrated her. **Reference:** 

<sup>1.</sup> Forensic Exams for the Sexual Assault Suspect by Joanne Archambault 2008.

Suspect Examines, Sexual Assault, DNA Collection

## D49 Examination of Sexual Violence Victims in Colombia: A New Approach

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After attending this presentation, attendees will learn the reality of the medical examiner's response to survivors of sex crimes in Colombia. The introduction of new elements in routine victim examinations will be proposed.

This presentation will impact the forensic science community by introducing new technical and technological tools that will provide medical examiners with new basic equipment for the collection of evidence from sex crimes survivors in Colombia. Three sexual assault cases evaluated by the Forensic Clinical Services of the Colombian Institute of Legal Medicine and Forensic Sciences will be described.

A large part of the Institute's casework is represented by forensic examinations of victims who report sexual abuse. According to the 2010 Masatugo publication, 73,395 sexual violence victims were examined between 2004 and 2008. These victims were primarily girls and female teenagers under 18 years of age. Forensic examinations of sexual abuse victims require a thorough search for physical evidence, which is essential for prosecution. Although sexual assault reports are less frequent, the special characteristics of these crimes, where physical force and contact between the perpetrator and the victim are involved, require expertise of medical examiners in terms of finding as much evidence as possible. The other element of the investigation is the investigators who work in both sexual abuse and sexual assault cases. Their efforts involve interviewing victims, understanding the context of the crime scene, and obtaining additional information to clarify the facts. Witness presentation helps prove the case beyond reasonable doubt. However, cases where no physical evidence exists are a huge challenge. Until recently, forensic examinations of sex crime survivors in Colombia were visually conducted by medical examiners. This promotes the loss of imperceptible evidence that examiners may miss. Obviously, this is not only detrimental to the victim, but it promotes impunity. Health centers in charge of sexual assault victims' attention should be equipped with new technical tools that help medical examiners minimize the loss of physical evidence. The first recommendation is the use of alternate light sources (ALS) for both the victim's physical examination and her/his clothes. Alternate light sources contribute to the detection of evidence frequently found in unusual places. The use of dermatologists magnifier glasses is also recommended. Toluidine blue staining and colposcopic examinations should be part of the process.

In these cases, alternate light sources, dermatologic magnifier glasses, Toluidine blue staining, and colposcopy were systematically used. These cases prove that these instruments may help medical examiners find relevant evidence. Consequently, these tools should be part of the basic equipment required by health care centers responsible for these cases. This new procedure will give the forensic community the arguments required to support the need for the routine use of these instruments.

Sexual Assault, Physical Evidence, Basic Equipment

# D50 Pictures From the Dark Side — Inaccurate and/or Biased Sexual Assault Examination Reports From a Defense Expert's Prospective

#### Theodore N. Hariton, MD\*, 65344 East Rocky Mesa Drive, Tucson, AZ 85739

The goals of this presentation are to: (1) better understand that sexual assault examination reports need to be carefully evaluated before and at trial; and, (2) to demonstrate that conclusions drawn by the sexual assault examiner may be inaccurate and/or biased and the examinations themselves incomplete with little or poor documentation to substantiate the sexual assault examiner's conclusions.

This presentation will impact the forensic science community by presenting the sexual abuse examination as an important tool in law enforcement that needs to be carefully guided to be a fair, honest asset to both defense and prosecutors in their quest for justice.

**Synopsis:** Actual examples of SART examination photos and statements by sexual assault examiners will be shown. They will include those that were used in trials that have lead to convictions in which the information given to the jury was not accurate. The interpretation of the photos themselves could be inaccurate or the information from the photographs was interpreted in such a manner as to suggest sexual assault had occurred. This could be whether this was an accurate interpretation or not and even if accurate ignored the fact that there were other possible causes that would explain the findings.

The interpretation of the sexual assault examination reports also leads police to make arrests and prosecutors to charge in situations that do not warrant these actions.

The issue as to the genesis of the inaccurate examination reports will be discussed. Is it just from the lack of experience and knowledge of the examiner or does advocacy become a major factor? Cases on point from inexperienced and very experienced examiners will be shown and discussed.

Problems in selecting candidates to become sexual assault examiners will be discussed as will the lack of consistency in the training and continued qualification of these examiners. The lack of consistency in standards for peer or case review will be discussed.

From experienced centers we see excellent reports which include quality photographs and a reporting of the findings in a professional manner with accurate dispassionate discussions of the findings and their possible interpretations. However, with the rapid expansion of the sexual assault examination program there is now a major difference in the quality of the reports from various centers. For example in the last six cases reviewed from one northern state no photographs were taken.

The manner in which sexual abuse examiner groups has escalated in the last twenty years with their own ability to qualify their examiners has lead to a marked differential in skill sets of the examiners in various parts of the country and in the same states. These groups are either privately owned, owned by emergency rooms or rape crisis centers and contract with local hospitals or local law enforcement agencies to deliver this service. This does lead to the consideration of a possible conflict of interest.

With the need to fill their staffs and meet their contracts, these sexual assault examiner groups at times must use less than experienced examiners with the expected results. This leads to situations in which a less experienced sexual abuse examiner's report is presented in court and is challenged leading to more senior member of the group rebutting the challenge to the report in a protective but less than accurate supplemental report. Examples of this will be shown.

Sexual Assault Examiner Qualifications, Conflict of Interest, Trial Preparation

## D51 Characterization of Soil Composition Using a Wavelength Dispersive Spectrometer X-Ray Mapping Method

Craig S. Schwandt, PhD\*, McCrone Associates, Inc., 850 Pasquinelli Drive, Westmont, IL 60559

The goal of this presentation is to describe an electron microprobe method using stage mapping, which is being developed to help quantify soil characterization. Example soils that highlight the issues involved with developing the methodology of this approach for soil characterization will be discussed.

This presentation will impact the forensic science community by helping provide additional statistically defensible compositional parameters for the comparison of soils.

An electron microprobe method using stage mapping, which is being developed to help quantify soil characterization will be described. Example soils will be discussed that highlight the issues involved with developing the methodology of this approach for soil characterization.

Adoption of this, or similar methods, once developed for the characterization of soils will help provide additional statistically defensible compositional parameters for the comparison of soils.

Comparison of soil types can be subjective and dependent of the experience and biases of the forensic microscopist or investigator. Descriptions of soil often include aspects such as color, size fractions, general type – sand, silt, clay, amount of organic matter, and mineral composition. Most of these aspects rely on the experience of the examiner for adequate description. Therefore data from different examiners, especially data provided by examiners from different localities or organization, are often not directly comparable. Efforts to develop a standard method for semi-automatic compositional characterization of soils using stage mapping methods in an electron microprobe with wavelength dispersive spectrometers (WDS) are ongoing. This approach is developing in tandem with an approach utilizing an automated scanning electron microscope with energy dispersive X-ray spectrometer (EDS).

Both the WDS and EDS approaches utilize a sieving process to portion out grains based on size, thus capturing statistics for the size of the soil particles. One size range at a time is characterized by composition. Additionally, morphology information can also be obtained. However, at this time research is focused on completing development of the algorithm for uniquely identifying the mineralogical composition of the grains. A sampling of one size fraction is prepared as a polished grain mount for analysis in the electron microprobe. The WDS approach utilizes element ratios and creates element ratio maps on a pixel by pixel basis created from elemental maps captured by the WDS system. The ratio of elements contained within different crystal structural sites of the soil minerals is unique to each mineral group. Therefore the abundance of the mineral types can then be determined from the element ratio maps. Each map can consist of millions of pixels, each representing a compositional analysis. The abundance of each mineral type for one size fraction can then be combined with the data acquired for the other size fractions of a soil sample to provide a mineralogical histogram for the soil sample as a whole. In other words a quantitative description of the soil sample is produced containing statistical information pertaining to the size and to the mineralogical composition.

The latest progress in our efforts to refine this method will be presented. Ultimately, adoption and use of this method would improve quantitative comparison of soil samples from different localities. This would reduce subjectivity involved with soil comparisons and potentially increase the forensic usefulness of soil comparisons.

Quantitative, Soil Comparison, X-Ray Mapping

# D52 The Application of *In-Situ* Reflectance Spectroscopy for the Detection of Mass Graves

Emily A. Norton, BSc\*, 8 Winchester Drive, Burbage, Hinckley, Leicestershire, LE10 2BB, UNITED KINGDOM

After attending this presentation attendees will be able to understand how in-situ reflectance spectroscopy could aid in the location and detection of single or mass graves, will appreciate the potential for further research to be conducted at human natural burial grounds within the United Kingdom, will recognize that remote sensing in the form of *in situ* reflectance spectroscopy has definite potential to be applied in a soil forensic context, and will recognize the importance of conducting research on human burials rather than simulated or animal burials.

This presentation will impact the forensic science community by offering a quick, non-invasive tool which can be utilized within the initial search, location, and detection of mass graves.

Research was conducted to investigate the findings published within the paper "The Application of Remote Sensing for Detecting Mass Graves: An Experimental Animal Case Study from Costa Rica" (Kalacska et al. 2009). Kalacska et al. (2009) conducted spectral measurements using both *in situ* reflectance measurements and hyperspectral analysis on two simulated mass graves (one containing cattle and the other, disturbed soil) over a sixteen month period. The original study was conducted in the tropics of Costa Rica and thus, a recommendation was made for further research to be conducted in alternative climatic zones to ensure that the extent of the application could be ascertained and determine whether this form of remote sensing could provide the international community with a preliminary detection tool for mass graves.

Therefore, research was conducted into the application of *in situ* reflectance spectroscopy, monitoring within the visible to near infrared (350-2500nm) to ascertain whether there was potential for this technique to be used as a quick, non-invasive method for the detection and differentiation of soil from grave and non-grave areas. The *in situ* reflectance measurements were collected using a portable fibre type vis-NIR LabSpec Pro, Near Infrared Analyser from Analytical Spectral Devices Inc (ASDI), USA, were conducted.

Therefore, to assess the application of in situ reflectance spectroscopy, two investigations were designed and undertaken to allow a new research methodology to be created; a controlled laboratory based pilot study and field work, focusing on four natural burial grounds within the United Kingdom.

The methodology developed for the controlled laboratory pilot study included simulated mass graves containing organic minced beef being exposed to 30°C over a period of six days. Reflectance spectra were obtained on days one and six of each of the experimental weeks; for statistical reasons the pilot study was repeated four times over four consecutive weeks. The collected spectra from the four pilot studies were subjected to Principal Component Analysis (PCA) to ascertain whether the spectra obtained from the soil from grave and non-grave areas on day six were significantly different to those collected on day one.

Consequently, it was found after subjecting all of the reflectance spectra obtained to PCA that within all of the similarity maps produced from the laboratory study, clear differentiation was observed between the spectra collected from grave areas in comparison to those from the nongrave areas on day six.

The second of the two investigations, the field work, was conducted at four Natural Burial Grounds within the United Kingdom. An operating procedure was devised which was employed for all of the twenty four graves measured; thirty three reflectance spectra were obtained from the soil from both grave and non-grave areas. The spectra collected from the natural burial grounds were also subjected to PCA, after which it was found that differentiation between the spectra obtained from the grave and non-grave areas was achieved within 75% of the graves measured, whereas, 25% of the similarity maps produced indicated that no differences between the spectra obtained existed.

In conclusion, it was found from conducting both a controlled laboratory study and also field work, that *in situ* reflectance spectroscopy monitoring within the visible to near infrared does have the potential to be utilized as a preliminary tool for the location and detection of single or mass graves during site assessment.

The aim of site assessment is to locate and define a potential area which may contain a mass grave; during which many disciplines are pooled to create a multidisciplinary team. Any information regarding the potential location of a mass grave is collected and investigated. The difference sources of information often utilized during area location are eye witness testimony, aerial imagery and geophysical survey; as these sources enable the investigation to become more focused on one particular area (Anderson et al. 2008).

Within literature it is documented that one of the most successful methods of mass grave location was found to be eye witness testimony; where evidence is often sought to corroborate the information obtained, to determine the mass grave location; this has been seen in countries such as Bosnia.

Due to mass graves becoming the stimulant for criminal proceedings increasingly over the past decade, the emphasis placed on recovering evidence utilizing forensic principles has also increased dramatically. Consequently, the first standard operating procedure for the investigation of mass graves was published during 2008 by Cox et al., within which the forensic principles of site integrity and continuity were emphasized. Particular weight was placed on the methods used to detect mass graves, where the least intrusive techniques are employed prior to the more intrusive methods, to ensure that the site's integrity and also the integrity of the evidence is maintained.

Consequently, it is intended that this application of *in situ* reflectance spectroscopy could be used as part of the process towards confirming the information obtained from eye witness testimony or aerial photography during initial assessment missions; particularly during the processes of area and site location. The method proposed within this research is non-intrusive, quick and the instrument is portable and would therefore be a valuable addition to the multidisciplinary methods currently utilised within the location and detection of mass graves.

Mass Graves, Humanitarian, Remote Sensing

### D53 Asian Forensic Sciences Network - Report on New Regional Collaboration

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After attending this presentation, attendees will have an appreciation of the Asian Forensic Sciences Network and developments in the forensic sciences scene in Asia. This presentation will impact the forensic science community by providing a better understanding of the network, which will help to open up collaborative and co-operative opportunities, and promote closer interaction between counterparts in the North American continent and those in Asia.

Regional associations, such as the American Society of Crime Lab Directors (ASCLD), the Academia Iberoamericana De Criminalistica Y Estudios Forenses (AICEF), Senior Managers of Australian and New Zealand Forensic Laboratories (SMANZFL), and the European Network of Forensic Science Institutes (ENFSI) have been formed over the years to bring forensic science communities in various regions together to discuss common issues.

In 1999, the United Nations International Drug Control Programme (UNDCP), forerunner of the United Nations Office on Drugs and Crime (UNODC), organized a regional Consultative Meeting for the Heads of Drug Testing Laboratories in Southeast Asia in Hong Kong SAR. This lead to the publication of an annual regional newsletter *DrugNetAsia*, this newsletter has served as a platform for information sharing among the drug testing laboratories in the region.

In 2006, DNA scientists in the region met at a regional Symposium on Forensic DNA and Population Statistics Workshop held in Singapore, and discussed issues of common interest. This was followed a year later, by the formation of a regional Forensic DNA Profiling Workgroup arising from the perceived need to share information efficiently after the 2004 Asian tsunami. In the same year, Dr. Barbara Remberg of UNODC vetoed the idea of forming a regional forensic science network during a regional UNODC project workshop on precursors and illicit drugs.

In October 2008, representatives from six national forensic institutes in the region converged in Singapore to discuss the issue of the formation of a regional forensic science network. The six institutes are: Department of Scientific Services, Brunei Darussalam; Department of Chemistry, Malaysia; National Bureau of Investigation, Philippines; Central Institute of Forensic Science, Thailand; Forensic Science Institute, Vietnam and Health Sciences Authority, Singapore. Dr Barbara Remberg of UNODC and Prof Jose Lorente, International Liaison Officer of AICEF were also present at the meeting. This meeting gave birth to Asian Forensic Sciences Network (AFSN) which will henceforth serve as a collective voice for the forensic science community in Asia.

In November 2009, the network was formally inaugurated in Kuala Lumpur, Malaysia with adoption of a formal constitution and election of the AFSN Board. Three technical working groups (DNA Workgroup, Illicit Drugs Workgroup, and Trace Evidence Workgroup), and the Quality Assurance and Standards Committee were formed.

The Second Annual Meeting of AFSN was successfully organized in Brunei Darussalam in May 2010. The program included a number of workshops and scientific meetings, with poster sessions as well as the business meetings and the annual general meeting. At the AGM, AFSN signed a memorandum of understanding with the International Forensic Strategic Alliance (IFSA), to work together with other regional networks to further strengthen collaboration and co-operation in the forensic arena globally. A toxicology workgroup was also formed to promote technical discussion and advancement in this discipline. The membership of the network has grown from the initial six institutes to a total of eighteen covering eleven countries in Asia.

The presentation will elaborate on the goals and objectives of the network, and a general introduction to the profile of its members and the ongoing work of the workgroups. The presentation will also discuss the challenges that members of the network face in providing quality forensic scientific services to their communities.

Regional Network, Asia, Forensic Sciences

# D54 An Overview on the Wood and Hair Digital Images Databank Project of the Brazilian Federal Police Anatomical Laboratory of Biological Samples

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After attending this presentation, attendees will understand details about the Brazilian initiative concerning the use of anatomical features to identify wood and wildlife.

This presentation will impact the forensic science community by showing some specific aspects of the anatomic analysis adopted by the Brazilian Federal Police Forensic Team that works with environmental crimes (deforesting and wildlife) to identify the seized biological material (wood and hair).

Brazil, as a mega-diverse country, has an ecological relevance with its great variety of ecosystems, some of them considered the richest of the world (e.g., Amazonia, Atlantic Forest, and Cerrado), but are seriously threatened by a sort of environmental crimes. In Brazil, the Federal Police has the duty of the judiciary police, established by the Federal Constitution. Among the several acting areas (such as fighting drug trafficking, money laundering, cyber crimes, among others), recently, the Federal Police has developed a specialized intervention investigating and performing various operations to combat environmental crimes in areas under the authority of the Federal Government (Indian Lands, Federal Conservation Units, Public Forests, and Public Lands). Great effort has been spent in selection, hiring and training of skilled professionals, as well as improving infrastructure and logistics.

This presentation describes the deployment of the Anatomical Laboratory of Biological Samples to aid in forensic examination of seized material (wood and hair) subject to federal criminal investigation, arising from arrests in cases of environmental crimes (under the Brazilian Environmental Crimes Law), such as deforestation, transport/trade of illegal flora products, wild animal trafficking, illegal hunting, etc. The National Institute of Criminalistics of the Technical-Scientific Directorate of Federal Police is a modern forensic complex, with over 20,000 m<sup>2</sup> of floor space, located in Brasilia. Its facilities are divided into sectors and laboratories that comprise the most different areas of expertise (accounting, ballistics, chemistry, computer science, and environmental, among others). The Anatomical Laboratory of Biological Samples is part of the Area of Expertise of Environment that, because of its recent and multidisciplinary character, has a great need and momentum of expansion and development. The exams of materials seized are performed on a workstation composed of a binocular microscope coupled to a high resolution digital camera, allowing evaluation and record of microscopic images of layers of biological samples, which is thus compared and feeds digital database hosted on the intranet of the Federal Police and accessible to federal experts distributed throughout Brazil. The Anatomical Laboratory is particularly useful in actions to refrain the illegal logging, when there is no possibility of dendrological identification by leaves, fruits, flowers and seeds, as well as the trafficking of wild animals, illegal hunting and other forms of crimes against wildlife, which demanding the identification of parts of animals by examining their morphological and anatomical structures. These crimes cause several damages to the society affecting soil, water, flora and fauna, and represent a usurpation of the Brazilian public property and a threat to the national genetic heritage. The anatomic identification of wood takes place, either by a naked eye exam, from the general characteristics (odor, color, grain, etc.) as by anatomical features (layout, distribution, density, size of vessels, parenchyma, fibers)

\* Presenting Author

visualized with increases of 10 to 80x. Similarly, the identification of wild mammals is done with the analysis of anatomical structures (cuticle and medulla) with species-specific features present in certain regions of the hair (rod and shield). In general, are also used increases of up to 80x optical with additional digital magnification (up to 500x). Examining the image of the wire capillary allows identification which occurs through the morphology of the scales of the cuticle (the hair surface region) and the characteristics of their bone marrow, located within the capillary structure. The records obtained feed electronic databases consist of anatomical and morphological information extracted from images generated by a set of optical stereoscopic vision with increased optical to 80x, plus a digital camera to record real-time image. This set also features dual light source and is guided and connected to a personal computer with software for viewing, measuring and storing images of anatomical elements, allowing the realization of statistical calculations and enhancement of images, as well as the information store. Operations to repress environmental crimes have intensified resulting in the arrest of several involved, but it is worth noting that, to convict the defendants, it is imperative to be demonstrated the materiality of the crime, namely that the material aspects to confirm the crime are presented. The fight against illicit environment acts depends on proper instruction of police investigation and lawsuits with the aid of good forensic expert reports. Wood Exam, Anatomic Identification, Hair Exam



**JURISPRUDENCE** 





# E1 Classifying Criminal Subjects: Clustering Based on Psychobiological Sciences and Italian Criminal Law

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After attending this presentation, attendees will be able to explain, via a complete examination of the criminal, all the elements necessary to fully understand the phenomenon described by the classification of criminals in appropriate clusters based on Italian case law and psychobiological sciences of criminal behavior.

This presentation will impact the forensic science community by allowing to easily examine the various characteristics of criminals and use the information with ease during their studies also in reference to the parameter of naming each cluster into where the subject is placed.

Prior to the presentation of the various clusters, for this study, the definition of the "active participants in the offense" (or "soggetto attivo del reato") which identifies the offense and brings the offender to be called a criminal, a person who violates a judicial or codified law. The concept itself is distinct from that of the deviant who is in fact the author of acts against the rules and social norms. Finally, the most suitable name to define the subject of the crime, or "deviant criminal," is identified as someone who deviates from social norms to damage constitutionally protected assets (Italian Constitutional Law). A list of requirements is then provided so that one can legally designate a human act as a "criminal act," even from the standpoint of U.S. law (actus reus, mens rea). All judicial parameters (Italian criminal law) such as the criminal ability, the penal ability, the ability to commit a criminal crime, the character of the offender, and the suitas (conscience and will of conduct) are taken into account in full harmony with the criminal psychiatry in order to describe the legal and psychological parameters that will allow us to collect the various criminal elements into predetermined clusters.

The first cluster is based on a breakdown of the criminals following a legal approach where the various benchmarks based on the Italian penal code are explained. Such benchmarks include the habitual crime alleged by law, the habitualness assumed by court, the professionalism in the offence, the tendency to commit crimes (as stated by Article 108 of the Italian penal code), and the seriousness of the offence.

The second cluster is based on a scientific setting collecting and describing the occasional criminal, the constitutional criminal who can be psychopathic, a neuropsychopathic or psychotic, or with a tendency to be a criminal.

In the third cluster criminals are gathered under the concept of diminished capacity and insanity under Article 88 and 89 of the Italian penal code. Moreover, an explanation is given on the concept of accountability related to one's ability to be in full possession of one's faculties and to mental illness. Within particular cases, this cluster includes: psychopaths, psychotics, constitutional criminals, criminals by passion, criminals who are insane, insane who are criminals, and the criminal by proclivity.

In the fourth cluster, criminals are grouped according to their social danger (intense capability to commit a crime), where the habitual criminal, the professional criminal, that with a tendency to be a criminal, the recurrent criminal, and the constitutional criminal can be found (B. Di Tullio, Judgement Court of Appeals, Trieste, October 5<sup>th</sup>, 2009).

The main clusters described will be of great importance for researchers of forensic science because they were confirmed in the very recent, and for the first time in Italy, decision of the Court of Appeal of Trieste (Italy) on October 1, 2009. In this case, the sentence was reduced because of the presence of certain MAOA genes (MAOA-L) in the defendant which can cause violent reactions within certain adverse environmental contexts, predisposing the subject to antisocial reactions. Finally, the four clusters are compared to a cluster of criminals used in the United States.

Criminals, Biology, Criminal Law

# E2 Admissibility Decisions Regarding Toxicology and Engineering Evidence: Judicial Review of *Daubert* Factors and Forensic Expert Qualifications 1993-2010

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After attending this presentation, attendees will be familiar with the *Daubert* factors that were most frequently reviewed by judges in 200 cases involving toxicological and engineering expert testimony (100 cases per each evidence type). Attendees will also become aware of the key expert qualifications that were discussed and how the *Daubert* factors discussed and qualifications that were reviewed influenced the admissibility decision.

This presentation will impact the forensic science community by providing practitioners with insight into the characteristics of evidence that judges evaluate, which will help those providing expert testimony provide information that addresses those characteristics. This information is relevant to members of the broader legal community that work with forensic experts. For example, if attorneys are aware that judges who consider engineering to be scientific evidence will examine the falsifiability and known or potential rate of error associated with the evidence, then the attorneys are better able to prepare their experts to testify about those characteristics.

This study was a content analysis on a systematic sample of 200 U.S. District Court cases published on Lexis between July 1, 1993 and March 1, 2010 in which the admissibility of expert testimony was at issue. These cases were selected from the two of the most frequently occurring kinds of forensic expert testimony: toxicology and engineering. One-hundred cases from each category were systematically selected by decision date so that they spanned the period of time from the July 1993 Daubert decision to the present. Data was rendered from these cases using standard content analysis techniques that have been used by the researchers in other socio-legal research and a three-stage data verification process. Codeable cases contained a substantive discussion of the admissibility of proffered expert testimony that included the rule(s) of evidence relevant to the analysis, and a discussion of how the evidence met or failed to meet the criteria for admissibility. Challenges to admissibility were substantive (e.g., related to the characteristics of the experts or the evidence), rather than procedural challenges in which the attorneys objected to the timeliness of the expert's report or other statutory issues. Cases in which no proffer of evidence was made (e.g., a party claims that a decision should be overturned because an attorney

failed to proffer expert testimony) were excluded because there was no evaluation of the evidence.

Of the 100 proffers of toxicology expert testimony, 22% were found to be admissible and 78% were found to be inadmissible. The most frequently mentioned expert qualifications in admissible cases were experience, skill/subject matter knowledge, and education. The most frequently mentioned expert qualifications for inadmissible cases was skill/subject matter knowledge, followed by education and experience. In twenty-six cases the expert's qualifications were not discussed. The Daubert guidelines and non-Daubert factors were mentioned infrequently in admissible cases. The most frequently mentioned Daubert guideline in the inadmissible cases was falsifiability, followed closely by general acceptance, existence or maintenance of standards controlling the technique or operation, peer review/publication, and error rate. The most frequently mentioned non-Daubert factor mentioned in admissible cases was the underlying facts/data/studies, followed by the use of facts/data relied upon by other experts, and reliance on verifiable facts/data.

Of the 100 proffers of damages expert testimony, 53% were found to be admissible and 47% were found to be inadmissible. The most frequently mentioned expert qualifications in admissible cases were skill/subject matter knowledge, experience, and education. Relatively few expert qualifications were mentioned in inadmissible cases, but the most frequently mentioned of these was experience. The *Daubert* guidelines were mentioned infrequently in both admissible and inadmissible cases. Most frequently mentioned in both instances was falsifiability, followed by general acceptance, and peer review and publication. The most frequently mentioned non-*Daubert* factors in both admissible and inadmissible cases were the quality of the underlying facts/data/studies, and the use of facts or data relied on by other experts. **Evidence Admissibility, Expert Testimony, Toxicology and Engineering** 

## E3 Medical Malpractice Litigation at the Policlinico Hospital of Bari (Southern Italy)

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After attending this presentation, attendees will be introduced to the concept of adverse outcomes and to the evaluation of claims and their consequent economic impact on the Sanitary System.

This presentation will impact the forensic science community by underlining the critical points in the organization of a Sanitary System and their costs and will propose strategies to reduce them.

From January 1, 2008 to December 31, 2009, the Policlinico Hospital of Bari (Southern Italy) received 49 claims for medical injury. During this period the total amount of the requests was  $8,417,430 \in (\$10,933,147.16)$ , of which  $6,080,875 \in (\$7,898,266.01)$  pertained to the year 2008 and only  $2,336,555 \in (\$3,034,881.15)$  to 2009, therefore showing a significant decreasing trend. In this study the 49 claims were divided according to clinical areas and specialties; a subsequent internal analysis was performed to determine in each case the existence, or not, of the right to the compensation and at the same time the economic amount was estimated. As a result, against the total requested amount of  $8,417,430 \notin$  only  $2,580,674 \notin (\$3,351,959.99)$  were considered justifiable payments, in detail,  $1,336,330 \notin (\$1,735,718.93)$  for the year 2008 and  $1,244,374 \notin (\$1,616,280.04)$  for 2009.

An estimation of claims distribution for each medical branch was also performed. The largest number of claims (seven) was filed against the Department of Ophthalmology; however, in only two cases were medical errors found. The other specialties in which a large number of claims identified were orthopedics, surgery, and emergency medicine. Even if this data is in agreement with previous studies in this field, comparing them to the national and international results, reveals that, Policlinico Hospital received a smaller number of claims, in relation with the number of provided services and to the weight of Diagnosis Related Group (DRG), but, at the same time, the requested compensation for every single of them was, on the average, higher than what was generally observed in the other reports.

In the light of all obtained results and to reduce risk, the decision was made to find strategies to expedite compensation payments in those cases in which the liability was unequivocal, with particular reference to injuries related to blood or hemoderivate transfusions, in which – according to the current regulations – the doctrine of *res ipsa loquitur* can be applied.

Overall, only a small proportion of the claims (about one-third of the total) were not attributable to a diagnostic or therapeutic error. In some of these cases, in consideration of the Italian normative system, compensation was paid anyway in order to avoid the eventuality of a litigation because of gaps noticed in the compilation of the medical records or in the creation of the informed consent forms. This is the reason why the Policlinico Hospital has put specific training and checking courses into practice. On the other hand, in the specialties in which a higher number of errors were detected, auditing methodology was applied. Audits were conducted with systematic reviews of the diagnostic and therapeutic pathways for each specific clinical area and this has allowed the rationalization of these processes and, at the same time, has guaranteed a better cohesion with standards of quality and excellence in medical practice.

The introduction of these preliminary procedures has resulted in a reduction in insurance premiums.

Medical Professional Liability, Clinical Risk Management, Insurance Aspects

## E4 Science 101: Accuracy, Reliability, and Validity – From the Lab to the Courtroom

Gerald M. LaPorte, MSFS\*, National Institute of Justice, Investigative & Forensic Science Division, 810 Seventh Street, Northwest, Washington, DC 20531

After attending this presentation, attendees will have a better understanding about the scientific concepts of accuracy, reliability, and validity.

This presentation will impact the forensic science community by providing an in-depth discussion about accuracy, reliability, and validity. It is critical that forensic scientists be able to demonstrate that these factors have been sufficiently tested prior to issuing an official report.

Science can be thought of as "any systematic knowledge-base or prescriptive practice that is capable of resulting in a correct prediction or reliably-predictable type of outcome. In this sense, *science* may refer to a highly skilled technique, technology, or practice from which a good deal of randomness in outcome has been removed." The methods and results used in all sciences are sometimes described as accurate, reliable, and valid, but these terms can have many connotations, and thus be interpreted quite differently. This presentation will provide a discussion about the concepts of accuracy, reliability, and validity as they pertain to forensic science methods. Moreover, forensic scientists should be prepared to demonstrate, when called upon in court, that the results attained in their testing have satisfied a working hypothesis. The conclusions that are reached must be based on the results and supported by the data.

Sometimes, forensic scientists can derive dissimilar conclusions when presented with the same results. This is not necessarily unusual from other science-based practices because the interpretation of data by humans is invariably complex and is often related to the type and extent of training and knowledge, years of experience, and the ability to make a decision given multiple types of information. However, it is imperative that attorneys understand the methodologies used to derive forensic conclusions and if they are valid and reliable.

Forensic Science, Accuracy and Reliability, Validity

#### E5 Computer Forensics and Digital Evidence for Attorneys and Investigators

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The goal of this presentation is to present attorneys and investigators a solid overview into what can and cannot be determined with computer forensics.

This presentation will impact the forensic science community by providing attorneys and investigators a better understanding of the capabilities, limitations, and opportunities in computer forensics and the examination of digital evidence.

The need for computer forensics and examination digital evidence has grown exponentially over the past two decades. Historically, this forensic discipline was focused on elaborate embezzlement schemes, high tech crimes like network intrusions, and possession/distribution of child pornography. With the growing popularity of smart phones, GPS devices, and social networking sites, digital media now captures a wealth of information about our daily activities. As a result, computers capture all manner of criminal activity. Sexual assaults are often preceded by frequent text messaging between the suspect and victim. Death investigations are often solved by the discovery of digital suicide notes, internet activity researching harmful chemicals, and even video clips of suicides and homicides; and drug deals are frequently conducted completely with digital communications. Liability and malfeasance in civil cases are often proven by the discovery of computer files that were altered or deleted. Finally, terrorists often rely on cell phones and email schemes to exercise command and control of their resources. As a result, just about every criminal investigation has digital media associated with it. Some of the key topics are described below.

**The Anatomy & Scope of an Exam:** Digital media, in general, has four types of stored data: (1) User created files like documents and spreadsheets; (2) Metadata (sometime called "data about data) that provides information about various files; (3) operating system files and file system data that direct and keep track of information about how and when programs are running; and, (4) latent information from files that were previously deleted. Attendees will have an opportunity to review some of the basics of computer science and how these principles are exploited to extract evidence from digital media.

Judicial Disposition and the Key Court Cases and Documents in Digital Evidence – Frye, the Daubert Trilogy, Melendez-Diaz, Ashcroft vs. Free Speech, ISO 17025, and several others: Attendees will explore several important legal aspects of computer forensics. Fundamentally, there is some confusion about whether computers are "searched" or "examined." Validation poses special challenges in computer forensics because hardware, firmware, and software are often changing. Finally, computer forensics has some special challenges in defining and applying common laboratory ideas like calibration, measurement uncertainty, and error rates.

How to Write the Ideal Lab Request and Understand a Computer Forensics Lab Report: Examination requests that take the form of, "please tell me everything about this computer," often result in delays and confusion. Attendees will learn how to tailor their request to get the information they are looking for and how to work with examiners to shape reports that make sense.

**Key Questions for Direct & Cross Examination:** (1) Validation Studies?; (2) Calibration?; (3) Protocol/Order of Exam?; (4) Role of Malicious Code?; (5) Attribution of Contraband?; and, (6) Electronic Discovery and the Role of Peer-to-Peer Networks and Social Networking?

Although this presentation is geared towards attorneys and investigators who are fairly new to computer forensics, it will contain material that will serve as a nice review for those with more experience.

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 Department of the Air Force, Department of the Army, Defense Intelligence Agency or the Department of Defense.

**Computer Forensics, Digital Evidence, Media Exploitation** 

#### E6 Interpretation and Presentation of Forensic DNA Evidence

Anjali R. Swienton, JD\*, SciLawForensics, Ltd., 25 Walnutwood Court, Germantown, MD 20874

The goal of this presentation is to provide a basic understanding of forensic DNA reports, terms, and data for the novice attorney.

This presentation will impact the forensic science community by providing a basic understanding of forensic DNA testing for the novice attorney so that testimony presented by DNA experts is valid, reliable, and understandable.

DNA evidence has changed over the years. While once requiring a stain the size of a quarter, DNA can now be detected in significantly smaller amounts. Further, with the proliferation of widespread training, law enforcement officials have become better educated about collecting evidence that could potentially yield viable DNA profiles. Due to this increased awareness of the types of evidentiary items that may produce usable DNA profiles and the increasing sensitivity of today's forensic DNA technology, DNA evidence is appearing in more and more cases.

What is the lawyer to do?

This presentation seeks to give the uninitiated attorney the basic tools necessary to start looking at DNA cases. It is no longer adequate as a prosecutor to rely on questions provided by the lab scientist. Defense attorneys cannot and should not simply use the questions asked by the last attorney to handle a DNA case in their office. Each case is unique, presenting its own set of facts and own special circumstances.

The first step is, of course, reading the report from the lab; however, that is merely a starting point, not the end point. Once the report has been read and understood, the lawyer must review the underlying data. This presentation will provide attendees with the information needed to competently review the work of the scientist, from identifying what samples were tested (and possible reasons why others were not) to comparing the results of questioned DNA evidence to the known samples. The information gleaned from lab notes and underlying data is also important and examples of what can be learned from case notes alone will be provided. The balance of the content of the DNA case file as well as other supporting and relevant laboratory documents (such as QA/QC records, equipment calibration logs and proficiency test results) will also be touched upon.

Once a lawyer has competently reviewed the file, decisions must be made about whether to hire an expert and what type of expert should be retained. Alternate theories of defense need to be considered. Plausible explanations of how DNA ended up in a crime scene sample must be explored by both the prosecution and the defense attorney.

Preparing to cross examine the other side's expert also requires a great deal of consideration and will be discussed. The final challenge,

presenting the DNA evidence effectively to the trier of fact, will also be touched upon. The use of slide shows, for example, can be an excellent way to draw the jury in. Learning how to convert scientific language into familiar, but still accurate language the jury will understand will also be discussed.

Additional types of specialized DNA testing protocols may be discussed to include Y STRs, Mitochondrial DNA, mini STRs, and SNPS.

DNA, Lab Reports, Testimony

# E7 Mitochondrial DNA Forensics for the Legal Practitioner: Critical Knowledge From Crime Scene to Courtroom

Terry Melton, PhD\*, Mitotyping Technologies, LLC, 2565 Park Center Boulevard, Suite 200, State College, PA 16801

After attending this presentation, attendees will: (1) understand the basic biological features of forensic mitochondrial (mtDNA) analysis; (2) understand the progression of a case from crime scene to courtroom; and, (3) and learn of interesting cases that capture the relevant issues that arise in courtroom presentation.

This presentation will impact the forensic science community by increasing the number of legal practitioners knowledgeable of forensic mtDNA analysis. The overall experience of the legal community with forensic mitochondrial DNA cases is limited due to the relatively small number of cases nationally. Through exposure to a distillation of the basics including critical facts and issues the legal practitioner will become conversant in this useful forensic method.

Recent high profile cases using forensic mitochondrial DNA (mtDNA) analysis include the murders of Laci Peterson, Samantha Runnion, and Danielle Van Damm, as well as the prosecutions of Michael Skakel, Jason Williams, and Gary Ridgeway, the Green River Killer. Although this form of testing is routinely accepted in the courtroom at state and federal levels, most defense and prosecuting attorneys have had limited exposure to it due to the relatively small number of cases annually. For current prosecutions and cold cases as well as post-conviction explorations of guilt or innocence; however, mtDNA can be a critical and highly useful piece of the crime scene puzzle. Naturally shed hairs, especially those found in highly probative locations at the crime scene or on the homicide victim's body, are frequent case samples for mtDNA analysis. Skeletal remains subjected to extreme environmental challenges are also frequently submitted for identity confirmation using this type of testing when nuclear DNA testing is unsuccessful. Laboratory analyses have been routinely carried out since the early 1990s and are typically straightforward. Major areas of utility have been forensic, military, and historical; however, criminal justice attorneys should have a "mtDNA toolkit" ready and waiting for the potential case. In addition, a basic awareness of the pitfalls and best practices is required of the attorney with a "mito" case.

Relatively simple biological basics underlie the principles of mtDNA analysis. Legal practitioners need to understand these at a foundational level, as well as how to explain and use this non-unique marker in a courtroom setting once the forensic laboratory has developed a failure to exclude with crime scene evidence such as a shed hair or skeletal remains. Relevant topics to become comfortable with include basic lab techniques, statistical context, mixtures, databases, contamination, and other challenges to the evidence. For example, contamination is more prevalent and likely when testing this naturally abundant DNA molecule, yet mitochondrial DNA analysis results can be reliably obtained when a contamination is present when the laboratory has comprehensively validated its protocols to include interpretation in situations when contamination is minimal. The FBI's on-line

mitochondrial DNA forensic database will be described in depth as a resource for understanding frequency estimates of individual mtDNA types. The difference between this "non-unique" form of DNA analysis that links maternal relatives and the "source attribution" form of nuclear DNA analysis, STR typing, will also be covered.

Using interesting litigated prosecution and defense cases to illustrate the courtroom basics of forensic mtDNA analysis, the above topics will be discussed. Cases that examine post-conviction testing and exoneration, successful trial outcomes for the state, small and old hairs, and skeletal remains will be highlighted. The attendee will also gain an understanding of why mtDNA analysis is not used in certain types of cases such as cuttings, stains, and paternity testing. In addition, the presentation will provide the legal practitioner with some simple resources for gathering further information that are readily available online. This presentation will provide the legal practitioner with brief and basic "need to know" information that can be augmented at any later date. **Forensic Mitochondrial DNA, Legal Education, Courtroom Issues** 

#### E8 Trace Evidence Overview for Attorneys: Sourcing and Resolution

Max M. Houck, PhD\*, West Virginia University, 1600 University Avenue, 208 Oglebay Hall, Morgantown, WV 26506-6217

After attending this presentation, attendees will gain a fundamental understanding of the principles of trace evidence, the nature of this core forensic evidence, and the concepts of sourcing and resolution in its analysis.

This presentation will impact the forensic science community by improving the criminal justice process, where attorneys have a better understanding of how forensic science works—particularly the concepts and issues surrounding trace evidence—how to best utilize their evidence in adjudication, and the use of evidence in investigative and intelligence work.

Trace evidence is the umbrella term for any evidence that because of its size or texture is easily transferred from one location to another and persists for some period of time, until it is lost, collected, or ignored. Trace evidence reveals associations between people, places, and things involved in alleged criminal activities. The fundamental precept in trace evidence, and arguably all of forensic science, is the Exchange Principle. Initially described by Edmund Locard, it posits that:

...none can act with the intensity induced by criminal activities without leaving multiple traces of his passing...The clues I want to speak of here are of two kinds: Sometimes the criminal leaves traces at a scene by his actions; sometimes, alternatively, he picked up on his clothes or his body traces of his location or presence. (Locard E., L'enquête criminelle et les méthodes scientifiques, *Flammarion*, Paris, 1920, page139. Translated by Frank Crispino.).

Thus, transfer can be one-way, two-way, or multiple; an increase in the number, types, varieties, amounts, and locales can disproportionately increase the incriminating or exculpatory value of the evidence.

All of the transfers of interest to a forensic scientist occurred during the commission of the alleged crime and, therefore, are past events. Forensic science uses the physical remnants of those past criminal activities to reconstruct events with as high a level of resolution and accuracy as the evidence, analysis, and circumstances allow (think "short-term archaeology"). Some evidence types have higher resolution than others (DNA vs. ABO blood groups, for example) although the quality of the evidence may change that resolution. It is critical to remember that simply because one method's result may have a lower resolution than another does not mean that the method is "bad"–within the limits of the method, it may have rendered the best possible answer ("That letter of the alphabet could be a "B" but it's fuzzy" vs. "It is an R.").

Trace evidence analysis encompasses chemical, material science, biological methods, and primarily microscopy. The analysis process occurs in two phases: identification and comparison. Identification is classical categorization and set theory: something is or isn't nylon, is or isn't bottle glass, is or isn't automotive paint, etc. This level of information is called class-level evidence. Once identified, crime-scene evidence is then compared to one or more known or reference samples to determine if there is a common-source between the two samples. In this context, "common source" has a wide range of potential meanings, including manufacturer, production facility, batch or lot number, to asused condition of the final item (damage, wear, alterations, etc.). Biologically, this level of sourcing (another way to think of resolution) runs the gamut from taxonomic class (as in animal hairs) to the individual (DNA). Other natural materials that occur as evidence, such as soil or pollen, have their own levels of resolution, depending on the processes that produced them (fill dirt vs. untilled alluvial soils).

More than "could have" evidence, trace materials occupy a critical link in the criminal justice system to reconstruct events, support, or refute allegations or statements, and to associate people, places, and things involved in criminal activities.

Trace evidence, Sourcing, Resolution

#### E9 What Lawyers Need to Know About Forensic Anthropology

Douglas H. Ubelaker, PhD\*, Department of Anthropology, National Museum of Natural History - MRC 112, Smithsonian Institution, Washington, DC 20560

After attending this presentation attendees will understand the fundamentals of forensic anthropology.

This presentation will impact the forensic science community by raising awareness within the Jurisprudence section of the nature of the discipline of forensic anthropology.

Although many think of forensic anthropology as a single discipline, the field actually involves a variety of applications with distinct complex methodology. Forensic anthropologists are routinely consulted on problems relating to human remains and legal issues requiring knowledge of human biological variation. Major areas of applications include recovery of remains, determining if materials are of human origin, and the assessment of age at death, sex, ancestry, living stature, and time since death. Central goals of analysis include personal identification and interpretation of evidence for foul play. Some forensic anthropologists also offer opinions on issues relating to living individuals, especially those involving personal identification and chronological age.

Forensic anthropology is research oriented and closely linked with academia. Certification and diplomate status are available through the American Board of Forensic Anthropology for experienced forensic anthropologists who hold the PhD degree and successfully pass examination. Although forensic anthropologists continue to find traditional employment in universities and museums, many also work within government agencies, medical examiner offices, and specialized programs focusing on the recovery and analysis of human remains.

Methodology varies greatly with each area of application but is closely linked with a substantial published literature. Interpretations are expressed in relation to that literature with appropriate levels of confidence. Although technological advances have become incorporated into recovery efforts, much of this work continues to rely upon traditional archeological techniques. Methods of assessment of age at death, sex, ancestry, and living stature rely extensively on published research conducted on museum collections, clinical data and information gleaned from documented forensic cases. Estimates of time since death have advanced with use of radiocarbon analysis, especially relating to the modern bomb curve.

Personal identification of human remains in forensic anthropology usually involves comparison of antemortem and postmortem radiographs (x-rays). Such identifications result when aspects of skeletal anatomy are observed to be present in both the antemortem and postmortem radiographs and are judged to be sufficiently unique.

Forensic anthropologists are especially useful in contributing to interpretations of foul play. Evidence of peri-mortem (at or about the time of death) alterations can suggest blunt force trauma, gunshot injury, sharp force trauma, or other forms of injury that can prove important in suggesting that foul play was involved. Such alterations have to be distinguished from developmental anomalies and antemortem injuries that the person sustained during their life as well as postmortem alterations reflecting taphonomic factors occurring after death. Anthropologists are especially valuable in such interpretations because of their knowledge of human variation in skeletal anatomy and experience with human remains recovered from varied contexts.

The rapidly growing field of forensic anthropology has become increasingly interdisciplinary and international in scope. Applications relate closely with those of other disciplines of the forensic sciences, especially forensic pathology, forensic odontology, and criminalistics. **Forensic Anthropology, Physical Anthropology, Fundamentals** 

#### E10 The Force of Narrative and Jury Perception

Katherine Ramsland, PhD\*, DeSales University, 2755 Station Avenue, Center Valley, PA 18034; and Gregory J. O'Meara, LLM\*, Marquette University Law School, 1215 Michigan Avenue, Eckstein Hall, Milwakee, WI 53233

After attending this presentation, attendees will understand how human cognitive processing impacts courtroom narratives and will understand how to construct sophisticated presentations that recognize the way the story-telling heuristic affects listening and logic.

This presentation will impact the forensic science community by using narratology and cognitive science to show attorneys how jurors perceive information and make judgments about evidence.

In every trial, there is a distinction to be made between the content of the testimony and the means whereby that content is delivered to the jury or the judge. Ordinarily, analysis focuses on the content itself. Like readers of British murder mysteries, we want to know "Whodunnit?" As we move from the realm of fiction to real life crime, judges and trial attorneys need to attend to not only the plot but also the way the story is told. We need to know "who said it?," an inquiry that invites a hermeneutic of suspicion – "Why did he say what he did?" Because the construction of nonfiction narratives is a complex, multi-layered, and nuanced process that undermines any claim that the past can be apprehended unproblematically, it is worth attending not only to the content of testimony but also to the various interactions among speakers and listeners that can color, and possibly corrupt, testimony presented to the jury.

The trial of *State of Wisconsin v. Jeffrey Dahmer* provides a marvelous vehicle for exploring the intricacies of narrative in the trial arena. Although his confession and extensive statements to mental health professionals provide almost all of the evidence in the case, the jury never heard Dahmer speak. Because the insanity trial focused not on Dahmer's actions per se but on his mental state, his evidence was wholly mediated through the testimony of other witnesses. In some ways, the experts in the case may have missed what the jury recognized: that Dahmer continued to manipulate his interlocutors even after his arrest in hopes of acquittal.

Trial attorneys use story-like narratives to direct their audience to certain conclusions, sometimes with words, other times by leaving things out. The subject matter is inextricably linked with *how* it is told, and

those who fail to understand human information processing (and thus leave holes for listeners to mentally fill) gamble with success. Anyone charged with devising a courtroom narrative should learn what research demonstrates about the influence on perception of such factors as the illusions of confidence, the nature of attention, and issues with memory.

What we think should be the result of a presentation in the minds of jury members can conflict with what does happen, because the human mind doesn't always work the way we think it does. The truth is that we mediate knowledge as we apprehend it, and the limits of our mental processing force us into cognitive efficiencies. Among these are internalized plots from cultural arenas such as film, news reports, and novels that can reshape facts and logic.

The centerpiece of courtroom presentation is a story, and recounting information in a credible narrative will bias impressions in the direction of the story line. A satisfying narrative confers an encoding advantage that makes the evidence easier for jurors to recall. If a credible story gets rolling, its psychological momentum can shut out all else. If it's not satisfying; however, it can undermine itself.

Attorneys and experts cannot assume that their logic will be sufficient to persuade; they must include the right elements for credibility, clarity, and closure. Research shows that if information is missing, ambiguous, or unpersuasive, listeners will "hear" the narrative according to what feels right to them. They may fill in holes with what they expected to hear or transform facts to suit their beliefs. Thus, if a narrative does not hold their attention and persistently satisfy, their personal frame of reference may override accurate recall.

Narrative, Jury Perception, Cognitive Illusions

# E11 The Current Use and Research Investigating Insect Succession for Determining the Postmortem Interval

Anne E. Nichols, BS\*, 1034 Ridgeway Avenue, Morgantown, WV 26505; Jeffrey D. Wells, PhD, West Virginia University, Department of Biology, Life Sciences Building, Room 3135, 53 Campus Drive, PO Box 6057, Morgantown, WV 26506-6057; and Neal H. Haskell, PhD, 425 Kannal Avenue, Rensselaer, IN 47978

After attending this presentation, attendees will be aware of how carrion insect succession is currently used in forensic casework, as well as the current deficiencies in forensic succession research that inhibit its utility in forensic cases.

This presentation will impact the forensic science community by outlining the research steps in process, that are necessary for developing a dataset that is suitable for postmortem interval statistics.

In forensic entomology casework, the use of blow fly and flesh fly growth and development is unequivocally the most common method for estimating a postmortem interval (PMI). In these cases, determining the PMI can be crucial to reconstructing the events associated with a suspicious death. The use of insect succession to estimate the postmortem interval is exceedingly rare, and is uncommon in published case reports. In 100 cases worked by a practicing entomologist, over a 10 year period, no cases utilized a classical succession approach.

Insect succession, in the classical sense, has meant the change in species composition inhabiting a corpse over time. This allows a forensic entomologist to estimate the PMI by associating the assemblage of species collected on a corpse with a certain time elapsed since death. A contributing factor to the virtual absence of the use of succession in forensic entomology casework is the lack of validation of this technique, as well as the lack of a standard method for its use.

Two shortcomings of published forensic insect succession research include the absence of using an Accumulated Degree Hour (ADH) model to describe succession and a lack of replication of appropriate decomposition models. An ADH model utilizes the linear relationship of an insect's growth rate and temperature, between an upper and lower developmental threshold, to describe the amount of time it takes for an insect to reach a certain developmental stage. The ADH model has been widely used in estimating a PMI from dipteran growth and development, as well as in the decomposition of human bodies. Similarly, an ADH model should be considered in developing a succession model. Using ADH to describe succession would allow this method to be used in future years when the temperature regimes significantly differ from the experimental period.

The domestic pig, *Sus scrofa*, is an accepted model for human decomposition. *S. scrofa* is an attractive surrogate for a human corpse because they are relatively cheap and easy to obtain in high numbers. Experimental succession studies using *S. scrofa* commonly used only 3 pigs per experimental condition. This sample size is too small to investigate the inherent variation in succession patterns. Without this knowledge it is impossible to have a high degree of statistical confidence in a PMI estimation from insect succession. This lack of replication leaves much succession research anecdotal and improper for forensic use.

In an effort to develop a data set suitable for determining a PMI from an insect succession analysis, a total of 53 *S. scrofa* were exposed to insect colonization during three consecutive summer periods (2 in 2008, 20 in 2009, 31 in 2010). This study took place in Rensselaer, Indiana, on a rural 800+ acre corn and soybean farm. Daily collections were made for 14 consecutive days during the months of July and August. Collections included sweep netting and hand picking of adult and larval specimens. All pigs were placed on the ground in full shade and covered with a wire cage to deter scavenging. Hourly temperature data for the field site was recorded throughout the entire experimental period, as well as daily temperature at each pig placement site.

While this data set has not been fully analyzed, preliminary analyses are of forensic importance. The precision and accuracy of a PMI estimate based on an ADH model of succession will be compared to that based on an absolute time model. The relative reliability of each insect species for succession-based PMI estimation will also be evaluated.

This research represents the largest succession dataset generated using *S. scrofa*, and supports a novel statistical approach to PMI estimation. This data will be immediately applicable to rural Indiana outdoor death scenes during summer. Future research is needed to determine the extent to which they can be applied to other areas or seasons. **Forensic Entomology, Insect Succession, Postmortem Interval** 

#### E12 Forensic Pathology - Basics for Attorneys

Lindsey C. Thomas, MD\*, Minnesota Regional Medical Examiner's Office, 1175 Nininger Road, Hastings, MN 55033

After attending this presentation, attendees will understand some of the basics of forensic pathology that attorneys should know, including what education and training forensic pathologists have, what documentation attorneys can expect, and some of the limitations in what forensic pathologists can determine.

This presentation will impact the forensic science community by providing attorneys with basic information about forensic pathology and assist them in working with forensic pathologists as expert witnesses.

This presentation will briefly address specific issues in forensic pathology. While a lawyer should certainly never substitute their judgment for that of a forensic pathologist, lawyers should have a fundamental understanding of various types of injuries and what they can and cannot tell us about a death. Injuries caused by firearms, sharp force and blunt force will be explored. Defensive wounds will also be discussed. Understanding how these injuries may be incurred, as well as the corresponding injuries that can be sustained can assist the lawyer in formulating a theory of the case – either prosecution (or the decision not to prosecute) as well as defense.

In a world where cases are tied up neatly in 49 minutes or less, it is crucial for lawyers to understand there are certain questions a forensic pathologist can't answer. Some possible "red flags" will be identified and discussed. For example, lawyers should be cautious of forensic pathologists who are inappropriately certain about facts which can't be known. Of course, the lawyer may not know "what can't be known." This presentation seeks to inform lawyers of areas wherein caution should be exercised, such as time of death or determining the order of wounds. Finally, lawyers should be cautious of the forensic pathologist who claims the ability to determine the number of assailants based on the wounds.

Finally, there is a difference between an autopsy report and a good autopsy report. This presentation seeks, in part, to outline what a good autopsy report should include. Of course, it's not enough to know what should be included; lawyers must also have the tools available to actually understand the report. Other documentation a forensic pathologist should provide will be briefly discussed.

Forensic Pathology, Expert Witness, Autopsy Reports

# E13 Justice for Ryan Revisited: Potential Exonerations in Shaken Baby Syndrome Convictions

#### John Plunkett, MD\*, 13013 Welch Trail, Welch, MN 55089

After attending this presentation, attendees will be able to: (1) describe the differential diagnosis for subdural bleeding, retinal hemorrhage, and encephalopathy in an infant or toddler; and, (2) describe the role of formal biomechanical analysis in apparent traumatic head injury.

This presentation will impact the forensic science community by illustrating an approach to potential post-conviction exonerations in Shaken Baby Syndrome cases.

Professor Tuerkheimer from DePaul University College of Law suggested that Shaken Baby Syndrome (SBS) should be "the next Innocence Project" in a 2009 Washington Law Review article. DNA has been the basis for almost all successful Innocence Projects exonerations to date. However, reversal of SBS convictions will require a different approach, based largely on "new evidence" or on "expert review" of existing evidence. The "new evidence" approach utilizes evolving understanding of the differential diagnosis for subdural bleeding, retinal hemorrhage, and encephalopathy in an infant or toddler, and developing appreciation of the role of formal biomechanical analysis in apparent traumatic head injury. The "expert review" approach is more problematical and less intellectually satisfying than "new evidence." It may be difficult for an "expert" panel to agree on anything. If the experts do not agree, the Court will have the responsibility to evaluate differing evidence, "critically evaluating the reasoning process by which the experts connect data to their conclusions...", and contrasting scientifically verifiable facts with opinion. This may not be an easy task.

This presentation describes a 1999 case in which a prosecutor charged a babysitter with murder 21 years after the death of the 5-monthold infant in her care. A jury convicted the babysitter of second degree murder. She was sentenced to ten years in prison under the Statute in place in 1978. She was paroled in 2005 after serving six years in prison. The Third National Conference on Shaken Baby Syndrome in Salt Lake City, September 26, 2000 highlighted the prosecution and conviction in a Plenary Session. However, a subsequent review of the records and autopsy photographs indicated that superficial cortical vein thrombosis, not "shaking," caused the infant's death.

Shaken Baby Syndrome (SBS), Innocence Project, Tuerkheimer

#### E14 Civil and Criminal Defense Trial Attorneys: Relevant Issues and Relationships With Forensic Pathologists

Cyril H. Wecht, MD, JD\*, Cyril H. Wecht & Pathology Associates, 1119 Penn Avenue, #404, Pittsburgh, PA 15222-4205

After attending this presentation, attendees will learn how to relate to coroners, medical examiners, and forensic pathologists in preparing for trials in which autopsy and other pathological findings are critical in understanding and effectively representing their clients.

This presentation will impact the forensic science community by encouraging and informing trial attorneys how to be more diligent and aggressive in their efforts to obtain critically important information from appropriate medical consultants prior to trial. Failure to utilize appropriate medical experts – in particular, forensic pathologists in death and serious injury cases – will often lead to disastrous results.

In handling civil cases (e.g., personal injury, product liability, wrongful death, medical malpractice) and criminal cases (e.g., homicide, drug deaths, rape/sexual assault, physical abuse), the trial attorney needs to fully understand the nature and extent of the relevant issues pertaining to anatomic, physiological, clinical, and pathological aspects of the disease processes or traumatic injuries sustained by the plaintiff (civil) or the victim (criminal).

In order to acquire and fully comprehend this fundamental and frequently critical information, the attorney needs to seek input from appropriate medical experts. Certainly, in death cases, such consultations should always include a pathologist. In criminal cases, this should be a forensic pathologist.

While most experienced, competent plaintiff trial attorneys pursuing a civil claim appreciate this point, and usually reach out to identify such specialists, meet with them, and utilize their expertise in depositions and trials—many criminal defense attorneys fail to do so. Understandably, the major problem is financial. The vast percentage of criminal defendants is unable to pay for such experts. Many times, public defenders, court-appointed attorneys, and occasionally even privately-retained attorneys simply fail to properly grasp the importance and necessity of utilizing such consultants. Public defenders and courtappointed defense attorneys should be extremely aggressive in requesting court approval for such consultations, with reasonable allowances for payment.

A very serious procedural deficiency among criminal defense attorneys is the failure to request a meeting with the coroner, medical examiner, or forensic pathologist who performed the autopsy in order to properly prepare for that individual's testimony. Medical examiner's and coroners are not (and definitely should not be permitted to perceive themselves as) an integral part of the prosecution team. Official governmental medical-legal investigative offices should be independent operations, whose diagnoses and opinions are based upon objective forensic scientific findings, and not tailored in any way to comply with the theories and decisions made by law enforcement officers and prosecutors. Therefore, the autopsy prosector should be willing to meet with a defense attorney and convey his findings and opinions in the same fashion that he would to the prosecuting attorney.

These points will be expanded upon and illustrated by references to and discussions about several prominent cases.

Forensic Pathology, Trial Preparation, Expert Testimony

#### E15 Junk Criticism

Thomas W. Vastrick, BS\*, 380 South State Road 434, Suite 1004-132, Altamonte Springs, FL 32714-3866; and Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of PA, Erie County Court House, 140 West 6th Street, Room 223, Erie, PA 16501

After attending this presentation, attendees will understand the problem of proffered testimony by those of limited technical background utilizing untested and unreliable methodologies contrary to accepted practices in an attempt to discredit various branches of the forensic sciences. Attendees will also understand the methods used to recognize junk criticism from appropriate criticism and how to distinguish the two.

This presentation will impact the forensic science community by bringing the issue of junk critics and their methods to the attention of the judiciary so all involved can be better prepared to address the issues on a scientific basis.

While the recent report on forensic science from the National Academy of Sciences has been the top conversation within the Academy since its release, criticism of forensic science is not new. Some criticism is warranted but some is based on a combination of innuendo, misrepresentation of facts, untrue statements, misapplication of statistical methodologies, and opinion pieces disguised as research – and the source of these biased, unfounded attacks may surprise you. This presentation will highlight the methods these critics have used against forensic document examination for over ten years and how the examiners have successfully dealt with the situation. This presentation will also take a careful look at rules of evidence and procedure in the light of how they address this phenomenon.

The purpose of this presentation is to educate the judiciary in the techniques used in junk criticism, how to differentiate legitimate recommendations of improvement from junk criticism, and options available to judges in handling these situations. The presentation will be made by a forensic document examiner who has faced the scenario of attack by junk criticism more than once and a sitting trial court judge with judicial academic credentials who has researched this subject. **Forensic Science, Criticism, Judiciary** 

## E16 Considerations in Selecting and Working With Forensic Document Examiners

Karen S. Runyon, BA\*, 400 South 4th Street, Suite 505, Minneapolis, MN 55415

After attending this presentation, attendees will be able to recognize the choices among advertised handwriting experts and forensic document examiners and be able to better understand the offered expertise levels that have developed due to the ease of internet advertising and the lack of barriers into the profession or regulation in forensic science. A review of empirical research findings on development of expertise will promote greater understanding of necessary considerations by those who engage or cross-examine experts in general. Educational programs in document examination will be reviewed to promote awareness and an ability to better select the desired type of expertise.

This presentation will impact the forensic science community by demonstrating how improved reliability will occur for attorneys seriously seeking a neutral, highly educated expert to competently analyze evidence, thus leading to lessened error.

Unfamiliarity with the history of the forensic document profession coupled with poorly informed knowledge concerning the examination of documents has led to frequent confusion among legal professionals who encounter this forensic discipline. Misconceptions exist concerning the examination of documents and specifically concerning the examination of handwriting patterns. While some confusion may be due to FRE 901 sanction of non-expert handwriting opinions, greater misinformation is due to the proliferation of online expertise. In spite of honed critical thinking skills, legal professionals are unprepared to wade through internet advertisements and the incredible and often false witness claims found there. How to find your way through the spectacular marketing of handwriting and document examination expertise? The differing paths to forensic document examination skill will be reviewed and evaluated to provide assistance to the legal professional in selecting experts as well as in encountering evidence and expertise claims. The best practice for working with an expert will be discussed to promote better understanding of the expert versus advocacy role with the goal of preventing reckless expert opinions and negligent presentation of evidence in legal motions or in testimony.

Forensic Document Examination, Expertise, Reliability

#### E17 Collect the Bugs - Pretty Please!

#### Neal H. Haskell, PhD\*, 425 Kannal Avenue, Rensselaer, IN 47978

After attending this presentation, attendees will learn the importance of insect collection from the death scene, the detrimental aspects of not collecting the evidence, and the major benefits the collection of insect evidence can make in the outcome of the case.

This presentation will impact the forensic science community by providing officers of the court with understanding of why and how the entomological evidence is necessary and must be collected as with any of the other forensic science tools requiring its retrieval.

Insect evidence is primarily used to determine the postmortem interval (PMI) and may also answer other questions such as location of death, presence of drugs, and the identity of the victim if the body has been removed. The use for PMI determination has been available for over a century, but has only come into common application over the past two decades internationally where it has been used in hundreds of case investigations. With this increased use, appearances by forensic entomologist experts have been increasingly beneficial to the courts. This is because the insects are going to be present in nearly every homicide, suicide, or natural death when the victim is outdoors and commonly even where there is some type of barrier such as when the victim is indoors, in a car trunk, or buried. Equally important is the absence of insect, because this situation may only be explained by very specific condition or circumstances. Thus, it is the forensic entomologist who can connect elements of the case investigation between the victim, a suspect, and a specific time and location by using an unbiased quantitative scientific methodology independent of other forensic sciences involved in the case investigation. Initially, the entomological evidence can be used to focus in on a suspect that has been generated by the detectives, but equally or even more importantly, it is the forensic entomological expert who will be a key witness at the time of trial to either illuminate or eliminate other elements of evidence whether it be testimonial or physical.

However, even with this increased usage, many jurisdictions across the country are unwilling to recognize or are still ignorant of the importance afforded by ubiquitous entomological evidence, resulting in the failure to collect forensically important insect specimens. It is unclear whether this decline is due to the economic environment at this particular time, from lack of funding for training, or the perception that expert analysis is cost prohibitive. If only the investigators would have collected the insect evidence which was present, we could have had the means to answer some of the most important questions of the case (e.g., time since death and those individuals related to the death). Consequently, a very solid and reliable position would have resulted to support either the prosecution or defense and a resolution found.

Several examples of how cases could have been argued differently for either side will be used to demonstrate these frustrating situations (for the forensic entomologist) and how conclusions could have yielded significantly different outcomes for the courts. To further highlight the importance of collection of entomological evidence, cases where insect evidence was brought into the case later and an immediate resolution to the case was then rendered will be presented. Also, even if the insect evidence was collected, elements of the analysis and conclusions may be erroneously determined by not seeking the most qualified forensic entomological experts available. These proper qualifications include: degrees (PhD) in entomology and forensic entomology; internship with a qualified and practicing forensic entomologist; extensive research in carrion insect (those specific insects which only feed on dead vertebrate animal soft tissue) study; several years of case and trial experience; presentation of case and research at regional, national or international meetings; a recognized publication record in peer reviewed journals and book chapters relating to forensic entomology in the forensic literature. In addition, case examples will be used to demonstrate shortcomings in analysis and major mistakes committed in the final conclusions resulting in justice not being attained.

It is ultimately the attorneys and judges who are responsible for using all the tools available at their disposal for finding the truth of a case. Why are these professionals not demanding the use of this unbiased quantitative tool at their disposal to help find the truth? Please collect the bugs!

Forensic Entomology Expert, Insect Collection, Maggots

#### E18 Maggot Length Versus Rice Length: Finding a Possible Correlation

Neal H. Haskell, PhD, 425 Kannal Avenue, Rensselaer, IN 47978; and Marissa Garbacz\*, 6233 South Neenah Avenue, Chicago, IL 60638

After attending this presentation, attendees will understand the critical importance of collecting the very small fly larvae from living patients and will have a greater understanding of elements of fly myiasis.

This presentation will impact the forensic science community by identifying the necessity of requiring immediate collection and preservation of insect evidence in health and elder care facilities for determining physical neglect/abuse through insect fly species identification in human myiasis cases.

Seldom, if ever are maggots (fly larvae) collected, photographed, or preserved for evidential or scientific purposes when medical personnel discover these organisms present on patients under their care. Entomological evidence, usually maggot infestations, is commonly used to identify a period when abuse or neglect may have occurred, and thus relevant and material to judicial proceedings. In elder care facilities a person may be found infested with necrophorus (feeding on dead tissues) or filth flies (house fly relatives) larvae, but in many of these cases the medical care givers fail to collect specimens from these insect infestations observed on their patients. The condition is known as human insect myiasis. Specifically, human myiasis refers to the infestation of fly larvae on living humans and includes the blow fly, flesh fly, house fly, and other groups. Case examples from nursing homes and elderly patient facilities will be used to illustrate the seeming lack of concern over patients having insect infestations. The only description given in several of these cases where specimens and photographs of specimens were absent was the larvae were white and about the size of a grain of rice. Therefore, analogous description of maggots to rice needed to be qualified and quantified.

A research study was initiated to determine the size of an average rice grain and found that grains of rice from different sources, as well as within sources, vary in size. A variety of commercially available brands and types of uncooked rice were purchased from a local grocery store and a Chinese restaurant. The three brands from the local grocery store were, enriched long grain white rice, whole grain brown rice, and medium grain white rice. The fourth brand, extra long rice, was served at the local Chinese restaurant.

A small portion of rice was placed into separate petri dishes for measurement of individual sample grains. Intact grains of rice were removed for further observation (as opposed to broken pieces). Two hundred grains of rice were measured from each brand. Of the 800 grains of rice measured, the total length ranged between 4.3mm to 7.5mm with mean lengths per treatment between 5.21mm to 6.33mm. The rice length data were then compared to the fly larvae length of known myiasis agents and was found to most represent larger mature second stage larvae or even early third stage larvae from the blow fly group, or older larvae of some "gnat size" fly larvae groups. The perception of most people would be that a grain of rice is relatively small when compared to a string bean, a carrot, or an ear of corn. Thus, it is obvious that the description provided by the observer is relative to an object being small and is not definitive of the life stage or size of maggots observed. This research demonstrates that a grain of rice may not be as small as perceived and if taken literally may be drastically misleading to the forensic entomologist who is trying to evaluate the age of the insect larvae.

Even though generalized visual descriptions cannot provide a specific entomological identification, use of a "rice grain" analogy may provide a contextual description referring to a larval specimen which is quite small. It is incumbent upon medical personnel and health care facilities to incorporate mandatory insect specimen collection, (photographs and preservation) in their SOP manuals and regulatory routines. Failure to collect and preserve insect evidence in health and elder care facilities must be rectified.

Maggot Length, Rice Length, Maggot Identification

#### E19 Using a Faceless Murder Victim to Illustrate Crap Tests, Quackery, and Incompetence in Using or Not Using Forensic Entomology

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After attending this presentation, attendees will learn what entomological evidence can or must be collected from crime scenes, how it should or shouldn't be analyzed, limitations of evidence and interpretation, and the implications of deficiencies in any of these processes.

This presentation will impact the forensic science community, with emphasis on the legal community in forensic science, by indicating how and when entomological evidence must and must not be used, and how to recognize key limitations of such evidence. Ultimately such understandings will help improve and increase the use of entomological evidence.

The discovery of a faceless, decomposing homicide victim in a ditch provides an ideal example of how entomological evidence should be used in a criminal investigation. Entomological evidence in this example was obtained at the scene by entomologists and was valuable (though not essential) in establishing the cause and time of death. Through an examination of this case, key issues will be explored in obtaining, processing, and analyzing entomological evidence. In particular, what should be known about this process will be illustrated to increase appreciation of the weight (or weightlessness) of entomological evidence. Among the issues the crap test will be considered – methods to immediately identify incompetence in CSIs, experts, (and lawyers) and quackery – methods used by the incompetent to mask their deficiencies. Our underlying theme is that unless non-scientists gain the skills to skeptically evaluate scientific evidence – sort the maggots from

the mealworms – the use of forensic entomology and other areas of forensic science will likely remain tainted by incompetence. Entomology, Decomposition, Homicide

# E20 Firearms Identification: A Shot in the Dark?

Kenneth E. Melson, JD\*, Bureau of Alcohol, Tobacco, Firearms and Explosives, 99 New York Avenue, Northeast, Suite 5S 100, Washington, DC 20226

After attending the presentation, attendees will have a better understanding of the issues related to firearms identification and the expression of an expert's opinion.

This presentation will impact the forensic science community by supporting the Jurisprudence Section's Forensic Science 101 initiative.

Critics of forensic science have been taking aim at the firearms identification discipline for a long time, and their target has recently been more clearly defined by the 2009 National Academy of Sciences Report (NAS), *Strengthening Forensic Science in the United States: A Path Forward.* Nevertheless, the courts continue to admit testimony concerning the traditional task of confirming that a particular cartridge or bullet was fired from a particular gun or that two or more cartridges or bullets were fired from the same gun. However, the more recent judicial trend has been to limit the opinion testimony to non-probabilistic terms. The difficulty is determining how to express the expert's opinion. This presentation will give attendees a primer on the comparison of bullets and shell casings. In addition, the nature of the firearm identification expert's opinion and terminology will be discussed in light of the NAS report and the ASCLD/LAB accreditation criteria on report writing.

**Firearms Identification, Ballistics, Firearms** 

## E21 The Systemic Scientific Problems With Firearms and Tool Mark Identification

Adina Schwartz, JD, PhD\*, John Jay College of Criminal Justice, CUNY, Department of Law & Police Science, 899 10th Avenue, New York, NY 10019

The goal of this presentation is to alert attendees to the severe scientific problems with the discipline of firearms and tool mark identification.

This presentation will impact the forensic science community by stimulating critical reflection on the scientific status of firearms and tool mark identification and, more generally, the traditional forensic identification sciences

There are grave, systemic scientific problems with firearms and tool mark identification (often improperly termed "ballistics"). Firearms and tool mark examiners' identity conclusions are not grounded in adequate statistical empirical foundations. Disagreements among experienced examiners about when identification conclusions are warranted make it impossible to calculate a day-to-day error rate for the discipline of firearms and tool mark identification. Further systemic problems are the absence of rigorous and blind proficiency testing and the confirmation bias that permeates both firearms and tool mark examiners' day-to-day practice and the "studies" that purport to show that their identification conclusions are warranted.

Firearms, Tool Mark, Identification

#### E22 Laboratory Support in Child Protection Litigation

A.R.W. Forrest, LLM\*, University of Sheffield, Centre for Analytical Sciences, Department of Chemistry, Sheffield, South Yorkshire S10 1DA, UNITED KINGDOM

After attending this presentation, attendees will have a better understanding of the support that laboratorians, in particular toxicologists, can provide to children, families, investigators, advocates, and the courts who have to deal with the emotionally fraught issues implicit in child protection cases. Attendees will also appreciate the need for high quality scientific support in such cases and the problems that can arise from a "one size fits all" approach.

This presentation will impact the forensic science community by emphasizing the need for an individual approach to each and every case when child protection issues are before the court.

Questions frequently arise about current misuse of drugs and/or alcohol by caregivers. Continuing alcohol misuse may be investigated in some cases by simply asking for a liver function test profile and mean cell volume (MCV) despite the known lack of sensitivity and specificity of these tests for detecting continued alcohol misuse. At the other end of the scale, measurement of urinary ethyl glucuronide (EtG) on a Monday may be sensitive enough to detect the ingestion of alcohol by an Episcopalian taking holy communion on a Sunday. Other approaches to detecting continued alcohol misuse by a caregiver, such as the measurement of fatty acid ethyl ester (FAEE) in hair are also imperfect. The direct commissioning of such tests by social workers from commercial laboratories, sometimes located in other countries outside the jurisdiction of the case, may lead to a less than critical assessment of the significance of the results being presented to the trier of fact.

When issues of continued drug misuse by the caregiver arise, the issues surrounding the interpretation of laboratory investigations is usually better understood by advocates and the courts, given the experience that jurists and advocates inevitably acquire of drug abuse related issues. Even so, over-interpretation of unconfirmed and nonspecific screening tests can still occur. Advocates often seem to be unaware of the form of words such as, "The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical results" are universally incorporated in the packet inserts of reagents immunological screening tests for abused drugs in urine. A numerical result printed on a piece of paper is all to frequently naively over interpreted by professionals of all disciplines who do not have a laboratory background. An understanding of the imprecision inherent in laboratory testing and the inherent limitations of tests is a necessary precursor to being able to assess the probative value of such tests in context. The way in which reports are written ought to aid, but sometimes hinders, the interpretation of test results by the courts.

A particular class of cases where difficulties can arise is the investigation of allegations of drug administration to young infants. Such cases are often confounded by the mother's use of drugs during pregnancy. This is particularly the case when hair samples are collected from an infant in the first few months of life. There is still much basic research to be done on hair growth and the deposition of drugs in hair *in utero* and in early post-natal life. At present, in such cases, professional opinions can often only be based on anecdote, either personal or case reports in the peer-reviewed literature.

This presentation will highlight the need for a critical approach to the results of laboratory investigation and the need to interpret them together with all of the other evidence available to the trier of fact.

Child Protection, Toxicology, Holistic Approach

### E23 Limitations of the Assay: Gastroesophageal Reflux in BrAC Measurements

Justin J. McShane, JD\*, 4807 Jonestown Road, Suite 148, Harrisburg, PA 17108; and Wanda Marley, PhD\*, 4341 Cobb Lake Drive, Ft. Collins, CO 80524

After attending this presentation, attendees will come to understand how gastroesophageal reflux (GER) and an incompetent lower esophageal sphincter (LES) during a forced expiratory volume (FEV) maneuver limits the accuracy, reliability, and validity of a breath alcohol test result. Every analytical assay has limitations. Breath alcohol testing for breath alcohol concentration (BrAC) is no different.

This presentation will impact the forensic science community by increasing understanding of how the effects of GER disease (GERD) on a BrAC test is of paramount importance, in order to ensure that only those who are truly guilty are found guilty. This year's theme is "Relevant, Reliable, and Valid Forensic Science." As such, the validity of the BrAC quantification in an ethanol-based per se driving violation is crucial.

Key anatomical and physiological features of GERD will be presented and show how a forced exhalation can exacerbate gastric reflux through the lower esophageal sphincter and spuriously increase the BrAC test result. Since GER affects at least 10% of the U.S. population and up to 75% of affected individuals demonstrate few symptoms, many BrAC test results can be covertly inflated. Despite pharmacological treatment, the retrograde flow of gastric alcohol vapors into the esophagus occurs due to diffusion gradients and mechanical factors. The orifice between the esophagus and the stomach is normally regulated by involuntary smooth muscle (the LES) which controls the reflux of gastric contents/vapors. With chronic GERD the LES is weakened, allowing either constant or intermittent passage or ethanol vapors into the esophagus. These vapors combine with alcohol vapors that are exhaled from the lungs and airways as they merge together in the hypopharynx (throat). Gastric alcohol vapors are typically much more concentrated than those exhaled from the lungs and airways, which significantly inflate the cumulative exhaled ethanol concentration from both sources. The net effect depends on the type, amount, and concentration of ethanol consumed, as well as the presence/type of food in the stomach. The partition coefficient between gastric alcohol and its vapor is considerably lower than that for pulmonary capillary blood and alveolar air. Equations that employ these partition coefficients demonstrate that even a teaspoon (~5 ml) of gastric alcohol vapors from 5% (w/v) beer can raise a BrAC result from 0.07% to 0.09%. The inflated result would expectedly be even greater with hard spirits (40% w/v).

Combined alcohol vapors from the stomach and lungs are not adequately detected by the slope detector algorithms of evidentiary breath alcohol testing (EBT) instruments, because they are mixed together before they are exhaled. Unlike a belch, which may trigger an error message on EBT instruments, the insidious chronic release of gastric alcohol in GERD elevates the expiratory ethanol slope to a higher plateau than expected from lung-derived alcohol alone. Making matters worse, a deep inhaled breath, followed by a forced exhalation is required to provide an adequate breath alcohol sample to the EBT instrument. This maneuver alone causes the LES to become even less patent, because it increases intra-abdominal pressure which pushes against the stomach and LES. By its very design, the procedure for BrAC testing predisposes the GERD patient to even greater reflux, which goes undetected by the EBT device. Wider recognition of this problem will hopefully reduce the false charges of driving under the influence of alcohol, by educating the public that a *blood* alcohol test would be the forensically acceptable choice for persons affected by GER or GERD.

Breath Alcohol Content, GER/GERD, LES

#### E24 The NSC-CAOD's Source Code Resolution: Scientific Nonsense, Legal Misrepresentations, and Political Bias

Gil Sapir, JD\*, PO Box 6950, Chicago, IL 60680; and Mark G. Giangrande, JD, 4827 Pine Avenue, Hammond, IN 46327

After attending this presentation, attendees will understand the ethical improprieties, political bias, legal misrepresentations, and scientific nonsense promulgated and promoted by the National Safety Council's Committee on Alcohol and Drugs (NSC-CAOD).

This presentation will impact the forensic science community by promoting the use of reliable source code software in breath alcohol testing equipment and exposing political motives to remove related evidentiary standards.

The National Safety Council is a non-profit organization devoted to developing and fostering public policy on safety. On February 16, 2009, the NSC-CAOD enacted its "Position of the NSC Committee on Alcohol and Other Drugs on the Source Code of Evidential Breath-Alcohol Analyzers" (hereafter, Resolution). The Resolution addresses breath alcohol test evidence used in the prosecution of driving under the influence of alcohol (DUI) cases. The source code is the basis of all computer programs and consists of a structured set of instructions that cause a computer or microprocessor-equipped device to perform specific operations. Removal of the source code causes the device to stop functioning. Questioning of the source code program in court proceedings has exposed various flaws.

The Resolution states in part, "software of an evidential breathalcohol analyzer is not pertinent, required, or useful for examination or evaluation of the analyzer's accuracy, scientific reliability, forensic validity, or other relevant characteristics, or of the trustworthiness and reliability of analysis results produced by the analyzer."<sup>1</sup> It defies the practice of good science, technology, ethics, and current law.

The Resolution was published as a political statement to commercially benefit breath alcohol-analyzer manufacturers and affect policy positions on drunk driving by unilaterally declaring it a nonissue.

Breath alcohol test (BrAC) results are collected solely for the purpose of prosecution. Too often the BrAC result is the only significant evidence of impairment. The proponent must establish the validity of the computer program that generated the BrAC statement.<sup>2</sup> The reliability of the device's source code, based upon a proper legal foundation (critical impact or crucial evidence) is a question for the jury, not a predetermination by the NSC-CAOD or any other party.

The Resolution selectively relies upon self-serving scientific and legal citations which are obscure, relatively difficult to obtain, and improperly referenced. The Resolution repudiates the integrity of the measurement process (metrology), including potential sources of undetected software errors, quality assurances for intended purpose, and validation techniques by the manufacturer. It ignores that analysis of the source code provides a recognized means for validating error handling routines. For example, normal quality assurance and quality control procedures do not test for, or validate, a unit's ability to detect and properly respond when an internal component failure occurs.

The legal references within the Resolution were cited for narrow propositions taken out of context or are non-authoritative. The cases and articles, cited by NSC-CAOD, are actually indicative of a judicial trend to maintain a defendant's constitutional right to confront the evidence used against them.

The Resolution disregards conflicts annunciated in the National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*,<sup>3</sup> including those between the needs of law enforcement, forensic science, and criminal defendants. By all practical measures, the Resolution selectively rejects relevant material against its position. The Resolution is intended to be accepted without question.

The NSC-CAOD sought and obtained validating recognition for its Resolution through the Journal of Analytical Toxicology (JAT). The Resolution was published in June, 2009 through JAT, without apparently subjecting it to editorial review, peer review process, or accompanying disclaimer.<sup>4</sup> Publication was based upon JAT's previously unpublished expanded editorial or relaxed publication standards.<sup>5</sup>

The NSC-CAOD should not attempt to limit constitutional and evidentiary standards under the guise of a scientific statement. Courts should take into account the motivations behind this Resolution and maintain their independent views when it comes to source code material. The NSC-CAOD source code resolution is a political statement, not a scientific one, and should not be given any legal credence.

#### **References:**

- <sup>1.</sup> Journal of Analytical Toxicology, vol.33, no.5, p.287-290 (June, 2009)
- <sup>2.</sup> Edward J. Imwinkelried, This Is Like Deja Vu All Over Again: The Third, Constitutional, Attack On The Admissibility Of Police Laboratory Reports In Criminal Cases, 38 N.M. L.Rev. 300, 320 (Spring, 2008)
- <sup>3.</sup> Harry A. Edwards and Constantine Gatsonis, Strengthening Forensic Science in the United States: A Path Forward, National Academy of Sciences, The National Academy Press, Feb. 2009, ISBN 0-309-1313-6, 254 pages (2009); www.nap.edu/catalog/12589.html.
- <sup>4.</sup> Journal of Analytical Toxicology, vol.33, no.5, p.287-290 (June, 2009)
- <sup>5.</sup> Journal of Analytical Toxicology, Editor's Note, vol.33, no.9, p.16A (Nov./Dec., 2009)

Source Code Software, National Safety Council, DUI/DWI

# E25 Reliability of the Drug Recognition Exam: Admissions by the Suspect Increase the Chances of Getting It Right

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After attending this presentation, attendees will be able to recognize the limitations of the Drug Influence Evaluation (DIE) conducted by law enforcement, compare the methods of administering the Horizontal Gaze Nystagmus (HGN) Test and the test for the Romberg Sign between physician's and law enforcement officers, and contrast the reliability of the DIE when the suspect has and has not admitted to prior drug use.

The presentation will impact on the forensic science community by demonstrating the lack of reliability of DIE testing and how the results of urine drug testing are misused by prosecution witnesses to confuse prior use with current impairment.

When suspects stopped by police did not admit to prior drug use, the incidence of correct or partially correct conclusions (regarding the drug or class of drugs allegedly consumed by the suspect) reached by socalled "Drug Recognition Experts" (DREs) decreased from 87% to 66% and the incidence of incorrect conclusions increased from 13% to 34%. The Drug Influence Evaluation tests ("DIE") conducted by DREs lack reliability and reproducibility in the field. Medical doctors correctly identify drugs in patients only 47% of the time (Brett, 1988) and were incorrect 40% of the time (Teitelbaum, 1977). Medical tests like Horizontal Gaze Nystagmus (HGN) and the test for the Romberg sign are administered by police and DREs in such a way as to elicit a positive response. In two studies, based on standardized Field sobriety tests, police consistently rated sober people as impaired (Tharp et al, 1981; Cole and Nowaczyk, 1994). Unprincipled prosecution witnesses portray *Williams v. State*, 710 So.2d 24 (Fla. 3d DCA, 1998) was an appeal by defendant Frederick Williams of his conviction for Driving Under the Influence of Drugs (DUID). Williams was stopped at a field sobriety checkpoint. After failing a series of field sobriety tests, a breath test registered a Blood Alcohol Concentration (BAC) of 0.07%, an amount under the legal threshold for intoxication in Florida. A Drug Influence Evaluation test ("DIE") conducted by a DRE, without knowledge of Mr. Williams' prior drinking experience, led the officer to conclude the defendant was under the influence of cocaine and cannabis. Williams was arrested for DUID. A urine sample subsequently tested positive for inactive marijuana and cocaine metabolites.

At trial, over the objection of the defendant, the State was allowed to introduce the results of the DIE. The defendant moved to exclude the evidence under *Frye v. United States*, 293 F. 1013 (DC Cir. 1923) arguing that the scientific evidence generated by the DIE was not generally accepted in the relevant scientific community, and that admitting the testimony of a minimally trained officer referred to as a DRE misled the jury and prejudiced the defendant. The court ruled that the DIE protocol was not scientific, *Frye* did not apply, and the tests were easily understood by the jury. The appeal failed.

To prove its claim of unreliability, the defendant subpoenaed in all of the DRE examinations conducted during the prior year in Dade County. The data were first separated into two distinct groups: (1) suspects who had admitted to drug use (often this included the drug(s); and, (2) suspects who had not admitted drug use. In 110 cases out of 114 arrests, a urine drug test also was performed. When suspects did not admit prior drug use, the incidence of correct or partially correct conclusions decreased from 87% to 66% (24%) and the incidence of incorrect conclusions increased from 13% to 34% (160% increase). The drop in accuracy from suspects who admitted to a drug in comparison to those who did not was statistically significant  $X^2(2) 8.07 = p < .05$ .

Unfortunately, material potentially exculpable, scientific evidence regarding the fact that urine drug tests cannot be used to infer impairment, only to demonstrate prior exposure (Benjamin, ToxTalk, March 2010), and that up to 10% of the population may suffer from HGN as a result of an inner ear infection, vertigo, labyrinthitis, or water in the ear never reached the jury. Moreover, urine tests for THC and cocaine detect only the non-psychoactive metabolites of these drugs, THC acid and benzoylecgonine (BE). Testimony regarding the presence of metabolites in the urine should be limited or suppressed pursuant to FRE 403 or risk misleading the jury, confusing prior use with current impairment, and unduly prejudicing the jury.

Reliability, Credibility, Disingenuous

# E26 Dr. Cop: The Need to Examine Validation & Reliability Standards for Specialized Law Enforcement Knowledge Testimony

Rafael E. Silva, JD\*, 5950 SW 120 Avenue, Miami, FL 33183

After attending this presentation, attendees will understand inherent problems in validating the reliability of specialized knowledge.

The presentation will impact the forensic science community by illustrating the unreliability of police officers as expert witnesses.

In 2009, the National Academy of Sciences issued a report criticizing serious deficiencies in the nation's forensic science system and the necessity for major reformation (Report).<sup>1</sup> It discussed a lack of

necessary comprehension of science by judges and lawyers, vague standards for evaluation of non-scientific experts, and adversity to change. The Report severely condemned a law enforcement culture that induces wrongful convictions. Although the Report addressed judicial practices and evidentiary standards for expert testimony, its primary focus was improvement within the forensic science community.

The important issue of police officers and federal agents as experts was tangentially addressed. Law enforcement personnel are routinely "qualified" as experts and their opinion testimony is admitted as evidence. Unlike scientific and technical experts, police undergo intense, but very short term training. In three months, brief classes presume to teach them knowledge and competence of scientific evidence, constitutional law, and crime prevention. Their expertise is superficial compared to other experts. There is no prerequisite of scientific training. A junior college associate's degree suffices. Yet, courts are quick to declare them experts.

Expert witness testimony is the most persuasive evidence.<sup>2</sup> Common witnesses can only testify to what they directly observe. Only experts are permitted to state opinions based on observations – and police experts greatly speculate. They are imbued with respect and admiration of courts and society. Their departments then give them prestige titles (for example, "Special Agent," "Inspector," or "Drug Recognition Expert") that do not of themselves ensure truthfulness or reliability. Upon being qualified by a judge, they receive an imprimatur that further enhances their credibility with uninformed jurors, who have a distorted view of the criminal justice system formed by art and not reality. Police are not neutral examiners of evidence. They have a deep bias to convict those they arrest.

Experts in law exist because of Federal Rule of Evidence 702. Three types are recognized: scientific, technical, and "other." Initially interpreted by the Supreme Court in the Daubert case,3 for expert testimony to be admitted requires that it be helpful for the court to understand evidence or the jury to determine a fact at issue. It must also be reliable. In the Kumho<sup>4</sup> case the Court decided that these standards apply to all experts alike. They left this up to trial judges, and said the factors that prove reliability are flexible, but police are not exempt from scrutiny just because they are outside the realm of science. Since Daubert and Kumho these vague standards have resulted in massive confusion. An intergral difficulty of finding applicable factors for "other" experts is the complete lack of comprehension (and often disdain) of science by lawyers and judges. Factors that prove reliability in scientific fields may not precisely apply to police. However, scientific methodology is specifically designed to meet the goal of reliable knowledge. The less scientific police practices are, the less reliable their knowledge or expertise. Ergo, the oxymoron "Dr. Cop."

Courts initially avoided applying *Daubert's* admissibility standards to "other" experts. When *Kumho* confirmed the judicial "gatekeeping" function of protecting jurors from unreliable expert testimony must be applied to all experts, courts bogged on the vagueness of the factors. Many relied on the history of police expert testimony as irrefutable, and took judicial notice of it. The Supreme Court gave great deference to trial judges, and effectively shielded them from any review when it decided in *Joiner*<sup>5</sup> that "abuse of discretion" had to be shown to reverse a trial decision on admissibility.

The pervasive habit of past practices is very difficult to stop. Presumed police expertise is simply not expertise at all. It is based on shallow knowledge and is highly unreliable. It does not demonstrate the basic constructs for reliability. Police are not experts of any kind. Their testimony merits no higher consideration than a lay witness. If police aspire to expert witness status, they should abide by those requirements. Judges and lawyers may not know these standards, but any good theoretical or practical scientist does.

#### **References:**

<sup>1.</sup> Strengthening Forensic Science in America: A Path Forward, National Research Council of the National Academies, The National Academies Press, Washington D.C., 2009.

- Daubert v. Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579, 595 (1993).
- <sup>3.</sup> *Daubert*, supra
- <sup>4.</sup> Kumho Tire v. Carmichael, 526 U.S. 137 (1999)
- <sup>5.</sup> General Electric Co. v. Joiner, 522 U.S. 136 (1997)

Expert Witness, Daubert, Evidential Reliability

## E27 The Development of a Scientific Working Group for Breath Alcohol Analysis

Brian M. Lutmer, BS\*, Missouri Department of Health and Senior Services, 2875 James Boulevard, Poplar Bluff, MO 63901

After attending this presentation, attendees will understand the importance uniformity has in breath alcohol testing and importance of Scientific Working Groups (SWGs) in establishing and perpetuating the practice of good science in breath alcohol testing.

This presentation will impact the forensic science community by recognizing a need for standardization in breath alcohol testing and outlining a strategy for implementing it through the use of a SWG.

The National Academy of Sciences (NAS) Report: *Strengthening Forensic Science in the United States: A Path Forward* made several recommendations for the advancement of the forensic sciences. Specifically, Recommendation 6 references Scientific Working Groups (SWGs) working with the federal government to establish standards within the relevant forensic communities.

SWGs, originally founded by the FBI in the early 1990's, have been instrumental establishing standard protocols and guidelines within forensic disciplines (e.g., Firearms and Tool Marks, Bloodstain Pattern Analysis, DNA Analysis Methods). Although the SWGs have no enforcement capabilities, they nonetheless are a valuable source for creating and improving recognizable standards.

In October 2009, the Forensic Toxicology Council, with funding from the National Institute of Justice, founded the Scientific Working Group on Toxicology (SWG-TOX). The founding of SWG-TOX reflects the NAS Report's impact and recognition of the need for standardized ethics, training, and methodologies in forensic toxicology. However, SWG-TOX does not anticipate addressing the field of breath alcohol testing due to significant differences in foundational criteria.

Exclusion of breath alcohol testing by SWG-TOX is understandable. The significant program differences include education, training, operation, certification, and administration. Accreditation for breath alcohol calibration laboratories (reference solutions) under ASCLD-LAB/ISO 17025 standards became available in 2008. Unfortunately, only three governmental agency programs in the United States have been accredited. Disparity of personnel qualifications exists between programs. Proficiency testing in alcohol testing is generally non-existent. Furthermore, the Forensic Specialties Accreditation Board does not offer certification for individuals strictly employed in alcohol toxicology.

Standardization of breath alcohol testing must be competently and effectively addressed. The societal and legal ramifications of drunkdriving convictions are profound. Over one million breath alcohol (BrAC) tests are conducted annually in the United States. BrAC results are collected solely for the purposes of license revocations and criminal prosecutions. Frequently the BrAC result is the only evidence of impairment. Accordingly, good science must be practiced in BrAC testing.

Forensic breath alcohol testing must implement established standards through creation of its own scientific working group, the Scientific Working Group on Breath Alcohol Testing (SWG-BAT). Membership would include representatives from the private sector, academia, legal community, governmental agencies, and international bodies. This SWG would establish recommended standards and discipline for forensic analysis of breath alcohol testing. The objectives of the SWG-BAT must at a minimum include:

- Specifying requirements for operator's knowledge, skills, abilities and competency;
- Promoting professional development;
- Applied ethical standards and viable sanctions for operators and program administrators;
- Providing minimum standards for analyses and reporting of results;
- Long term retention of records and download data (calibration, maintenance, testing, references standards etc.);
- Establishing quality control requirements for testing;
- · Proficiency testing for all testing sites and instruments;
- Traceable measurements of uncertainty (error analysis); and,
- Operator and repair technician certifications recognized by the Forensic Specialties Accreditation Board.

Many of the necessary scientific standards for adoption by SWG-BAT are included in the historic recommendations of the National Safety Council's Committee on Alcohol and Other Drugs. Guidance for adoption on other issues is also readily available, from groups such as ASCLD/LAB on operational issues, NIST on the proper handling of measurement uncertainty, and AAFS on issues of ethical conduct and professional development.

The time has arrived for creation of a practical scientific working group for standardized requirements and protocols in breath alcohol testing. The tenets and practice of good science must be applied to breath alcohol testing as in other forensic science disciplines. It is in the legal and forensic science community's best interests to implement SWG-BAT.

Scientific Working Groups, Breath Alcohol, Standardization

#### E28 The Scientific Process of Blind Verification

Kathryn Suchma, BA\*, Federal Bureau of Investigation Laboratory, 2051 Investigation Parkway, Quantico, VA 22135

After attending this presentation, attendees will be able to distinguish between verification and blind verification procedures, explain the purpose of blind verification as a scientific procedure, discuss the effectiveness of blind verification, discuss the capabilities and limitations of the policy, and discuss how quality measures are related to cognitive bias and error.

This presentation will impact the forensic science community by sharing an understanding of how blind verification can be used within the fingerprint discipline as well as other forensic disciplines and will assist all members of the forensic community when discussing bias and the impact on policy. A clear understanding of the capabilities and limitations of blind verification and how it relates to error and conflict resolution policy, will assist the forensic community in addressing the topic of bias and policy within the court system. Scientists can assist the legal and judicial community by clearly articulating the role quality assurance policies play.

The 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward* cited research which explores the extent to which cognitive bias may be present and may impact forensic science examinations. The roles that various psychological factors may play in forensic pattern recognition have been discussed and the importance of being aware of the potential for bias has become widely acknowledged within the forensic science community.

In 2004, the FBI incorrectly identified a latent print on an item of evidence associated with a bombing in Madrid. In the wake of the Madrid case, thorough investigations of the latent print unit were conducted, including reviews by an internal review team, an international review panel, and the Office of the Inspector General. After reviewing the documentation and procedures in place at the time of the Madrid case, several recommendations were made as to how to improve practices within the latent print unit. One of the recommendations included the suggestion of employing blind testing, or blind verification, within the fingerprint examination process.

While the presence of bias or the potential for bias does not relate directly to error, often the topic of bias and blind verification seem to be associated by many with error.

The FBI and other laboratories have since implemented, and subsequently improved, blind verification procedures in many forensic disciplines, including fingerprints, questioned documents, firearms, and trace evidence. The procedures and protocols developed to this end are offered, as forensic disciplines strive to be clear and transparent within the legal system. As forensic disciplines continue to receive increased attention within the court system, the need to clearly articulate procedures and protocol becomes increasingly important. Both an accurate description of such policies and the role the quality assurance policies play are important to a fair and equitable criminal justice system.

This discussion seeks to explain the scientific nature of blind verification, as well as the role blind verification can play, both in the fingerprint discipline as well as other forensic disciplines. Of course, for purposes of clarity of the discussion, definitions of "verification" and "blind verification" will be discussed. Procedures used in both "verification" and "blind verification" will be reviewed to give the audience an understanding of where the procedures vary, as well as an understanding of how verification fits into scientific testing methods. The capabilities, as well as the limitations, of both "verification" and "blind verification" will be discussed.

As scientists, it is not enough to accept recommendations to improve the science. Rather, we must ask, "Has this modification in procedure resulted in improved effectiveness and outcomes?" This seemingly simple question requires a complex answer, taking into consideration several performance measures. As such, effectiveness of quality assurance policies and the specific role blind verification plays in an attempt to reduce bias will be explored. Finally, information as to how this strategy has been implemented within the FBI Laboratory, specifically the latent print unit, will be presented.

**Blind Verification, Policy, Bias** 

# E29 A Strategic Approach to Improving Forensic Science Performance: Sufficiency as an Example

Anjali R. Swienton, JD, SciLawForensics, Ltd., 25 Walnutwood Court, Germantown, MD 20874; Randall Murch, PhD\*, Center for Technology, Security and Policy, Virginia Polytechnic Institute, 1101 King Street, Alexandria, VA 22314; and Bruce Budowle, PhD, Forensic & Investigative Gen, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, EAD 310, Fort Worth, TX 76107

The goal of this presentation is to begin a discussion on developing an overarching strategic plan for enhancing the forensic sciences and providing confidence in specific disciplines and the enterprise as a whole.

This presentation will impact the forensic science community by opening discussion on issues that have been addressed by the recent National Academy of Sciences (NAS) Report related to improving forensic science performance using sufficiency in latent print examinations as an example.

Most disciplines of the forensic sciences compare a profile derived from an evidence sample with that of a reference sample(s) to determine whether or not the two samples could have had the same origin. Over the last 100 years many of the original practices were based on pattern comparisons (e.g., latent prints, handwriting, hairs, tool marks). They were often developed in an *ad hoc* manner and based their validity and

reliability on experiential, subjective observations, and inferences. Several factors have impacted the perceived foundations of these experiential-based disciplines: (1) today's forensic scientists are far more sophisticated than they were a century ago; (2) scientists in other fields are having greater input in the forensic sciences; (3) the legal field is more adept at challenging the admissibility of scientific evidence; (4) legal admissibility standards have been augmented; and, (5) a recent report by the NAS calling for improvements in a number of areas in the forensic sciences. The basic assumptions of the science applied and the interpretation of results, as well as the education and training of forensic scientists, are increasingly being called into question. There are some justifications for such criticisms; we all should strive to improve processes, better define attributes and limitations, and establish improvements to interpret and communicate findings. Clearly research is needed in several of the aforementioned forensic disciplines. For example, examiners analyzing friction ridge patterns employ the term "sufficiency" to describe the minimum amount of information required for applying ACE-V to a sample. There are no defined quantifiable data defined for stating that a sample meets the criterion of "sufficiency." Thus, it is difficult to ensure that a minimum acceptable performance is being practiced in the latent print examinations and comparisons and for that matter what is the minimal accepted standard. In the spirit of the recommendations of the recent NAS Report, a plan will be proposed, using friction ridge pattern analyses as an example, to move forward in an effective manner to instill education, quality, validation, and (of the utmost importance) reliability in the results obtained and decisions made from analyses, and then interpretations and opinions espoused in reports or in the courtroom. While there is likely a science component in defining sufficiency and other portions of an analysis that must be addressed, there are fundamental training requirements that all forensic scientists in the experiential-based, as well as the analytical-based, disciplines should obtain. Education (including continuing education) and training are essential components to maintaining high quality and reliable performance. The training should include: forensic ethics, statistics, quality assurance, validation, critical conduct of science, and problem solving. The training requirement should apply to all new forensic scientists as well as to all current practicing forensic scientists. There should be no grand fathering as these topics should be part of the fundamental repertoire of forensic science education and practice. This basic training will better prepare forensic scientists in their respective fields, more intelligently question current practices, and better embrace legitimately alternative viewpoints. Once imbued with these qualities, a plan of action can be prepared to address the scientific short-comings that all disciplines have. It is essential to have a well-vetted research, development, validation, and technology transfer plan in place. Otherwise, stakeholders, sponsors, and policy-makers will not take the forensic sciences seriously and support to improve will be limited. Lastly, whatever criteria are developed must be effectively communicated to the legal community and will therefore become subject to critical review, but will become part of the routine requirements for performing analyses. It is simply not acceptable to consider that if a methodology is accepted in the courtroom that it is reliable, as well as the converse that if a methodology is not accepted in the courtroom that it confers unreliability. Using sufficiency as the example, the plan we describe is a starting point for discussion. It is anticipated that this will stimulate further thought and input in developing an overarching strategic plan for enhancing the forensic sciences and providing confidence in specific disciplines and the enterprise as a whole.

Quality, Sufficiency, Validation

# E30 Special Challenges in the Assessment of Jihadists

Michael Welner, MD\*, The Forensic Panel, 224 West 30<sup>th</sup> Street, Suite 806, New York, NY 10001

After attending this presentation the attendee will learn of the challenges unique to Jihadist cases and their defendants: welcome sources of information, approaches that aid in the better understanding of an often-elusive examinee, and approaches to cutting through the fog of war. The lecture respects the boundaries of national security but offers lessons gathered from a long war's early days, its entrée onto the soil of American courts, and the psychological lessons for trial and corrections. This presentation will impact the forensic science community by examining special challenges in the assessment of Jihadits.

The Jihadist, in the person of a foreign born defendant affiliated with al-Qaeda or other nihilistic terror groups, may increasingly face evaluation in civilian settings. Contrary to current presumptions, combatants hail from multiple continents and derive from distinct pathways. With a number of cases referred to federal courts, forensic psychiatrists and psychologists working in both the civilian and military settings encounter unique challenges in the assessment of these offenders.

From confession statements to diminished capacity, to potential mitigating influences, custodial arrangements, future dangerousness, and even capacity to be executed, the Islamist defendant poses peculiar cross-cultural and interview challenges. Controversies from the Guantanamo and Bagram legacy, the prevailing political climate, veils attached to national security, even the influence of the news media layer additional complexities. Mythology of numerous "scholars" of terrorism introduces others confounders to accurate assessment.

Islamist Terror, Al-Qaeda, Forensic Psychiatry

# E31 Relevant, Reliable, and Valid Forensic Science in Continental Europe Criminal Justice Systems

Christophe Champod, PhD, School of Criminal Sciences, University of Lausanne, Batochime, Lausanne, 1015, SWITZERLAND; and Joelle Vuille, LLM\*, Institute of Criminology and Criminal Law, University of Lausanne, Internef, 323, Lausanne, 1015, SWITZERLAND

After attending this presentation, attendees will understand how legal systems from Continental Europe appraise the relevance, validity, and reliability of forensic evidence. The basic differences between inquisitorial and accusatorial criminal systems will be outlined and the position and role of scientific experts in European criminal procedures will be discussed. Finally, the consequences in terms of procedural rights of the accused will be debated.

This presentation will impact the forensic science community by raising awareness of the similarities between inquisitorial and accusatorial criminal justice systems when scientific evidence is assessed. In fact, it is often postulated in the Anglo-American scientific and legal literature that the appointment of scientific experts by the courts (as in inquisitorial systems) could solve many problems encountered when expert witnesses are hired by parties. It will be shown that such is not always the case and that inquisitorial procedures raise their own set of problems when scientific evidence is to be evaluated.

Contemporary European legal systems are familiar with the principle of freedom of evidence, meaning that a court may consider any type of evidence to establish the facts. There is; however, an exception to this rule: evidence cannot be considered when, despite its (hypothetical) reliability, it was adduced contrary to statutory provisions (for instance, searches without a valid warrant).

Contrary to American law, where admissibility is subject to fairly precise rules, the scientific admissibility of evidence is seldom addressed in European legal writings, and continental legal systems seem rather uncommunicative on the subject. The question of scientific reliability is seen as intrinsically linked with the assessment of the actual evidence, that is with the determination of its probative value. Magistrates are left to their own devices in taking these decisions, with the risk of disparate practices developing, of unreliable evidence being admitted, or new methods being rejected despite their reliability.

In practice the situation is unsystematically regulated, traditional types of evidence being admitted because they always have been (and because it is thought that their reliability has long been proven), while calls for expert testimony concerning "outlandish" subjects are rejected. In borderline cases, the judge will appoint the expert and decide as to the probative value of the expert testimony according to the intelligibility of the report and in the light of the other facts of the case.

Such "laissez-faire" attitude is justified in the legal literature by the structure of these legal systems, which, it is thought, set up enough formal and informal barriers to the admittance of invalid scientific evidence: the formal accreditation of experts, fact-finding being addressed by a professional judge instead of a lay jury and the duty to give reasons for decisions, are seen as adequate safeguards against the taking of unreliable and irrelevant evidence.

Yet, problems encountered by inquisitorial systems when assessing scientific evidence are numerous and often poorly addressed because the judicial community has great trust in its court-appointed scientific experts and lacks awareness as to the questions raised by such kind of evidence. It remains unanswered then whether inquisitorial systems can under such circumstances safeguard a defendant's right to a fair trial.

Admissibility, Probative Value, Continental Europe

# E32 Minnesota's Advanced DNA Institute – A Training Model for Defense Counsel

Pamela A.W. King, JD\*, 400 South Broadway, Suite 15, Rochester, MN 55904

After attending this presentation, attendees will understand how a statewide public defender system addressed concerns about insufficient training for lawyers in forensic science disciplines, raised in the National Academy of Sciences Report, by developing a comprehensive and cost effective training model in DNA litigation.

This presentation will impact the forensic science community by showing how in-depth training of criminal defense attorneys even in times of lack of funding and resources, can raise the quality of complex DNA litigation in the courtroom.

The National Academy of Sciences Report found that lawyers "often lack the scientific expertise necessary to comprehend and evaluate forensic evidence." Providing quality training to teach lawyers about science so they can properly evaluate the forensic evidence in individual cases is an important part of continuing to improve the state of forensic science in the courtroom. Providing comprehensive training in forensic disciplines for practicing attorneys through continuing legal education programs presents challenges that are unique from those faced with training new lawyers in a law school setting.

Most continuing legal education programs are short. Many last no longer than a week, others cover the span of a weekend and address multiple topics. Often they provide more of an overview than addressing advanced issues that present in individual case work. Finally, comprehensive advanced trainings on particular forensic topics are often expensive. They require extensive time away from work and often involve out of state travel making them cost prohibitive. This leaves many lawyers in a position where they only learn areas of forensic science one case at a time over a life time of practice. Yet, with forensic science and in particular DNA typing being used more and more in court, it is imperative that organizations like a statewide public defender system have lawyers with advanced experience with forensic issues to serve as consultants, train other lawyers and handle the unique issues that forensic science cases often present.

The Minnesota State Public Defender System has previously attempted to offer advanced training opportunities to their lawyers, both locally, and until budget issues prohibited out of state travel, in other states. Many of these opportunities, like much of the continuing legal education training, were short and/or very expensive. On at least one occasion, a more advanced option was made available through a week long course where many skilled DNA lawyers and scientists participated in teaching DNA to Minnesota Public Defenders. However, these skills were not regularly used by those who had received this training. Even after this training, some still lacked confidence in their ability to spot issues and work with experts on these complex issues. With these considerations in mind the Minnesota State Public Defender's office created a new training model to assist lawyers in DNA cases.

In 2009, the State Public Defender, working with a small group of lawyers, developed a year long training program to teach thirty lawyers DNA using a combination of intensive lecture, small group and one on one tutoring. Each lawyer worked with a pending criminal case from around the state to allow these lawyers to immerse themselves in DNA both in a classroom setting and a practice setting. In developing this program, the Public Defender Office also sought the advice and assistance of the Minnesota Bureau of Apprehension's Biology Section.

This presentation will give a detailed explanation of how the program was structured and developed, the costs involved, and provide information about the outcomes this program has seen to date.

Training, Lawyers, DNA

#### E33 Forensic Science Academy for Lawyers!

Jody M. Wolf, MS\*, Phoenix Police Department, Laboratory Services Bureau, 621 West Washington Street, Phoenix, AZ 85003; and Marna McLendon, JD\*, Arizona Attorney General's Office, 1275 West Washington, Phoenix, AZ 85007

After attending this presentation, attendees will have a better appreciation for the need to educate lawyers in forensics science disciplines and an understanding as to identifying opportunities and methods to create a forensic science academy.

This presentation will impact the forensic science community by recognizing the value of having attorneys who work with forensic science experts being trained specifically in the scientific method and the basic fundamentals of the various forensic science disciplines. The focus will be on lawyers and criminalists working together to provide such a training opportunity.

With the publication of the National Academy of Sciences 2009 Report on Forensic Science: Strengthening Forensic Science in the United States: A Path Forward, increased focus has been directed on the training and certification of forensic scientists. But other justice system participants, namely lawyers and judges, have also become increasingly aware of the vacuum in their training regarding forensic science and science in general. The report itself recognizes that the judicial system is encumbered by "judges and lawyers who generally lack the scientific expertise necessary to comprehend and evaluate forensic evidence in an informed manner..." With the publication of the report and, the following year, the legislative mandate to change from Frye to a Daubert standard, Arizona lawyers and criminalists found themselves faced with a variety of education challenges. A solution to increase attorneys (prosecutors, defense attorneys, and judges) fundamental understanding of the scientific method as well as the scientific principals and processes of a variety of disciplines in forensic science was identified. By creating a Forensic Science Academy, prosecutors and defense attorneys would train on scientific principles and then develop direct and cross-
examination skills by working with criminalists in that discipline. Criminalists from several crime labs could join in the mock courtroom training and, in that fashion, gain competency, and skills regarding courtroom testimony.

This presentation will provide the roadmap that Arizona is using to provide attorneys with fundamental scientific concepts and a familiarization of the different forensic science disciplines. Focusing on topics such as scientific method, basic statistical analysis in scientific research, DNA, drug analysis, forensic biology, firearms and ballistics, fingerprints, digital forensics, arson, and death investigations, attendees will have a chance to become aware of the basic principles in each discipline and an opportunity to develop and practice predicate questions for experts as well as cross-examination skills. Included during the sixmonth curriculum are tours of various crime labs, the Maricopa County Medical Examiner's Office, and the Arizona Computer Forensic Lab. The information provided to lawyers will also give them an advantage early on in their review of a case to better assess the value of the evidence.

At the Academy, launched in January, 2011, senior criminalists serve as the instructors. The following week, they would serve as the expert witnesses in the mock trial portion; this session would be open to any criminalists interested in a better understanding of the dynamics of courtroom testimony. Cross training and additional dialog will be encouraged.

Such education may develop into a certification program providing additional credentials for practitioners as the Arizona State University is one of the planning partners in the new effort.

Academy, Training, Forensics

#### E34 A 21st Century Way to Educate Lawyers and Judges About the Use of Forensic Sciences Evidence in Their Courts

Bernard A. Raum, JD\*, Levin College of Law, University of Florida, PO Box 1149, Newberry, FL 32669

After attending this presentation, attendees will learn of a new online program designed to educate them in the admissibility, use, and reasonable expectations of forensic sciences as evidence in court.

This presentation will impact the forensic science community by introducing a new online program which will teach the fundamentals of forensic science and the issues involving the admissibility of such evidence.

This presentation will introduce lawyers and judges to a new online course that is specifically designed to teach the proper use of the forensic sciences as evidence in court. In recent years it has become obvious that there exists among lawyers and judges a substantial lack of understanding regarding not only the fundamental issues which surround the admissibility of forensic science evidence but also a lack of understanding of the scientific principles which underlie the creation of such evidence by the various forensic sciences professionals. As a result when they are confronted by evidence which has been generated by forensic science experts, many lawyers and judges are unable to effectively analyze not only how such evidence was created but also whether such evidence is proper in their particular case. Furthermore, lawyers in particular may lack sufficient knowledge of the particular scientific subject matter to effectively conduct either direct examination or cross examination. Lastly, both lawyers and judges need to be able to recognize the fundamental legitimacy of proffered forensic science evidence.

To fill this need an on-line course was designed in 2007 to offer a fundamental education to lawyers and judges in the field of the forensic sciences. As the basis for the lectures, the course utilizes a noted textbook, *Forensic Science: Introduction to Scientific and Investigative* 

*Techniques*, *3<sup>rd</sup> Edition*, James & Nordby (CRC Press:Boca Raton:2009). Lawyers and judges who participate in the course are introduced to the science and the fundamental principles of many of the various fields of the forensic sciences. In addition to the forensic sciences, course participants are also presented with a discussion concerning the admissibility issues that surround the use of evidence generated through the application and use of the forensic sciences. Attendees will be shown examples of portions of some of the lectures taken directly from the course. **Lawyers, Judges, Education** 

#### E35 The National Clearinghouse for Science, Technology & the Law: An Online Forensic Resource

Carol Henderson, JD, Stetson University, College of Law, 1401 61st Street, South, Gulfport, FL 33707; and Anjali R. Swienton, JD, SciLawForensics, Ltd., 25 Walnutwood Court, Germantown, MD 20874\*

After attending this presentation, attendees will become familiar with the free resources available from NCSTL.org, the website for the National Clearinghouse for Science, Technology and the Law at Stetson University College of Law.

Funded by grants from the Department of Justice and part of Stetson University College of Law, the National Clearinghouse for Science, Technology and the Law this presentation will impact the forensic community by providing information about the National Clearinghouse for Science, Technology and the Law, it with the world's only online resource containing information about the nexus of science, technology, and law that is completely open to the public and available at no cost.

Attendees will understand how to use NCSTL.org to learn what's new, find a conference or seminar, prepare for trial, attend an online lecture, connect with professional organizations, find out about expert witnesses, research a topic, and locate online resources related to scientific evidence and expert witnesses.

The relationship between law, science, and technology is both an essential alliance and a reluctant embrace. One reason for this tension is the lack of a free flow of information between the legal and scientific communities. Worldwide developments in science and technology are occurring at a rapid rate. Legal challenges are being made to emerging fields like biometrics. Even scientific evidence that has been relied upon for years, such as fingerprints, is facing challenges. The forensic science community is overwhelmed by the amount of information required to meet these challenges.

In response to these challenges, the National Clearinghouse for Science, Technology and the Law at NCSTL.org provides one centralized source that allows a forensic researcher to navigate all relevant case law, journals, reports, and resources necessary to conduct effective investigations and litigation. NCSTL.org's award-winning database is a free resource offering one-stop-shopping for references to resources intersecting science, technology, crime scene investigation, and the law. Forensic researchers, such as scientists, lawyers, judges, investigators, and anyone with a need for forensic-related information, can explore information in over two dozen topics that are vital to their profession, such as DNA, toxicology, and expert witness testimony. The database offers bibliographic information for over 100,000 legal and scientific resources, as well as references to popular literature, organizations, and educational opportunities. Using the NCSTL.org database, forensic researchers can easily track developments in science and technology related law from the legislature and courts, and keep up with the latest theories and trends from both legal and scientific literature.

In addition to the searchable online database, the National Clearinghouse for Science, Technology and the Law at Stetson University College of Law builds partnerships with universities, agencies, and professional associations. The National Clearinghouse for Science, Technology and the Law provides many educational opportunities, such as seminars and training workshops, such as its recent seminar in Forensic Science for Capital Litigators. Educational opportunities are often first presented live, with video archives made available on NCSTL.org. NCSTL.org also offers some special collections of resources, such as its "Cold Case Toolkit," its collection of resources related to the National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, and a subset of its database focusing solely on multimedia resources, such as forensic-related podcasts and interactive lessons.

**Database, Website, Information** 

#### E36 Judges as Students of Science in the Law

Joseph J. Maltese, JD\*, New York Supreme Court, 355 Front Street, Staten Island, NY 10304; and Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of Pennsylvania, Erie County Court House, 140 West 6th Street, Room 223, Erie, PA 16501

After attending this presentation, attendees will have a better understanding of the various educational outlets that are available to educate judge on matters of science and technology.

This presentation will impact on the forensic and legal community by providing an example where if judges can go back to school to learn about science and technology, then forensic specialists and attorneys should also keep current on scientific matters in a formal educational setting.

The National Academy of Sciences (NAS) Report on "Strengthening Forensic Sciences: A Path Forward" recommended amongst other items that in addition to forensic scientists obtaining additional formal education, that judges, attorneys and law students be educated on scientific matters. In this presentation, two state trial judges will discuss judicial education opportunities available for judges in the area of science and technology. They will discuss their first hand experiences of participating in these judicial education opportunities, which include the congressionally sponsored program known as, the Advanced Science Technology Adjudication Resource Judge Program (ASTAR) which has provided numerous seminars and training sessions for judges from various state and federal courts. The U.S. Congress mandated an education program for judges to study the impact of the human genome project on the courts over a decade before the NAS Report was issued on the role of forensic sciences. The ASTAR program includes judicial education on the entire scope of science, technology and forensics likely to be introduced as evidence or issues in the trial and appeals of complex cases. Judges participate in a three day science bootcamp, followed with required reading and writing assignments and two years of seminars totaling 120 hours of judicial education training and other assignments in various scientific and technological subjects.

The ASTAR curricula includes subjects taught by medical physicians, forensic scientists, and PhDs in numerous disciplines. Studies include updates on forensic science, but also medicine, genetics, evolutionary biology, nano-technology, environmental studies, and the study of diseases, as well as the conduct of evidentiary hearings and the reading of studies and reports. The purpose of the ASTAR Program is to create a group of scientifically trained judges to handle complex scientific matters and to be a resource judges to other judges within their home jurisdictions. Approximately 244 judges have graduated from the ASTAR program and are designated as ASTAR Fellows. In 2010, a new cohort of approximately 300 judges began the two year program.

In addition to ASTAR, this presentation will provide information on other judicial education opportunities which include Master's degrees as well as a PhD program in Judicial Studies at the University of Nevada at Reno (UNR). At UNR, students are full-time judges currently serving on the bench and who have graduated from ABA-accredited law schools. For the Master's degree program, judges may apply for one of two academic majors - the Trial Court Judge major or the Juvenile and Family Court Judge major. This Judicial Studies Curricula is intended to provide judges a formal academic setting in which trial judges (including administrative law judges) or juvenile and family court judges can integrate technical studies of the judiciary with more academic courses to provide an intellectual assessment of the role of the American judiciary.

The University of Nevada, Reno (UNR) provides specially designed courses for judicially-related issues which include the humanities, social, behavioral, and natural sciences and communications. On the campus of UNR are the National Judicial College (NJC) and the National Council of Juvenile and Family Court Judges (NCJFCJ) which also provide a series of courses treating more technical subject matter such as courses in Scientific Evidence and Handling Complex Litigation.

The UNR provides academic degree programs having a national scope and impact for judges and the judiciary and has created a new academic discipline: Judicial Studies which provides judges challenging and stimulating intellectual opportunities to become resources in teaching and research in various academic communities. Judges are provided structured, interdisciplinary academic curricula to encourage them to be active in teaching, planning and administering judicial education while providing them experience (by virtue of the Master's thesis and PhD dissertation requirements) for conducting and publishing their own research of interest applicable to their judicial systems. Judges study in courses such as Scientific Research Methods, Law and the Social and Behavioral Sciences, Medical Legal Issues, Science in Law, and Law and Economics.

Judicial education programs are also available at the Ohio Supreme Court's Judicial eCademy, which is providing online education curricula for busy state trial court judges who are unable to leave their jurisdictions to attend out of state education programs due to heavy caseloads. This format includes distance education by internet derived through both asynchronous and synchronous deliveries. Instructors include scientists and PhDs, who teach in the fields of addiction treatment technologies, nanoscience, neuroscience, DNA, forensic technologies, modern genetics, genomics, computer science, and internet crime as well as the issues of admissibility of such scientific evidence in court proceedings.

Other judicial education programs are offered to judges through entities such as the American Association for the Advancement of Science (or AAAS), an international non-profit organization which promotes cooperation between scientists, defends scientific freedom, encourages scientific responsibility, and supports scientific education and science outreach for the betterment of all humanity. The Judicial Division of the American Bar Association (ABA) has encouraged its judicial members to attend the AAAS's cutting edge neuroscience courses where present and future neuroscience research efforts are provided involving the validity and reliability of various types of brain scans as applied to potential evidentiary issues in their courtrooms.

**Students, Judicial Education, Science** 

#### E37 Terrorism and Policing in the 21st Century: Are We Ready For Suicide Bombers?

Sheri H. Mecklenburg, JD\*, Office of the United States Attorney, Northern District of Illinois, 219 South Dearborn Street, Chicago, IL 60604

After attending this presentation, attendees will learn about how current local law enforcement policies and practices affect the ability to deal with local terrorism and how those policies and practices must be adapted to effectively confront the continuing threat of local terrorism, particularly suicide bombers. This presentation will impact the forensic science community by assessing its ability to deal with the increasing threat of terrorism. Local law enforcement and local crime laboratories, which develop policies and practices based upon traditional crime fighting policies, must continually assess how those policies and practices must develop to deal with the continuing threat of terrorism.

In recent years, as terrorism has come from local sources, local law enforcement faces a continuing threat of terrorism, and must adapt its traditional crime fighting policies and practices to address the unique aspects of confronting terrorism here at home, particularly suicide bombers.

In the years since September 11, the threat of terrorism has increasingly come from those living among us, in our own cities and even in our own neighborhoods, rather than training camps and terrorist cells in far-away lands. The internet has come to serve as a recruiting tool and training ground for individuals sitting in their own American living rooms, disenfranchised from American society. As a result of the reality of local threats of terrorism, local law enforcement agencies are more likely than ever to find themselves in the position of confronting local terrorist cells, including suicide bombers intent on public destruction. As local law enforcement refines its strategies to combat urban crime, potential suicide bombers represent a challenge that traditional local law enforcement policies, practices and training are particularly inadequate to address.

This presentation will briefly review the history of terrorist threats of suicide bombers in the United States, and will discuss the increasing threat of local cells of terrorism in the United States. The presentation will review the lessons learned by local law enforcement from the Madrid and London train-bombing incidents, the terrorist attempts in London and Glasgow, and the more recent terrorist plots in New York City. The suicide bombings in Madrid and London leave no doubt that the war on terrorism will be fought in the streets of major urban areas, with local law enforcement as a primary combatant.

The threat of suicide bombers from a local law enforcement perspective will be addressed, including how the current police policies, training and practices, particularly regarding the use of force, which are followed by most local law enforcement in the United States, are not well suited to confronting a suicide bomber, and may even make the confrontation more dangerous and more likely to result in tragedy. This presentation also will touch upon how the current local law enforcement policies on crime scene investigation might affect evidence collection in the event of a suicide bomber. This presentation will discuss how local law enforcement's current policies, practices, and procedures will have to be adapted in order to address the threat of suicide bombers on United States soil. The presentation also will raise the issue of how any change in policies, practices, and procedures to effectively deal with terrorism and suicide bombers inevitably will be influenced by public acceptance and politics.

Given the future likelihood of suicide bombers in our cities, local law enforcement must immediately evaluate how to adapt its policies and practices, and how to train its rank-and-file, to address this horrific and possibly inevitable crime. This presentation will suggest policy and training changes that local law enforcement must consider to successfully address the threat of suicide bombers, and whether those policy and training changes will be acceptable to law enforcement as well as to the public which they serve, and what factors will influence such acceptance.

Terrorism, Suicide Bombers, Local Law Enforcement/Police

#### E38 Redux: AAFS President's Speech to the American Bar Association Criminal Justice Section Conference at the Fordham University School of Law, June 4, 2010, New York

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The American Academy of Forensic Sciences and the American Bar Association Criminal Justice Section co-sponsored a conference entitled, "Prescriptions for Criminal Justice Forensics." The AAFS President was invited to deliver an address at this conference. The goal of the ABA presentation was to address some controversial issues and conflicts which exist between lawyers and forensic scientists, and presented suggestions for coming together to minimize these conflicts. Lawyers and scientists must be willing to acknowledge the views of the other side and to examine the worlds of science and the law through parallel prisms.

This presentation will impact the forensic science community by examining suggestions for finding solutions aimed at strengthening the forensic sciences by looking at ways scientists in the laboratory and lawyers in the courtroom evaluate one another's actions and motives. There is enough culpability for failure on the shoulders of all who play a part in the presentation and evaluation of forensic science testimony at trial. This paper will also examine possible shortcoming in the arguments of those who have a responsibility for the formulation and management of forensic science testimony, those who are responsible for the admission of this testimony, and those who must argue against the validity of forensic science methods and conclusions both pre-trial and in trial.

Shortly after the 2010 American Academy of Forensic Sciences meeting in Seattle, an article appeared in *Newsweek* magazine which took a direct shot "across the bow" at forensic science. The President of the Academy responded to this article with the "other side of the story." That response (which *Newsweek* did not print) was disseminated across the internet and later appeared in publications of forensic science organizations in the United States. Responses were received by the AAFS President from as far away as Australia with the same message: Thank you for finally responding to the attacks on forensic science.

That response to *Newsweek* formed the basis for the speech, presented June 4, 2010, at the ABA conference. This presentation will include a synopsis of the speech with an opportunity for questions and comments from the audience.

American Academy of Forensic Sciences, American Bar Association, Newsweek Magazine

### E39 Legal and Management Challenges Raised by the Current Changes in the Fingerprint Community – Are the Requested Changes Backfiring?

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After attending this presentation, attendees will realize that the changes rightfully imposed on the fingerprint community are now impacting the legal and management communities. Some of the challenges for these communities are presented, supported by three multi-year research projects.

This presentation will impact the forensic science community by making managers and actors of the legal community aware of the upcoming challenges awaiting them because of the changes currently undergoing in the fingerprint community. Challenges regarding missing evidence, the reporting of probabilistic statements, training of staff, etc., will be addressed.

Since the early 1900's, it has been suggested that fingerprint evidence could be presented probabilistically to express the uncertainty associated with the inference of a source attribution to a questioned impression. However, this approach never gained widespread acceptance from the practitioner community. In fact, the forensic fingerprint community has generally eschewed, even banned, the use of probabilities to express fingerprint evidence, asserting that the inherent biological uniqueness of friction ridge skin prevented duplication of ridge arrangements. Any use of probabilities would thus allow for "some probability" of duplication. Practitioners have also noted that proposed theoretical models did not correctly or completely capture expert processes, and thus use of such statistical tools was limited or inaccurate.

Recently, the fingerprint community in particular, but also the wider forensic community, has been put under increasing pressure to reassess old paradigms. Most arguments are based on the lack of existing measurements of the validity, reliability, and real evidential value of fingerprint evidence and its examination and comparison processes.

The availability of new technology, the pressure of the scientific and legal communities, and evolution from within the profession are triggering fundamental changes. More research is being done, training programs and policies are being updated. And most recently, following a two-year project from one of the committee of the International Association for Identification (IAI), two resolutions from 1979 and 1980 banning the use of probabilistic conclusions for fingerprint were rescinded. Instead, the IAI has now opened the door to the use of probabilistic statements.

Through the use of results from three recent research projects, this paper will discuss the impact of these changes from the criminal justice system point of view and from a laboratory management point of view.

Indeed, the demonstration of the strong evidential value of fingerprint evidence, even with low number of features (Project 1), may open the flood gate in terms of number of evidence to collect from the crime scene and consider in the lab, at the risk of missing evidence (Project 2). On the other hand, training and competence of staff is a critical issue that needs to be addressed to answer current and future challenges in terms of quality of evidence, and reporting framework (Project 3).

Ultimately, the changes, rightfully requested from the fingerprint community, are now in turn impacting the legal community and are creating new demands on laboratory management. These challenges are currently faced by fingerprint examiners, their customers and managers, but will soon expand to most other evidence types.

Management Policy, Legal Impact, Fingerprint

### E40 AFIS vs. CODIS: Why Did the DNA Match When the Fingerprints Didn't?

Melissa Mourges, JD\*, and Martha Bashford, JD\*, New York County District Attorney's Office, One Hogan Place, One Hogan Place, New York, NY 10013

After attending this presentation, attendees will understand why latent fingerprints might not "hit" in the Automated Fingerprint Identification System (AFIS) even though there has been a Combined DNA Index System (CODIS) match to the same perpetrator who has left his prints at the crime scene.

This presentation will impact the forensic science community by illustrating deficiencies or "quirks" in the AFIS system that prevent latent fingerprint identifications even though the suspect's inked prints are in AFIS, and why it is important to re-search AFIS after getting a CODIS match.

Cold case investigators sometimes find that after a CODIS match, when they run the suspect's prints in AFIS they get a match to crime scene latents that didn't match before. This raises speedy trial issues: if the defendant's inked prints were in AFIS, why didn't they hit sooner? What went wrong? Why is the defendant facing charges many years after the crime when he might have or could have or should have been identified when the latents first entered the AFIS system?

Lawyers may be surprised to learn that approximately 25% of the time, latent prints fail to match inked prints in the AFIS system. Factors such as the quality of the inked prints, the fact that inked prints may be entered only once instead of after every arrest, the surface area of the latent vs. the surface area of the inked print, and whether the "minutia" of the latent print are plotted by a live fingerprint examiner or the AFIS computer will affect the likelihood of a match.

The Manhattan District Attorneys Office Forensic Sciences/Cold Case Unit will present a case study of a CODIS "cold hit" rape case where AFIS failed to initially make a match. After the cold hit, prosecutors asked the latent print examiners to take another look at latents lifted from the crime scene, and to re-submit them to AFIS. This resulted in a match to the same defendant identified through DNA. After the defense made speedy trial objections, prosecutors researched the AFIS system. A demonstration of a latent print search showed that the skill of the latent print examiner in plotting the minutia of the latent print affected the likelihood that the computer would offer the correct perpetrator in the list of candidates for a match.

Latent Fingerprints, AFIS, CODIS

#### E41 A Prosecutor-Led Interagency Cold Case DNA Project: An Alternative to "Blindlabbing" the Backlog

Ted R. Hunt, JD\*, Jackson County Courthouse, Jackson County Prosecutor's Office, 415 East 12th Street, Floor 7M, Kansas City, MO 64106

After attending this presentation, attendees will understand a new paradigm in cold case identification, review, and investigation that is unique among American jurisdictions – a prosecutor-led interagency cold case DNA project.

This presentation will impact the forensic science community by explaining and promoting a new model of cold case review that directs front-end legal and factual prosecution review of all cold case files before DNA analysis or police investigation is approved and initiated. This model firmly rejects traditional yet inefficient models of cold case review.

Traditional models often result in "blindlabbing" – testing cases solely because they are part of a crime laboratory's cold case DNA backlog, rather than based on the prosecutor's present-day assessment of the legal and factual merits of a case. Traditional models also often result in inefficient and ineffective investigative efforts by police. This stems from the fact that these models bypass front-end approval of forensic testing and investigation by the one official who has the final say over whether or not the case will ever be pursued in court – the prosecutor.

Before the Jackson County (Kansas City, Missouri) Prosecutor's Office Cold Case Unit began its work on June 1, 2009, each of the three participating agencies (the others being the Kansas City Police Department Sex Crimes Cold Case Unit and the Kansas City Police Crime Laboratory) met and agreed to a systematic methodology for the identification, review, and investigation of violent cold case crimes.

Phase 1 of the project involved cold case evidence identification. This phase was effectuated by the completion of a then-ongoing

inventory of all evidence retained by the lab in unsolved sexual assault cases. The prosecutor's office Cold Case Unit completed this exhaustive inventory. At the conclusion of the inventory, 2,551 cases had been reviewed in a single month. Based on this review, it was determined that there were 1,835 cold cases in Kansas City with evidence amenable to DNA testing between the years 1979 and 1992.

Phase 2 of the project involved cold case investigative file review. Only those files previously determined by the inventory to have associated evidence at the lab that was amenable to DNA analysis were reviewed. The prosecutor's office reviewed cases from 1979 (the first year legally permissible under Missouri's statute of limitations) forward in time. The police department conducted case review from 2006 backward in time.

Although both the prosecutor's office Cold Case Unit and the police department concurrently reviewed cases from separate years, *only* those cases approved by the prosecutor's office were tested by the lab. This model of cold case review maximizes laboratory and investigative resources because the prosecutor *and only the prosecutor* has the final word as to whether charges will be filed in any case. Prosecution "pre-approval" for DNA analysis is the equivalent of a conditional commitment that charges will be filed in the event that a database DNA match is obtained. With this case review model, scarce investigative and laboratory resources are not wasted or misdirected on cases that have not received – and may never receive – the prosecutor's approval. As a result, collective laboratory and investigative resources are much more focused and efficiently directed.

As of mid-July, 2010, the prosecutor's office Cold Case Unit had reviewed a total of 1,181 cold sex crimes cases. Of these cases, 1,003 had been rejected for DNA analysis and 146 were approved for immediate testing, with 32 cases approved for delayed testing. Thus, 85% of the backlogged cold case load at the lab was disapproved for DNA testing for either legal or factual reasons, or both.

This model of case review resulted in a substantial preservation of laboratory and investigative resources and concomitantly focused these resources on cases with a high potential for future prosecution. Time, effort, and scarce systemic resources were not wasted, and project morale was thereby enhanced. The overall focus of this project has been to coordinate interagency resources so that each participating entity is working smart rather than simply working hard.

Prosecutor, Cold Cases, DNA Backlog

## E42 Forensic DNA Policy Developments: The United States and Abroad

### Lisa Hurst, BA\*, Gordon Thomas Honeywell Governmental Affairs, 1201 Pacific Avenue, Suite 2100, Tacoma, WA 40502

After attending this presentation, attendees will have a better understanding of new developments in forensic DNA policy and how such policy decisions will impact future demands for DNA analysis at crime laboratories. The presenters will provide attendees with an overview of significant changes in U.S. policy and funding as it relates to forensic DNA programs, as well as a global perspective on the current status as well as proposed growth to DNA programs in countries throughout the world. There will also be obvious implications for how DNA is utilized in criminal investigations and in prosecutions.

The presentation will impact the forensic science community by providing a broader understanding of how responsibilities and workloads of DNA laboratories are impacted by policies discussed by legislators. A better appreciation of such developments will in turn assist the forensic community in anticipating and managing the impact of such decisions in the future

Forensic DNA programs and corresponding databases have seen tremendous growth in recent years, in both the United States and abroad. In the United States, approximately half of all states have expanded their DNA database programs to include certain arrestees, although most laws have vast differences in scope and other implementation issues such as collection point, expungement requirements, and funding. There is also continued interest in Congress and throughout the country in the status of backlogged rape kits and other rape kits that have never submitted for DNA analysis (including those kits from acquaintance rapes). Between database program expansion and potential new requirements regarding rape kit testing, actions taken by state legislatures and Congress will ultimately have a significant impact on the incoming workload at public crime laboratories. At the same time, the constitutional authority for arrestee DNA collection and sample retention is being challenged in United States and international courts, and matters such as familial searching are increasingly on the radar of policy makers throughout the United States.

Globally, the international community has also seen drastic expansion of DNA database programs as well as interest in increased regional database sharing. While most countries have initiated forensic DNA programs for analysis of crime scene evidence, a significant number of countries are only just beginning to give serious consideration to laws for DNA collection and databasing of offender and/or suspect samples. The growth of such databases will have a significant impact on the increased use of DNA in solving crimes without suspects and crimes with incorrectly identified suspects. As with the United States, there is a significant variation between countries in the extent of their DNA programs, and the existence and scope of databases. Politicians at both the national as well as state (or equivalent) levels are exerting enormous influence on how the criminal justice systems utilize DNA in forensic investigations. Additionally, sharing DNA data across borders is of growing interest for many countries, including throughout the European Union where the Prum Treaty has resulted in the production of new standards for cross-border DNA sharing.

DNA, Policy, Legislation

### E43 Understanding Asian Youth Gangs From Graffiti to Gang Enhancement Laws

Cliff Akiyama, MA, MPH\*, PCOM, Department of Forensic Medicine, 4170 City Avenue, Philadelphia, PA 19131/1694

After attending this presentation, attendees will be able to recognize and interpret various tattoos and graffiti associated with Asian gangs.

This presentation will impact the forensic science community by helping them understand, while keeping themselves safe from gangs, especially Asian gangs.

Every single day we often hear of someone who has fallen victim to gang violence. Youth gangs throughout the United States continue to terrorize the neighborhoods that they claim as their own, causing the citizens in these gang infested neighborhoods to live in constant fear of their lives every single day. As a result of the recent influx of gang violence and gang related homicides in all communities, the safety of those first responders and investigators at the scene are put in jeopardy, leaving the medical examiners/coroners, death investigators, and detectives as possible targets of intramural shootings just because they are at the scene. Throughout the United States gang violence has risen over 20% over the last year. Sadly, every single state has gangs and the problem is getting much worse in areas that would never have thought about gangs a year ago. Gangs are not just an urban problem, but a suburban and rural problem too. With the population of Asian and Pacific Islander Americans (API) continuing to rise in the United States, so do their needs. Unfortunately, not all Asian Americans are as uniformly educated, acculturated, and financially stable, as the myth of the "model minority" would have us suggest. Although adults from many nationality groups between Asian and Pacific Islanders have adapted well to life in the United States, serious problems have emerged among Asian American youth. In particular, youth gang violence in the

Asian and Pacific Islander community has dramatically increased in the last few years by nearly 35% nationwide according the U.S. Department of Justice, Office of Juvenile Justice and Delinquency Prevention. In Los Angeles County, California alone, there are currently 165 Asian youth gangs, with a total gang membership of over 6,000. In neighboring Orange County, California, gang involvement has reached an all time high with over 70 documented gangs and a membership of over 2,000. Demographics show gang member (male and female) age average of 15 with a range of 8-22 years. Even more disturbing is the increase of Asian females involved in gang activity. In Orange County, where the Asian gang population makes up 12%, there are 150 Asian female gang members, up 70% from last year. Other surrounding counties in California, Philadelphia, Pennsylvania, Fairfax County, Virginia, and Portland, Oregon have seen similar trends in the rise of Asian youth gangs.

The purpose of this paper is to present timely data on API youth gangs; offer strategies on how to recognize and interpret various tattoos and graffiti associated with these gangs, which could assist the medical examiner/coroner and death investigator in the positive identification of the decedent out in the field and/or in the autopsy room. This paper will also discuss some of the recent gang enhancement laws that California and Virginia have in place to help tackle this deadly problem.

Youth Gangs, Asian American, Gang Prevention

### E44 Impact of the Implementation of the New Adversarial Criminal System on the Prosecution and Conviction of Serial Sex Offenders in Colombia

Juanita Montes, BSc\*, Fiscalia General de la Nacion, CalleE 35 No. 4-31 PISO 5, Bogota, COLOMBIA

After attending this presentation, attendees will understand the experience of a Colombian prosecutor and the task of investigating and prosecuting serial sex offenders, as well as the obstacles posed by the recently adopted criminal system within a framework of tolerance towards sexual assault.

This presentation will have an impact on the legal community, as well as on investigators, detectives, and forensic experts involved in the process of investigating and prosecuting sexual predators. These offenders skillfully evade the action of the law and mingle with society; they are usually protected by procedural assurances that hinder their identification and prosecution. The experience obtained in the area of sex crime investigations will be described. This presentation will impact the forensic science community by sharing knowledge acquired in trying to overcome the obstacles that characterize the shift from an inquisitive criminal system to an accusatory criminal system. The following include some of the problems that must be overcome by the new system: old paradigms and differences of opinions among judges, prosecutors, victim advocates, and defense attorneys. Although these changes have occurred in the context of a culture that resists change, they have provided knowledge and created skills that will be useful for future criminal litigation.

It has been difficult to sort out situations where multiple interpretations of the new statutes have created an abundance of case law. The claims submitted by the public have had a significant impact, which ranges from taking justice into their own hands, sometimes promoting lynching of sexual predators and claiming life sentences for sex criminals. The above has changed the legal reality between one procedural situation and another or between one trial and another. This has become a challenge not only for those involved in the criminal process, but also for Colombian investigators and detectives.

Conducting investigations in an overly lenient legal framework, where automated searches for information in computer systems, such as CODIS, are restricted and subject to prior approval by a judge; where questioning a suspect or offender is still a taboo; where the arrest of the suspect must comply with requirements that hinder the effectiveness and efficiency of police activities; all have forced investigators, detectives, and prosecutors to develop skills and abilities that will be discussed in this presentation.

This study will show how a case was closed when a judge accepted the allegations of the defense attorney and granted home arrest to a serial rapist who had raped at least five victims in a time period of six months. Arguments were submitted to demonstrate that the sex crimes committed by the same person on different dates, in different areas, against different victims, and with different evidence were not connected and therefore should not be prosecuted in one trial. Therefore, the perpetrator received the benefit of one conviction.

Among the cases presented here, how the case of a sexual predator was solved will be shown. The offender accepted responsibility for one sexual assault, while he was also considered a suspect in five additional assaults. Strangely, DNA testing overruled him as a potential donor of the sperm found on the victims. This occurred when the case was to be arraigned and a few days before the plea agreement and sentencing. Was this the conviction of an innocent person or the acquittal of a culprit?

The above mentioned examples will show the dilemma between the assurance of sexual offenders' fundamental rights or the rights of their victims. Both investigations and prosecutions should become more effective and efficient in order to respond to society at large. **Sexual Predator, Investigation, Prosecute** 

E45 Child Pornography and Risk Assessment for Contact Offenses

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After attending this presentation, attendees will acquire a greater understanding of evaluations of risk assessment for defendants charged with possession or distribution of Internet child pornography.

This presentation will impact the forensic science community by increasing awareness of penalties and criminogenic factors associated with child pornography.

Child pornography refers to any visual depiction of a minor engaged in sexually explicit conduct [Title 18, USC, Section 2256(8)]. Over the past ten years, due to easy availability of child pornography and ease of trading files from the Internet, greater numbers of cases of child pornography are being prosecuted. Acquisition and trading of child pornography from the Internet involves interstate commerce; consequently, these cases are prosecuted in federal courts. Federal statutes mandate increased prison sentences when possession involves larger numbers of image files (e.g., an extra five years is added to the sentence for possession of more than 600 images) [Title 18, USC, sect.2G2.2(b)(7)(D)]. Distribution, a charge stemming from trading files, is a separate enhancement of five years [Title 18, USC, sect. 2G2.2(b)(3)(B). Ease of internet access and the use of public domain software facilitate the acquisition (possession) of child pornography and trading of files (distribution) from the internet.

The purpose of a risk assessment is to provide information to court regarding risk for future criminal activity. To conduct a risk assessment, a range of empirically based factors associated with general criminal activity is reviewed in a written report for the court. Customarily, the defense provides this information to the court for mitigation. When sentencing a defendant for possession or distribution of child pornography, a question arises concerning the risk of a future contact sexual offense by the defendant is upon release. Risk assessments in child pornography cases involve two questions: (1) future risk related to the recidivism specific to possession or distribution of child pornography; and, (2) risk related to the possibility of a contact sex offense with a minor.

Extensive research exists concerning risk of recidivism in contact sex offenses. By comparison, research of risk posed by defendants in child pornography cases regarding risk for future contact sex offenses is nominal and unreliable. Empirical findings to date are limited to correlations derived from surveys and anecdotal accounts of contact sex offenders during mandatory treatment programs while incarcerated.

The connection between child pornography and contact sex offenses may lie in both the function and use of child pornography. The widespread availability of child pornography from the Internet enables an offender to rationalize the use of child pornography. The use of child pornography for sexual arousal may be secondary to its use in grooming. An offender may test a minor's reaction upon being shown pornographic material. This provides clues about the minor's receptivity. Empirical findings have not confirmed the assumption that sexual arousal from child pornography increases risk for a contact offense.

When compared with contact sex offenders, data on defendants charged solely with possession or distribution of child pornography has shown these individuals to have fewer factors associated with increased risk of criminal recidivism in general. Factors that reduce risk of recidivism include the absence of criminal histories, absence of destabilizing mental health difficulties (e.g. substance or alcohol abuse), the presence of a stable family support system, and a history of stable employment. These factors account for the lower rates of recidivism for individuals convicted solely for possession or distribution of child pornography.

Based on the information available, risk assessment for defendants in cases of child pornography must be based on factors common to risk of general criminal behavior and risk for future contact cannot be based solely on sexual arousal from child pornography.

Risk, Recidivism, Child Pornography

#### E46 International Child Abduction Cases and Problems in the Turkish Legal System

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After attending this presentation, attendees will understand the current situation on the child abduction cases and related international problems as well as the problems in Turkish Legal system.

This presentation will impact the forensic science community by alerting attendees to child abductions internationally, the Hague Convention, and the Turkish legal system in reflection to that.

Children are affected by the divorce of their parents. They threaten each other about doing their best so that one of them cannot be able to see and have the child. One day the child finds him/herself in an environment with a different language, people, food, clothes, etc. The child asks for his/her mother or father and the answer is sharp that there will not be the mother or father in the child's life anymore. The child is confused, lonely, and afraid afterwards. Each year, this drama happens in many parts of the world. Parents use children as a weapon, more powerful than atomic bombs and turn them into tools of war. In a globalizing world, the number of children whose parents are from different countries, increase each year. International child abduction came to the forefront at the end of the 1970s, and unfortunately the economic, cultural, technological changes and developments increase the number of the abducted child. The rising rate of divorce, changing patterns of child custody, and general societal concern about the welfare of missing children also have contributed to the recognition and criminalization of parental abduction of children. Parental abduction of children is estimated to occur 350,000 times a year or more depending in part on the definition used. The Hague Convention on the Civil Aspects of International Child Abduction was enacted in 1980 and entered into enforcement for the United States on July 1, 1988. Since that time, the Convention has proven to be the most effective tool available for Left Behind Parents (LBPs) to potentially reunite with their abducted children. The Convention is an international treaty that provides a civil mechanism to bring about the prompt return of children who have been wrongfully removed from or wrongfully retained outside the country of their "habitual residence" in violation of the LBP's "rights of custody" under the law of the country of habitual residence. The United States accepted Turkey's entry to the Convention in 2000. Turkey was a noncompliant country in 2004. Although only nine cases have been submitted for return of children to the United States, the problems experienced in those cases indicate that Turkey is not fulfilling its responsibilities under the Convention. Applications for return of children to the U.S. are subject to long and repeated court delays, and courts allow consideration of issues unrelated to Convention criteria when adjudicating return applications. There have also been indications of the use of political influence over the courts and other government officials involved in case processing. Turkey has not implemented the Convention into its domestic law. In the 2004 compliance report, Turkey was cited as "noncompliant." Locating children continues to be problematic and Turkish law requires the prosecutor to locate the children before a court case can be opened in that geographic district. As part of a new criminal code passed for European Union entry that went into effect in June 2005, Turkey criminalized parental child abduction for the first time. Turkey has also consolidated abduction cases into new family courts, which are more familiar with all aspects of family law, including the Convention. During the reporting period the EU sponsored two training programs on Convention implementation for Turkish judges and prosecutors. Turkish attorneys and judges are becoming more familiar with the Convention and any efforts to expand judicial education would be encouraged. The USCA has upgraded Turkey's compliance rating from "noncompliant" to "not fully compliant." Despite the discouraging reports, there are serious developments and cases which will be discussed and presented in this study. Furthermore, it is a positive step that these cases caused positive results and the Turkish courts are dealing with the abduction cases more carefully and with a positive attitude. Some of the cases will be discussed in this presentation including the award winning International Child Abduction case by the U.S. State Department on November 2008 for the outstanding work.

Child Abduction, Hague Convention, Turkish Legal System

#### E47 Life After Melendez-Diaz: Confrontation Accomplished Through Technical Review Testimony

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After attending this presentation, attendees will understand the legal landscape that has developed in reaction to the United States Supreme Court's decision in *Melendez-Diaz vs. Massachusetts*, 557 U.S. —, 129 S.Ct. 2527 (2009). *Melendez-Diaz* held that the admission of a laboratory analyst's certificate of analysis in a drug case without the benefit of live testimony from the analyst violated the Confrontation Clause of the Sixth Amendment.

This presentation will impact the forensic science community by explaining a post-*Melendez-Diaz* avenue of admissibility for the opinions and conclusions of expert witnesses who did not physically manipulate forensic samples or operate analytical instruments, but who did conduct the technical review of the non-testifying analyst's work or who otherwise reviewed the data and materials generated from the test at issue.

In the wake of the Melendez-Diaz decision, numerous courts have addressed whether and to what extent a testifying expert witness may testify to the procedures followed, the data generated, and the results obtained by a non-testifying expert. The vast majority of courts in the post-Melendez-Diaz era have held that although the Supreme Court's decision does not permit the introduction of a testimonial certificate absent live witness testimony, the common law rule embodied in Federal Rule 703, which permits experts to testify based on "facts or data . . . reasonably relied upon by experts in the particular field in forming opinions or inferences," allows a testifying expert to state his or her independent conclusions and opinions based upon data generated by a non-testifying expert. Post-Melendez-Diaz state and federal courts have widely held that eliciting an expert's testimony through this evidentiary method does not violate the Confrontation Clause. Thus, the independent opinions and conclusions of a testifying analyst who conducts the technical review of a non-testifying colleague's work should be admissible. The path to admissibility under this rationale; however, is nuanced and legally specific.

After the *Melendez-Diaz* decision, the United States Supreme Court denied *certiorari* and let stand decisions from the Indiana Supreme Court in *Pendergrass v. State*, 913 N.E.2d 703 (2009), *cert. denied*, — S.Ct. — (2010), and the California Supreme Court in *Geier v. California*, 161 P.3d 104 (Cal. 2007), *cert. denied*, 129 S.Ct. 2856 (2009). However, in *State v. Crager*, 879 N.E.2d 745 (Ohio 2007), *cert. granted*, *judgment vacated*, *and remanded by*, 129 S.Ct. 2856 (2009), and *People v. Barba*, No. B185940, 2007 WL 4125230 (Cal. Ct. App. 2007), *cert. granted*, *judgment vacated*, *and remanded by*, 129 S.Ct. 2857 (2009), the Court granted *certiorari*, vacated the judgments, and remanded the cases for reconsideration in light of *Melendez-Diaz*.

Each of the four cases involved a Sixth Amendment challenge to the testimony of a DNA analyst who testified at trial but who did not personally manipulate the samples or operate the analytical instruments. The bench notes, worksheets, raw test data, and laboratory report in each case were generated by a non-testifying expert. Analysis of the underlying opinions from the respective state courts reveals a salient factor that may explain the Supreme Court's disparate treatment of these cases.

In the *Pendergrass* and *Geier* cases, the testifying expert relied on the non-testifying expert's report, documentation, and data as the basis for his or her opinions and conclusions. However, the laboratory reports and the underlying materials were apparently *not* admitted into evidence as exhibits. By contrast, in the *Crager* and *Barba* cases, the laboratory reports that contained the non-testifying analysts' opinions and conclusions *were* admitted into evidence as business records. Further, the *Crager* court explicitly held that scientific tests that qualified as business records were non-testimonial in nature. The *Barba* court made the same finding. These findings were in direct contravention of *Melendez-Diaz*, which held that a "certificate of analysis" was testimonial in nature. Thus, the admission of the lab report into evidence in the latter two cases seems to be the salient factor that led to vacation of the judgment and remand.

The U.S. Supreme Court's treatment of the cited cases, as well as numerous post-*Melendez-Diaz* state and federal decisions, indicates that there is life after *Melendez-Diaz* for prosecutions in which laboratory witnesses are no longer available to testify. Accordingly, it appears that a defendant's right to confrontation is not violated if: (1) the testifying expert has based his or her opinion on "facts and data" generated by a non-testifying expert that are "reasonably relied upon by experts" in that particular field; (2) it is clear that the opinion offered belongs to the testifying expert; (3) the non-testifying expert's opinion is not offered or elicited; and, (4) the underlying laboratory report is not admitted into evidence.

Expert Witness, Technical Review, Confrontation

## **ODONTOLOGY**



### F1 Disability and Dental Neglect: A Population-Based Study of 70 Patients

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After attending this presentation, attendees will learn more about child dental neglect. This abuse does not involve physical infliction of pain, but exposes nevertheless, the individuals to harm.

This presentation will impact the forensic science community demonstrating how a disability predisposes sufferers to dental neglect although preventive dentistry and appropriate diet greatly contribute to better oral health.

The American Academy of Pediatric Dentistry has defined dental neglect as the "willful failure of the parent or guardian to seek and follow through with treatment necessary to ensure a level of oral health essential for adequate function and freedom from pain and infection." Dental caries, periodontal diseases, and other oral conditions left untreated in children will have a negative effect on nutrition and facial growth. Dental neglect can be observed in the disabled in children and adults with severe physical or mental disorders where personal hygiene is the responsibility of the parents or guardians. In these individuals the severity of the disability often results in the inability of autonomous oral care. Parents and/or guardians should assist any the personal hygiene which these individuals are not able to conduct alone, including brushing teeth and appropriate diet.

To ascertain the association between patients with disabilities and dental neglect a population study of 70 patients with severe disabilities were observed. Dentists and dental hygienists, with the collaboration of nurses and psychologists, visited these individuals in a rehabilitation centre in Noicattaro (Bari) where they spend the day on educational and rehabilitation programs. The goal of the oral examination was to assess the condition of teeth and oral soft tissue. Parents and/or guardians were also interviewed in order to register and evaluate cultural and psychosocial backgrounds, together with knowledge of oral hygiene and tooth brushing regimes.

The results of the clinical observations showed an association between disability and dental neglect. There is evidence that having a disability predisposes sufferers to dental neglect although preventive dentistry and appropriate diet greatly contribute to better oral health. Cultural attitudes and poor knowledge of basic oral hygiene appear to be associated with dental neglect, but also insufficient provision of dental services specifically tailored to disabled patients' needs. Many caregivers neglect their oral health themselves, visiting a dental office only when in pain for emergency treatment. The Italian National Health System provides free dental care to patients with disabilities but few dental clinics are adequately equipped for disabled patients. Also community dentistry is given a low priority and there seems to be insufficient prevention and educational programs on these issues.

It is the opinion that child dental neglect is an underestimated phenomena and that oral health professionals should increase their knowledge of community dentistry and child maltreatment. Finally families should receive specific oral hygiene instructions tailored to those with disabilities.

Disability, Child Abuse, Dental Neglect

#### F2 Bitemarks in Domestic Violence Cases: An Epidemiologic Study in Brazil

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After attending this presentation, attendees will understand and appreciate how bitemarks can contribute to domestic violence investigation.

This presentation will impact the forensic science community by serving as a reference for those dental practitioners and other experts who may be requested to provide a testimony before the court where bitemarks are the main crime evidence.

Bitemarks may be discovered in association with crimes of violence. Such evidence, left by both perpetrator and victim, has included burglaries, domestic violence, murders, and assaults. The domestic violence includes any form of physical, sexual, or emotional abuse. Physical abuse can include slaps, kicks, scratches, and bites. In some cases, bite injuries can be the principal link between the victim and perpetrators.

The forensic dentist can assume an important role in the resolution of these crimes, especially when bitemarks are present on the victim or suspect's body. In many cases these are the main physical evidence available to investigators.

The goal of this study was to demonstrate the bitemarks prevalence in domestic violence crimes involving physical aggression in a Brazilian State.

Data was collected in the police station of the Woman's Defense in Araçatuba (São Paulo State-Brazil). Analyzing 7,550 forensic issues from the period of 2001-2005. The collection occurred over a sixmonth period.

Once selection was complete, domestic violence assaults totaled 1,856 cases. Among these, 42 cases that included bitemarks injury were selected.

Data was analyzed, the results reported, and organized in the following categories: bitten victim distribution by gender and age, bitemark distribution by location, and number of bitemarks. The results were processed and analyzed by statistical software. A difference with a *p*-value <0.05 was considered statistically significant. Percentages in the study data were rounded out.

Forty-two cases were found involving bitemarks with a total of 56 bitemarks: 33 bitten subjects were victims (31 female and 11 male) and 9 were assailants (all male).

The mean age of the bitten population was 36 years (range 15-53 years). Sixty-nine percent (n=29) of the bites occurred within the 18 to

40 year old group. The majority number of bites cases occurred in female in all age groups. There was a significant difference concerning the age between the male and female victims.

In over half the cases, the violence occurred at home. On seven incidents, the assailant was either a sister, father, mother, brother, family acquaintances, and a boyfriend, 26.1% the perpetrator were a former spouse, but in 57.1% the spouse was the assailant.

Comparing bitemark location reveals that 88.9% of the bites on the assailants were unique and was most frequently on hand/fingers, while in the violence victims 7.1% was multiple locations and 34.8% occurred in arms. Females were bitten victims in 73.8% (n=31) of cases and the bites were on the arms, face, and hand/finger in descending order of frequency. Males were most frequently bitten on hand/fingers, arms, and legs. About 80.0% of male bitemarks victims were themselves the perpetrators of the domestic violence and around 30.1% of bite injuries were the principal link between the victim and perpetrators.

Bitemarks can be found on all anatomical regions of the body, but some sites are significantly more likely to be bitten, and the frequency that an area may be bitten will vary with the crime type. Sex and age of the victim may also impact location and bite frequency.

The results shows human bitemarks can be found at almost every anatomical location, with the arm being the anatomical site most often involved, although there is clearly a trend toward certain areas in domestic violence assaults.

A bitemark could be the only piece of physical evidence linking the suspect with the victim. In addition, among forensic practitioners, the validity of whether or not there are unique characteristics present within bitemarks remains controversial. In the courtroom both sides may disagree as to whether or not a particular injury is indeed a bite mark.

Forensic Odontology, Bitemark, Domestic Violence

#### F3 Bitemark Analysis in Brazil: Four Case Reports

Jeidson A. Morais Marques, PhD\*, Feira de Santana State University (UEFS), Fanco Manoel da Silva, 437,CIdade Nova, Feira de Santana -Bahia, 44053-060, BRAZIL; Jamilly Oliveira Musse, PhD, and Luis C. Cavalcante Galvão, PhD, Feira de Santana State University (UEFS), Franco Manuel da Silva Avenue, 437, Feira de Santana, 44053060, BRAZIL; and Moacyr da Silva, PhD, University of São Paulo, Rua Lineu Prestes, 1235, Cidade Universitária, São Paulo, 05508-000, BRAZIL

After attending this presentation, attendees will understand and appreciate how bitemarks can contribute to child abuses investigation and violence cases.

This presentation will impact the forensic science community by serving as a reference for those dental practitioners and other experts who may be requested to provide a testimony before the court that bitemarks can be the main crime evidence.

Bitemarks are circular or ovoid areas of abrasion or contusion, occasionally with associated indentations. It may be composed of two U-shaped arches that are separated at their bases by an open space. Newer techniques that have enhanced bitemark identification include application of electron microscopy and computer enhancement techniques.

Bitemarks may be found at the scene of a crime and their analysis has been used for many years as an aid in forensic investigation. Bitemarks can occur on the skin of a victim or on other objects, including foods.

In Brazil, some investigative cases involving bitemarks on skin and bitten foodstuffs: apple, cheese, and chocolate bar, associated with crime scenes, were used as important evidence factors which resulted in convictions. Given this information, along with other relevant evidence, the judge or jury is likely to find that the perpetrator of the bite also committed the rape, murder, or other criminal act. The goal of this presentation is to report four bitemarks cases in Brazil.

**Case 1:** A seven month-old Brazilian female infant was admitted to the Medical Center Pediatric Intensive Care Unit with multiple injuries. A pattern of suspected abuse was established and Child Protective Services was notified. After clinical examination, numerous ovoid and circular pattern injuries were observed by the physician who recognized them as human bitemarks. She immediately notified the forensic odontology department to request a forensic bitemark examination and photographic documentation of the injuries. Among five suspects, the mother was determined to be the individual responsible for the injury and during the trial, she confessed the crime.

**Case 2:** The "Maniac in the Park" was a famous serial killer case in Brazil. Young female bodies were found on a forest reserve on the out-skirts of São Paulo. The suspect in the case was a 31-year-old motorcycle courier who confessed to raping and killing nine women. The bitemarks on the victim contributed to his conviction.

**Case 3:** A domestic assault where the victim was a female. A bitemark on the chest was the main evidence that linked the unique suspect with murder.

**Case 4:** During a fight between two women, the aggressor bit the ears of the victim. In one, there was partial removal of the ear. The suspect denied having caused the bites, but after the assessment of the bitemarks, was charged with injury.

The Brazilian experience showed that early recognition of bitemark evidence can play an important role in criminal investigation. Bitemarks cases have increasingly occurred in Brazilian territory and several cases have been successfully solved. Some scientific research has been undertaken using simulated bitemarks in foods and pig skin. Bitemark evidence has been increasingly accepted by trial courts in Brazil.

Forensic Odontology, Bitemark, Skin

#### F4 Dosimetric Testing of a Hand-Held Dental Radiation Source: Implications for Correct and Practical Use in a Forensic Setting

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After attending this presentation, attendees will be aware of the dosimetric testing of a hand-held dental x-ray device in both its use in a clinical setting and on skeletal remains. Proper radiation hygiene relative to the use of these devices will be reviewed so that the attendee will be able to safely use these devices in a forensic setting such as a mortuary and in the field in multiple fatality incident situations.

It has been shown that hand-held dental radiation generators emit very little radiation to the operators when used as directed by the manufacturers; however, the pattern of radiation emission when being used in a traditional forensic setting where multiple adjacent persons might be working in close quarters has yet to be plumbed. This dosimetric study will impact the forensic science community by showing that while the device is perfectly safe when used as directed, the safety of this machine does not obviate the need for prudent simple radiation hygiene measures.

A dosimetric verification human phantom with head with the oral cavity prepared to hold dental image receptors was used to provide a realistic test object for the measurement of scattered x-radiation in-vitro. An image receptor was placed in the radiation phantom's oral cavity and the collimator was aligned in the standard fashion so that the central ray was perpendicular to the plane of the film. All measurements were performed at 60kV,2.5mA and 1 second exposure (390mR) to provide a high enough primary exposure such that the accuracy of measurement of any scatter radiation could be maximized. Operator exposure could then

be scaled by dividing the exposure time by a suitable factor for various film or digital image receptors. The phantom was placed in a dental chair in a dental operatory that had lead-lined walls. An 1800cc air chamber and a 2026 C control unit in "integrate" mode with recent calibration was used to measure exposure. The ion chamber was placed at various locations relative to the x-ray generator. Extremely low occupational exposures (7,000 images per year using F speed film) were observed for the hand holding the x-ray generator 0.06mSv; a hand placed on top of the phantom's head 0.8 mSv; the operator's eye 0.02mSv; and the operator's groin 0.03mSv. Higher measurements were observed to the operator's groin if no lead apron was used on the phantom 45mSv; and for personnel standing opposite the primary beam behind the patient 26mSv; or at ninety degrees relative to the primary beam 0.4mSv. It is therefore recommended that an operator use the device while standing in its protective cone and not use the device with an assistant that cannot be positioned behind them within this protective cone. The device, like most x-radiation devices is safe when used according to manufacturers instructions. It is important that in a multiple fatality situation or in forensic case work that both the operator and any assistants obey simple rules of radiation hygiene as well as manufacturer's instructions.

Dental Radiology, Dosimetry, Safety

#### F5 Dimples, Pimples, and Operator Error: Impact on Dental Identification

Diane Penola, MA\*, 54 Fayson Lakes Road, Kinnelon, NJ 07405

After attending this presentation, the attendees will become familiar with the orientation of dental radiographs as a function of the victim identification process.

This presentation will impact the forensic science community by alerting ID team members to the possibility of film reversals in antemortem records.

Mass disaster victim identification, as well as individual cases, require accurate and complete antemortem records. Dental radiographs have proven to be of significant value in this task. Orientation of the film for left and right sides of the oral cavity is crucial to successful interpretation of the deceased victim's dental records.

While many practices have made the conversion to digital sensors in their offices, there are many that continue to use film. In addition, it seems reasonable that for some time into the future the ID team will be receiving ante mortem film even if they are using digital technology postmortem. Many times the antemortem data received represents dental examination and/or treatment that took place some time in the past; anywhere from several weeks to several years. Even as new technology becomes more prevalent in general and specialty practices, the dental identification team will continue to see the results of traditional methods.

Traditional intra-oral dental film has a small dot in one corner that helps orient the film. Most dental offices read intra-oral radiographs with the raised dot toward the viewer. This makes the teeth on the viewer's right actually the left side of the patient's mouth. When mounting newly exposed radiographs it is important to pay careful attention to the direction of the dot. The literature also calls this feature a raised bump, an occlusal dot, or a button. Conversely, this same feature on the opposite side of the film may be referred to as a depression or concavity.

Operator error in the placement of the dental film will result in a confusing situation with the dot appearing backwards. Fortunately, a telltale pattern in the lead foil of the film packet will show up in the processed image. Visualization of the pattern is the clue that the film needs to be reversed.

The components and packaging of the intra-oral dental film are such that their details are significant. The emulsion coated film is of

primary importance. The film is protected by a wraparound sheet of black paper that folds over the film and a piece of foil. The outer most protection is the light and moisture proof plastic envelope, which is actually two layers (light and dark) fused together. The periphery of the plastic packet is hermetically sealed. Frequently it is this careful seal that is responsible for patient complaint and discomfort.

Inserted between the black paper and the outer packet is a single sheet of lead foil. The foil is the same size as the film sheet and protects the film from secondary radiation, assuring a higher quality radiograph. It is this foil that has become the focus of this investigation.

In preparation for this presentation the literature was reviewed and professionals were consulted for historical information about the composition and packaging of dental radiographic film. It was discovered that changes were made many years ago that have never received much publicity.

In addressing the dental professionals in the audience, they will be asked to recall the early days of their careers when the exposure of dental radiographs was new. The "herringbone" pattern on dental films was an indicator of a mistake. This presentation will explore the misconceptions of operator error and expose possible ante mortem ambiguities.

Dental, Radiographs, Antemortem

#### F6 The Dental Identification of a U.S. WWI Service Member

Calvin Y. Shiroma, DMD\*, Joint POW/MIA Accounting Command (JPAC), , 310 Worchester Avenue, Building 45, Hickam AFB, HI 96853

This presentation will provide attendees the opportunity to view a case involving the identification of a U.S. service member who was lost in France during WWI. Upon the completion of the presentation, the audience will be able to appreciate the effort made by the U.S. Government and their quest to account for service members missing as a result of our nation's past conflicts, witness the dental care that was performed in the early 1900s, and to learn from the thought process that progressed through the case.

This presentation will impact the forensic science and dental communities by providing an example of a positive identification of a previously unidentified U.S. service member who was lost over 90 years ago and how the obstacles that were presented while working on this case were overcome. The knowledge gained was very basic, but was essential to support his opinion while writing the forensic odontology report. Multiple hurdles which had to be met included: obtaining personnel/dental records, properly interpreting these records, and researching/reviewing standard dental care of the early 1900's.

The mission of the Joint POW/MIA Accounting Command (JPAC) is to achieve the fullest possible accounting of all Americans missing as a result of the nation's past conflicts. The majority of the forensic cases at the JPAC Central Identification Laboratory (CIL) involve losses during the Vietnam War, Korean War, and World War II. Thus, the opportunity to analyze remains from a WWI casualty is rare.

In September 1918, the Expeditionary Forces led by General John J. Pershing was engaged in the first U.S. led offensive of WWI. Of the 7,000 allied lives lost, 2,000 were American service members, with 11 U.S. Marines being listed as unaccounted for. In September 2009, French Nationals (relic hunters) reported they located a burial site containing the remains and artifacts believed to be those of an American service member. The men notified the proper French government authorities which lead to the contact with officials from JPAC. In October 2009, a JPAC team recovered the remains and material evidence. The remains were accessioned at the Central Identification Laboratory on October 26, 2009. The recovered dental remains consist of an attached maxilla and near complete mandible. Restorative, oral surgery (extractions), and endodontic procedures were all evident in the

remains. Multiple teeth are restored with various dental materials (amalgam, porcelain, screw posts, and gold restorations). The dental remains were compared to the available antemortem dental information resulting in the following concordances: 15 unrestored teeth, 8 restored teeth, and three missing teeth. All lines of evidence (historical, anthropology, material evidence, and dental) corresponded to the associated casualty and circumstances of his loss. On April 1, 2009, the recovered remains of the WWI service member were positively identified at the JPAC CIL. On June 23, 2010, the remains were buried at Arlington National Cemetery with full military honors.

WWI, Dental, Identification

#### F7 The Development of A Protocol – New York City OCME's Large Scale Initiative to Upload "Cold Cases" to National Databases

Richard M. Weledniger, DDS\*, 931 Walt Whitman Road, Melville, NY 11747-2297; Roy H. Sonkin, DDS, 45 Eagle Chase, Woodbury, NY 11797; Lawrence A. Dobrin, DMD, OCME-New York City, 471 East Westfield Avenue, Roselle Park, NJ 07204; and Kenneth W. Aschheim, DDS, 44 East 67th Street, New York, NY 10065

After attending this presentation, attendees will understand the requirements as well as the difficulties with implementing a large scale data and image entry system to transfer Office of the Chief Medical Examiner (OCME) archival data to National databases such as NCIC and NAMUS.

This presentation will impact the forensic science community by demonstrating the importance of strict data entry requirements to assure reliable results. In addition, this presentation will impact the forensic community by helping to provide closure for friends and families of unidentified persons and as an aid for forensic odontologists to participate in the nationwide effort to locate and identify unidentified persons and bodies.

The goal of this presentation is to give an overview of the efforts being made by the New York City's Office of Chief Medical Examiner in the development of procedures to transfer archival "cold case" data of unidentified postmortems to National databases. Through a National Institute of Justice (NIJ) grant in conjunction with the NYC OCME Forensic Odontology, Anthropology, and Biology Units, a pilot program was established to aid in this effort. The goal of this large scale project is to enter complete forensic data and images into the Unified Victim Identification System (UVIS) Dental Identification Module (UDIM). This consortium will optimize future National Crime Information Center (NCIC) and National Missing and Unidentified Persons Systems (NamUs) database analysis.

The grant allowed the NYCE OCME Anthropology team to initiate procedures to analyze 448 unidentified persons from 1998 through 2009 and enter information and data into both NCIC and NamUs databases. After confirming that the remains were human, data files were examined. Any incomplete data required exhuming the body and the necessary data was reentered. The files were then transferred to the Odontology Unit for review of the previously coded dental information.

The Odontology Unit reviewed the data files for dental charting accuracy as well as the accompanying radiographic information. In addition this project was used as an "in-service" that would allow for training of the team on the UVIS/UDIM module. Coding was converted to the UDIM format which uses different modifiers than the traditional WinID codes.

All too often, inaccuracies in interpretation of radiographic data can lead to ambiguities in dental coding. During the presentation some of these ambiguous situations will be enumerated. The protocol that was put in place will be discussed as well as the formation of "coding committee" rules that were set up to insure that interpretation occurred in a consistent fashion. It is hoped that these rules will be incorporated into future versions of forensic odontology comparison software. This review process validates the premise that a second set of eyes is an asset in coding as it minimizes the possibility of misinterpretations or missing key evidence that is vital to making matches.

Since all the radiographs accompanying these cases were analog (film) format, the need to set up a digitizing protocol was also necessary. The radiographs were scrutinized for their mounting and orientation. Ambiguous or erroneous mountings were corrected. Once corrected, radiographs were scanned utilizing imaging processing software. The formatted images were then imported into the UDIM database to be integrated with the reviewed dental charting. Images were exported as jpegs as both a Full Mouth Series (FMS) and individual images as well, to help in the facilitation of export into NCIC and NamUs.

The next step in the process was to translate the data to NCIC/NamUs compatible codes. It is anticipated that a data export program to convert the data into an acceptable file structure for direct importation to NCIC database would be developed for this project. However, this module will have to be included in a future version of the software. Currently, this process is performed manually.

Additionally, protocols are being developed to upload this information into the National Dental Image Repository (NDIR). The NDIR was established in May, 2005 by the FBI's Criminal Justice Information System (CJIS) to help facilitate the identification of Missing, Unidentified, and Wanted persons. The NDIR permits law enforcement agencies to store, access and supplement dental records which are currently housed in the Missing, Unidentified, and Wanted Persons files in the National Crime Information Center (NCIC) system.

This presentation will conclude with several cases demonstrating examples of the diligence required when documenting and recording information to be used in the identification process.

Forensic Odontology, NAMUS, Unidentified Persons

### F8 ANSI/ADA Specification No. 1058: The Forensic Dental Data Set –What Does It Mean to the Forensic Dentist?

#### Kenneth W. Aschheim, DDS\*, 44 East 67th Street, New York, NY 10065

The goal of this presentation is to familiarize the attendee with proposed American National Standards Institute/American Dental Association Specification No. 1058 Forensic Dental Data Set.

This presentation will impact the forensic science community by demonstrating the importance of consistent means of communicating dental information, not least in electronic format. The use of partnership working between forensic and regulatory/professional groups is also described.

The United States government has set a target date of 2015 to complete the National Health Information Infrastructure Initiative which would mandate the use of Electronic Health Care records in both medicine and dentistry. To prepare for this initiative the American Dental Association in June 2007 appointed the Electronic Health Record Workgroup and the SNODENT (Systemized Nomenclature of Dentistry) Editorial Panel to help formulate the dental component of this standard. A working group of the ADA Standards Committee on Dental Informatics (SCDI) was set up to develop the standard. Its mandate was to create the framework necessary for dentists to communicate information in electronic patient records to all health care providers. This standard became known as the American National Standards Institute/ADA Specification No. 1000 Standard Clinical Data Architecture for the Structure and Content of an Electronic Health Record.

As part of this initiative, a Joint Working Group was establish to address the needs of the forensics odontology community. This group

became known as Joint Working Group 10.12 Forensic Odontology Informatics Subcommittee and was given a mandate to "create a technical standard concerning the collection and electronic transference of dental forensic information." Its role was to ensure that that forensic dental data would be included in the standard.

It is vital that forensic odontologists are familiar with the proposed standard whose final draft was approved by the ADA Council on Dental Practice in June 2010 and was sent for balloting by to the SCDI for final approval. This presentation will impact the forensic science community by furthering the forensic odontologist's understanding of how the standard will impact the field as the United States moves toward the 2015 implementation of the Electronic Health Record.

In 2005, the ADA established the NHII Task Force to establish the role of the ADA in developing access, content, standards, and code vocabularies for dentistry in the electronic health record. As part of this initiative the ADA House of Delegates, in 2006, mandated the reviewing and updating of the Systematized Nomenclature of Dentistry (SNODENT), the vocabulary of electronic health and dental records.

The ADA SNODENT Editorial Panel mandate was to update the clinical terminology of SNODENT and to be certain that it is interoperable with the rest of the electronic health record. Numerous code sets were identified as being an essential part of an electronic dental record. Because the establishment of a positive identification requires submission of supporting documentation from both the antemortem treating dentist as well as the forensic odontologist, it was decided by the ADA to include a forensic odontology data set as part of the standard. It was anticipated, that at some point in the future, this information (e.g., radiographs, charts, and progress notes) would be electronically submitted directly or through a clearinghouse. The goal was to create a standardized electronic format to transfer this information. Not only would the application of information technology standards and electronic transactions reduce the time required for data transfer and the costs associated with it, but it would also reduce errors and confusion regarding what data needed to be transferred.

Current forensic odontology comparison software is based on the concept of computer comparison and ranking with final determination made by the forensic odontologist. Utilizing numerous dental descriptors, comparison and/or elimination queries, and advance sorting algorithms creates a ranking of possible matches. A final identification is based on the evaluation of similarities and differences of the individual based on these descriptors. Supporting biometric and familial radiographs, and visual information, support the likelihood of a match. Standardizing the descriptors used to code this information increases the likelihood of identifying human remains, as well as, reducing errors that come with ambiguous descriptions of conditions.

The SCDI Joint Working Group 10.12 on Forensic Odontology Informatics was formed in October, 2006, and first met in San Francisco in October, 2007. Representatives from all major Forensic Odontology organizations and numerous government and private agencies were represented. Six working groups were created with the ultimate goal of creating a uniform nomenclature for the description of forensic dental data and to define a standardized set of terms to convey this information. In addition, the standard created the ground work for the standardized electronic transmission of this information to all compatible software. From the onset, the goal of the Standard was not to define the extent of information collected, but to be certain that that there is no ambiguity in the meaning of common terms used to aid in human identification. This presentation will cover the ADA standard and familiarize the attendees as to how it will be implemented.

Acknowledgement: This specification was a joint effort of all of the members of the SCDI Joint Working Group 10.12 on Forensic Odontology Informatics.

ANSI/ADA Specification No. 1058, Forensic Dental Data Set, SCD

### F9 Computerized Dental Ranking: A Look at a New Coding Strategy and an Optimized Ranking Algorithm

Bradley J. Adams, PhD\*, New York Office of the Chief Medical Examiner, 520 1st Avenue, New York, NY 10016; and Kenneth W. Aschheim, DDS, 44 East 67th Street, New York, NY 10065

The objective of this presentation is to present a new dental coding system and sorting algorithm that can be used with computerized dental ranking software. Currently, software systems are based on detailed coding strategies. Although previous studies have tested these systems utilizing a simpler coding scheme, some degradation was noted especially on larger databases. This study addresses some of those shortcomings by adding a few additional novel codes and by utilizing optimized ranking algorithms. A large reference database was used to accomplish this research. The benefits and shortcomings of this new system will be discussed.

Computerized dental ranks are a powerful tool to assist in the victim identification process. This study will impact the forensic science community by presenting an objective evaluation of a new coding format and optimized ranking algorithm. This evidence-based research will impact the field of forensic odontology by providing solid recommendations on coding and algorithms for use with computerized dental ranking.

For many mass fatality incidents, dental comparison serves as a primary means of victim identification. In order to expedite the comparison of antemortem and postmortem records, computer software is often used to provide a rank of possible matches. In the United States, WinID3 is commonly employed for this purpose due to its proven utility and acceptance in the field. These computer generated ranks provide forensic odontologists with an objective "best-match" tool from which to undertake a more in-depth review of the dental records. It goes without saying that the computer ranks simply provide a starting point for the comparison of dental records. The final evaluation and victim identification should be performed by an experienced forensic odontologist.

Within the field of forensic odontology there is not one universally agreed upon coding system that is used for documenting tooth conditions. The goal of this research is to compare two different coding options and two ranking algorithms. WinID3 codes and ranking algorithms (most hits and least mismatches) were compared with a simplified coding system and concurrent ranking algorithm being tested at New Office of Chief Medical Examiner (OCME).

Intuitively it makes sense that "more is better" with coding since more detailed codes imply a greater level of precision in documenting the dental status and potentially, greater accuracy in the resulting ranks. In addition, more detail in the coding offers the ability to perform "focused" searches (e.g., find all records with a root canal and crown on tooth #30 and #31 but a post only in #30). However, the potential pitfalls of detailed coding may include data entry errors, lack of compliance/understanding, and a slow/tedious charting process. The important consideration for computerized ranking is to utilize a coding format that provides the best results with the least amount of effort.

With WinID there are 36 possible primary codes that are used with the ranking algorithms (Table 1). Most of these codes pertain to the various combinations of surface restorations. There are also 12 secondary codes that can be used along with the primary codes to describe dental status. With the simplified coding system there are only 7 primary codes and no secondary codes (Table 1). The goal with the simplified codes was to develop a system that was easy to interpret, lacked ambiguity, was expedient to code, and would provide some degree of "focused" search capability.

Table 1. Different Coding For	e 1. Different Coding Formats		
WinID Primary Code	OCME Simplified Code	Description	
V	V	Virgin (tooth present with no restorative treatment)	
U	V	Unerupted	
M, O, D, F, L (31 possible conhinations)	F	Filled (Tooth present with "nontine" filling(s)).	
Crowns and Root Canals are not part of the WinID primary codes	s	Special Treatment (Tooth present with "special" treatmentsuch as a Root Canal and/or Crown A filling with a root canal would receive only the "S" code.)	
x	x	Missang Antemotem (This code is used regardless of whether or not the location has been replaced with a denture or bridge.)	
x	1	Implant (Entire tooth is missing antemortem, but has been replaced with an implant inserted into the bone.)	
J	P	Tooth is/was present, but is not fully observable as to the extent of breatment (e.g., postnortem fracture of crown, empty sockst, externe coronal decay, not fully observable in x-ary).	
1	N	Absolutely no information (e.g., portion of mandible missing, or no antemortem information available).	

The role of computer ranking programs is to compare antemortem and postmortem records and quantify the number of matches, mismatches, and possible matches between records. The computer then ranks the various records based on a sorting algorithm (e.g., most matches followed by least mismatches). Matches occur when the code is identical in both the antemortem and postmortem record. Mismatches and possible matches occur due to charting discrepancies which can come in two types: explainable and unexplainable. Explainable discrepancies could be the result a logical progression in dental status (e.g., an occlusal filling (O) could progress to a mesial-occlusal-distal filling (MOD)). Unexplainable discrepancies are physical impossibilities in the progression of dental status (e.g., an extracted tooth (X) cannot become a virgin tooth (V)). Explainable discrepancies would result in a "possible" match in the computer ranking program, while unexplainable discrepancies would result in a "mismatch" in the computer ranking program. Due to charting errors and/or outdated records, both explainable and unexplainable discrepancies are commonly encountered in records pertaining to the same individual.

For this study, a large sample of adult dental data was compiled from numerous National Health and Nutrition Examination Studies (NHANES). The available data consist of approximately 33,000 records. These databases allowed for systematic changes to be made order to test the different coding and ranking methodologies. In order to explore the effect of various levels of coding discrepancies, the NHANES data were systematically modified to reflect various rates of coding discrepancies (Table 2).

Explainable Discrepancies	Unexplainable Discrepancies	Combination (exp / unexp )
Nocharges	No changes	No changes
2 tee fh per record	2 teeth per record	1 exp. /1 unexp.
4 tee fn per record	4 teeth per record	2 exp. /2 unexp.
6 tee fn per record	6 teeth per record	3 exp. /3 unexp.

The data were analyzed using the WinID3 coding format (Table 1) and the WinID3 ranking algorithms for Most Dental Hits and Least Dental Mismatches. Since it is possible for there to be ranking ties (e.g., several records all ranked #1), correct ranks were recorded as the number of records "tied with or better than" the correct match. For example, if 27 records were all ranked #1 and the correct match fell somewhere in this group of 27, the "tied with or better than" rank would be 27.

The same data were then converted into OCME's simplified coding format (Table 1) and were run through an optimized set of ranking algorithms. It should be noted that some coding discrepancies will be "self-corrected" when the detailed codes are converted to simpler codes. For example, the simplified format just has one code for a restored tooth irrespective of the surface location, so any discrepancy based on restored tooth surfaces would be "self-corrected" during the conversion. These results will help forensic odontologists make sound decisions on the coding detail needed for computerized ranking, as well as the optimal sorting algorithms.

Dental Ranking, Dental Coding, Computer Algorithms

#### F10 Haiti Earthquake 2010: DMORT Response

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After attending this presentation, attendees will have an understanding of the mass fatality incident response to identify American citizens who died in the aftermath of the 2010 Haiti earthquake.

This presentation will impact the forensic community by reviewing the challenges faced in the first international DMORT mission to identify American citizens in a joint effort between the U.S. Department of State, Department of Defense, and Department of Health and Human Services.

On January 12, 2010 a powerful earthquake measured at 7.0 on the Richter scale hit Port-Au-Prince, the capitol city of Haiti. According to estimates, over 222,570 people were killed, 300,000 were injured, and 1.3 million people were displaced. Estimates also stated that 97,294 houses were destroyed and 188,383 houses sustained damages. Both the physical and political infrastructure was seriously impaired.

An estimated 45,000 American Citizens were in Haiti at the time of the earthquake. Those included dual-national citizens, aid workers, multinational corporations' employees, embassy staff, and military personnel.

The U.S. Embassy in Port-Au-Prince served as the central hub for the accounting of American Citizens. During multiple press briefings over the course of the next several weeks, the United States Department of State reported evacuation and recovery efforts to the media. International medical and food aid efforts were hampered by the damage to both the seaport and the airport.

Family members in the United States grew increasingly concerned regarding the health and welfare of their loved ones in Haiti as the fatality numbers grew. Under the direction of the U.S. Department of State, in communication with the government of Haiti, a joint mission was established to recover, identify, and repatriate remains of U.S. citizens. Organized under the U.S. Department of Health and Human Service, within the National Disaster Medical Services, the first international mission of the Disaster Mortuary Operational Response teams was undertaken.

A *Family Assistance Center* opened in Miami, Florida to take the reports of those concerned family members within the United States. Those reports, sent to Haiti, would begin an *Investigative Element* that potentially would lead to a *Recovery Element*. Recovered remains would be processed by the *Morgue Operations*. Approximately 4,100 reports were generated, but 90% were unfounded by the ensuing investigations. Operations were combined between DMORT members and soldiers in the U.S. Army 11<sup>th</sup> Quartermaster Company (Mortuary Affairs), 49<sup>th</sup> Quartermaster Group out of Fort Lee, Virginia. Elements of the U.S. Army 82<sup>nd</sup> Airborne Division provided security during recovery operations.

The most significant site of recovery efforts, due to the concentration of American Citizens' living quarters, focused on the Hotel Montana. The five-story 145-room, terraced hotel perched atop a mountain, collapsed in a pancake fashion. Excavation was painstakingly slow.

Deployed DMORT members, staged first in Atlanta, received medical clearances as well as briefings regarding the risks associated with the Haiti Earthquake mission. Diseases, uncommon in the United States but endemic in Haiti, included malaria, dengue fever, tuberculosis, anthrax, HIV, hepatitis B, lymphatic filariasis, and typhoid fever. Essentially, the warning was "if you have a medical emergency, you will die" due to the austere conditions.

The morgue compound was juxtaposition to the Port-Au-Prince airport, consisting of numerous tents with generators providing electricity. The morgue consisted of two tents in an "L" formation. The admitting tent had stations for data input, personal effects processing, photography, and radiology. The main tent had stations for pathology, anthropology, odontology, DNA sampling, and fingerprinting. The morgue operations processed 121 sets of human remains, identifying 119 individuals. About 50% were returned to the United States and 50% were interred in Haiti. Estimation was made that no more than 400 U.S. citizens were left in mass graves. The government of Haiti would not allow any recovery from mass gravesites.

The Demobilization Rotation had the responsibility of taking down the Morgue Compound. Inventorying, disassembly, cleaning, and packing of morgue components prior to inspection by U.S. Customs was required. Equipment to be sent back to the United States was loaded into containers for transport via ships. Some equipment, such as tents, was donated to USAID. Partial pallets of disposable supplies such as hand sanitizer were donated to the Miami University field hospital.

Haiti Earthquake, Mass Fatalities, DMORT

#### F11 A Retrospective of Forensic Cases: Identifications Aided by Medical Diagnostic Imaging

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The goal of this presentation is to provide the attendee with an alternative option to assist in the process of identification when dental charts and radiographs are not available. Forensic cases will be presented that will exhibit how varied forms of antemortem evidence were needed to assist in several different investigations.

This presentation will impact the forensic science community by providing alternate forms of antemortem data which may be useful to assist in a positive dental identification when antemortem dental radiographs may not be available.

The forensic odontologist, when called upon to assist in the identification of unidentified remains, is tasked to document any remaining dentition and surrounding oral structures. This would include a visual oral examination, charting of the dentition, dental radiographs, and photography of the case. Often this may require resection of the jaws to allow these tasks to be adequately performed. Obtaining this postmortem information is only the first step. Having adequate antemortem information then allows for comparisons to be made, which may result in an exclusion, inclusion, or positive dental identification. Forensic odontologists rely on the medical examiners or coroners and their investigators to provide this important antemortem information. There are situations where it is not possible to obtain antemortem dental information. Whatever antemortem records available may then have to be utilized to assist in this endeavor.

This presentation will exhibit several cases where this has occurred and successfully assisted in a positive identification.

Two case presentations involve missing persons cases. The first case is of a missing girl with a bite on the hand of the suspect. The detective was told that the teeth of the missing girl were needed to make a comparison. The suspect apparently realized this after his hand was photographed. When the girl was finally found, no teeth were present in the jaw. The dental identification was made by a unique maxillary sinus combined with a curved root socket.

The next case is that of a young woman who disappeared with her baby daughter in January of 2003. Her car was found in the middle of the night, abandoned on a bridge. In April of that same year, remains were washed ashore near the area of the bridge. Antemortem dental records of the missing woman were not available. However, antemortem CT and MRI scans of the brain, taken years earlier, were provided and used to assist in a dental identification. This example was assisted by the unique features of her upper arch when compared to a cross section of the maxilla on the CT and MRI scans.

The remaining cases are related to a discovery of multiple bodies in a "House of Death." The victims of a serial killer were discovered piled upon each other as well as strewn within debris in a residence that the killer was forced to vacate due to a horrendous odor emanating from the apartment. Many of these identifications were made using hospital medical radiographs, as the victims were "women of the street," and had often been beaten and abused and taken to a hospital for treatment.

These cases will present practical examples of employing alternative antemortem resources to assist in a dental identification. **Dental Identification, Antemortem Records, Medical Radiographs** 

### F12 Can We Handle It? Creating a Reference Database to Test the Limits of Current Forensic Software?

#### Kenneth W. Aschheim, DDS\*, 44 East 67th Street, New York, NY 10065

The goal of this presentation is to familiarize the attendee with the methodology utilized in the creation of a reference database for use in dental research on victim identification.

As part of the mass disaster identification team, the forensic odontologist has become a key player in the identification process. However, recent mega disasters such the Asian tsunami of 2005, and the Haitian earthquake of 2010, has shown that the odontologist's role is often limited by the scarcity of dental records and the extremely large size of the victim pool. Although disasters such as Hurricane Katrina and the World Trade Center Attacks have shown that the current modalities work adequately for a victim population in the low thousands, a key unanswered question is what would happen if the number of victims that needed to be identified dentally were 10 or even 100 times the number of victims seen previously? How is this affected by introducing body fragmentation?

In order to test these questions, a reference database needs to be created to objectively evaluate the limits of current forensic odontological software. This presentation will impact the forensic science community by describing the methodology involved in creating a reference database of dental data based on real world data. In addition, by characterizing previous dental disaster data and carefully controlling characteristics of the antemortem to postmortem changes in the reference databases the ability to improve matching algorithms could be explored.

Part of New York City's goal for disaster preparedness includes the ability to handle thousands and possibly tens of thousands of victims. Although current modalities have served well in the past, questions still arise as to what would happen if an even greater magnitude mass fatality incident were to occur. In addition, questions arise outside of the mass disaster realm as to how effective current dental matching algorithms are with large national databases of missing/unidentified individuals.

One of the dictates of evidence-based decisions is the ability to test these questions in a controlled, objective manner. Therefore, a realistic reference database needed to be created that would allow for benchmarking of current forensic odontology systems. Currently, New York City dental forensic reference databases, which are used for testing, contain up to 3000 entries. The goal of this project was to create a database of at least 10 times that size based on real world data and controlled manipulation of key parameters.

For the purposes of this project, a large sample of dental data was compiled from numerous National Health and Nutrition Examination Studies (NHANES) as well as the 1994 and 2000 Tri-Service Comprehensive Oral Health Survey (TSCOHS) military data. The NHANES data that was utilized for this study consists of approximately 33,000 records of adults aged 17 and above, in four databases of the U.S. population, compiled between the years of 1988-2004. The TSCOHS used for this study was compiled as part of a congressional directive to evaluate the dental care system. It consisted of standardized protocols developed by dental epidemiologists to assess the oral health of over 20,000 Army, Navy, and Air Force personnel. The NHANES and TSCOHS data provide over 50,000 records of dental data. The two databases are a useful source of "real world" data; however, since it gives only a single "snapshot" of tooth condition it could only serve as a starting point for database creation. The first step in the conversion process was to analyze the NHANES coding system. Unfortunately, the type of dental data collected and the coding methodology used changed over time. It was then necessary to find the "least common denominator" for the data to ensure that a single database could be created. Methodology was developed to create statistically valid dental data when the NHANES coding system lacked the information. Finally, conversion algorithms were created to translate NHANES coding to a more universally accepted coding system.

In order to create a transition from antemortem to postmortem, different methodologies were explored. A look-up table was created to define all possible "explainable" and "unexplainable" discrepancies based on the software's codes. Software was then created that could alter the data in order to create random control changes to allow for this transition. For example, if the software was set to place "1 explainable discrepancy per record" the selected antemortem codes for "M" would randomly be changed to either "MO," "MOD," "MODFL," "X," etc. on the postmortem side. If the software was set for a "1 unexplainable discrepancy per record" the antemortem code for "M" code would randomly be changed to either a "OD," "V," "FL" etc. on the postmortem side.

The result of this project has been to build software that creates an infinite number of scenarios to mimic mass fatality incidents of various parameters. The creation of a very large reference database that can be manipulated in a controlled manner is a vital first step in the testing of forensic odontology software and our ability to utilize evidence based techniques. In addition, an added benefit of the software was its' ability to quantify the degree of explainable and non-explainable discrepancies that occur in "real world data," which will also be presented. Finally, having a reference data set is a vital first step in testing the performance of new coding formats and ranking algorithms in order to advance the field.

Multiple Fatality Incidents, Reference Database, Forensic Odontology Software

## F13 Reliability in Dental Coding: Strengthening the Chance of Victim Identification

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After attending this presentation, attendees will have a greater appreciation of the necessity for accuracy and quality control in the recording of dental codes for unidentified and missing persons as recommended in the 2009 National Academy of Sciences (NAS) Report. This presentation will impact the forensic science community by serving as a reminder to those involved in unidentified and missing persons cases that review and interpretation by qualified individuals, and the accurate recording of the scientific evidence are key to increasing the possibility of a victim identification.

The New Jersey State Police Forensic Anthropology Laboratory Dental Initiative is an on-going initiative to centralize and digitize all New Jersey unidentified and missing person's dental records. A scientific identification is paramount in law enforcement investigations. One of the most reliable and scientific means of positive identification of the unidentified or missing person is that of dental comparison. Dental information is recorded by means of dental codes in national databases, such as the National Crime Information Center (NCIC) and the National Missing and Unidentified Persons System (NamUs). Recommendation #8 of the NAS Report, states that "Forensic laboratories should establish routine quality assurance and quality control procedures to ensure the accuracy of forensic analyses and the work of forensic practitioners." The initiative objectives are to: (1) systematically review and code, in detail, all of New Jersey's unidentified and missing persons dental records; (2) to determine the accuracy of the dental coding already in all dental databases; (3) take corrective action in the dental coding if necessary; and, (4) to ultimately offer a secure, centralized dental database where the records are digitized and accurately coded. The initiative required a comprehensive and standardized protocol, which was developed utilizing two forensic odontologists, appropriately trained and experienced, to provide the quality control needed to ensure accuracy of the entries.

It became apparent through this review that inaccuracies existed. Tracking the inaccuracies and following up to see where they occurred has revealed that potential matches would have been excluded in the NCIC system. This initiative reinforces the work performed by the forensic odontologist as stated in the NAS recommendations.

Accurate database coding in national databases is a prerequisite screening mechanism of antemortem and postmortem information. Positive identification or exclusions can only be made from accurate data. In order to promote valid scientific data, it is also recommended that the application of these codes be standardized. Once accuracy is attained, all national databases can be populated with the goal of additional positive identifications.

This presentation will present the results of the data accumulated from the ongoing review of the dental coding for New Jersey's unidentified and missing persons. Based on this data, recommendations will be presented to ensure accuracy and quality control.

**Coding, Identification, NCIC** 

### F14 Use of Digital Photo Enhancement Software as an Aid in Edentulous Victim Identification

#### Henry J. Dondero, DDS\*, 2 Emerald Drive, Glen Cove, NY 11542

The forensic odontologist must be able to utilize all devices and methods available in the quest for victim identification. The goal of this presentation is to present the use of digital photo editing software as an aid in accentuating boney landmarks.

This presentation impact the forensic science community by encouraging the forensic odontologist to be aware of the various investigative modalities available.

The forensic odontologist may not be able to identify every victim he or she encounters due to a multitude of reasons. One situation arises when a victim presents with few or no restored teeth. It is particularly challenging when a victim is edentulous. To receive antemortem radiographs that date back more than ten years further compounds a difficult situation. The following case is an example of such an identification. What is particularly unique is that the boney identifiers were highlighted through the use of digital photo editing software.

This case involved partially skeletonized remains found on the muddy bank of a lake located in a nearby state park. Initial examination revealed a badly fragmented maxilla and associated cranial structures with no teeth present. The victim's mandible was also fragmented. There was complete bilateral fracture of the left and right angles. Both condyles and associated ascending rami were missing. The remainder of the mandible; however, was intact. Clinical charting of the mandible revealed the presence of tooth #27 with a full crown aesthetic restoration. Tooth #22 was missing and appeared to have been evulsed postmortem. The remainder of the mandible was edentulous with well healed bony ridges. The maxilla was badly fragmented. There were numerous missing structures and it was impossible to determine an accurate evaluation of the dentition. Whatever cortical bone was present appeared to be well healed and edentulous. The assumption was made that the victim's entire maxilla may have been edentulous.

This case was ruled a homicide and the State Police suspected that the victim was an ex-convict. The last known dental record was a panoramic radiograph taken when the victim was incarcerated fourteen years earlier. The x-ray was taken as a matter of course and no subsequent treatment was rendered. This radiograph was requested from the Department of Corrections. Examination of the radiograph revealed an underdeveloped copy of a rather grainy original panoramic film. The maxilla was edentulous and the mandible showed bilateral edentulous saddles with teeth #21-28 present. Postmortem radiographs of tooth #27 were taken and compared to the antemortem panoramic x-ray. This offered no definitive points of identification. Several radio-opaque and radio-lucent areas were noted in the mandible and an attempt was made to compare these areas with the antemortem radiograph. Because of the poor quality of this x-ray a process of digital photo enhancement was undertaken. The radiograph was scanned at a high resolution and saved as a TIFF file. This file was then opened in a digital photo editing software application and adjustments were made to the brightness, contrast, and sharpness of the image as well as enlarging certain areas of interest.

The resultant enhanced images afforded the opportunity to more accurately compare ante- and postmortem radiographs sufficient to establish identity. Areas of increased calcification, possibly retained root fragments, were compared as well as several radiolucent areas containing specific trabeculation.

Radiograph, Digital, Enhancement

#### F15 Odontologic Identification Reporting – Forms and the Interpol Form System

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After this presentation attendees will become aware of the forensic report in odontologic identification cases. A number of formal requirements and also descriptive data should be included. Attendees will also be more aware of the Interpol form system for odontology reporting and final identification.

This presentation will impact the forensic science community by increasing awareness of the importance of the report and hopefully more professional reports from forensic odontologists.

There are a number of requirements for a forensic legal report. These requirements are more or less universal and vary little for instance between the United States and Norway. The requirements are also similar for a technical police report, a forensic medical report, or a forensic odontology report. Experiences with the reports in identification cases, for instance from the tsunami identification in Thailand, seem to indicate that forensic odontologists around the world are not always aware of what is to be expected from a professional forensic report. Sometimes one has the feeling that dentists look upon the written form in identification cases only as working documents. As a matter of fact, some dentists claim that they only report the conclusion of the comparison to the police or other authorities. Due to the quality control system, most Norwegian reports should be acceptable with focus on writing professional reports.

Some of the more important aspects of a legal report as well as the most important odontology registration in an identification case will be discussed. Basically all legal reports should be able to stand alone. That means that after reading the report there should be a basic understanding of the background of the case and especially facts that might be important for odontology findings and comparison. The condition of the body and especially the face and the teeth should be noted. Any injuries to teeth and oral structures should be noted as well as an attempt to explain those in connection with the death. Most odontologists give a good description of each tooth with restorations, but often forget to note the condition of teeth without restorations. This leaves to the reader only to guess the condition even though a sound six-year-molar may be more characteristic and thus more important for the final comparison and conclusion than a restored one. Assessment of age should always be an integrated part of a dental description in an identification case. In skeletal findings also an assessment of sex based on the teeth only should be given by the forensic odontologists. In cases of reconstructive identification an attempt should be made to assess the ethnic or geographic origin, occupation, or habits.

Interpol has since the 1960s been interested in identifications, especially after disasters as they often involve victims from many countries. International communication and co-operation between different countries' police authorities are important to obtain optimal results. Interpol has thus developed guidelines for international cooperation, a guide to identification work and forms for registration including dental registrations. A presentation of the forms for dental registrations as they are and the previous changes will be given. A discussion if they are compatible with a professional legal report will be given. In addition, forms for comparison and for the Identification commission's final declaration of identification will be presented. In Norway, there is long tradition and experience in the use of the Interpol form as, since 1980, they have always been used in reporting even in single identification cases.

**Identification, Reports, Interpol Forms** 

#### F16 Cone-Beam CT as a Practical Tool in Forensic Dental Identification – A Preliminary Study

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After attending this presentation, attendees will understand how cone-beam computed tomography-derived images of the maxilla or mandible are similar enough to conventional radiographic images to allow for forensic odontological comparisons. Attendees will gain knowledge of cone-beam computed tomography (CBCT) imaging and will be familiarized with how CBCT volumes can be analyzed in multiple planes.

This presentation will impact the forensic science community by demonstrating how CBCT imaging offers a rapid postmortem imaging technique for use in dental identification. This technique could be utilized under circumstances when conventional techniques would be prohibited or difficult. It is hypothesized that CBCT-derived images of the dentition are similar enough to conventional dental radiographs to allow for forensic comparisons in dental identifications.

Biological imaging techniques have been improving at a rapid rate over the last decade with improvements in ease of use, speed of imaging, and image resolution. In medicine, CT imaging is being used for virtual autopsy (virtopsy) in the forensic setting.<sup>1,2,3</sup> Conventional computed tomography (CT) has been investigated for dental profiling with limited results, mostly due to artifact seen from metal containing dental work.<sup>4</sup>. In dentistry, cone beam CT technology is becoming more accessible and utilized than ever before. Many individual dentists have installed CBCT machines in their offices and use CBCT volumes for a variety of clinical applications, including treatment planning for dental implant placement, pre-surgical evaluation, and 3D orthodontic diagnosis and treatment planning.

CBCT data sets can be displayed in multiple ways and even in 3D. This unique ability is one which is proposed to apply in forensic odontology. There are times when it is impossible, difficult, or unsafe for forensic odontologists to take postmortem radiographic series; such situations may include the presence of toxic chemicals or radiation. CBCT scans can be acquired postmortem without the necessity to open the cadaver's mouth, and possibly even without removing the victims from a body bag. Here, images will be shown that are similar to conventional or digital periapical or bitewing radiographs can be generated from CBCT volumes of jaw specimens from cadavers previously used in anatomic training.

Digital dental periapical and bitewing images of human cadaver half heads and cadaver mandibles were taken utilizing a Gendex 770 dental x-ray unit and a Schick Elite digital sensor. Exposure settings were 70 kVp and 1/20 sec. Separately, CBCT scans of specimens were acquired with an i-CAT Platinum CBCT unit. Panoramic images of the jaws were reconstructed from each CBCT volume using i-CAT Vision software. Panoramic reconstructions were reformatted utilizing a developed protocol to repeatedly and rapidly generate images with the same anatomic coverage as typical periapical and bitewing images of the alveolar processes. CBCT-derived periapical, bitewing and panoramic images are presented side-by-side with intraoral periapical and bitewing images to demonstrate the similarity.

The CBCT images carry sufficient anatomic information for conclusive comparison with antemortem intraoral radiographs and that, in practice, images derived from CBCT volumes according to our protocol can be compared with antemortem images of the same area for forensic identification.

The presentation will demonstrate that CBCT technology can be used as an alternative to conventional and digital radiographic studies for comparison of dental radiographic images.

This pilot study lays the ground work for additional studies currently underway which will demonstrate the ease and accuracy of using CBCT derived images for dental identification.

It is believed that CBCT imaging is a viable time saving and resource sparing technique in such situations as mass disasters and for dental identification of cases which can not safely be imaged with conventional techniques (e.g., hazardous chemical, toxin or radiation contamination).

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Cone Beam CT, Postmortem Imaging, Dental Identification

### F17 A Positioning Device to Aid the Odontologist in a Morgue Setting

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The goal of this presentation is to show the forensic odontologist that the use of a positioning device while performing a dental autopsy can make the task easier, faster, and safer as well as improve the quality of the postmortem records.

The presentation will impact the forensic science community by illustrating the benefit of the application of a new "hands free" positioning device. The device aids the forensic odontologist, especially the solo investigator, by providing a helping hand to lessen the ardent tasks of radiography, photography, and visual recording of dental records in a morgue setting.

Dental autopsies are accomplished in many facets due to the nature of circumstances: (1) the state of the remains, (fragmented, skeletonized or intact); (2) the range of the postmortem records (those deemed necessary to be collected); and, (3) the objective or limits set by the Medical Examiner/Coroner (ME/C) directing the case. The compliment of available help in a morgue setting also varies greatly. Morgue staff may or may not be a resource. Depending on the manpower or time of day when the forensic odontologist chooses to do the examination, morgue staff or auxiliary staff of the investigating dentist may not be readily available. This lessens the ease of one's record gathering. The practice of dental autopsy is hands on and the use of a versatile, near infinitely posturable positioning device could prove useful. The following paragraphs provide several examples for its intended use.

This positioning device can provide stable, "hands free" assistance, acting as a clip to hold an ABFO #2 rule; held on its proper plane and parallel orientation to a photographed specimen. The device can function as a clamp to hold a UV light source for constant illumination of dental restorations for easier dental inspection and charting of the subject.

The task of postmortem radiography is often difficult to accomplish alone. The supine position of a subject on a gurney and the inability of the subject to facilitate the odontologist are contrary to radiography in a viable dental setting. Exposing the jaws through tissue manipulation, tissue displacement or tissue release allows the operator access to place a dental film or digital sensor; however, tissue rebound, tissue resistance, or lack of supporting structure hampers stability of the imaging medium. Proper angulations for good radiologic results are difficult. Hence, for fixed specimens, the positioning device can hold a dental film packet or digital sensor in a position comparable to a holding device used in the living. This eliminates the out stretched hand of an auxiliary or the operator; the positioning device holds the film or digital sensor with resistance to tissue rebound and maintains stabile angulations during the exposure. Whether using a standard fixed mount or portable X-ray generating source, the operator or auxiliary is not involved with the path of the ionizing radiation.

Jaw removal, whether partial or complete, or fragmented jaws yields specimens in a spatial relationship different from the normal. For excised or fragmented specimens this positioning device is able to suspend or orientate oral structures in a more suitable position to offer better radiologic results. Suspending a specimen, raising it off a hard surface, can give way to better film or digital sensor positioning and can give room to manipulate an X-ray head for better angulations prior exposure. A better postmortem radiograph leads to a better antemortem/postmortem comparison.

Also, with minor variations, this positioning device can be set on a jig and used to suspend simultaneously both excised upper and or lower jaws in their proper planar orientations for orthopantograph imaging. Lastly, this device can be formed into a "skull cradle" and placed on the same jig for imaging of a complete skull with its associated maxilla and mandible. This positioning device with its articulating segments can replicate the upper portions of the spine for very good orthopantograph positioning, i.e., the Frankfort plane, which could be helpful in identifications as well as research endeavors of the skull.

Positioning Device, Hands Free, Odontology

#### F18 Identification of David Koresh by Dental Records

Roger D. Metcalf, DDS, JD\*, Tarrant County Medical Examiner's District, 200 Feliks Gwozdz Place, Fort Worth, TX 76104

After attending this presentation, attendees will have an understanding of the process of identification of unidentified human remains by dental records as applied to the Mount Carmel incident near Waco, Texas, in 1993.

This presentation will impact the forensic science community by providing a review of one forensic science discipline's contribution to the resolution of a significant mass-fatality incident.

Eighty-six people died as a result of the Mount Carmel incident: ten people in the initial raid on the compound on February 28, 1993 (six residents of the compound and four BATF agents), and 76 people died in the fire that subsequently occurred at the compound on April 19, 1993.

The Texas Justice of the Peace with jurisdiction in this matter ordered the decedents to be taken to the morgue at the Tarrant County Medical Examiner's District (TCMED) in Fort Worth, Texas, for autopsy and examination under the direction of Chief Medical Examiner. The Director of the TCMED Human Identification Lab at the time was Rodney Crow, DDS. The "Dental Disaster Squad," composed of many members of the Fort Worth District Dental Society had been well prepared for such a mass-fatality incident by: (1) training at the Southwest Symposium on Forensic Dentistry at the University of Texas Health Science Center at San Antonio Dental School; and, (2) participation in actual, local incidents involving Delta Airline flights 191 (1983) and 1141 (1985) at D/FW International Airport in Fort Worth/Dallas, Texas. Approximately 40 to 50 unpaid volunteers from the dental society donated almost countless hours of professional services extending over a period of three months.

Dental ID, Mount Carmel, David Koresh

#### F19 Development of a Colorimetric Scale as a Visual Aid for the Time of Bruising in Blunt Trauma and Bitemark

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After attending this presentation, attendees will learn more about color of bruise versus age of bruise and how a colorimetric scale may be visual aid for the assessment of the age of bruising.

This presentation will impact the forensic science community by introducing two prototype colorimetric scales with and without linear measurement, each with six bruising colors, three circles with black and white calibrators to be used for forensic photography of white European population

Medical examiners and forensic odontologists are frequently asked to establish the age of a bruise on a living or deceased individual. Injuries may be the result of bitemarks or of non-accidental traumas, thus having a medico-legal significance in the field of child abuse. In June of 1996, persons investigating child abuse and neglect were mailed a pamphlet from the U.S. Department of Justice entitled, "*Recognizing When a Child's Injury or Illness is Caused by Abuse*," with a specific part dedicated to aging of bruises. The pamphlet gave a very clear cut description of color of bruise versus age of bruise, as follows: Red 0-2 days; Blue, Purple 2-5 days; Green 5-7 days; Yellow 7-10 days; Brown 10-14 days. However, a colorimetric scale for forensic photography based on the bruise colors has never been proposed, as photographic color reproduction is unreliable and depends on several factors, like camera, lighting, printer, and photo-editing color calibration.

The purpose of this study is to propose two prototype colorimetric scales with and without linear measurement, each with six bruising colors based on RGB color model, three circles with black and white calibrators to be used for forensic photography of skin injuries of white European population, during different stages of healing. The prototype scales were employed during forensic photographic imaging of cases of blunt trauma and bitemarks.

This study does not attempt to give a definitive account of the different scientific methods available for the assessment of the age of bruising. This presentation will present an opinion that a color aid when analyzing photos could assist with the interpretation and accuracy of estimation of bruise age, especially when the analysis it made directly on digital images prior to printing. Such an aid would give a reliable standard condition and allow color calibration. It is essential that the colors within the image represent colors within the bruise under standard and reliable conditions.

Observation on a large sample of blunt trauma and bitemark injuries applying the proposed colorimetric scales is needed to verify and validate the preliminary results obtained, although bruise age estimation remains an expert opinion with several degrees of accuracy and variability. For this reason colors within the bruise have to be analyzed by experienced and confident observers along with every and any relevant findings and observations in order to prevent errors or misjudgment.

A synergy between medical examiners and odontologists is also advisable for a more acceptable forensic interpretation in order to assess the correction parameters to be used in the proposed colorimetric scale. **Bruise Age Estimation, Forensic Odontology, Bitemark Analysis** 

#### F20 Forensic Identification of Flight AF 447 Disaster Victims

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After attending this presentation, attendees will learn more about DVI in a multinational scenario applying INTERPOL recommendations.

This presentation will impact the forensic science community by demonstrating how every identification method should be employed when human identification is requested.

Air France flight 447 departed on schedule at 19:30 (22:30 GMT) on May 31, 2009 from Rio de Janeiro for Paris. On board were 228

people (216 passengers and 12 crew members), 59 Brazilians and 169 others belonging to 32 different nationalities. About three hours after take off there was loss of radio signal from the aircraft. It is unlikely that the cause of the disaster will ever be established, as the black boxes were lost in the Atlantic Ocean. Only 50 bodies were recovered and identified, 20 of which were Brazilian and 30 of other nationalities. Of those recovered 25 were adult females, 24 adult males, and 1 male child.

Forensic victim identification was performed by Brazilian Federal Police staff with the technical support of INTERPOL and French personnel. Several antemortem and postmortem teams were employed for the identification process. An advanced PM Base located on Fernando de Noronha Island, a main PM base located in Recife; a federal police PM DNA lab in Brasília; and an AM team in Rio de Janeiro. The advanced PM team located on Fernando de Noronha Island was tasked with photographing the bodies and human remains recovered, with the initial registration and archiving of clothing, personal belongings and obtaining fingerprints and a biological sample for DNA profiling. The body bags were shipped to Recife and DNA samples to Brasília for further analysis. The medico-legal Institute in Recife performed medical, dental, and radiological examinations, taking additional photographs, DNA, and fingerprints samples. These operations were performed by Brazilians forensic pathologists and odontologists, with French and INTERPOL delegates as observers representing the international Community.

DNA, odontology, fingerprints, as well as personal belongings/findings (tattoos, jewelry, piercings) and clothing were used by the reconciliation teams in Recife and Brasília. The forensic management of victim identification differed little from the one used after the 2004 tsunami in Asia. Due to the large number of nationalities involved (33), and the difficulties in processing antemortem data, it was decided to use INTERPOL DVI protocols and software, to ensure an international standard and quality control.

The recovery and identification of AF447 victims required 2 months work, largely due to the timescales involved in receiving ante mortem data from the many countries involved through their police agencies.

During DVI process identifications were made by following methods: DNA, odontology findings/fingerprints. Although more methods were used in many instances, the order largely reflects the percentage of each method used, DNA being used in more cases. However, the identification of the non-Brazilians victims was mainly done via DNA and odontology data, as a result of the absence of fingerprints AM data for the non-Brazilians casualties.

Human rights, quality control, and Interpol DVI recommendations (ratified by 187 countries) require that in a multinational identification exercise, all primary identification methods should be employed. This opinion that the combination of DNA, odontological data, dental x-rays, and where possible fingerprints will permit the establishment of an international standard ensuring certain and fast results.

Forensic Science, Forensic Odontology, Disaster Victim Identification

# F21 Contribution of New Technologies to the Bitemarks Study

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After attending this presentation, attendees will understand how new technologies especially laser scanner using 3D modeling of dental casts can enhance the study of bitemarks. The sectioning performed allows the approach to optimistically further development in the bitemarks study to help investigators and magistrates to identify the perpetrator of these acts to find the truth.

The technique mixing data processing and forensic science is an original tool in the fight crime and is currently being tested within Forensic Science Institute of the French Gendarmerie (IRCGN). This presentation will impact the forensic science community by presenting practical applications which can represent guidelines available to a wide population of specialists.

Bitemarks are caused by the mechanical action of the teeth accompanied or not by the pressure of tongue or lips. Thus bitemarks induce a physical deterioration of soft tissues characterized by a print whose contours vary mainly according to the strength of the pressure exerted on the tissue, the mandibular movements of the perpetrator, and the movement of the victim. Naru and Dykes (1996) showed that the manual sketching copying dental arches of a suspect vary perceptibly if they are not performed by the same technician. The imprecision of the copying depends of the dexterity, sensitivity, and the tiredness of the operator. There is a consensus among experts in bitemark studies who consider that data processing is an ideal tool to copy objectively the contours of teeth of the suspect. But according to the strength of indentation, the marks of incisal edges or occlusal surfaces don't show all the morphological characters used to identify the aggressor. Charles Georget (2003) developed a method based upon sections made from plaster dental casts. This method provides a more accurate representation of the dentition. Nevertheless, it requires a specific material made for the experimental process. The data is produced by the assessment of the dental arches of the aggressor in situ and the molding of the cast in white plaster. Around the white plaster cast, a form work is made and filled with black plaster. The sections and the pictures of the dental casts are entered into a database. The tool used to produce the sections is commonly found in prosthodontic laboratories. It guarantees the orthogonality of this assemblage and the equidistance between two sections. The tool is made to create a controlled removal of plaster every millimeter. After a section is ground the cast is dismantled from its support. It is photographed or photocopied next to an ABFO N°2 scale. However, this method is limited because it is destructive and the number of sections is limited. It is also a time consuming process. The contribution of new technologies and especially the use of laser scanners assist in the production of fast and easy 3D models of the dental cast. This none destructive method enables to production of sections according to the needs of the odontologist. The digitized dental cast is available for further examination due to the non-destructive nature of the methodology.

**Bitemark, 3D Modeling, Plaster Section** 

### F22 A Modified Method for Bitemark Overlay Fabrication

Barbara L. Needell, DMD\*, Den-Care West, 5280 North University Drive, Lauderhill, FL 33351

After attending this presentation, attendees will learn an alternative method for fabricating bitemark overlays. Attendees will determine if this method is valuable in bitemark analysis and expert witness testimony.

This presentation will impact the forensic science community by providing an alternative method and a useful tool for bitemark overlay fabrication.

The method of acetate overlay fabrication is a critical part of bitemark analysis and expert witness testimony. It began as a magic marker tracing of teeth edges over models made from impressions of a suspected biter and progressed to the more current usage of graphic editing program software and other similar computer programs. The purpose is to accurately represent the biting edges of teeth. The overlay has undergone much study. An error in fabrication can lead to disastrous mistakes in assessing innocence or guilt. In addressing a jury, the forensic dentist should make his or her opinions clear and easy to duplicate. Because many innocent people have found themselves behind bars due to inaccurate forensic analyses, odontologists need to be meticulous and exact in their analyses and their presentations in the courtroom.

There are several methods of overlay fabrication. No one method is always better than another. The method described in *Digital Analysis* of *Bitemark Evidence* by Raymond J. Johansen, DMD and C. Michael Bowers, DDS, JD is one of the most comprehensive to date (*Forensic Imaging Services, 2000*). This presentation will make use of instructions from this text when fabricating the alternative overlays. Several cases will be shown utilizing this method and comparing it to the traditional method.

Traditional overlays display the edges of the incisors, canines and bicuspids. These are then placed on top of a photograph of the bitemark for comparison. This can be done with a hard copy, or acetate transparency, over the actual photograph, or else performed on a computer software program. Either method is usually acceptable in court. The jury has to imagine that the elliptical or rounded area is the incisal edge of a tooth. This alternative method will reflect the entire tooth over the bitemark photograph. It will help the odontologist, as well as members of a jury, visualize the entire tooth over the bitemark.

A step by step presentation of the alternative method of bitemark overlay will be presented. By the end of the presentation, each attendee should then have the expertise necessary to reproduce this method and the understanding of whether this method is applicable to his or her forensic cases.

**Overlay, Bitemark, Expert Witness** 

### F23 Statistical Shape Analysis of Bitemark Distortion in Human Skin

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The goal of this presentation is to explore the range of distortion possible in bitemarks created on human skin using shape measurement tools coupled with multivariate statistical analysis.

This presentation will impact the forensic science community by addressing one of the two fundamental principles of bitemark analysis, that is, transferability of the shape of the human dentition to skin.

Distortion in a bitemark in human skin is unavoidable. What is poorly understood is the extent of distortion that is possible. Skin is a less than optimal recording medium as it undergoes visco-elastic, anisotropic non-linear response to stress. Prior studies have shown that these factors create a situation in which the deformation will show both intra- and inter-arch variation in multiple bites, even if all are created with the same dentition. However, these studies employed a metric approach.

Traditional methods of exploring this problem have used metric measurements in an attempt to quantify mesial to distal, intercanine, and angulation differences of the teeth. Nonetheless, metric measurements provide no overall description of shape changes. Nor do they provide any formal statistical analysis with regard to biological form.

A well-established statistical shape method used to describe biological form is Geometric Morphometic analysis (GM). GM

methods allow for a quantitative analysis of shape by capturing the geometry of morphological structures of interest and preserving this information through statistical analysis.

Shape information can be visualized by plotting landmark positions in Procrustes distance superimposition. Procrustes distance is a measure of the closeness in shape of Procrustes superimposed specimens and is recognized as a general-purpose measure of specimen similarity in the geometric morphometrics framework. Procrustes distances can be used to summarize variations in populations, express the degree of similarity of individual specimens, means of populations, or to search for matches between bitemarks and dentition.

Among the tools available for statistical analysis is Principal Component Analysis (PCA) with which the principal variations of shape can be plotted and visualized. This allows for determination of which shape aspect is responsible for the most variation. Canonical Variates Analysis (CVA) can also be used to determine if shape information can distinguish between different categories of data.

Thus, the use of shape change analysis software allows a multivariate statistical approach to explore one of the principal tenets of bitemark analysis; *transfer of the dentition to skin.* 

All necessary Human Subject Institutional Review Board (HSIRB) procedures were completed and exemption was granted. Eighty-nine bitemarks were created on unembalmed cadavers. The cadavers were stored at 4°C and allowed to come to room temperature prior to bite infliction. The bites were created both perpendicular and parallel to skin tension lines. Bitemarks were also created in wax for comparative purposes.

For bitemark infliction, a single dentition was used. The dentition of a volunteer was impressed with polyvinylsiloxane and then poured in light viscosity metallographic epoxy resin. The models were mounted on a hand-held vice grip. The opening diameter was set to 40mm (opening diameter of the volunteer).

Each bite was photographed with a #2 ABFO scale in place. Landmark points were placed on each digital image that described the mesial to distal endpoints, intercanine and angulation of each of the 6 anterior teeth with freeware. Landmark points were also placed on the ABFO scale as an internal reference. Intra operator error threshold was also calculated. Following landmark data point extraction, statistical analysis was completed with another freeware program.

PCA, CVA, and Procrustes distance was determined, and used to demonstrate the non-equality of measured images of the dentition, wax impressions, as well as the range of distortion of bitemarks in skin. In addition the bitemarks were compared to a population of 410 dental models to determine the closest match. Results showed that none of the bitemarks matched the dentition that caused them within measurement error and that two unrelated dentitions matched more closely.

The data presented will allow the forensic community to understand the range of distortion possible in bitemarks created on human skin.

Bitemarks, Bitemark Research, Skin Distortion

#### F24 Diagnosing and Reporting Abuse in the Dental Office: Recommended Procedures in Switzerland

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After attending this presentation, attendees will be able to evaluate a method of reporting abuse cases diagnosed in a dental office. This presentation will impact the forensic science community by showing the importance of training dentists in detecting and reporting an increasing problem of public health.

Diagnosing a case of abuse can be a difficult task for dentists and their staff. It requires knowledge and familiarity with signs that might indicate violence without jumping to conclusions. Diagnosing physical abuse is the first step of a systematic procedure. It needs a particular specialized training and knowledge to link detected injuries and the behavior of the patient and/or the accompanying person(s). The etiological factors of the injury must be determined as to be either accidental or non-accidental event. The delicate initiation of a discussion with the patient regarding a situation of abuse should include pertinent questions in a professional atmosphere of confidentiality with an evaluation of the discerning capacity of the presumed victim.

Reporting such a situation has to be systematic and compatible with the local and/or national legal requirements. Any report of suspected child abuse should include exact information regarding the victim with diagrams and photographs of the injuries. All observations should be clearly mentioned and commented. However, the author of the report should avoid any personal investigation and/or conclusions.

Responding to a situation of domestic abuse to an adult requires an evaluation of the cognitive ability of the presumed victim. The interview should include documented assistance referrals and contacts to seek help and support about violence.

Dentists are generally confronted with patients whose age range from children to the elderly. They see their patients on a regular basis and/or during an emergency service. They are in the front line to diagnose signs of abuse as 65% or more of the lesions linked to abuse appear in the head, face, neck and other visible parts of the body such as hands and arms. Unfortunately, some studies and results show that reports coming from dentists are very scarce compared to those coming from other health professionals such as physicians, nurses, caretakers, medical examiners or social workers. This may be due mainly to a lack of basic training during the undergraduate and during most of the postgraduate training programs.

This lack of training should be addressed as child abuse and domestic violence among partners and older people has become an alarming issue in public health.

The Swiss federal law requires the duty to report cases of child abuse by health professionals and dentists belong to this category. In some of the 26 different cantons of the country, it is even an obligation to report. Additional law on the assistance to offended victims came into effect in 1993. This law encourages the creation of violence prevention programs and specific consultation for victims of interpersonal violence. These centers inform victims on rights and duties and evaluate risk.

As abuse cases are increasing, dentists should become more aware of the risk of being confronted to a non-accidental problem. Careful observation of a patient's symptoms, his or her behavior, appearance, and ability to communicate is a very important part of any dental visit. **Forensic Odontology, Abuse, Dental Traumatology** 

### F25 A Bitemark Identification by Geometric Pattern: A Medico-Legal Problem

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After attending this presentation, attendees will learn to appreciate the ABFO guidelines and the necessity to use accurate metrics in analyzing bitemarks involved in criminal cases. The ramifications of such testimony can be serious. This presentation will impact the forensic science community by showing that, in light of last year's National Academy of Sciences (NAS) Report, the standard of courtroom testimony has been raised. Now, more than ever, testimony has to be supported by accepted science. This presentation will discuss the lack of proper use of ABFO guidelines, of proper metrics, of the *Daubert* rules and the loss of being an independent reporter of scientific fact.

This is a case of child abuse, not alleged child abuse. The question is who is the abuser? The victim lived with her mother, her 7-year-old brother, and her mother's boyfriend. The maternal grandmother was her babysitter. In January 2007, the  $3\frac{1}{2}$ -year-old little girl presented at the New Bedford, Massachusetts Hospital. The grandmother stated that two days earlier the little girl fell in the bathtub hitting her lower face and chin. Upon examination, the doctors found a single midline external puncture of her maxillary lip with internal mucosal tearing and small punctures on the underside of her chin. However, further inspection indicated large bruises on the child's torso and apparent bitemarks on both the right and left arms. Several photographs were taken. Only one bitemark photo had a ruler in it. The hospital dispensed antibiotics for the child to the grandmother and they were released.

April 2007, the child again presented to the hospital. Neither the mother nor the grandmother gave the child the antibiotics. The injury to the lip deteriorated to mimic a cleft lip forcing reconstructive surgery. Approximately 5 months later, the mother's boyfriend was arrested for child abuse. When the trial began, the central issue focused on the accused as the perpetrator by bitemark identification. The prosecutor alleged the injuries to the maxillary lip, the chin, the torso, and the arms were all bitemarks caused by the accused. A forensic odontologist testified for the prosecution on the pattern injury on the child's left arm and her chest. He testified that he obtained plaster imprints of the teeth of the mother, the grandmother, the accused, and the brother. From these he made wax bites and overlays. The odontologist limited these four subjects as the only possible biters. He stated that, while he was unable to perform a metric analysis of the bitemarks due to the limitations of the evidence provided, he determined that, by evaluating the "geometric pattern" of the bitemarks on the arm and side of the chest, the accused was the biter.

A forensic odontologist testified for the defense. He rebutted the prosecution's testimony entirely. He pointed out that an examination of the only bitemark with a ruler in it excluded all four people studied by the prosecution. This automatically opens up the pool of possible biters. He showed that the wax overlays presented by the prosecution had no tooth designations to identify alleged matches. He pointed out the prosecuting odontologist's error in aligning the bitemark on the left arm. He brought into question whether there was a bitemark on the torso as opposed to bruising on the child's torso. When the defense odontologist attempted to contradict the geometric pattern theory by invoking the *Daubert* ruling the presiding judge stopped him from testifying further. The requirement for not only class characteristics (geometric pattern) but individual characteristics (not illustrated) was disallowed by the judge. The accused was found guilty and sentenced to 12 to 15 years in prison. The case is being appealed.

Daubert, Bitemark, Geometric Pattern

### F26 A Perfect Storm: Is There a New Paradigm to Keep Bitemarks Afloat Or Will They Sink?

C. Michael Bowers, DDS, JD\*, Ventura Coroner's Office, 2284 South Victoria Avenue, Suite 1G, Ventura, CA 93003

After attending this presentation, attendees will appreciate prosecutorial, defense, and appellate strategies used in current bitemark cases.

This presentation will impact the forensic science community by addressing the issues associated with insufficient scientific literature supporting bitemark evidence, the problems of traditional judicial acceptance which battles the 2009 National Academy of Sciences Report, systemic difficulties exposed in exoneration cases, and compelling new research that suggests a paradigm shift. The use of this information in the current peer review commentary and judicial review will be explored.

Bitemark analysis is based on the following two concepts or assumptions. First, the dental characteristics of anterior teeth involved in biting are unique in all individuals. Second, that the asserted uniqueness is transferred and recorded in the injury thus allowing distinguishing features in the patterned injury to be related with some level of certainty to a given dentition.

The historical question in bitemark analysis has been what, if anything, is "unique" regarding teeth. Recent publications have pointed to the fact that as human beings we share a common biological form and that when considering large populations, tooth positions and shapes can overlap. Consequently, dental matches can be found.<sup>1</sup> In light of this, the concept of dental uniqueness is not scientifically, nor statistically, supportable.

Another confounding variable in studying bite skin injuries is that most show as only bruises and discolorations, effectively reducing resolution and allowing more than one dental pattern to fit the injury.<sup>2</sup> With regard to the second assumption of bitemark analysis, the notion that a bitemark in skin could have been made by a particular person (i.e., someone with teeth like the defendant's), something that is commonly stated by odontologists to law enforcement investigators, the forensic community, and the court, can be called into question.

The research discussed in Miller et al,<sup>1</sup> Bush et al,<sup>2,3</sup> a 2009 scientific methods review by the U.S. Congress,<sup>4</sup> and the use of DNA, no longer suggests using the old method of trying to "match" teeth to bruises in the skin.

The lack of scientific research to support that "everyone's teeth are unique" (an impossible challenge to prove in relation to bitemarks) and distortion/bruising with regard to skin makes forensic bitemark opinions an obvious out-of-step use in forensic science. Even the assertion that a bitemark could have been made by one person in particular ignores the problems in the scientific reliability of bitemark identification when DNA has not been recovered from the location of the bitemark.<sup>5</sup> In fact, uniqueness is never used as a conclusive opinion in the much more scientifically based discipline of DNA.<sup>6</sup>

In addition to these events, there is obvious proof that bitemark opinions have contributed to the wrongful conviction in ten cases in the United States in the last decade. Law enforcement, prosecutors, and the courts should now realize that bitemark analysis is *not* the same as DNA in either function (giving the court reliable proof of guilt or innocence) or scientific accuracy.<sup>7</sup> Also, bite skin injuries often show only as bruises and discolorations, which can make analysis ambiguous and opinions discordan.<sup>8</sup>

The scientific and most rational approach to take is to follow the National Academy of Sciences and have bitemark identifications follow the road similar to lie detector examination and limit its use to pre-arrest investigations where it may assist in development of leads for search warrants and persons of interest. The new concept is an obvious one. The best identification evidence from a bitemark is DNA obtained from the saliva of the biter.<sup>9</sup>

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Bitemark, Paradigm, Shift

### F27 The Correlated, Non-Independent Nature of the Human Dentition: Why the Product Rule Cannot Be Used

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This goal of this presentation is to show that the human dentition has correlated, non-independent features which render the use of the product rule invalid as a means of determining dental uniqueness.

This presentation will impact the forensic science community by demonstrating how the use of the product rule is an improper means to statistically analyze the human dentition.

One of the fundamental principles of the bitemark analysis assumes that the human dentition is unique. However, there are very few published studies that explore this issue. One off-cited paper that attempted to prove the uniqueness of the dentition was published in 1984.<sup>1</sup> In this work, the product rule was used to determine that the number of possible combinations of human tooth positions in the lower jaw alone is on the order of  $6.08 \times 10^{12}$ . This study ignored the possibilities of biological correlation and also assumed a uniform distribution of tooth position to make this claim.

The questions are thus: Are the features of the dentition correlated? Are they uniformly distributed? Do the biological features of the human dentition demand more nuanced approaches than the product rule?

Two data sets of the human mandible were randomly collected. HSIRB exemption was approved for each set. One set consisted of 172 3D laser scanned models. The second set consisted of 344 2D models that were scanned on a flatbed scanner. Landmark points were first measured on the dentitions. The center position of each tooth and the angle the tooth made in a horizontal plane was calculated. The 2D or 3D nature of the source was immaterial as the information extracted was independent of the third dimension. The arches were oriented such that the distal of the canines touched a baseline and a perpendicular line was drawn from the baseline to the mesial of the right central incisor. This resulted in a set of three measurements per tooth, x and y coordinates measured with a resolution of +-1 mm and angles measured to +-5 degrees. The data distribution was then recorded.

Two simulation tests were performed to examine the effects of correlation and non-uniform distribution. The first simulation used was a permutation test. In this procedure, a simulated data set was created using the original tooth measurements, but randomly assigning measurements to specimens using a random number generator. The x, y and angle measurements were permuted independently. This procedure preserves the distributions of individual measurements, so that histograms of the individual measurements (x, y position or angle values) are identical to the histograms seen in the original data. However, the permutation test as used here destroys all the *correlation* between measurements that was present in the original data. So the permutation test allows one to see how important correlation is in the data set. This simulation was repeated 1,000 times.

The second simulation used was a Monte Carlo simulation that assumed uniform distributions of all measurements over the observed measurement ranges, which is the assumption made implicitly in Rawson's model. To generate such a simulation, the range of possible tooth positions was calculated from the empirical observations in the datasets. Then simulated specimens were assigned measurements *randomly distributed* over the observed range with no correlation between measurements. As in the permutation test, the simulation was repeated 1,000 times.

Results show that the features of the human dentition are highly correlated and show a non-uniform distribution.

Conclusions indicate that the use of the product rule is an invalid means of describing the human dentition and should be avoided. **References:** 

 Rawson RD, Ommen RK, Kinard G, Johnson J, Yfantis A. Statistical evidence for the individuality of the human dentition. J Forensic Sci 1984;29(1):245-53.

Bitemarks, Bitemark Research, Dental Uniqueness

#### F28 Assessment of Bitemark Severity and Willingness to Assess

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After attending this presentation attendees will: (1) understand the relationship between bitemark severity and forensic significance; (2) recognize the degree of agreement between experts; (3) recognize the importance of linking severity with the degree of analysis that can be undertaken; and, (4) understand the risks of assessing injuries of low significance.

This presentation will impact the forensic science community by highlighting the need to be cautious in assessment of bitemarks and will suggest a threshold at which such injuries should not be compared to a suspect's dentition.

There is a clear link between the severity of a bite injury at presentation and its forensic significance. For example, a bite injury that presents as a diffuse, non-discrete bruise is unlikely to possess unique characteristics suitable for analysis resulting in the positive identification of the perpetrator. However, on the other end of the severity spectrum, very aggressive, avulsive injuries are frequently poor candidates for analysis. A combination of factors including the loss of tissue, tearing and distortion of wound margins, and the need for urgent medical treatment generally render such injuries poor candidates for analysis. Bite injuries that present in the middle of these extremes, i.e., injuries made up of discrete, individual bruises, small abrasions and lacerations frequently and considered by odontologists to present the highest level of significance and many will enable the exclusion and inclusion of potential suspects.

A novel index, relating severity to forensic significance has been previously developed. A total of 37 suspect bitemarks images were selected and a range of questions asked of each odontologist completing the questionnaire. In each case the severity scale was shown to the examiner and they were asked if the injury was or was not a bitemark and secondly to rate its severity. Supplemental questions were asked of the degree to which the examiner would pursue the bitemark in terms of forensic analysis. 20% of respondents were asked to repeat the exercise to provide intra-examiner reliability.

Initial results suggest that there is a high degree of agreement between the odontologists on which injuries are bitemarks (kappa = 0.92) and to which level of the scale they should be assigned to (kappa = 0.88). Agreement is reduced when considering the action to be taken in regard to the bitemark, with bitemarks rated as high severity having the largest degree of disagreement (kappa=0.63). Those bitemarks that fall within the middle of the scale have the highest level of agreement with the majority of respondents stating that they would analyze the injury further. Intra examiner reliability is also high with kappa values of 0.96 for determination of injury as a bitemark, 0.91 for scale assignment and 0.78 for analysis action.

Bitemark, Odontology, Agreement

## F29 Pitfalls of Bias and Bitemarks: Where Does One End and the Other Begin?

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After attending the presentation, greater awareness to the types and introduction of bias in scientific investigation will become a protocol for the investigation. The goal of the presentation is to help the bitemark investigator isolate himself/herself from the introduction of bias.

Becoming aware of the many types of known biases in the scientific investigation associated with bitemark analysis is critical. This presentation will impact the forensic science community by making the investigator aware of the presence of potential sources of bias and working to prevent the introduction of these biases is an absolute requirement in bitemark analysis.

Bitemark analysis is a very complex investigation that often involves many different pieces of information collected from many different venues. Interactions by the bitemark investigator with the sources of the information can begin a chain reaction cascade of events that cause the investigator to move from the application of scientific methodology to bias-based assumptions. The introduction of these biases interferes with the objective science-based process and the associated findings, which can lead to erroneous results ranging from the false positive to the false negative, with everything in between.

Generally speaking, bias takes on two forms: conscious and subconscious. Conscious bias, for example, can be something as simple as being told by law enforcement that there are only three known suspects in the bitemark homicide with the inference that one of the three is the actual biter. The bitemark investigator simply has to figure out which one of the three is the biter.

Subconscious bias is often much harder to detect and avoid. An example of subconscious bias would be the bitemark investigator collecting the actual bitemark evidence and then subsequently collecting evidence from the suspected biters, one of whom looks and acts like a criminal. Subconsciously, the bitemark investigator will form bias toward the individual "criminal acting" suspect, possibly leading to a false positive.

Knowing that the presence of bias exists prior to beginning an investigation helps to isolate its introduction. The most common types of bias in scientific investigation will be presented with explanations of how the bias can be introduced and how to avoid it. Bitemark analysts must develop protocols for the investigation that work to identify, define and remove bias if there is any chance of reaching an opinion based only on the science of the investigation. To do otherwise invalidates the investigation and all of its associated findings.

Bitemark, Bias, Bitemark Analysis

### F30 Evaluation of Affine Methods in Bitemark Analysis: Why Mathematical Models of Distortion Correction Should Be Used With Caution

H. David Sheets, PhD\*, Canisius College, Department of Physics, 2001 Main Street, Buffalo, NY 14208; Peter J. Bush, BS, Laboratory for Forensic Odontology Research, School of Dental Medicine, SUNY at Buffalo, B1 Squire Hall, South Campus, Buffalo, NY 14214; and Mary A. Bush, DDS, SUNY at Buffalo, B1 Squire Hall, 3435 Main Street, Buffalo, NY 14214

This goal of this presentation is to describe a range of Affine mathematical methods and demonstrate how these methods relate to bitemark analysis.

This presentation will impact the forensic science community by providing an understanding of the limitations of mathematical distortion correction of a digital image of a bite mark, as well as a method for comparing the quality of the attempted distortion correction.

The biomechanical properties of human skin allow for a wide range of distortions in a bitemark. This deformation may be varied within the bite itself, so that distortion is not uniform across an image. The largest factor responsible for localized distortion is the anisotropic nature of the tissue. Since skin is anisotropic, the tissue possesses varying degrees of tightness. This will cause the non-uniform degree of distortion typically seen within the bitemark.

Attempts have been made to try to describe and quantify this distortion mathematically in anticipation of scientifically matching a dentition to a bitemark. Affine transformations have been suggested as a possible method to achieve this goal. However, if skin is anisotropic, affine transformations may not be an entirely effective method of analysis and subsequent correction of the distortion. A range of affine methods are examined as well as approaches to quantifying attempts at removing distortion.

The set of affine transformations of a plane (2D) image are all the deformations of that shape that leave initially parallel lines parallel after the deformation. There are six possible affine transformations (vertical, horizontal, rotation, scaling, stretching, and shearing). A study of the effects of using three distinct combinations of affine approaches (fixed scale, variable scale, and full affine) to match dentitions to bitemarks was undertaken.

Human subject review exemption was granted for all phases of this project. Thirty-six bitemarks were inflicted on unembalmed cadavers. The bites were created with a set of epoxy resin dental models that were mounted onto a hand held vice grip. All 36 bites were made with this single dentition.

The bites were photographed with a #2 ABFO scale in place. The set of dental models that inflicted the bites were scanned on a flat bed scanner at 300 dpi, also with a #2 ABFO scale in place. Using freeware,

landmarks were placed on the resultant digitized bite images (300 dpi) as well as that of the dentition.

The following criterion for matching a dentition to a bitemark was adopted. If the Euclidean distance D between dentition and the bitemark is greater than twice the RMS scatter value for repeated measures of the bitemark after an affine matching procedure, then the difference between the bitemark and the dentition could not be reasonably attributed to chance (with a p-value of roughly 5%), and the two do not match. If the dentition is within twice the RMS scatter, than the difference may be attributed to measurement error, and the two are a match.

The digitized results of the thirty six bitemarks were then compared to the digitized dentition that created the marks, to determine if affine transformations could explain the distortion in the bitemarks on skin.

Results showed that high levels of distortion in the bitemarks were not attributable to affine deformations or measurement error, suggesting that non-uniform anisotropic properties of skin mostly contribute to the distortion seen, thus concluding that bitemark distortion cannot be corrected by using affine transformations.

**Bitemarks, Bitemark Research, Affine Transformations** 

#### F31 Is There a Consensus Between Forensic Dentists on Whether Bruising is Useful in Determining the Amount of Pain Caused by a Bitemark?

Helen York, BSc\*, Dental Health Unit, 3A Skelton House, Manchester Science Park, Lloyd Street North, Manchester, M15 6SH, UNITED KINGDOM; and Iain A. Pretty, DDS, PhD, Dental Health Unit, Skelton House, Lloyd Street North, Manchester Science Park, Manchester, M15 6SH, UNITED KINGDOM

After attending this presentation attendees will: (1) understand the drivers for court requests for assessment of pain and distress in family proceedings; (2) appreciate that empirical research cannot answer these questions; (3) understand the consensus view on pain and bitemarks; and, (4) understand the limitations of the research design.

This presentation will impact the forensic science community by highlighting a common area upon which forensic dentists are asked to comment and recognize the difficulties in providing an empirical answer to the problem.

Forensic dentists are often called upon to comment on the degree of pain or discomfort that a child may have experienced following a bitemark. Such issues are often of importance in family court proceedings. Anecdotally it is believed that bitemark injuries that are visible on young children several hours after infliction are likely to have caused immediate and continuing pain. However, there is little in the forensic or biomedical literature to support this belief. Given that empirical research is impossible in this area a study was undertaken to look at the view of a range of professionals.

This was achieved through a questionnaire, which was sent out to a target group consisting of forensic odontologists, community pediatricians, and emergency room staff. A collection of bitemarks on children were combined with scenarios including the age of the child and the time since the injury was inflicted. Using the Wong-Baker pain face scale, the respondents were asked to say how much pain the victim would have experienced at the time of the injury. It was the opinion of the respondents that all injuries would cause some amount of pain and discomfort to the victim. The perceived pain was compared to the average severity and significance score of the bitemarks and this demonstrated a significant positive correlation between the perceived amount of pain and the severity of the wound.

While the intra-class correlation coefficients demonstrated that there was only moderate agreement on pain levels for each individual injury (between all professional groups) there was good agreement that each injury would have been painful and that the pain would have been felt immediately and for some time after. These data are helpful when responding to questions of caregivers knowledge of an injury.

**Bitemarks, Pain, Agreement** 

## F32 Self-Inflicted Bitemarks in a Drowning Death

Gina R. Pittenger, DDS\*, 2604 Baugh Road, Thompson Station, TN 37179; J. Michael Cisneros, DDS, 4660 Trousdale Drive, Nashville, TN ; and Michael P. Tabor, DDS, 310 23rd Avenue North, Nashville, TN 37203

After attending this presentation, attendees will be shown the techniques and materials used to analyze a postmortem bite mark. In addition, attendees will observe how comparisons of dental impressions are made between the family members in an effort to rule out persons not responsible for the bitemark.

This presentation will impact the forensic science community by giving an example of an uncommon characteristic of a postmortem bitemark and by establishing the method and procedures used to determine who made the bitemark on the victim.

Although most bitemarks found on homicide victims and abuse cases are left by the perpetrator of the crime, there are instances of selfinflicted bites. The reasons for these self-inflicted wounds are not always clear, but in an investigation of possible homicide when a selfinflicted bitemark can help explain the frame of mind of the deceased, it becomes necessary to try and prove who created the bite. In this particular case, the decedent's body was discovered by her husband in a nearby pond. The Tennessee Bureau of Investigation investigator reported that the husband and wife were high on crystal meth and had been in a fight earlier that day where he reportedly struck her in the head and neck area. He claims he did not kill her, and that she had made statements earlier that week that she wanted to kill herself.

On March 29, 2010, Dr. Tom Deering, Forensic Pathologist from Forensic Medical and the State of Tennessee Office of the Medical Examiner contacted Dr. Mike Tabor, Chief Forensic Odontologist to evaluate a patterned injury on the left forearm of the decedent. Dr. Tabor contacted Dr. Gina Pittenger and Dr. Michael Cisneros to assist in the analysis of the bite mark.

The primary objective in this case was to rule out other family members present on the day of the victim's death, and to determine if the decedent inflicted the bite on herself. Since there was a suspicion of suicide versus a homicide, the TBI agent was trying to establish a frame of mind of the decedent by saying that if she had inflicted the bitemark to herself, it would answer questions about whether or not she committed suicide. These family members included the decedent, her husband and her six year old son. The techniques used to analyze the bitemark were dental tracings taken from dental casts made from each individual involved. Intra-arch measurements were made on each of the three suspects, from cuspid to cuspid. Polyvinyl siloxane and alginate were used to make the dental impressions of each suspect. Stone casts were made from these impressions and used for study models. The materials and devices used for the dental tracings were celluloid overlays, and a digital copier machine. Each model was photocopied on a 1:1 magnification ratio and printed on a clear piece of acetate. This allowed the doctors to outline the incisal edge position of both arches of all three suspects. Due to some unilateral obscurity in both arches the life-size transparences were of little value in determining which of the three committed the bite. Detailed and further study revealed and incisal edge bite in each arch that was composed of the distinct and

recognizable lobes instead of a smooth brick like central incisor shape. The decedent was the only one of the three who still had mamelons that would have made the three lobe shape on the bite mark.

The factors that played a role in determining the origin of the bite will be presented in this case, and it will be shown that the decedent was the individual responsible for the bitemark inflicted upon herself.

Self Inflicted Bitemark, Drowning Death, Mamelons in Bitemarks

## F33 What Happens When Your Dog Kills Someone?

#### Michael P. Tabor, DDS\*, 310 23rd Avenue North, Nashville, TN 37203

After attending the presentation, the attendees will be able to recognize the importance of understanding local and state animal control ordinances.

This presentation will impact the forensic science community by highlighting important points in animal bitemark analysis.

To what degree is a dog owner directly responsible for the actions of his animal? Research will show that liability issues differ from state to state. To further complicate the issue, what if several dogs, all licensed and registered jointly participated in a single incident resulting in 200-300 individual dog bites and the ultimate death of a human? If it becomes unclear which dog was the primary perpetrator of the homicide, does that relieve that dog's owner of some of the responsibility or liability?

These type questions may not be frequently entertained in traditional bitemark analysis or animal bite cases. In this particular case, the victim was taking her routine and traditional afternoon walk in a rural town in middle Tennessee. Although there were no witnesses to the incident, her badly mangled and mutilated body was found some hours later in the front yard of her neighbor, friend and family physician. The well known local librarian was readily identified, even though all her clothes had been torn away from her body, which had even been partially eaten. There was forensic evidence indicating she put up a struggle that was manifested by the blood splatter and fingerprint residue found on the unlocked door of the doctor's unlocked auto in the driveway, as she evidently tried to get away from the attacking dogs.

When her body was found by a passerby, a group of four dogs, two of which belonged to the property owner, hovering over the shredded human body. Local law enforcement officials were so confused initially, they originally investigated this case as a possible assault and battery or homicide case. It was noted that the dogs all had evidence of the woman's blood on their coat. When forensic odontologists determined that this incident was completely explained by animal activity, local and state officials immediately began the process of evaluating Tennessee laws pertaining to the degree of responsibility that the dog owner(s) may or may not have.

This presentation will present a time line sequence of details that lead to dental opinions regarding the perpetrators of the attack. Each of the four dogs was anesthetized and detailed measurements were made of their dentition, as well as wax bites of their occlusion and impressions of each animal while were asleep. Details will be presented which differentiate obvious human bites from animal bites, to include cuspid dominance, differing number of upper and lower incisors, as well as the other distinguishing factors.

When overlay templates were fabricated over life-size photos of the most readable and representative of the bitemarks, two of the dogs could be eliminated as perpetrators of that individual wound. It would not be possible, however, to determine their involvement in other wounds on her body as their number was too numerous and many were not of evidentiary value.

The owners of these two dogs voluntarily euthanized their pet even though this isolated comparison appeared to exclude them as possible attackers. The physician who lived in the home where the attack occurred was also the owner of the remaining two dogs. He denied his pets were involved with the attack even though forensic evidence was leading in another direction.

Additional forensic research was obtained by hiring a veterinary forensic DNA lab to evaluate stomach contents of the animals which proved to be non-conclusive. Without more definitive forensic evidence, the local district attorney was reluctant to implicate the remaining two dogs or the owner with any criminal involvement. Civil litigation soon followed.

This case may very well become a landmark case in the state of Tennessee which addresses specific liability issue on the owners of a dog attack.

Dog Bites, Animal Bites, Bitemarks

#### F34 Effects of Combining Radiological Third Molar and Cervical Vertebrae Development on Human Age Estimation

Patrick W. Thevissen, DDS\*, Dendermondsesteenweg 483, Sint-Amandsberg, Oost Vlaanderen, B-9040, BELGIUM; Jasdeep Kaur, BDS, Katholoieke Universiteit Leuven, Kapucynenvoer 7, Leuven, B 3000, BELGIUM; and Guy Willems, PhD, Katholieke Universiteit Leuven, School of Dentistry, Kapucijnenvoer 7, Leuven, B-3000, BELGIUM

After attending this presentation, attendees will be informed on combining dental and skeletal age related variables for age predictions.

This presentation will impact the forensic science community by providing an improvement of age predictions combining third molar developmental data obtained from orthopantograms and cervical vertebrae data collected from encephalographs.

For the sub-adult age category, dental age estimation is most commonly based on third molar development. Third molar development has, compared to all other developing teeth, the highest human variability. This results in age predictions with wide prediction intervals. Therefore the accuracy of age predictions combining information of third molar development collected on orthopantograms with information of skeletal development collected on cephalographs is evaluated.

In a pilot study the skeletal variable and the method providing most information about age was searched on cephalographs. Cephalographs of 500 individuals from central India (238 F, 262 M) with known chronological age between 4 and 25 year were collected. All cephalographs were imported into a graphics editing software program and scored or measured following the techniques described by Baccetti (B), Hassel and Farman (HF), Caldas (C) and Rai (R). Regression models with age as response and each of the four scoring or measurement values as respective explanatory variable were established. To compare these models the proportion of the variability in age explained by the explanatory variable (R-square) and the magnitude of the age prediction error (Root Mean Squared Error, RMSE) were calculated.

In the main study orthopantograms and cephalographs, taken from the same individual on the same date were evaluated. Retrospectively 460 Belgium individuals (236F, 224M) with chronological age between 3 and 25 years were selected from the dental clinics of the Katholieke Universiteit Leuven. All radiographs were imported into a graphics editing software program. On the orthopantograms third molar development was scored following a modified Gleiser and Hunt (GH) technique. On the cephalographs cervical vertebrae development was scored following the B and the HF technique. Regression models with age as response and information from GH, GH, and B, and GH, B, and HF as explanatory variable(s) were fitted. The stages of the left and right third molars were highly correlated. Therefore, in the GH models the stages of the left molars were used, reducing the multicollinearity issue. Because missingness of third molar values at lower age may contain information about age, missingness patterns were included in the GH models. R-square and RMSE were calculated for comparison of the age predictions of each of these three regression models.

In the pilot study, the variability in age was explained for 58%, 55%, 26%, and 3% for respectively the B, HF, C, and R scorings or measures. The detected RMSE were 3.19 (B), 3.28 (HF), 4.22 (C), and 4.83 year (R). Therefore in the main study B and HF were retained for further evaluation.

In the main study detected R-square and RMSE were respectively 0.38 and 3.59 year for the models using GH, 0.87 and 1.67 year for the models combining GH and B and 0.88 and 1.57 year for the models combining GH, B, and HF. The inclusion of information from the cephalographs based on the B technique drastically improved the age predictions in this sample, compared to predictions based on only GH scorings. Additional inclusion of scores based on the HF technique almost didn't further improve these predictions.

Forensic Odontology, Age Estimation, Third Molar and Cervical Vertebrae Development

### F35 Dental Age Estimation and Determination of the Probability an Individual has Reached the Legal Age of Majority

James M. Lewis, DMD\*, 577 Hughes Road, Madison, AL 35758; and Paula C. Brumit, DDS, PO Box 608, Nocona, TX 76255

After attending this presentation, attendees will understand how to calculate age probabilities from previously published techniques and associated data that utilize progressive morphologic changes in age estimation and measure variability in standard deviation.

This presentation will impact the forensic science community by assisting legal authorities and court systems in determining if an individual has reached the jurisdictional legal age of majority.

In the legal system, the term "minor" is used to refer to an individual who is under the age one legally assumes adulthood and is legally granted the rights afforded to adults by society. Not only in the United States, but throughout the world, this age varies depending upon the jurisdiction and application. Federal immigration and death penalty laws in the United States set the age of adulthood at age eighteen. The contractual and criminal laws that establish the legal age of majority are determined by each individual state. With exception of Nebraska, Alabama, and Mississippi, the contractual legal age of majority is eighteen. From a criminal standpoint, the age at which an individual can be tried as an adult, and if convicted, sentenced as an adult, differs greatly from state to state. Functions that may affect the ability to try a juvenile as an adult include: (1) the age of the juvenile; (2) the type of offense charged; (3) the extent of the juvenile's past history of delinquency; and, (4) whether the district attorney invokes the district court's original jurisdiction or seeks to transfer a pending juvenile court proceeding to the district court.

It has been documented that dental techniques that use progressive morphologic changes to estimate age are reliable and are considered to be the most accurate methods for estimating the ages of infants, children, and adolescents. At any given stage of tooth development, the random variation of age of the individual conforms to a particular probability distribution known as the "normal distribution." A normal distribution can be completely specified by two parameters, mean and standard deviation. If the mean and standard deviation are known, then one essentially knows as much as if one had access to every point in the data set. Therefore, probability of an individual being any given age can be calculated from previously published statistical data that calculates mean age and standard deviation.

Because the age of majority varies based upon the circumstances, probability calculations using published dental age estimation techniques need to be understood and charts fabricated to assist the forensic odontologist in calculating the probability of an individual reaching the jurisdictional legal age of majority.

Forensic Odontology, Dental Age Estimation, Legal Age of Majority

#### F36 Third Molar Development: Comparison of Nine Tooth Development Scoring and Measuring Techniques

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After attending this presentation, attendees will be informed on which tooth development scoring or measuring technique is most promising to use as tool for age estimation

This presentation will impact the forensic science community by explaining the influence of the amount of stages in a tooth developmental scoring technique on age predictions.

Forensic dental age estimation, particularly applied to advice about the age of unaccompanied young asylum seekers, is most commonly based on methods using third molar development. The degree of third molar development is classified using diverse tooth development scoring techniques and a measuring method. The scoring techniques consider observed anatomical tooth parts and (or) make predictions of crown or root lengths to establish divers developmental stages. The measuring method developed by Cameriere measures the inner side of open tooth apices and normalizes them by the corresponding tooth length. The goal of this study is to compare nine different tooth development classification techniques and to explore which technique is most promising to use as tool for age estimation.

Each accessible third molar on 1,199 panoramic radiographs of 591 female and 608 male individuals from the North Indian population with known chronological age between 4 and 33 years, was scored or measured following nine different tooth development classification techniques described by following authors: Gleiser and Hunt (GH), Haavikko (HA), Demirjian (DE), Raungpaka (RA), Gustafson &Koch (GK), Harris & Nortje (HN), Kullman (KU), Moorrees (MO), Cameriere (CA). Therefore the images were imported in a graphics editing software program. This allowed to perform the CA measurements digitally and in case of doubt between two adjacent scoring stages lengths of the concerned third molar and its preceding second molar were compared.

Spearman correlations were used to detect associations amongst the scoring and measuring techniques and between each technique and age. Regression models with age as response and the scores or measurements as predictor were developed separately for each of the nine classification techniques. The CA score is entered as a continuous predictor. To allow for a nonlinear relation, for this measuring technique restricted cubic splines were used on the log-transformed score. From each obtained model the proportion of variance in age explained by the scoring system (R-square) was calculated. Root mean squared errors (RMSE), reflecting the mean absolute error made in age prediction, were reported.

Additionally, a test sample of 239 panoramic radiographs of 131 female and 108 male subjects with age between 16 and 23 year was collected from the same population for validation of the two most to age related tooth development scoring techniques (MO, GH) and CA.

High Spearman correlation coefficients amongst the tooth development classification techniques (between 0.90 and 0.99) and between the different techniques and age (between 0.65 and 0.70) were determined, except for the relations with CA. The tooth development scoring techniques based on an equal or a similar amount of stages are not best related to each other. The R<sup>2</sup> values varied between 0.38 and 0.51 the RMSE between 3.4 and 3.9 year. All results were found similar with best performances detected for MO and GH, worst for CA. The validation of MO, GH, and CA resulted in RMSE reflecting less optimistic mean absolute error in age prediction (respectively 3.56, 3.57 and 4.04 years).

Choosing a tooth development scoring technique with a higher amount of stages is not inherently providing better age predictions. The CA measuring technique performs less than all studied scoring techniques.

Forensic Odontology, Age Determination By Teeth, Third Molars

#### F37 The Trabecular Bone in Identification

Robert Dorion, DDS, Laboratoire SJML, Edifice Wilfrid-Derome, 1701 Parthenais, 12ieme, Montreal, QC H2K 3S7, CANADA; and Sylvain Desranleau, DDS\*, 273 Boul Laurier, Mont St-Hilaire, QC J3H 3N8, CANADA

After attending this presentation, attendees will acquire new information regarding the use of trabecular patterns in the mandible for the establishment of positive identification.

This presentation will impact the forensic science community by providing new scientific evidence regarding the positive identification by trabecular patterns taking into consideration their variations in morphology and a method of calculating its significance.

According to Berkeley's Orthopedic Biomechanics Research, the trabecular bone can be classified as a porous cellular solid, consisting of an irregular three-dimensional 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.

Dental identification compares postmortem to antemortem records. It involves the analysis of different factors such as: the presence and the absence of teeth, crown and root morphology, and their interrelationships, the evaluation of the periodontal status, the type and extent of restorative and endodontic materials, fixed, removable and implanted prosthetics, tori and sinus configuration, anomalies and pathologies of teeth and bone, as well as trabecular pattern morphology.

Few studies have been completed on the statistical reliability of trabeculae bone patterns for identification purpose. Mann's research indicated that radiolucencies and radiodensities in the distal femur and proximal tibia are valid individualizing features for establishing a positive personal identification in human remains,<sup>1</sup> Hiss and Kahana used the densitometric analysis of the trabecular bone pattern as a sole means of identification that was confirmed later with two other methods,<sup>2</sup> Kahana, Hiss, and Smith's research concluded that the trabecular architecture is unique to each individual and stable enough to be used as a forensic marker for positive identification of human remains,<sup>3</sup> and, Couture, Whiting, Hildebolt, and Dixon studied the alveolar trabecular bone in radiographs.<sup>4</sup>

The current research focuses on trabecular bone pattern comparison as a viable and empirical method of positive identification.

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Positive Identification, Bone Trabeculae, Trabecular Bone Patterns

#### F38 Age Assessment: Use of Chartier Digital Colorimeter

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After attending this presentation, attendees will understand how the color of the dental root is an indicator for age assessment. This digitalized tool helps to provide a fast and easy first intention age assessment, especially as part of mass disaster identification.

The technique mixing image processing and forensic science is an original tool in the dental age assessment. It is currently being tested within Forensic Science Institute of the French Gendarmerie (IRCGN). This presentation will impact the forensic science community by presenting practical applications which can be used as a guideline available to forensic odontologists.

Age assessment is a crucial stage in dental forensic examination. It is often required by magistrates and investigators at the time of victim identification. Many assessment methods are based on dental measures (Lamendin, Solheim), others lean on different databases (Ubelaker, Nolla, Demirjian). The method we are going to address in this report requires a dental shade capture.

In 1957, Brudevold correlated the change in dental shade with the age. In 1972, Ten Cate, who observed dental roots of persons of differing ages, noticed that an age assessment should be possible using dental root shade. In the 1980s, Bequain selected teeth of known ages in order to create an assessment of dental root coloration, going from lighter to darker. In 1988, Solhein introduced the shade assessment score of the radicular dentin in some of his age assessment formulae. In 1995, Collet created a natural "shade chart" of dental roots based on a selection of teeth extracted from 45 individuals, aged from 8 to 93 years. Age assessment is carried out by comparing a tooth with the shade chart. This process seems simple but the major problem resides in the fact that there is only one shade chart and therefore cannot be easily used by other forensic odontologists. Recognizing the availability of IT systems Laurent Chartier designed MAORI software which allowed automated age assessment of dental root colorimetrics in the HSB (hue, saturation, brightness) space.

The current study undertook to constitute a database from teeth collected during dental extractions. Simply, MAORI software provides a simple user interface for assessing root color. The system is simple; first, the user takes a digital photograph of the dental root with a graded

test card. The image is taken in artificial (white light) or natural light. Secondly, the user loads the image into the software. The algorithm decodes the exact shade of the dental root color by comparison with the graded test card. As such, any differences in illumination are controlled. This comparison carried out in relation to a preprogrammed standard. Of course, this standard must be evolving. In increasing the quantity of samples, the standard equation increases in accuracy. Unlike the shade chart, it offers a better repeatability. The accuracy, which is totally independent of expert subjectivity and visual acuity, is in the order of +/- 5 years. Given the modularity and the possible evolutions, this first version should rapidly lead to a more stable version able to be deployed more widely in the future.

Age Assessment, Dental Root, Chart Color

#### F39 Identifying Bitten Victims: A Case Report of Child Abuse in Brazil

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After attending this presentation, attendees will understand and appreciate how bitemarks can contribute to child abuses investigation.

This presentation will impact the forensic science community by serving as a reference for those dental practitioners and other experts who may be requested to provide a testimony before the court where bitemarks can be the main crime evidence.

Bitemarks in children represent child abuse until proven differently. They are rarely accidental and are good indicators of genuine child abuse. Human bitemarks are identified by their shape and size. They have an elliptical or oval pattern containing tooth and arch marks.

The goal of this presentation is to report a child abuse case in Brazil. A seven-month-old Brazilian female infant was admitted to the Medical Center Pediatric Intensive Care Unit with multiple injuries. A pattern of suspected abuse was established and Child Protective Services was notified. After clinical examination, numerous ovoid and circular pattern injuries were observed by the physician who recognized these as human bitemarks. She immediately notified the forensic odontology department to request a forensic bitemark examination and photographic documentation of the injuries.

There were more obvious bites inflicted to the leg and chest. There were fifteen other bites noted elsewhere on the child's body. It was determined that the bite on the leg would be the most useful as evidence for comparative purposes.

A two year-old male child suspect was indicated. After evaluation and comparison of dental arches with the injuries, the offender was excluded from the possibility of having caused the injuries. He had an anterior open bite and incompatible arches with the teeth marks. Four new suspects were presented by police; two neighbors and the parents of the child. Stone dental casts were made of each person's teeth.

With the use of an imaging software, overlays of the biting edges of the 12 anterior teeth were made for comparison to life-sized images of the bite mark. Among the suspects, the mother was indicated as the responsible individual and during the trial, she confessed the crime.

Bitemarks are found in a significant number of child cases. Most reported cases are the result of attack bites and are recognized and documented only when the victim is examined by a medical examinercoroner.

Emergency room personnel, family practitioners, and law enforcement personnel can identify and preserve bitemarks in living victims. Bitemark identification entails several cognitive steps – recognition of the wound, documentation, and interpretation. Early recognition is critical if valuable evidence is to be preserved in child abuse cases. Successful bitemark identification is dependent on a high index of suspicion. Unlike most other crimes against persons, there may be no scene evidence whatsoever, aside from the victim.

The Brazilian experience shown that early recognition of bitemark evidence and its significance in suspected child abuse is possible, and successful prosecution probable, when the primary health officer is alert and responsible. Bitemarks cases have increasingly occurred in Brazilian territory and a lot of cases have been successfully resolved. **Forensic Odontology, Bitemark, Child Abuse** 

#### F40 Forensic Dental Aspects of Bitemarks in Food Caused by Dental Prostheses

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After attending this presentation, attendees will understand and appreciate how bitemarks caused by dental prostheses can be successfully used for crime investigation.

This presentation will impact the forensic science community by serving as a reference for dental practitioners and other experts who may be requested to provide testimony before the court that bitemarks can be the main crime evidence, especially when caused by a dental prostheses user.

Bitemark analysis constitutes the most common form of dental evidence presented in criminal court. Bitemarks tend to have a double horseshoe pattern showing the six anterior teeth of the upper jaw and the corresponding six teeth in the lower jaw. Those made in food are usually well defined; bitemarks made in flesh are usually less well defined. Bitemarks can be left by human teeth, animal teeth, or objects that mimic teeth, like dental prostheses, which can produce a bite impression.

Bitemarks may be found at the scene of a crime and their analysis has been used for many years as an aid in forensic investigation. Investigation of bitemarks in foods may be an important part of a criminal investigation to include or exclude suspect.

Cases have included bites on apples, cheese, and chocolate bars, and have been associated with successful convictions. Given this information, along with other relevant evidence, the judge or jury is likely to find that the perpetrator of the bite also committed the rape, murder, or other criminal act.

The goal of this study is to evaluate the identification viability of bitten foods by dental prostheses.

The sample was composed for 10 (ten) dental prostheses pairs, produced in a laboratory and mounted in a joint occlusion device. A sample was set randomly created and bites produced in foodstuffs (four chocolates, four cheeses and two bananas), without the knowledge of the researcher.

From suspects study casts of the upper and lower jaw were taken. The registration of the casts performed with an articulator. A metric analysis technique was employed involving the measurement of the diameter of each tooth in the food models and prostheses, using one digital measuring device.

Bitemarks in foodstuffs were investigated making a positive model of the impressions using plaster. Study casts of suspects were used for pattern-associated comparison of the bitemarks.

It was possible to positively identify the biter in seven of the assessed cases. However, in cheese samples 1 and 2, and chocolate 3 it wasn't possible to identify the biter, but to exclude 8, 5, and 8 suspects

respectively. The results suggest that the bitemarks in food stuffs, produced by prostheses, may be possible.

Forensic Odontology, Bitemark, Dental Prosthesis

#### F41 A Comparison of Bitemarks in Vital Tissue

John P. Demas, DDS\*, 8814 Fort Hamilton Parkway, Brooklyn, NY 11209; and Joann M. DeLeonibus, DMD, 139 Clinton Street, Brooklyn, NY 11201

After attending this presentation, attendees will appreciate the predictability, or lack thereof, of a given dentition to transfer its impression (i.e., bitemark) consistently to vital skin.

This presentation will impact the forensic science community by demonstrating the need for further research into both the response of human skin to biting and the relative certainty with which odontologists ascribe bitemarks to individual dentitions.

It has, it seems, been accepted by the forensic odontology community with regard to bitemark pattern injuries that the pattern (impression) the anterior dental arch (be it maxillary or mandibular) leaves in wax or styrofoam or a similar inert medium capable of recording the shape of the incisal edges/cusp tips and the positional relationship of the teeth to each other is also the pattern which is left in human skin when a bite is inflicted and that the pattern is identifiable. It is also accepted technique that hollow volume overlays created using computer programs (Adobe Photoshop) are used to identify, rule in/rule out suspected/potential biters by comparing (i.e. overlaying) the overlay to the photographed pattern injury.

Research performed on cadaver models over the past several years has shown that distortion of skin, position of bite (relative to Langer lines), non-uniform height of the teeth, and other not yet identified factors all contribute to the pattern which is left on skin and that this pattern is not necessarily identical to the overlays or the patterns left in inanimate recording media.<sup>1,2</sup> Additionally, it has been shown that positional distortions of bitemarks occur.<sup>1</sup>

This project was undertaken to evaluate a very small number of bite injuries made in vital tissue by actual teeth (i.e., not dental casts).

Multiple bites from a single biter (not casts) were inflicted upon a vital subject, these were photographed, a cast of the maxillary arch of the biter was made, and a hollow volume overlay was made (photography, cast fabrication, and overlay creation all as per ABFO guidelines). The overlays were compared to the bitemark images for similarities and dissimilarities.

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Forensic Odontology, Bitemark, Vital Human Skin

### F42 Readability of Oral Radiographic Age (Bone and Dental Age) to Determine Chronologic Age: Preliminary Results on an Italian Population

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After attending this presentation, attendees will appreciate the importance of the use of oral radiographic age to determine the chronologic age in critical forensic cases, especially those where young people are involved.

This presentation will impact the forensic science community by presenting a different method to determine the chronologic age is proposed in critical forensic population.

It is always an ongoing task to establish the chronological age of unknown individuals who are guilty or under suspicion for a specific crime. In these cases the judge need to ascertain the exact age to proceed in the trial, especially when young people are involved. The most used method to study the maturation degree is to consider the bone age, which means the examination of the shape and the mutual position of the bones that can be observed in different development degrees at different ages. But it is well known that the correspondence between skeletal and chronological age is very rarely established appropriately. The aim of this research is to evaluate if Oro-Cervical Radiographic Score (ORS), obtained by the combination of cervical vertebral maturation method and dental age, correlates with chronological age (CA) in an Italian sample.

**Material and methods**: 60 Italian individuals (21 males, 39 females) from 8 to 25 years old, divided respectively into three groups according to their chronological age (G1=8-14; G2=14-18, G3=18-25), were enrolled. Two different researchers, blindly, retrospectively examined panoramic X-rays (OPT) and lateral cephalometric radiographs (LCR) already taken for orthodontic reasons. Chronological age (CA) of the individuals was already known. Radiographic exams have been selected according to quality and presence of all left lower elements. The ORA was determined for each participant combining Demirjian's method for dental age calculation,<sup>1</sup> third molar development for age estimation,<sup>2</sup> and **c**ervical vertebral maturation method for skeletal age calculation (CVM)).<sup>3,4</sup> A liner regression model was used to evaluate the correlation between ORS and CA.

**Results:** The mean CA was 15.74 years (Standard Deviation (SD) = 4.80; range 8-25 years), while the mean ORS was 2.71 years (SD=1.93: range 0-5). There was a significant correlation between the ORA and CA (Slope=0.213, p<0.001, R-squared= 0.24). The correlation remained significant when the sample was stratified by sex (Female: Slope=0.23, p<0.001, R-squared= 0.356 - Male: Slope=0.2, p<0.05, R-squared= 0.07).

**Conclusions:** In this sample, ORS correlates with CA and can be a useful tool for forensic medicine. Maturation stage of left lower third molar is mandatory when determining the age of subjects older than 14 years, but this evaluation can be influenced by several individual variations (i.e., agenesia, malformations, impaction). The introduction of CVM can add more information especially for those individuals with the third molar missing. Further studies must be carried out to enlarge the sample and to determine the influence of many possible confounding factors (i.e., race, socio-economical status, nutrition).

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Forensic Radiology, Oro-Cervical Radiographic Score, Chronological Age

#### F43 Morphometric Analysis of Third Molar Development: A Comparison of Albanian and Italian Sample Populations

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The goal of this study was to investigate the differences between third molar root development in Italian and Albanian populations when determining the age of adults.

This presentation will impact the forensic science community by underlining the importance of using morphometric analysis in age estimation while also taking into account the differences that exist between various ethnic groups

**Introduction:** Determination of adult age by tooth analysis is an important issue in forensics and has significant implications in determining criminal liability. It also plays a critical role in issues regarding young illegal immigrants and refugee children. Moreover, the results of such analyses play a substantial role in areas related to school attendance, social benefits, adoption procedures, employment, and marriage as related to international protections guaranteed by the United Nations High Commissioner for Refugees (UNHCR).

The study was conducted on digital orthopantomographs (OPG) and was based on identification criteria using morphometric analysis with the goal of overcoming the limits associated with using morphological analysis alone. This investigation also served to verify the existence of differences in third molar development among Italian and Albanian populations.

The goal of the study was to test the possibility of applying the results of a previous morphometric analysis conducted on an Italian sample population (Forensic Sci. 2008 Jul; 53(4): 904-9) to an Albanian sample population: Albanians are the second most populous ethnic group in Italy after Italians.

**Materials and Methods:** OPGs were obtained by systematic digital analysis using specialized dental software. In the first phase of the study, the confidence intervals obtained from the Italian sample were

applied to a sample of 140 Albanians whose OPG ages were between 16 and 19 years. This was done in order to ascertain the feasibility of using these confidence intervals. Preliminary results revealed the risk of age overestimation in 15% of the sample when these confidence intervals were applied to the Albanian sample. As a result, another analysis was carried out on a sample of 100 third molars with fully developed roots, all belonging to Albanians, in order to investigate the existence of a constant crown-to-root ratio (C/R).

After recording the measurements, a statistical analysis aimed at identifying the arithmetic mean of the ratios, and standard deviation was carried out. This resulted in an average C/R ratio value equal to 0.560, slightly higher than the average values obtained from the Italian population, and a standard deviation of 0.07.

In the second phase of our study, 494 third molars were analyzed with developing roots belonging to Albanian individuals (from Tirana and Valona) aged between 16 and 19 years, and subdivided by sex. The values obtained from the first phase of the study were used in the subsequent phase, thus establishing that when the crown height (h) of the third molar, with developing roots, is known, the root length (lt) of the molar, when it is fully developed, may also be known.

After establishing the length of the developing root, and based on the constant ratio established in the first phase, the lengths of the incomplete roots (ir) were calculated and the complete roots according to growth estimates (cr). Final Ratio (fr): ir/cr incomplete root/complete root beginning with root size values, a preliminary macro identification was made for both sexes that distinguished minors from those whose ages were 18 years or older. Analysis was made with 90%, 95%, and 99% confidence intervals, obtaining two limit values (lower and upper) of the ir/cr ratios in subjects under 18 (i.e. 16 and 17 years old), and over 18 in our sample, according to sex.

A comparison of the results obtained from the two populations was carried out.

**Results**: The Albanian sample showed higher developmental asymmetry of the left and right inferior third molars than the Italian sample. The Albanian sample showed a higher crown-to-root ratio as well as a higher growth index than the Italian sample. There was not an over estimation of the predicted root length in any of the cases. This is an important consideration when seeking to avoid the risk of over-estimating age. **Age Estimation, Third Molar Development, Forensic Odontology** 

#### F44 Carbon Isotope Analysis of Dental Enamel Provides Precise Birth Dating and Clues to Geographical Origin

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After attending this presentation, attendees will understand how dental enamel produced in the past 55 years can be dated using the radiocarbon bomb-pulse. Attendees will also learn how the carbon-14 (14C) content of dental enamel can be used to determine year of birth of persons born after 1942.

The presentation will impact the forensic science community by showing how isotopic carbon analysis of enamel offers a precise age determination with geographic information that can be applied in forensic casework, particularly to assist in investigations of unidentified human cadavers.

Determining the age of an individual is an important step in identification and a common challenge in forensic medicine. Age

determination can be performed with high precision up to adolescence by analysis of dentition, but establishing the age of adults has remained difficult. The enamel of individual permanent teeth is formed at distinct, well-characterized time points during childhood. After formation there is no turnover of enamel, and its <sup>14</sup>C concentration reflects the level in the food at the time of enamel formation. Atmospheric testing of nuclear weapons doubled the global <sup>14</sup>CO<sub>2</sub> level between 1950 and 1963. After cessation of atmospheric tests in 1963, the level of atmospheric <sup>14</sup>CO<sub>2</sub> started to decrease exponentially with a mean life of about 16 years due to transport into large carbon reservoirs such as the oceans. The enhanced level of <sup>14</sup>C worked its way up the food chain from CO<sub>2</sub> so that all living things are labeled with the pulse.

The concentration of <sup>14</sup>C in tooth enamel was measured of 95 teeth from 84 individuals from around the globe and related it to the known concentration in the atmosphere from 1955 to present to establish the time of tooth formation. The use of the stable isotope <sup>13</sup>C was investigated and used as an indicator of geographical origin of the individual. Using established ages of tooth formation, the dates were then used to estimate the year of birth of the person. The crown of the tooth was cut away from the root at the level of the cervical line. The crown was then immersed in 10N NaOH, and placed in a water-bath sonicator. The enamel was then washed with DDH2O and re-submersed in 10N NaOH every 24 hrs for 3-5 days until only enamel remains. Samples were rinsed with DDH2O and shipped overnight for isotope analysis. Upon arrival enamel samples were pretreated in 1.0N HCl for 1 h, rinsed 3 times with DDH<sub>2</sub>O and placed on a heating block at 95°C to dry overnight. Enamel splits were hydrolyzed to CO<sub>2</sub> in individual reaction chambers, evacuated, heated and acidified with orthophosphoric acid at 90°C. The evolved CO<sub>2</sub> was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors. Graphite targets were measured for <sup>14</sup>C content by accelerator mass spectrometry (AMS).

The technique of analysis of <sup>14</sup>C content in enamel matched known age during the rising part of the pulse (1955-1963, N=12) and after the peak (post 1963, N=66) with average absolute errors of  $1.9 \pm 1.4$  and 1.3 $\pm$  1.0 years, respectively. Geographical location had no effect on the precision of <sup>14</sup>C enamel birth dating. Much of the variability can be attributed to inter-individual differences in tooth formation and possible variations in carbon food sources at the time of enamel formation. Enamel formed prior to 1955 contained no <sup>14</sup>C elevation above atmosphere at the time in 16 of 17 cases. Analyzing multiple teeth with different formation ages from a single individual can place date of birth on the ascending or descending side of the anthropogenic <sup>14</sup>C spike and improve the temporal precision. In 46 teeth, measurement of <sup>13</sup>C was also performed. Scandinavian teeth showed a substantially greater depression in average  $\delta^{13}C$  (-14.8) than teeth from subjects raised in Japan (-13.5), Middle East and North Africa (-12.7) and South America (-10.9). The differences in  $\delta^{13}$ C are due to differences in plants and diets in the different regions and thus can provide important information about the geographical origin of an individual.

Isotopic carbon analysis of enamel offers a precise age determination with geographic information that can be applied in forensic casework, particularly to assist in investigations of unidentified human cadavers.

Date of Birth, <sup>14</sup>C Bomb Pulse, <sup>13</sup>C

#### F45 Demonstration of the Fishman Method of Evaluating Hand-Wrist Radiographs and Its Forensic Application

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After attending this presentation, attendees will see a demonstrated technique for evaluating hand-wrist radiographs for the purpose of evaluating skeletal maturity. This method is commonly used in the field of orthodontics to estimate peak growth.

This presentation will impact the forensic science community by sharing a method commonly employed in the field of orthodontics and discussing its value as applied to age estimation for forensic purposes.

The hand-wrist film is an important diagnostic tool for predicting skeletal maturation. There are a number of methods used to assess skeletal maturation from these images.

In the 1930s, Professor T. Wingate Todd published an atlas of skeletal development with hand wrist radiographs. Following in 1959, Greulich and Pile revised the hand wrist atlas. This atlas is still in use today. During the 1970s and 1980s, several papers were written with the goal of synthesizing the data to a uniform and easily managed assessment. The Fishman analysis was first published in 1982. This method has since been utilized by orthodontists to predict peak pubertal growth.

The Fishman approach is easy to utilize. It reviews six anatomical sites. From these sites, four developmental categories are evaluated. The result is eleven stages of skeletal maturation. Assigned to each stage of skeletal maturation is an age range with standard deviation as seen in both males and females. The process of determining sites, assigning developmental categories and staging the image will be demonstrated.

Applying this method to hand wrist radiographs for forensic age estimation not only provides an average age for adolescents for the particular stage but will provide an age interval and confidence interval. **Forensic Odontology, Age Estimation, Hand-Wrist Radiographs** 

### F46 The Whole Tooth and Nothing But the Tooth – A Case Report

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Traditionally, forensic odontologists have been called upon to match a decedent to postmortem dental remains. However, in this interesting civil malpractice case, the forensic dental team was asked to prove just the opposite. The goal of this presentation is to highlight how, with the use of computerized tools, the forensic dentist can rapidly and cost effectively prove the provenance of extracted teeth.

On a daily basis in medical examiner's offices throughout the United States, forensic dentists are typically called in to examine postmortem remains, chart them, and take radiographs. This presentation will impact the forensic science community by demonstrating how forensic dentistry, along with fingerprinting and DNA, are the significant modalities for identification in mass disaster situations as well. Each one of these disciplines can be used for identification. In many cases, all of these modalities may be incorporated into the identification process.

In this civil malpractice case, the forensic dentist was presented with three extracted third molar teeth and asked to verify that they were indeed the teeth extracted from the plaintiff. According to the plaintiff's attorney, he was given these three teeth by an auxiliary working in the oral surgeon's office post extraction. They were wrapped in gauze, paper During depositions, prior to any contact with the forensic dentist, the oral surgeon presented his four-page medical treatment record of the extraction of three third molars. A detailed account of the "sectioning" of the two lower third molars was included in this record. The plaintiff then produced the three "whole" third molars in his possession. The oral surgeon challenged the origin of these teeth and claimed that these were not the teeth he extracted. Subsequently, the teeth were presented to the forensic dentist for examination, photography, and radiography. The attorney for the plaintiff also provided a panograph of his client taken just prior to the extractions. The plaintiff presented for upper and lower post surgical impressions of his dentition.

The use of computerized tools in conjunction with accepted forensic odontology techniques positively identified the three whole, unsectioned teeth as those belonging to the plaintiff. The plaintiff's attorney, armed with this information, consulted the forensic odontologist with regard to using DNA as an additional modality for positive identification. The trial commenced with the forensic odontologist appearing as the expert witness in the case.

Antemortem, Postmortem, Odontology

# F47 The Forensic Odontologist Role Beyond Identification and Bitemarks

Richard R. Souviron, DDS\*, Miami-Dade County Medical Examiner's Office, Number One on Bob Hope Road, Miami, FL 33136

After attending this presentation, attendees will learn of the expanding field of forensic odontology beyond identification and bitemarks, will learn age, race, sex and trauma determinations.

This presentation will impact the forensic science community by documenting the effects of dental aging vs. actual age and the relationship to long term neglect, malnutrition, and abuse.

An expanding of the knowledge of forensic dentistry in a shifting of the paradigm to include oral trauma evaluation as to time of injuries. The dental aging of a victim can be documented scientifically to prove the affects of long term malnutrition and starvation.

The forensic odontologist usually works under the direction of the coroner or chief medical examiner and is required to assist with body identification where other means of identification are not possible or practical. Examples of such cases are the obvious-skeletal remains, non-viewable bodies, decomposed, incinerated or mutilated victims. The teeth and oral structures are an accurate, inexpensive and usually rapid means of confirming an identification. In addition the forensic odontologist is called upon to assist in pattern injury interpretation primarily, bitemarks.

There are cases where the odontologist and medical examiner need to think beyond the obvious "think outside the box." A good example of this is the case of "baby lollypop." The body of this child was discovered November 2, 1990 on a trash pile outside a home in Miami Beach. Medical Examiner Case#90-3091 Unk.-W/M. The Medical Examiner called for odontology evaluation not just for dental records but for age estimation, tooth loss pattern, natural or traumatic and an opinion as to oral trauma. This clearly went beyond just dental records for comparison purposes. The victim was subsequently identified as Lazaro Figueroa a 3 year one month old white male. The victim was missing two maxillary deciduous central incisors, the maxillary and mandibular frenums were missing and were replaced with scar tissue. Dental x-rays of the pre molars were compared with dental aging charts. It was determined that this individual was two years of age. Not three years, one month, which was his chronologic age. The dental injuries and dental development assisted the medical examiner and prosecutor to prove to a jury that this child had suffered from severe malnutrition over a long period and that the traumas had been inflicted periodically over the years.

Odontology, Paradigm, Dental Aging

## F48 Homicide in South Carolina - NCIC and NDIR

Delora L. Fletcher, DDS\*, 6140-303 Calle Mariselda, San Diego, CA 92124

After attending this presentation, attendees will have an understanding of the value of detailed gathering of antemortem data and input into two FBI databases: (1) Missing Persons file, begun in 1975, of the National Crime Information Center; and (2) National Dental Image/Information Repository, established May 2005.

This presentation will impact the forensic science community by reviewing a criminal case and how forensic odontology can contribute to resolution. As with any database, the usefulness is enhanced with proper data input.

On September 3, 2008 a 31 year-old Registered Nurse, Aretha "Maria" Fernandez, finished her shift at a nursing home in the city of Union, South Carolina leaving to meet her boyfriend, Jamal Good. They had resided together with their five year old son at a home owned by Good's father. Recently, they had separated and Maria was looking to purchase her own home.

She was reported missing to the Union County Sheriff's department three days later by her mother. The investigating detective entered an initial report into the FBI Criminal Justice Information Services (CJIS) Missing Persons file in the National Crime Information Center (NCIC).

The boyfriend, an independent self-employed truck driver, drove routes along the east coast. He, or anyone in his family, refused to speak to the Sheriff's department without an attorney present from the very beginning of the investigation, raising suspicions. The case had potential to go across jurisdictional boundaries.

In October 2008, the NCIC system generated a \$.K message to the originating agency regarding the case. A \$.K message is a reminder that additional information is needed for the file. The detective, along with a mentor, determined that dental records may be the missing information. Ms. Fernandez had recently been seeking to improve her life, upgrading her education from a licensed practical nurse to a registered nurse just one month before she went missing. She had also undergone orthodontic treatment, completing the treatment one year prior. The orthodontist attempted NCIC dental characteristics coding using the NCIC Missing Person File Data Collection Entry Guide. Unfortunately, the system would not take the coding.

Requesting the assistance of a trained odontologist, the investigating detective obtained additional records from Maria's general dentist. Recoding the dental characteristics, plus digitizing the necessary records for the National Dental Image/Information Repository (NDIR), prepared the data for input to the FBI's databases. The NDIR, established May 2005, simplifies retrieval of dental information to facilitate the analysis of reports generated by NCIC. When a possible match between Missing Person files and Unidentified Person files by computerized query between the databases in NCIC occurs, a \$.M message is sent to the originating agencies. Comparing cases between agencies located in different jurisdictions, the NDIR speeds up the process of identifications. The NDIR records include: (1) the Missing and Wanted Person Submission Form; (2) digitized NCIC record; (3) NCIC Person Dental Condition Worksheet; (4) NCIC Person Dental Report Form; (5) digitized treating dentist's treatment records; (6) digital scans of all available radiographs; and (7) miscellaneous records such as scanned dental models or photographs.

In this particular case, additional challenges due to a software glitch in the state's feeds to NCIC terminals again thwarted efforts to upload to the FBI database. The NCIC dental characteristics codes are V, /, X, M, O, D, F, and L. At the terminal, the operator saw the codes A, F, G, I, P, X and numbers 0-9 for the tooth surfaces.

Meanwhile, investigations continued into the mystery of Maria's whereabouts. Her remains were found January 7, 2009 near train tracks in a wooded area in Union County. A utility worker looking for fallen lines came across her partial skeletal remains. She was identified through dental records. Cause of death could not be determined and was officially declared unknown. A pathologist testified that there were no signs of trauma to the bones.

Jamal Good was arrested outside his home on February 9, 2009 and charged with the murder of Maria Fernandez. He was held without bail due to flight risk. The trial took place in December, 2009. The jury deliberated less than 45 minutes, returning a murder conviction. He was sentenced to life in prison without any possibility of parole.

Missing Person, NCIC, NDIR

## F49 Campfire Murder – Identification From the Ashes

William C. Rodriguez III, PhD\*, Armed Forces Medical Examiner's Office, 1413 Research Boulevard, Building 102, Rockville, MD 20850

The objective of this presentation is to provide details on the investigation, recovery, and examination of burnt and highly fragmented human remains which were utilized in the identification of a serial murder victim that of a nine year old male. Examination procedures involving sorting fragmented and burnt human elements will be discussed in addition to identifying specific features of both dental and skeletal remains which provided identification of the victim.

This presentation will impact the forensic science community by providing present and future forensic investigations insight as to the possibilities of forensic analysis of highly burnt human remains. The forensic community will benefit from the knowledge that even very small fragmentary remains both dental and skeletal can provide useful information in reference to the identity of a victim as well as the type of fire and temperatures involved in their breakdown and destruction.

Reconstruction of highly burnt and fragmented remains of a body is one of the most difficult tasks in forensic investigation. As the result of exposure to an extremely hot fire many time all that remains of a body is calcined bone and tooth remnants. Proper recovery of such remains requires much care during the collection process in addition to treatment of the remains with various polymers to insure their preservation.

The case to be presented involves the kidnapping and brutal murder of Dylan Groene, a nine year old male, who was kidnapped along with his eight year old sister, Shasta Groene. On May 15<sup>th</sup>, 2005 a previously convicted sex offender Joseph E. Duncan III along with a female accomplice broke into the Idaho home of Dylan and Shasta Groene. Duncan killed the mother, older brother and mother's fiancé of Dylan and Shasta, and abducted both of the children.

Duncan and his female accomplice evaded law enforcement efforts to arrest them until July 2, 2005 when they were apprehended. Shasta Groene was still in their company but Dylan was missing. An interview of Shasta by law enforcement revealed her younger brother had been murdered earlier by Duncan while they were staying at a campground. According to Shasta, Dylan had been repeatedly sexually and eventually shot and killed by Duncan. In order to dispose of the body, he put Dylan in the campfire and burnt his remains while Shasta watched. Duncan threw the burnt bones and teeth down a nearby gutter.

FBI agents later conducted a search of the campground and gutter which led to the recovery of numerous calcined bone and tooth fragments. Forensic examination of the remains by staff of the Office of the Armed Forces Medical Examiner revealed the hard tissue material recovered to consist of a total of 1752 bone and tooth fragments weighing approximately 424 grams. An inventory of the fragments revealed 37 to be dental, 126 to represent cranial portions, and 1589 to represent postcranial or undetermined fragments. Anthropological study found no evidence of anatomical duplication indicating a minimum number of individuals of one. Several unfused epiphyseal surfaces were observed within the assemblage as well as shallow tooth crypts of mandibular and maxillary fragments, indicating a sub adult individual.

Odontological examination of the dental remnants revealed the presence of deciduous and developing adult dentition. Based upon the dental development of the tooth remnants an upper and lower age estimation was established which was consistent with the age of Dylan Groene at death. The condition of the dental and skeletal fragments indicated they had been exposed to temperatures in excess of approximately 1400 degrees Fahrenheit. The degree of fragmentation and burn patterning exhibited by the remains was noted to be the result of continual stoking in a campfire.

As a result of the forensic evidence presented in reference to the murder and burning of the body of Dylan Groene, Joseph Duncan was sentenced to death on August of 2008. In total, he received three death sentences and nine life sentences for the Idaho crimes.

Anthropology, Burnt Remains, Odontology

### F50 Report on Results of Questionnaire of American Society of Forensic Odontology Members

Edgar W. Turner, DDS\*, 410 Farino Way, Somerville, TN 38068; Barbara L. Needell, DMD\*, Den-Care West, 5280 North University Drive, Lauderhill, FL 33351; Harry H. Mincer, DDS, PhD, University of Tennessee, 875 Union Avenue, Memphis, TN 38163; and Mark Scarbecz, PhD, 875 Union Avenue, Memphis, TN 38163

After attending this presentation, attendees will learn how members of the American Society of Forensic Odontology responded to a questionnaire about their participation in various aspects of forensic dentistry.

This presentation will impact the forensic science community by reporting results for an online survey performed in order to learn how our colleagues are providing forensic odontology services.

**Introduction:** The purpose of this survey was to determine the activities of American forensic dentists in key areas of forensic odontology such as human identification, bitemark analysis, civil litigation cases, and age estimation.

**Materials and Methods**: Members of the American Society of Forensic Odontology who are forensic dentists practicing in the United States were surveyed in 2009 using Surveymonkey.com, a commercial website for survey deployment. Analysis: participants were categorized by whether members were board certified, geographic region of practice in the U.S., year of graduation from dental school, and year of board certification. Responders who did not specify a home state were excluded from additional analysis. Outcome variables of interest were the mean number of identification cases, bitemark cases, civil litigation cases and the number of age determination cases performed in the previous twelve months and in the past five years. Identification cases were further divided into those in which the responder performed the resections, those in which medical examiners (or technicians) performed resections, and those in which no resection was done. p<.05 indicates a statistically significant difference in means between groups.

**Results:** There were 135 respondents to our survey. Sixteen were diplomates of the American Board of Forensic Odontology, 116 were not board certified; 3 did not answer. 26 responders were board eligible, 96 were not and 13 did not answer. 33 responders were from the Northeast United States, 20 from the Midwest, 17 from the Southeast, nine from the Midsouth, and 14 from the West. Forty-two gave no home state. There

were 18 responders who graduated from dental school from 1990-2009, 36 from 1980-1989, 38 from 1970-1979, and 14 prior to 1970, with 29 non-responders.

The mean number of identification cases performed in the previous five years for board certified responders was 134.75 (n=15) and 30.09 (n=75) for non-certified forensic dentists (p<.05). Regarding identification cases in the last 12 months, the means were 32.87 (n=15) for board-certified respondents and 6.00 (n=76) for non-certified (p<.05). The mean number of bitemark cases performed in the last five years was 3.73 (n=15) for board certified dentists and 1.22 (n=74) for non-certified (p<.05). In the previous 12 months, the means were 2.07 (n=15) and .43 (n=74)(p<.05). The mean number of civil liability cases in the last five years for board certified forensic dentists was 6.40 (n=15) and 2.40 (n=75). For the previous year, the means were 2.00 (n=15) and 0.51 (n=75) cases. For age estimation cases done in the previous 5 years, the mean for board certified dentists was 26.00 (n=14) and 2.257 (n=72) for non-certified (p<.05). For age estimations made during the prior year, means were 27.29 (n=14) for board certified and 0.44 (n=73) for noncertified forensic dentists (p<.05).

More detailed statistics and analyses—as a basis for subsequent reports will be presented. This information as to what, how and where our work is being accomplished will hopefully be useful to ourselves and to other forensic professionals in determining the future directions of forensic odontology.

Survey, Odontology, Report



### **PATHOLOGY/BIOLOGY**





#### G1 Fatality Involving Complications of Bupivacaine Toxicity and Hypersensitivity Reaction: A Case Report

Mary H. Dudley, MD\*, Jackson County Medical Examiner's Office, 660 East 24th Street, Kansas City, MO 64108; Steven W. Fleming, MS, Children Mercy Hospital, Toxicology Laboratory, 2401 Gillham Road, Kansas City, MO 64108; Uttam Garg, PhD, Children's Mercy Hospital, Department of Pathology, 2401 Gillham Road, Kansas City, MO 64108; and Jason M. Edwards, PhD, University of Missouri Kansas City School of Medicine, 2411 Holmes Street, Kansas City, MO 64108

After attending this presentation, attendees will understand the complications from use of bupivacaine local anesthesia when administered in the head and neck region, including CNS, cardiac sequelae, and death.

This presentation will impact the forensic science community in understanding the possible adverse effects of local nerve block anesthesia when administered in the head and neck region, the actions, pharmacokinetics, contraindications, and toxicity of bupivacaine are detailed. In addition a hypersensitivity reaction may result from bupivacaine as measured by postmortem tryptase. The importance of a complete forensic autopsy and forensic toxicological analysis to accurately certify the cause and manner of death is emphasized.

This case represents unusual findings of elevated bupivacaine and tryptase concentrations following local anesthetic, bupivacaine, administered as a scalene nerve block for elective rotator cuff repair surgery. The patient exhibited almost immediate seizure activity, bradycardia, and cardiac arrest following bupivacaine injection. Resuscitative efforts including cardiopulmonary bypass restored a cardiac rhythm. However, the clinical medical status of the decedent progressively declined and he died seven hours following administration of the local anesthetic. An autopsy was performed and various biological specimens were collected for toxicological analysis. Autopsy revealed several abnormalities of the heart including cardiomegaly, myocardial bridging, and lipomatous hypertrophy of the intra-atrial septum. The cardiac findings may have contributed to bradycardia and arrhythmia. Autopsy findings associated with hypersensitivity reactions such as urticaria or laryngeal edema were not observed at autopsy. The absence of these findings alone does not rule out a suspected case involving a hypersensitivity reaction.

Postmortem toxicology results revealed an elevated cardiac bupivacaine and tryptase concentration. An elevated concentration of bupivacaine in the blood taken seven hours post-injection is indicative of an intravascular injection. When taking into account that the patient was alive for seven hours post-injection of bupivacaine and the half-life of bupivacaine is about two hours, it was estimated that the subclavian blood concentration of bupivacaine was most likely much higher at the time of seizure activity than at the time of sample collection. However, the postmortem cardiac blood analyzed had a similar bupivacaine concentration at the time of seizure activity due to intraventricular blood stasis resulting from cardiopulmonary bypass for approximately five hours.

Patients receiving local scalene nerve block anesthesia that is in close proximity to the carotid artery may be at greater risk of CNS and cardiac toxicity due to a greater risk of inadvertent intravascular injection or an injection into a highly vascular tissue area. This would result in rapid absorption of the local anesthetic into the systemic circulation causing cardiac and CNS sequelae. Therefore, this type of injection may increase the risk of adverse effects including seizures, bradycardia, and cardiac arrest as seen in this case.

Postmortem toxicology also included analysis of tryptase. This analysis revealed an elevated cardiac total tryptase concentration and a normal subclavian total tryptase concentration. The discrepancy between the cardiac and subclavian tryptase concentrations may also be due to intraventricular blood stasis resulting from cardiopulmonary bypass; whereas subclavian blood was actively circulating throughout intervention. Furthermore, tryptase peaks within 15 to 120 minutes post exposure to the allergen and follows first-order kinetics with a half life of 1.5 to 2.5 hours; therefore, approximately 3 half-lives had elapsed between symptomatic onset and blood collection. Thus, obtained subclavian serum tryptase concentrations are expected to be much lower than values at symptomatic onset if in fact an anaphylactic reaction occurred. The moderately elevated cardiac tryptase concentration in conjunction with the cardiac arrest and rapid onset of seizure activity post injection of bupivacaine indicates the possibility of an anaphylactic reaction. However, it is possible that the moderate increase in cardiac tryptase is due to lysis of mast cells in the tissue of the chest. At autopsy the chest had massive hemorrhages due to prolonged cardiopulmonary resuscitation.

In summary this unintentional death of a 37-year-old male during elective shoulder surgery was determined to be due to complications of bupivacaine. The moderately elevated cardiac tryptase concentration raises the possibility of anaphylaxis that may have contributed to the cause of death.

Forensic Pathology, Bupivacaine Toxicity, Postmortem Tryptase

#### G2 Undiagnosed, Untreated Natural Disease Mistaken for Lethal Child Neglect: Liability of the Family in Determining Child's Death

Lucia Tattoli, PhD\*, Sabrina Leonardi, MD, and Biagio Solarino, PhD, Section of Legal Medicine - University of Bari, Pizza Giulio Cesare 11, Bari, 70124, ITALY

After attending this presentation, attendees will understand that in cases in which there is a potential component of neglect or abuse, careful scene investigation, review of medical records, complete autopsy with skeletal survey, toxicology, chemical and metabolic testing should be requested.

This presentation will impact the forensic science community by emphasizing the fact that even if lethal neglect is a rare cause of death in industrialized countries, natural disease being mistaken for child abuse is rare too. As a matter of fact there are many potential organic diseases which may mimic neglect or abuse and an appropriate histological examination of all organs should be undertaken to assist in ruling out organic disease.

A case is presented of a 4-month-old infant who was found unresponsive at home and transported at a local hospital, where he expired in the Emergency Department. Physicians noted child's cachectic state; the mother stated he had fever, vomiting, and diarrhea for seven days but she was afraid to seek medical care because was fearful of legal action against her. Further investigations revealed a completely inappropriate and inadequate diet of meat, homogenized milk, and oatmeal from his birth. The infant was never breastfed.
Crime scene investigation showed the extremely poor living conditions of the apartment where the 22-year-old mother lived with her parents and her sons. The family was occasionally followed by social care workers. The infant had never been followed by a pediatrician.

The child weighed 4,000 g and was 62 cm long. His clothing and bedding were urine-soaked and vomit-covered. Whole body radiographic examination showed no fractures. At autopsy, there were clear signs of malnutrition and dehydration, like skin tenting and wrinkled loose skin, sunken fontanels and ocular globes, depressed cranial sutures, focal alopecia, prominent ribs and bony planes, and dry serosal and mucous membranes. Partial lack of subcutaneous and deep fat deposits with a severe atrophy of skeletal muscles was found. Brownish material was found in gastrointestinal tract. There was a severe atrophy of skeletal muscles, heart, liver, spleen, and kidneys; the small intestinal wall appeared swollen, with reddish discolored mucosa.

Further histological examination showed a T-cell lymphoblastic massive infiltrate of the liver, kidneys, and other organs with multiple foci of bronchopneumonia in lungs, and sporadic evidence of aspiration. Immunocytochemical studies confirmed the diagnosis of acute lymphoblastic leukemia of childhood. Toxicological examination revealed no substances in blood or urine. The cause of death was attributed to an Acute Lymphoblastic Leukemia (ALL) – related cachexia, worsened by malnutrition and dehydration.

The ALL is rare under one year and the youngest infants (ages 0 to 6 months) have the worst outcome. At diagnosis of childhood ALL, anorexia-cachexia syndrome may occur, presenting with anorexia, weight loss, wasting of muscle and adipose tissue, hyperlipidemia, and other metabolic abnormalities.

In the case presented here, an early recognition with appropriate treatment of ALL would probably have given the child a chance of survival. In fact, despite the progressive improvements in outcomes achieved for the children treated on chemotherapy, the outcome is positive in less than 25% of cases.

Cases of suspected child abuse which ultimately are determined to result from natural diseases are extremely rare. Moreover, although it is important to suspect child abuse when the history and examination are consistent with the diagnosis, it is equally important to think of other potential diagnoses, considering legal medico-legal aspects related the liability of the parents in determining child's death.

Lethal Neglect, Acute Lymphoblastic Leukemia, Malnutrition

#### G3 Sudden Unexpected Cardiac Deaths: An Autopsy Based Study From Mangalore, South India

### Tanuj Kanchan, MD\*, Kasturba Medical College, Department of Forensic Medicine, Light House Hill Road, Mangalore, 575 001, INDIA

After attending this presentation, attendees will be able to identify with the pattern and trend of sudden cardiac deaths in a coastal region of South India.

This presentation will impact the forensic science community by developing an understanding of the burden of sudden cardiac deaths in the coastal region. This presentation defines the problem status of sudden cardiac deaths in the region and emphasizes the importance of forensic pathologists in diagnosing the same at autopsy.

Forensic pathologists deal not only with unnatural deaths, but also with a wide range of natural deaths, especially, if the death occurs suddenly in apparently healthy individuals. Cardiovascular diseases are reportedly the most important cause of sudden natural deaths. Sudden cardiac death is defined as death due to cardiac causes, heralded by abrupt loss of consciousness within one hour of the onset of acute symptoms, in an individual who may have known preexisting heart disease but in whom the time and mode of death are unexpected. This autopsy based retrospective research was conducted to determine the causes and the epidemiological aspects of sudden cardiac deaths in Mangalore, a coastal township in South India. The study was conducted at the Department of Forensic Medicine, Kasturba Medical College, Mangalore. All the cases recorded in the departmental file as sudden deaths from January 2005 to December 2007 were included in the study and autopsy case files of the same were studied in detail. The data was analyzed using statistical software.

During the study period a total of 1864 autopsies were conducted, of which 207 cases were classified as sudden unexplained deaths. Sudden cardiac deaths constituted of 39.6% of the total sudden deaths during the study period (n=82). Males were predominantly affected (91.5%). Age of victims varied from 19 to 80 years, mean age of the victims being 49.96 years. Majority of deaths were reported in the 5th and 6th decade of life. Mean BMI was 20.8 kg/m2. The monthly distribution revealed that most of the sudden cardiac deaths were reported in May followed by February. Weight of heart varied from 210 to 560 grams (Mean=335.4 grams). Coronary artery diseases remained the most common cause of sudden cardiac deaths followed by cardiac hypertrophy, cardiomyopathy, myocarditis, and valvular diseases. More than 50% occlusion of the coronary arteries was evident in approximately half of the cases. Left anterior descending artery was the most commonly affected. Atherosclerotic changes were observed in the great vessels in most of the cases.

Cardiac causes are responsible for most of the sudden deaths in this region and coronary artery diseases are responsible for most of the cardiac deaths. Atherosclerotic changes were observed in the great vessels in most of the cases in our study. Atherosclerosis is responsible for significant cardiovascular morbidity and mortality worldwide. Medicolegal autopsies are an important source of epidemiological data that should effectively be used in planning the preventive strategies. Modifying the stressful life style and screening those at high risk are the measures to be emphasized to prevent such deaths.

Sudden Death, Cardiac Death, Coronary Artery Disease

### G4 Periventricular Leukomalacia in a 2-Month-Old Infant Who Was Born With Cocaine Addiction: A Case Report

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After attending this presentation, attendees will be aware of the neuropathologic sequela in a 2-month-old infant born with addiction to cocaine. The prenatal history and the scenario surrounding the infant's death will be presented. Common outcomes of the effects of cocaine on the fetus and newborn are reviewed.

This presentation will impact the forensic science community by demonstrating that cystic periventricular leukomalacia is one of the irreversible neurological complications that directly or indirectly may occur in cocaine-exposed fetuses and the dilemma in considering the cause and manner of death of the similar cases will be discussed.

**Case presentation:** The decedent was a 2-month-old African American female who was born precipitously in an ambulance at 30 weeks gestational age to a 29-year-old mother who had a history of cocaine and marijuana abuse and used crack cocaine the day she gave birth. She had no prenatal care for this pregnancy. She had given birth to three live children, including the decedent, and all three were born addicted to cocaine. She also gave birth to one stillborn (female). This decedent's birth weight was 1,034 grams and length was 19 inches. She was diagnosed with cocaine addiction and respiratory failure, was on

mechanical ventilation for the first two days of her life, and stayed in hospital for 20 days before being discharged home. The discharge diagnosis includes bilateral periventricular leukomalacia. The infant was adopted by her biological mother's sister. The decedent's condition was stable and she was fed with formula. At 20:04 on the day of the incidence, when lying supine on the couch in the living room with her adoptive mother, the decedent suddenly exhaled and stopped breathing. The adoptive mother started to gently rub the decedent's chest and the decedent still did not get breath. 911 was called and the decedent was transported to a hospital by ambulance with an admission diagnosis of cardiac arrest. CT scans/X-rays revealed a large right pneumothorax and pneumoperitoneum. An emergency chest tube placement and exploratory laparotomy were performed in the operating room. The decedent was taken to the intensive care unit and her condition deteriorated. Pronouncement was made at 13:40 the next day after the incident.

Autopsy findings included a poorly developed 2-month-old black female with less than 5<sup>th</sup> percentile of body weight and length. No traumatic injuries were identified. The lungs exhibited atelectasis. The brain exhibited bilateral cystic periventricular leukomalacia and severe hypoxic/ischemic encephalopathy. Accessory tests were noncontributory.

Discussion: Effects of cocaine on the developing central nerve system of a fetus may cause different pathologic changes, such as germinal matrix hemorrhage or cystic changes, introventricular hemorrhage, and periventricular leukomalacia. However, those changes are difficult to interpret as the sole consequence of the effects of cocaine because risk factors in cocaine abusing pregnant women tend to cluster together and interact, such as multiple drug use, poor maternal nutrition, lack of prenatal care, infectious disease, placental insufficiency, impaired fetal oxygenation, fetal intrauterine growth retardation, and premature birth. All the above CNS pathological changes can also be present in the premature newborn without intrauterine cocaine exposure. In addition, the premature infants with or without intrauterine cocaine-exposed tends similarly to be poorly grown, easily susceptible to infection and vulnerable for sudden infant death. Cause of Death: Although a definitive cause and effect relationship between these conditions and cocaine use is difficult to reach, the fact of intrauterine exposure of cocaine could not be ignored in this case. The cause of death was the complication of premature born with addiction to cocaine associated with cerebral cystic periventricular leukomalacia and severe hypoxic/ischemic encephalopathy. Manner of Death: Detailed history of the mother's cocaine abuse and the circumstances surrounding the decedent's addiction to cocaine at the time of birth were unclear, even though thorough investigation was performed and the death occurred two months after birth. In addition, constitutional issues may preclude criminal prosecution in many of these kinds of cases. The manner of death was classified as "Undetermined."

Cocaine, Fetus, Periventricular Leukomalacia

### G5 A Man Without a Head: Postmortem Decapitation by German Shepherd Dogs

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After attending this presentation, attendees will understand that in cases of postmortem animal depredation of human corpses or remains, physicians and crime scene investigators not experienced in the field of forensic medicine are often unable to attribute the questioned injuries to their true origin. This presentation will impact the forensic science community by emphasizing that postmortem injury by domestic animals is only rarely documented in the literature therefore lacking knowledge regarding morphologic features and the criteria for the differentiation of such postmortem soft tissue destruction may cause considerable complications in clarifying the cause of death. This is particularly true when postmortem animal depredation is caused by a domestic dog's activity.

A case of a 55-year-old man will be presented who was found putrefied in the bedroom of his apartment; head and neck of the body were missing. The rest of the body, in particular the hands, was intact. Also his well-feed two German shepherd dogs (8 and  $1^{1/2}$  years old) were in the flat. The flat was locked with the windows closed. He had been seen for the last time one week before the incident. The public health service had been called one year earlier because the man and his two dogs had not left the dwelling for eight weeks. Policemen were called to the scene as the dogs had been barking for four days. In the hallway and the living room they remarked several remnants of small supposed human bone parts and crowned teeth in puddles of feces and vomitus. The flat was in messy condition with garbage, emptied alcohol bottles, and moldy food in every room. Lots of accessible dog food was also found. The dogs are brought to pet asylum, and an autopsy of the incomplete body is ordered. Autopsy showed a fatal gastrointestinal bleeding by rupture of esophageal varicose veins (while head and neck were still missing). Furthermore, signs of chronic alcoholism could be determined. Toxicological examinations led to no specific findings. At postmortem, animal depredation signs, canine-like bite traces and tissue defects were found surrounding the collar region. The right pleural cavity was opened by animal depredation; parts of the right pulmonary lobe were missing as well as the cervical vertebral bodies 1-6. The clavicles, the scapulae and 7th cervical vertebral body showed extensive gnawing traces. After autopsy, the apartment was searched again for head and neck of the man by forensic scientists and police; still the missing parts could not be found. From forensic point of view, it must be presumed that the dogs ate head and neck of the corpse completely.

Postmortem Injuries, German Shepherd Dogs, Animal Depredation

#### G6 Responses of Mast Cells in the *Dura* to Traumatic Brain Injury in an Animal Model

Kazuhiko Kibayashi, MD\*, Tokyo Women's Medical University, Department of Legal Medicine, School of Medicne, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, JAPAN; and Ryo Shimada, PhD, and Rui Furutani, PhD, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjyuku-ku, Tokyo, 162-8666, JAPAN

After this presentation, attendees will understand the responses of mast cells in the *dura* to traumatic brain injury (TBI), the histamine-mediated brain damage after TBI, and the significance of histological examinations of the dura in cases of head trauma.

This presentation will impact the forensic science community by providing evidence for changes in the dural mast cells after TBI and the role of dural mast cells in the development of brain injuries. This presentation will also emphasize the need for histological examinations of the dura in autopsies of head trauma cases.

Mast cells secrete stored histamine in response to extrinsic stimuli. Histamine plays a role in the formation of brain edema and induces histamine receptor expression in the brain. Histamine receptors exert a protective effect against histamine neurotoxicity. Because the *dura* contains mast cells, it is hypothesized that blunt force to the head activates dural mast cells, leading to the release of their histamine and exacerbation of brain injury. Therefore, the time-dependent changes in dural mast cells and histamine receptor expression in the brain after TBI in a rat controlled cortical impact model was investigated. Male adult rats (7-10-weeks-old) weighing 200–310 g were used in this study. Under general anesthesia, a craniotomy of 6.0 mm in diameter was performed over the left parietal bone taking care not to penetrate the dura. A blunt force impact was applied to the craniotomy site using a pneumatic impact device and generated a cortical contusion on the left cerebral hemisphere. In sham-operated rats, the same surgical procedures were performed, but no impact was applied. Rats were perfused transcardially with phosphate-buffered saline under general anesthesia at 1, 4, 7, or 14 days after the surgery. Toluidine blue staining for mast cells and immunohistochemistry for histamine receptor H3 were performed on paraffin sections of the dura and cerebrum. Real-time PCR analysis of histamine receptor H3 mRNA expression was performed on total RNA extracts from the cerebrum.

The number of toluidine blue-stained dural mast cells at the site of impact was significantly decreased at one and four days after the trauma. The immunoreactivity and mRNA expression of histamine receptor H3 at the cortical contusion of the cerebrum were significantly increased at one and four days after the trauma. A previous report showed that activated mast cells release histamine-containing vesicles and appear unstained with toluidine blue. Therefore, the present results indicate that blunt force to the head causes dural mast cell degranulation and induces histamine receptor H3 expression in the cerebrum. The findings further indicate that a decreased number of toluidine blue-stained mast cells in the dura provide evidence of head trauma, suggesting that histological examinations of the dura may help to diagnose blunt force impacts to the head.

Forensic Neuropathology, Head Injury, Dura

#### G7 Plastic Bag Asphyxia: Suicide and Literature

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The goal of this presentation is to show: (1) the importance of a careful autopsy in cases of asphyxia; (2) the importance of the death scene traces; and, (3) the influence of the literature in some cases of suicide.

This presentation will impact the forensic science community by showing how the literature and media could influence and help someone to commit a suicide.

Asphyxial deaths using plastic bags are not common. Most frequently classified as suicide or accident (usually involving children, volatile inhalants, and autoerotic situations), they also can have a homicide origin. The death may be caused by mechanisms such obstruction of the external air passages, usually called smothering, and/or oxygen deprivation, included in the general group of mechanical asphyxia by suffocation.

The cases of suicide using plastic bags have increased with the publication, in March 1991, of the book *Final Exit: The Practicalities of Self-Deliverance and Assisted Suicide for the Dying* written by Derek Humphry. The book describes this method of suicide, in combination with drugs, as a painless way for those suffering from a terminal illness to end their lives. In New York City asphyxia deaths using plastic bags increased by more than 300% immediately following the publication of the book. However, these deaths have only been responsible for less than 5% of all suicides in the year after the book was released. In many other countries, like Portugal, this method of suicide is however rarely used.

The death scene investigation may be crucial to determine a suicidal aetiology. As a matter of fact, if the plastic bag has been removed, and as in such cases the external evidence of injury could be minimal, the death may be initial understood as a natural death.

A case of suicide using plastic bag is presented, in which the victim, a retired translator, left nearby the book *Final Exit* open to the chapter

*Suicide Using Plastic Bag.* Some pills were also found, as well as letters expressing his suicide intentions and last will.

The need, in such situations, of a high index of suspicion for the diagnosis of this entity is emphasized. When numerous petechiae are present, particularly in the conjunctivae, an attempt to identify their origin should be made to exclude other manner of the death, such strangulation. So, a full and careful autopsy, including toxicological analysis, combined with the investigation of the evidence at the death scene is mandatory in these cases.

Plastic Bag, Asphyxia, Suicide

#### **G8** The Bone Collector: When Reality Overcomes Fantasy

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After attending this presentation, attendees will understand the importance of the application of a multidisciplinary approach in challenging cases of identification of human remains.

This presentation will impact the forensic science community by demonstrating that a case that may apparently seem simple may instead reveal great methodological and interpretative challenges, making it imperative to use a multidisciplinary approach with methods that require specific professional expertise in various specialties (e.g., pathology, genetics, anthropology, physics, chemistry).

All who work in the forensic field know that the more crucial the biological samples to be analyzed are (charred remains in an advanced state of decomposition, fragments of tissues, bones, etc.), the more complex personal identification is. In these challenging cases it is extremely important to apply a multidisciplinary approach for identification.

A case that came under their observation in July 2007 in Rome will be presented. A skeleton was discovered by firefighters after extinguishing a fire in a grassy field. The skeleton was almost complete and its right side was charred because of the flames. Beside the skeleton, a bag containing a bunch of keys and an identity card was found, fortunately not destroyed by the fire. These items belonged to an elderly man who disappeared in that area four years before.

Genetic tests were performed on a left femoral bone sample in order to confirm the presumed identity of the skeleton and instead provided a genetic profile that was not compatible with the sons of the missing man.

Thus, other samples were taken from different bones and examined resulting in five different genetic profiles, corresponding to three women and two men, and none of them was compatible with the sons of the missing man.

Therefore the prosecutor asked for an anthropological expertise, who confirmed morphologically that the skeleton was composed by bones belonging to different individuals and could also give a range for the approximate age of these individuals at the time of death.

Thus, the prosecutor asked for the time-of-death estimation of these individuals and, at this request, specific investigations on the bone remains were carried out based on the measure of the isotopic ratio of <sup>14</sup>C in lipids and collagen by Accelerator Mass Spectrometry (AMS), which can provide a dating for the remains in exam.

So far, five DNA profiles have been identified but not all the bones available have been genetically examined yet, so it is possible that the genetic profiles, and therefore the number of individuals involved in the case, may be more.

Identification, Multidisciplinary, Approach

# **G9 Cancer Patient mtDNA Forensic Identification: A Case Report**

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After attending this presentation, attendees will understand how to manage a forensic identification case in a cancer patient, when only neoplastic tissue is available for the genetic analyses.

This presentation will impact the forensic science community by demonstrating that, because of the frequency of mutations in mtDNA is higher than in nuclear DNA in a variety of human cancers (as suggested from several studies), the mtDNA profiling should not be applied as the unique analysis in cases of forensic identification of cancer patients when only neoplastic tissue is available. Moreover, direct automated sequencing lacks adequate resolution to detect mtDNA heteroplasmy when, as in cancer cells, the somatic mutation tend to homoplasmy.

Mitochondrial genome mutations are described in many kinds of human malignancies, including lung cancer. These mutations can be base substitutions, insertion, or deletions, and the 1.1 kb d-loop region has been recently identified as a mutational "hot spot" in the mitochondrial DNA (mtDNA) of neoplastic tissue. Cancer cells harbor homoplasmic rather than heteroplasmic mutations; therefore, somatic mutant mtDNA appears as a single copy among a majority of wild-type mtDNA molecules and becomes dominant in the cancer cell probably due to the growth/survival advantages that such mutation confers to the cell.

A case of forensic identification will be presented in which a widow claimed medical malpractice by the physicians that had taken care of her husband, who was affected by a malignant lung disease. The wife thought that he had been wrongly diagnosed with cancer and, therefore, he had undergone massive and inappropriate therapies that finally led him to death.

In this case, the prosecutor ordered the seizure of the neoplastic histological samples attributed to the deceased and the comparison of the genetic profile obtained from these samples with those of the relatives, in order to establish the presence or absence of genetic compatibility among the neoplastic tissue and the relatives of the deceased.

To this end, autosomal markers were analyzed and compared with those of the two daughters of the deceased, while Y-chromosome markers and mtDNA were analyzed and compared with those of his brother.

While both autosomal and Y-chromosome markers confirmed the correspondence of the histological samples to the deceased, in the case of mtDNA a difference at nucleotide 16093 of HVRI region has been highlighted: in fact the brother had a C while the lung tissue examined showed a transition from C to T. In order to ascertain the full genetic compatibility it was therefore necessary to study the nature of this nucleotide difference by cloning of PCR products.

Sequencing of PCR cloning products thus allowed highlighting a heteroplasmic site (tending to homoplasmy) at nt.16093 in tumor cells with respectively 75% of mutated mtDNA and only 25% of germ-line mtDNA compatible with the brother reference sequence.

mtDNA Profiling, Heteroplasmy, Neoplastic Tissue

#### G10 Method of Concealment of Corpses in Mafia Related Homicides: Melting in Strong Acids

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After attending this presentation, attendees will learn about some awful methods of concealing corpses used by Mafia criminal organizations.

This presentation will impact the forensic science community by showing experimental data to help attendees better understand how strong acids and bases could melt a body; in particular, it will be shown using sulfuric acid activated with water, both soft tissues and bones could colliquate in few days, in contrast with pure sulfuric acid.

The criminal organization called "Cosa Nostra" has implemented brutal approach to commit murders over the past years, with dramatic symbolic implications, in order to prolong the agony of the victims, extract useful information, and no only to cause death as quickly and bloody as possible, but also to facilitate the concealment and the disappearance of the corpse itself. In the mafia, ritual murder must be view beyond the event itself, even the meaning of such gestures, take value as a warning to strike terror. Regarding the concealment of the corpses, nothing was known until the "pentiti" (those Mafiosi who turned informant) began to tell the dramatic episodes of which they witnessed or participated in from time to time, shedding light on a particular aspect of the phenomenology in Mafia's homicides known as "lupara bianca" (literally "white shotgun"): the disappearance of a subject who was known to be dead, but without knowledge of where his corpse was.

To hide the bodies of the victims tortured and killed, criminals used various methods, like burial in land, immersion in seawater with weights tied to the victims to get them to the bottom, disposal of corpses in natural caves or wells, or burning of bodies in ovens or cars. But the most chilling and ingenious method was destruction by "melting" by using strong acids or bases. In particular, some pentiti spoke about a "death's chamber," property of the criminal clan of Brancaccio and his boss, Filippo Marchese, called "u milinciana" (the eggplant, because of his skin's color), where the police found some tanks full of acid, torture instruments, and human remains.

This report's goal, therefore, is to verify experimentally the use of strong acids (sulfuric acid) for the dissolution of biological tissues animals, and also observing the macroscopic changes that the soft tissues and bones undergo over the time, in order to verify the claims of the Mafia's "pentiti" in their statements. In particular, two different tests were conducted: in the first, dipping a pork knuckle weighing 160 grams in a glass bowl containing pure sulfuric acid of known concentration, highly caustic, water-soluble, and able to carbonize organic matter. In this case, after only 30 minutes, the piece of pork appeared to be "cooked," with brownish color, in two days the muscle structures were loose, while after only six days, the bone began to be eroded, although it remained essentially integrated. The second test was made by dipping the knuckle of pork into a bath of 700 cc of sulfuric acid activated with water. In this case, the muscle-cartilaginous component disappeared after only 12 hours and after two days the bone appeared dissolved in the liquid component.

These experimental test made have thus demonstrated that it appears unlikely that an entire body can be dissolved in few minutes (as reported by some "pentiti") using the normal commercially available sulfuric acid, but in any case it's likely that, in several days, a corpse could be colliquated and made unrecognizable.

Mafia's Homicide, Concealment, Melting

#### G11 When Ribs Penetrate the Heart in Blunt Chest Wall Trauma

#### Tanuj Kanchan, MD\*, Kasturba Medical College, Department of Forensic Medicine, Light House Hill Road, Mangalore, 575 001, INDIA

After attending this presentation, attendees will be able to identify with the possible mechanism of penetrating trauma caused directly to the heart by the fractured ends of the ribs in run over traffic mishaps.

This presentation will impact the forensic science community by illustrating a type of injury that is well known but rare. The injury is reported for the first time in a run over traffic accident. Cardiac lacerations caused directly as a result of rib fractures although a rare phenomenon in blunt force trauma to the chest, its possibility should be explored so that prompt and early treatment saves the patient from a fatal outcome.

Cardiac damage in non-penetrating chest trauma is uncommon. Direct penetrating injuries to the heart are commonly observed in stab and gunshot wounds. The fractured ends of the ribs are very rarely reported to cause penetrating injuries to the heart. One such rare case where the sharp ends of fractured ribs has caused extensive damage to the heart in a run over vehicular accident is reported. The reported rare case illustrates the possible mechanism of direct cardiac injuries from broken sharp jagged fractured ends of ribs in blunt force trauma to the chest in run over traffic mishaps.

A 45-year-old male fell from a moving bus while trying to get off. By the time brakes were applied, the moving bus had run over the left side of his chest, neck, and head. The victim died instantly and the body was subjected to medicolegal autopsy. On external examination, the head and face of the victim was deformed. Underlying comminuted skull fractures were palpable. No external injuries were evident on the chest region. Avulsed lacerations were present on the lower limbs. Internal examination revealed multiple fractures of the cranial vault and base of the skull with diffuse subdural and subarachnoid hemorrhage, intraventricular bleeding, and extensive brain damage. Fractures of the 2nd to 6th ribs in anterior axillary line on the left side, and fracture of 1st and 2nd ribs in mid-clavicular line on the right side with corresponding chest wall muscle contusions were present. Pleura contained 300 and 400 ml of frank blood in the right and left sides respectively. Pericardium was torn and extensive damage to the left ventricle was evident. The heart weighed 280 grams. Transmural lacerations of the left ventricle were present, corresponding to the pointed fractured ends of the ribs on the left side. Peritoneal cavity contained 200 ml of blood. Multiple lacerations over the right liver lobe were present. All visceral organs were pale on cut section. Lungs escaped any major trauma in the reported case.

The rib cage acts as a protection for the thoracic organs and support for the vertebral column. Penetrating injuries to the heart in blunt chest trauma thus remain uncommon. Even when the ribs are fractured recoil of the intercostal muscles keeps the architecture of the rib cage intact preventing subsequent injuries to the thoracic organs. Fractured ribs at times may act as a weapon of offense causing damage to the underlying organs directly. In the present case of a run-over traffic mishap, no external injuries or deformity were apparent on the chest wall. On internal examination, intercostal muscle contusions were present but apparently the rib cage had retained its shape due to recoil of the intercostal muscles. It was only on further dissection that the major insult to the pericardium and the heart was observed. It is illustrated how the sharp jagged ends of the fractured ribs move medially on external pressure to cause penetrating injuries to the heart. It is proposed that as a consequence of the transient phenomenon of deformation of chest cavity under pressure in run over traffic mishaps, the projecting fractured ends of the ribs penetrate the underlying thoracic organs causing fatal injuries.

#### Ribs, Heart, Run Over Traffic Mishap

#### G12 Examination of Sexually Abused Children: Presentation of the First Danish Center for the Investigation and Care of Abused Children

### Annie Vesterby, MD, DMSc\*, Institute of Forensic Medicine, University of Aarhus, Brendstrupgårdsvej 100, 8200 Aarhus N, DK-8000, DENMARK

After attending this presentation, attendees will understand how the first Danish Child Protection Center was organized, and the importance of the participation of the forensic department and the forensic pathologist.

This presentation will impact the forensic science community by showing the importance of the involvement of the forensic pathologist in the investigation of child sexual abuse.

The prevalence of sexual abuse of children in the Nordic countries is unknown, but has been estimated to be around 5%. Very few cases of sexual abuse are reported to the police. The police may request a medical examination to document or verify the child's testimony.

Until now, the child and the child's family have had to go to the police station to give a videotaped interview to the police, go to a medical or a forensic doctor with examination rooms located elsewhere, followed by pediatric evaluation and treatment and psychosocial followup at yet another place.

In November 2007, the first Danish centre for the protection of abused children was established at Aarhus University Hospital, Skejby.

The Center, which receives all kinds of child abuse cases, is located in a building neighboring the Department of Forensic Medicine, Aarhus, and headed by a steering group with representatives from the Pediatric Department, the police, and the Department of Forensic Medicine; the Center is managed by a pediatric consultant.

Videotaped interviews by the police are performed at the centre as well as the forensic medical examination, pediatric and psychosocial evaluation and follow-up.

Experience and perspectives from the first Danish child protection centre for the forensic community will be presented.

Sexual Abuse, Child Protection Center, Forensic Pathology

#### G13 An Innovative Proteomic Approach for the Identification of Novel Plasma Biomarkers in Patients With Brugada Syndrome

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After attending this presentation, attendees will understand how plasma potentially carries important information whose knowledge

could help to improve early disease detection and prognosis in Brugada syndrome.

This presentation will impact the forensic science community by providing potential new tools for the correct diagnosis of "at risk" individuals with Brugada syndrome carrying specific gene mutations. The molecular signature obtained by the study of plasma proteome will complement genomic information therefore increasing the chance of disease detection in these individuals who are exposed to a dramatic risk of sudden cardiac death.

Brugada syndrome (BS) is a polygenic inherited cardiac disease characterized by life threatening arrhythmias and high incidence of sudden death. In the family enrolled in the present study, the disorder is caused by Q1118X-mutation in the SCN5A gene, encoding the cardiac sodium channel. 2D-PAGE was used to investigate specific changes in the plasma proteome of BS affected patients and family members sharing the same gene mutation, compared to healthy controls, with the goal to identify potentially specific disease biomarkers.

In order to reduce plasma sample complexity, the combinatorial hexapeptide ligand libraries were used.<sup>1</sup> The use of the beads prior 2D-PAGE enabled detection of many new protein spots and increased resolution and intensity of low abundance proteins.

Approximately 900 protein spots were detected in each gel. Proteins, whose expression was significantly different among the two groups, were excised, trypsin-digested and analyzed by LC-MS/MS.

Data showed that the levels of several proteins were significantly altered in BS patients compared with controls. In particular, Apolipoprotein E, Prothrombin, Vitronectin, Complement-factor H, Vitamin-D-binding protein, Voltage-dependent anion-selective channel protein 3, and Clusterin were considerably increased in plasma sample of BS patients, whereas Alpha-1-antitrypsin, Fibrinogen, and Angiotensinogen were considerably decreased; moreover, posttranslational modification of Antithrombin-III was detected in all affected individuals.

In the light of these results, it is hypothesized that these proteins might be considered as potential markers for the identification of disease status in BS. Further analysis is being conducted in our laboratory in order to validate these findings in a larger number of cases and to elucidate the pathogenetic role of these proteins in this specific cardiac disease.

#### **Reference:**

<sup>1</sup> Boschetti E, Righetti PG. The ProteoMiner in the proteomic arena: anon-depleting tool for discovering low-abundance species. J Proteomics. 2008 Aug 21;71(3):255-64. Epub 2008 Jun 20. Review

Brugada Syndrome, Plasma Biomarkers, Proteomics

#### G14 A Case of Lethal Peripartum Eosinophilic Myocarditis

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The goal of this presentation is to report an uncommon case of lethal peripartum cardiomyopathy in a young woman. A complete forensic approach was performed through autopsy, histological, and microbiological examinations and final results showed that the cause of death was due to an Eosinophilic Myocarditis (EM).

This presentation will impact the forensic science community showing that eosinophilic myocarditis is a rare, potentially fatal disease if left untreated.

Eosinophilic myocarditis is a histological diagnosis characterized by a mixed inflammatory cell infiltrate containing a variable amount of eosinophils within the myocardium. This phenomenon may be associated with a variety of disease such as idiopathic hypereosinophilic syndrome (IHES), hypersensitivity myocarditis, giant cell myocarditis, toxic myocarditis, Churg-Strauss syndrome, or parasitic infection.

Clinical presentation includes a wide spectrum of nonspecific signs and symptoms: chest pain, fever, shortness of breath, chills, cough, but they are not always present at the same time and sometimes unusual symptoms, such as epigastric pain, can be the only indication of a pathological state. They can be also associated with peripheral eosinophilia and transient or persistent left ventricular dysfunction.

EM is considered, together with coronary heart dissection, one of the clinical presentations of peripartum cardiomyopathy that usually occurs one month before to six months following delivery. EM etiology and pathogenesis are unknown: eosinophils may be present and activated because of the systemic hormonal perturbation occurring during the period of uterine involution.

A major problem is that EM is rarely recognized clinically and is often first discovered only at postmortem examination.

A correct diagnostic approach in these patients should include an echocardiogram study (with evidence of low ejection fraction and decreased left ventricular systolic function) and an endomyocardial biopsy (confirming eosinophils as a major inflammatory cell component).

If successfully diagnosed, EM can be treated with beta-blockers and ACE inhibitors to support heart failure and corticosteroids to reduce the inflammatory process that is involving the myocardium. Prognosis is strictly linked to ventricular function recovery because those patients with severe myocarditis-induced heart failure have less survival chances if normal cardiac function is not restored.

Few EM cases are reported in literature and most of them are based only on autopsy diagnosis.

A case is reported of a 29-year-old woman who was admitted to critical care unit in respiratory and cardiac failure, three weeks after giving birth. Patient clinical history was non-existent for allergy or autoimmune diseases. The third day after birth, she complained of thoracic pain but echocardiogram was negative. During hospitalization physicians treated her with antacids and gastric inhibitors and then she was discharged with prescription of proton pump inhibitors with the suggestion of gastroenterology visit. The following three weeks where characterized by growing anterior and back thoracic pain associated with general discomfort, but neither specific symptoms nor peripheral eosinophils increase were present; only inflammatory indexes (velocity of erythrocyte sedimentation, VES, and creatine kinase, CK) were slightly increased. With progressive and worsening clinical symptoms, she was finally sent to emergency room in critical condition: dyspnea, confusion, fever, and tachycardia. Echocardiogram showed severe left ventricular systolic dysfunction and 25% of ejection fraction; chest radiograph and TC displayed pleural effusion with general edema. The young woman died after seven hours of cardio-respiratory failure and no medical approach was effective. External examination of the body was completely negative. Autopsy revealed bilateral pleural effusions, increased lung weights, and hepatomegaly. Heart was normal in size and shape, but myocardium and papillary muscles showed malacic areas. Histological examination pointed out massive eosinophilic infiltrates, more evident in cardiac samples. The cause of death was indeed attributed to peripartum eosinophilic myocarditis.

The role of "peripartum" in the etiopathogenesis of such cardiomyopathy as well as possible medical liability in lacking diagnosis and treatment of myocarditis will be discussed.

Eosinophilic Myocarditis, Peripartum, Heart Failure

#### G15 The Bodies of Two Missing Children in an Enclosed Underground Environment

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The goal of this presentation is to illustrate a little-known but noteworthy case concerning the wrongful conviction of a Southern Italy father whose two missing children were found dead in enclosed environment after approximately 1.5 years since their disappearance.

This presentation will impact the forensic science community by warning and improving search operation, methods of investigation, and indictment process, based on the autopsy findings and physical evidence collected on the scene and from the bodies.

Two young kids, 13- and 11-years-old respectively, originally from a small town in Southern Italy, were missing on June 5, 2006 (06:30 p.m.). Soon after their disappearance, a "missing child" search began. Broadcasters promptly aired a description of the missing children pushing the entire community to assist in the search and safe recovery of the child. But every effort was in vain for more than one year. During the search, the investigators collected enough evidence against the father who was arrested 17 months after the disappearance. He was indicted for kidnapping, homicide, and concealment of the two bodies. He never confessed the crimes and he claimed to be innocent.

Three months after the conviction, a fireman found the two corpses in a subterranean dry cistern next to a more than 20-meter-high well water. The bodies were well preserved, almost mummified with only few body-parts skeletonized. Based on dental records they were identified as those of the two children missing 1.5 years before. Signs of a very low insect activity were present, reasonably consistent with a rapid skin dehydration. The autopsy showed no signs of defense injuries or ligature consistent with strangulation or captivation except for fractures of the axial skeleton at a number of points consistent with a fall from a low-medium height. The body of the elder brother (CP) presented major injuries with signs of recent hemorrhages and gut content analysis consistent with the last meal which provided enough evidence to ascertain a very short survival time. The younger brother (TP) showed minor injuries, signs of old hemorrhage, gastric and intestine emptying time consistent with a longer survival time of approximately 3-4 days, spent alone in the dark and cold cistern. No evidence of body removal or corpse displacement following death was observed. A long postmortem interval (PMI) of approximately 20 months was estimated mainly from the pattern of insect succession. Based on such physical evidence, on March 4, 2008, four months after conviction and 40 days after the recovery of the two bodies, the father was released from prison and exonerated from previous indictment of homicide.

Missing Children, Wrongful Conviction, Postmortem Interval

### G16 Diagnosis Of Drowning: The Contribution Of Microbiological Investigations

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After attending this presentation, attendees will gain knowledge of the effectiveness of marine bacteria and/or bacterial indicators of water fecal pollution as a new marker of drowning. This presentation will impact the forensic science community by introducing an alternative test that could be useful in drowning diagnosis.

To investigate the effectiveness of marine bacteria and/or bacterial indicators of water fecal pollution as a new marker of drowning, an experimental protocol was performed to identify bacteria on samples of drowned victims recovered from umor vitreo (UV) and blood of different anatomic sites such as: right ventricular blood (RV), left ventricular blood (LV) and peripheral blood (P). The study, performed in 2008 and 2009, was performed on ten victims: six drowned victims (two cases in sea water, three in rivers or lake; one in rainwater collection tank) (study group), and four subjects who died from causes other than drowning (three cases of heart attack and one case of death by vehicle collision) (control group). From all groups at least 0.5 ml of each sample were obtained and the tests were calibrated by considering the water fecal pollution rates. Selective culture media were used to detect bacterial growth. Each samples of control or study groups (RV, LV, P and UV) or water samples (all 100 ml) were incubated in Tryptic soy broth (TSB) for 48 h at 37°C and 5% CO2. After incubation, evaluation of bacterial growth was assessed by plating 100 ml of each sample onto: Todd Hewitt and Marine agars, selective for marine bacteria, m-FC agar, selective for fecal coliforms (FC) and KF Streptococcus agar, selective for fecal streptococci (FS). The plates were incubated for 24 h at 44°C and for 48 h at 37°C and 5% CO2 to determine FC and FS growth, respectively. The presence of FC was indicated by the development of blue colonies, whereas the presence of FS was indicated by the development of red colonies. The absence of blue and red colonies indicated a negative result, i.e., no blood fecal pollution. The presence of marine bacteria was evaluated through the observation of their growth on selective culture media. Results showed that in the samples of drowned victims in sea water there is growth of marine bacteria, as evidenced by the presence of colonies on TH4% and MA culture media for LV and P blood samples, for the case 1, and for P and UV samples for the case 2. Moreover, the case 2 showed growth of FS and FC bacterial colonies. Regarding drowned victims in rivers or lake water, the analysis of case 4 showed the presence of marine bacteria from RV blood sample; on the other hand the case 5 resulted positive to marine bacteria and fecal streptococci. Surprisingly, case 3 was negative for marine bacteria and fecal streptococci. All anatomic sites of case 6, drowned victim in rainwater collection tank, resulted positive to all the bacterial species considered. Bacteriological analysis of RV, LV, P and UV samples of the control group evidenced a total absence of bacteria. This result showed the reliability of the microbiological test. All the water samples obtained from locations where corpses were found showed a bacterial presence according to samples obtained from the related victims. Applied method is sensitive since a very few bacteria aspirated at follow drowning can be evidenced. Positive results obtained for various anatomic sites (RV, LV, P, and UV) can be an internal control of the sampling procedure to avoid the possibility of bacterial contamination during blood and umor vitreo sampling. Notably, umor vitreo as a new sample for the microbiological test of drowning diagnosis was used.

Drowning Diagnosis, Microbiological Test, Umor Vitreo

### G17 Butane Inhalation and Sudden Death: A Case Report

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After attending this presentation, attendees will have learned about a case of sudden death due to butane inhalation in a young inmate. This presentation will impact the forensic science community by stressing the importance of combining autopsy data and the detection of volatile substances in blood and tissues in all cases of unclear death of young people.

This presentation will inform attendees of something they do not do correctly-the misdiagnosed problem of sudden death due to abuse of volatile substances, especially in adolescents and people living in remote communities. In the current practice, forensic pathologists don't often consider that volatile substances are very easily accessible, and lethal if abused. They should learn how to detect halogenated hydrocarbons, and when it is correct to analyze the concentrations of these volatile substances in blood and tissues. This kind of investigation should be performed in order to avoid mistakes, especially in cases of sudden death of young people with aspecific pathological findings or unremarkable histological examinations.

The deliberate inhalation of volatile substances has been reported from most parts of the world, mainly among adolescents, individuals living in remote communities, and those whose job gives easy access to these substances, with a higher incidence in Countries with large rural populations. Although it is less widespread than twenty years ago, inhalant use still remains a problem today ranging from 10% to 15% among U.S. teenagers and young people (M.R. Marsolek et al., 2010).

Solvents from contact adhesives, typewriter correction, dry cleaning fluids, cigarette lighter refills, petrol (gasoline), halogenated solvents, and aerosol propellants are commonly abused in this way, but cigarette lighter refills and butane-containing cans for portable cooking stoves are the most frequently abused ones. Although aliphatic hydrocarbons are considered safe as aerosol propellants, the acute inhalation of these substances, particularly n-butane, may potentially cause severe damage in healthy hearts (M. Ago et al., 2002).

Volatile substance abuse gives rise to dose-related effects similar to those of hypnosedatives. Small doses can rapidly lead to euphoria and other behavior disturbances similar to those caused by ethanol (alcohol), and may also induce delusions and hallucinations. Higher doses may produce life-threatening effects such as seizures, coma, and sudden death (R.J. Flanagan et al., 1994). The mechanism of sudden death directly related to volatile abuse includes cardiac arrhythmia, hypoxia, and respiratory depression.

Butane is a gaseous aliphatic hydrocarbon, also called n-butane, with the "n" designating it as normal butane. Its other isomer is isobutene, but the name butane is used collectively to denote both nbutane and isobutane (R.L. Myers, 2007). N-butane and isobutane have an anesthetic or narcotic effect on the central nervous system, and induce fatal arrhythmia at 0.5–15% concentrations in the air (H. Sugie et al., 2004). It has been reported that many n-butane or isobutane abusers experienced fatal ventricular fibrillation immediately after a sudden fright or intense muscular exercise such as running and sexual activity (C. Jackowski et al., 2005. H. Sugie et al, 2004). A few cases of suicide by propane-butane inhalation have been reported too (A. Gross et al., 2002).

A case of sudden death of a 22-year-old male inmate is described. He had a history of drug addiction, depression, and multiple selfinflicted superficial incised wounds. His cellmates reported that the body was found in the bathroom of the cell. The bathroom smelled of gas. The body was lying on the bidet, with his back leaning against the wall; a butane-containing can and a portable cooking stove were found on the floor adjacent to the body. A complete medicolegal autopsy was performed. The external examination showed marked livor mortis, nosebleed, and some parallel linear scars on the forearms; no signs of recent injuries or trauma were observed. The internal examination revealed marked lung congestion; the other organs showed no pathological findings, but evidence of congestion. Histological examinations were unremarkable. Blood samples were collected and analyzed for halogenated hydrocarbons and drugs, using gas chromatography. A concentration of about 0.5 µg/ml for n-butane, with traces of isobutane and butene was measured; drug screening revealed

therapeutic concentrations of benzodiazepines and 0,5 g/L of ethanol in blood samples. The cause of death was ascribed to n-butane poisoning inducing fatal cardiac arrhythmia.

In conclusion, abuse of volatile substances is a serious problem because it is not illegal and agents are easily available and cheap. Thus, the risk of sudden death due to abuse of volatile substances in an environment with no witnesses should be taken into consideration in all cases of unclear death of young people. It is recommended that medicolegal death investigators become familiar with the principles of detection of volatile substances in blood and tissues, especially in those cases with unspecific macroscopic and histological findings.

Volatile Substance Abuse, Butane, Sudden Death

# G18 Case Report of a Fatal Intoxication by Nucynta<sup>®</sup>

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After attending this presentation, attendees will learn about a case, in which, Nucynta<sup>®</sup>, a newly released analgesic, a Schedule II controlled substance, comparable to tramadol, was fatally ingested.

This presentation will impact the forensic science community by raising the awareness of the toxicity of novel drugs, which is essential for medical examiners and forensic toxicologists.

Tapentadol (Nucynta<sup>®</sup>) is a centrally acting opioid analgesic prescribed for the treatment of moderate to severe acute pain. Its efficacy is believed to be due to mu-opioid receptor agonist activity and inhibition of norepinephrine reuptake resulting in increased norepinephrine concentrations. Metabolism of tapentadol is via glucoronidation to inactive metabolites. There are no cases in the literature relating to the toxicity of this agent or reports of fatalities. This report documents a case in which tapentadol was identified as the cause of death. The decedent was a 40-year-old obese male who was found at home by his girfriend. He had been prescribed Nucynta<sup>®</sup> (tapentadol) for shoulder pain, Lexapro (citalopram), and amitriptyline. There appeared to be more tablets missing than expected. At autopsy, there were early decomposition changes and hepatomegaly with fatty change.

Routine volatile, therapeutic drug, and abused drug testing was performed on the heart blood in this case. This included: (1) methanol, ethanol, acetone, and isopropanol analysis by head space gas chromatography (GC); (2) acid/neutral drug screen by GC-nitrogen-phosphorus detection (NPD); (3) alkaline drug screen by GC-NPD; (4) acetaminophen and salicylate by color test; and, (5) morphine and benzodiazepines by enzyme-linked immunosorbent assay (ELISA). The blood ethanol concentration was 0.01 g/dL; the vitreous humor ethanol concentration was negative. The alkaline drug screen was positive for diphenhydramine (0.6 mg/L), amitriptyline (1.1 mg/L), nortriptyline (<0.1 mg/L), and citalopram (0.3 mg/L). All were confirmed by full scan electron ionization gas chromatography/mass spectrometry and quantified by GC-NPD.

Given the case history, the heart blood was sent to a reference laboratory for tapentadol analysis. Tapentadol was quantified by liquid chromatography – mass spectrometry/mass spectrometry (LC-MS/MS) using D5-tapentadol as internal standard. Extraction of tapentadol from blood involved addition of carbonate buffer followed by methyl-tert-butylether (MBTE). After taking the MTBE layer to dryness, methanol was added and then transferred to an autosampler vial for injection. The limit of detection (LOD) and limit of quantitation (LOQ) of the assay were 0.06 ng/mL and 0.5 ng/mL, respectively.

The therapeutic range for tapentadol is 5-300 ng/mL. The tapentadol concentration found in the heart blood submitted in this case was 6600 ng/mL; more than 20 times the upper limit of the therapeutic range. Possible mechanisms of death include respiratory depression, CNS depression, and serotonin syndrome.

Based on the scene investigation and autopsy findings in this case, the medical examiner determined that the cause of death was narcotic (Nucynta<sup>®</sup>) intoxication and the manner-of-death was undetermined. **Tapentadol, Nucynta<sup>®</sup>, Overdose** 

#### G19 Nasal Mucociliary Motility: New Forensic Tool for Estimating Time Since Death

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After attending this presentation, attendees will understand the potential contributions of postmortem investigation of nasal mucociliary motility in time of death estimation.

This presentation will impact the forensic science community by emphasizing the potential role of nasal scraping that could become a routine procedure in estimating time-since-death.

Postmortem interval (PMI) estimation is one of the most difficult issues in forensic medicine. Time-of-death is usually appreciated by recognizing early postmortem changes to the body prior to the onset of gross decomposition phenomena: algor mortis, rigor mortis, and livor mortis.

The study of these physical processes is strictly connected to the operator's subjectivity hence it can be source of confusion in estimating PMI. Moreover these body changes can be altered by several internal and external factors: body temperature at death time, subcutaneous fat, muscular mass, clothes, environmental temperature, humidity, and ventilation.

There have been many proposed innovative methods in attempts to avoid this trouble defining PMI objectively as possible. The goal of these new techniques is to find a link between PMI and objectively detectable values such as infrared tympanic thermography, skin fluorescence, electrolyte concentration in cerebro-spinal fluid, pericardial fluid or vitreous humor. All these samples, on the other hand, present practical difficulties in performing and require invasive methods and long time waiting.

Some studies have been published about nasal scraping role in clinical practice (ciliary dyskinesia, NARES, allergic rhinosinusitis), but no studies have never been performed in cadavers for PMI estimation.

A study concerning the examination of ciliary motility as residual life phenomenon, realizing a study on time of death evaluation using a new, rapidly available requiring substrate: nasal mucosa is presented.

Nasal mucosa is composed by numerous cell types (globet cells, basal cells, ciliated and not ciliated cells) and can be easily obtained by nasal scraping, a technique commonly used in otolaryngology; it consists of a curette crept on nasal mucosa and cells picked up in this way are then observed.

From June 2009 to June 2010, nasal scraping in 70 cadavers was performed. Age ranged from 24 to 95 years and the cause of death was most frequently due from ischemic cardiopathy, septic shock, and car accident. The only exclusion criteria of this study was nose bleeding.

A specimen of ciliated epithelium was obtained by scraping from the middle third of the inferior turbinate with a spoon-shaped nasal probe (Rhinoprobe). An *in vitro* evaluation of ciliary movement was performed. Ciliary beat frequency (CBF) was analyzed by phasecontrast microscopy. Three different samples at different postmortem intervals were carried out: between 4 and 6 h (T1), between 10 and 12 h (T2) and after 24 h (T3). Then CBF (beat number/second) was classified in: present (3-4/sec), hypo-valid (1-2/sec) and absent.

Results demonstrated that, except for those cases which showed fungal or bacterial infections, at T1 motility was present in the majority of cases; at T2 motility was still present, but it was hypo-valid in a higher percentage. Ciliary activity was absent at T3. It is believed that all these findings can be explained with progressive metabolic reserves lowering: the more time passes after death, the more ciliated cells loose energetic substrates for ciliary motility.

In conclusion, mucociliary motility seems to be linked to PMI and thus nasal scraping can be considered as a new, easy, cheap, and efficient objective tool in detecting PMI; further studies are required. **Nasal Scraping, Mucociliary Motility, Time Since Death** 

#### G20 Fatal Spontaneous Non-Traumatic Subdural Hematoma and Terson Syndrome

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After attending this presentation, attendees will learn that a ruptured cerebral aneurysm can cause a compressive acute subdural hematoma without concomitant subarachnoid hemorrhage.

This presentation will impact the forensic science community by expanding the attendees knowledge base by increasing awareness of causes of non-traumatic subdural hematomas and retinal hemorrhages.

This presentation will inform attendees of something that they do not know. While most acute subdural hemorrhages are the result of trauma, forensic pathologists must be aware that a ruptured cerebral saccular aneurysm can cause a spontaneous non-traumatic subdural hemorrhage along with associated retinal hemorrhages (Terson syndrome).

Cerebral saccular aneurysms frequently rupture into the subarachnoid space, accounting for 70-80% of non-traumatic subarachnoid hemorrhages (SAH); however, aneurismal rupture also may result in concomitant intraparenchymal, intraventricular, or subdural hemorrhage. Most acute subdural hematomas (SDH) in adults are due to traumatic head injuries, although less common causes include coagulopathies, non-traumatic intracranial hemorrhage, intracranial hypotension, or post-surgical complications. A ruptured cerebral berry (saccular) aneurysm causing only an acute SDH is rare, representing < 0.5 - 2% of all ruptured aneurysms in several large case series. In 1881, Litten first described intra-retinal hemorrhage associated with SAH. However, Terson's description in 1900 of vitreous hemorrhage following SAH is now associated with this syndrome. Although originally defined by the presence of vitreous hemorrhage in association with SAH, Terson syndrome now encompasses any intraocular hemorrhage associated with intracranial hemorrhage and elevated intracranial pressures.

A case of 46-year-old woman who died suddenly and unexpectedly at her residence is presented. Found on the bathroom floor, she had no obvious injuries. According to investigations by the medical examiner and law enforcement, she had a vague past medical history significant for hypertension but did not consume alcoholic beverages or use illicit drugs. Subsequent toxicological analysis did not reveal any licit or illicit drugs.

At autopsy, she appeared well nourished and had a body weight, length ,and body mass index of 49.1 kg, 160 cm, and 19.1, respectively. Postmortem monocular indirect ophthalmoscopy revealed bilateral retinal hemorrhages. The right and left fundi exhibited 25-35 and 15-20 flamed-shaped and dot retinal hemorrhages over the posterior poles, respectively. A 1.5 cm subscalpular contusion was left of the vertex over the parietal area. No subgaleal extravasated blood or skull fractures were present. Diffuse liquid and clotted subdural blood covered the cerebral convexities (R > L) and weighed 67 gm. The calvarial dura had adherent non-organizing blood over the right and left frontoparietal regions. The leptomeninges were thin and translucent without any extravasated blood. Compression of the midbrain involved the inferomedial temporal lobes and 2 x 1.5 x 0.3 cm dusky area of hemorrhage was in the inferomedial right temporal lobe (medial to the groove caused by transtentorial herniation). The arteries of the circle of Willis were in the usual anatomic configuration and patent. A 0.5 x 0.2 x 0.2 cm ruptured saccular aneurysm projected from the callosal side of the bifurcation of the left pericallosal and callosal marginal arteries. The brainstem contained Duret hemorrhages in the pons and midbrain.

Ophthalmological examination revealed bilateral diffuse optic nerve sheath hemorrhages and extravasasted blood within the perineural fat. The right and left fundi had 75-100 and 25-35 flame-shaped and dot retinal hemorrhages, respectively. These involved all four quadrants and extended past the equator but did not abut the ora serrata. The fundal hemorrhages were in all retinal layers and scant blood was in the vitreous of both globes.

A non-traumatic SDH can occur due to the rupture of cerebral saccular aneurysm. Most of these aneurysms are located on the internal carotid artery followed by the middle cerebral artery and anterior communicating artery, but only rarely arise from the distal anterior cerebral artery. Four mechanisms have been proposed by which blood from a ruptured cerebral aneurysm causes a SDH:

- 1. Successive small hemorrhages allow adhesions to develop and the final rupture dissects between the subarachnoid and subdural layers
- 2. The arachnoid membrane is breached by the rapidly accumulating blood from the rupturing aneurysm
- 3. A massive hemorrhage ruptures the cortex and breaches the arachnoid membrane
- 4. A carotid artery aneurysm located between the arachnoid layer and dura mater ruptures causing a SDH

Subarachnoid hemorrhage almost invariably develops following the rupture of a cerebral aneurysm and only extremely rarely does a SDH occur without an associated SAH. While most acute subdural hemorrhages are the result of trauma, forensic pathologists must be aware that a ruptured cerebral saccular aneurysm can cause a spontaneous non-traumatic SDH along with associated retinal hemorrhages (Terson syndrome).

Acute Non-Traumatic Subdural Hematoma, Cerebral Aneurysm, Retinal Hemorrhages

#### G21 A Fatal Complication of Vacuum-Assisted Vaginal Delivery

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After attending this presentation, attendees will learn of a fatal complication following vacuum-assisted vaginal delivery.

The presentation will impact the forensic science community by expanding the familiarity with a fatal complication of vacuum-assisted vaginal delivery, neonatal mortality, and conditions associated with retinal hemorrhages.

This presentation will inform attendees of something they do not know-that fatal neonatal subgaleal hemorrhage can result from birth assisted vacuum extraction and have associated extensive retinal hemorrhages.

An Edinburgh professor of obstetrics, James Young Simpson, first introduced a successful obstetric vacuum extractor in 1849. Technical difficulties limited its effectiveness and vacuum extraction (VE) fell from clinical interest until 1956 when the stainless steel cup vacuum device was introduced. While common in Europe, VE did not gain continued popularity in the United States until the 1980s. Early studies showed no significant traumatic complications attributed to VE when limited to 15 minutes and/or two "pop-offs" of the vacuum cap. The "pop-off" presumably served as a safety valve that would protect the neonate from excessive tractional forces. The development of the soft cup VE device with its implied safety caused VE to gain increasing popularity. Currently forceps and VE are used as delivery instruments, but over the past decade VE has replaced forceps as the main delivery instrument in assisted vaginal deliveries. However, controversy continues concerning which instrument is the best to use in specific clinical situations. VE remains popular because of its relative ease of use, lower maternal morbidity, and supposed safety. Nevertheless, severe neonatal complications can occur. The reported incidence of fetal death or severe fetal injury from VE ranges from 0.1-3 cases per 1,000 assisted deliveries. Three cases are presented of neonates who died from complications following vacuum-assisted vaginal delivery.

**Case 1:** Delivered by vacuum-assisted vaginal delivery, a 2.8 kg, 37-weeks-gestational age neonate had Apgar scores of 7 and 8 at 1 and 5 minutes, respectively. His initial hemoglobin (Hgb) was 17.4 gm/dL, but 5 hours later when he began grunting and developed hypothermia his Hgb was 6.8 gm/dL. He was transferred to a medical center with admitting diagnoses of subgaleal hemorrhage, anemia, hypotension, disseminated intravascular coagulopathy, and respiratory failure. After three weeks in the intensive care unit, the family withdrew care due to his increasingly poor prognosis. At autopsy, he had severe anasarca and hypoxic-ischemic/re-perfusion injury to his heart, liver, spleen, kidneys, and brain. An organizing subgaleal hematoma measured 15 cm and weighed 77 gm. No retinal hemorrhages (RHs) were identified by postmortem monocular indirect ophthalmoscopy (PMIO) and he had no documented clinical fundal examination.

**Case 2:** Born by vacuum-assisted vaginal delivery due to an arrested second stage of labor and shoulder dystocia, a 4.36 kg term neonate had Apgar scores of 0 at 1, 5, and 10 minutes and 2 at 15 minutes. She experienced immediate respiratory distress, hemodynamic instability, and presumed sepsis. Her initial Hgb was 14.9 gm/dL that later decreased to 10.7 gm/dL. An electroencephalogram demonstrated severe encephalopathy and her condition continued to decline until she died a day later. At autopsy, she had large subgaleal and subscalpular hematomas that were 20 cm in greatest dimension and weighed 54 gm. No skull fractures were present but she had bilateral subdural hematomas as well as subarachnoid hemorrhage. A clinical fundal examination was not done, but PMIO detected extensive bilateral multi-layered RHs.

**Case 3:** Delivered by cesarean section after a failed vacuumassisted delivery, a 3.8 kg term neonate had Apgar scores of 3, 5, and 7 at 1, 6, and 10 minutes, respectively. He had respiratory distress, hemodynamic instability, and developed disseminated coagulopathy. The parents withdrew care the following day. At autopsy, extensive subgaleal and subscalpular extravasated blood was present measuring 35 cm in greatest dimension and weighing 140 gm. No skull fractures, epidural, or subdural hemorrhage was identified. PMIO revealed extensive RHs in the right globe and 1 RH in the left fundus; however, no clinical fundal examination was documented in the medical record.

The most common extracranial injuries associated with VE are superficial scalp abrasions, lacerations, and hemorrhage that can occur in 10% of neonates. Two major types of scalp injury are the common, but clinically unimportant, cephalohematomas and the relatively rare, but potentially life threatening, subgaleal (SG) hemorrhage where extravasated blood dissects between the periosteum of the skull and the galea aponeurotica. The mortality rate of SG hemorrhage following VE is estimated at 20%. Vacuum-assisted vaginal delivery is a relatively common procedure and most often benign. However, forensic pathologists must be aware that a fatal SG hemorrhage can result from VE and have associated extensive RHs.

Vacuum-Assisted Vaginal Delivery, Subgaleal Hemorrhage, Retinal Hemorrhages

#### G22 The Relationship of Back Surgery to Overdose at Autopsy

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The goal of this presentation is to define the relationship between the presence of a scar in the midline of the back, indicating a history of laminectomy, with drug intoxication sufficient to cause death.

This presentation will impact the forensic science community by discussing how presence of a laminectomy scar is a powerful marker for a drug related death. In a practice where toxicology is not routinely performed on all cases, the presence of a laminectomy scar should lead to toxicological analysis for that case.

**Rationale:** Individuals who have died suddenly and unexpectedly in which examination reveals a scar in the midline of the back of the sort left by a lumbar or cervical laminectomy are regularly received. Because the cause of death in such individuals is frequently intoxication with some drug, it is hypothesized that death due to a drug overdose is more common in individuals with evidence of a previous laminectomy than in individuals with no prior laminectomy. Therefore, we tested the null hypothesis. There is no difference in the frequency of death from a drug overdose in a study group with a linear scar in the midline of the back when compared to the frequency of drug overdose in cases evaluated at the medical examiner office in which no scar is found on the midline of the back.

Methods: A retrospective case-control study of deaths in 2008 investigated by the Jefferson County Coroner/Medical Examiner Office, Alabama was conducted. The study group consisted of decedents 18 years of age or older who had a linear scar in the midline of the back; as determined by review of the autopsy protocol (body diagram or written report). The control group was chosen from all the decedents examined at the Jefferson County Coroner/Medical Examiner Office, Alabama in 2008. Controls were matched to the study cases by age, race, and sex. Race and sex were matched exactly. Age was matched to the same year in 21 cases, to within one year in five cases, and to within two years in three cases. When more than one control was available the control used was determined randomly the throw of a die. The charts of both the study group (back scar) and of the control group (no scar) were reviewed for the cause of death and evidence of intoxication. All toxicology results were noted in the decedents, including the presence of cocaine, any other drugs or medications, and ethanol. Bodies charred by fire (six cases) or recovered as skeletal remains (two cases) were excluded from the study. This project was approved by the medical Institutional Review Board of the University of Alabama at Birmingham.

**Results:** For all decedents 18 years of age or older in 2008, the likelihood of death being due to a drug overdose was 12.8%. The study group of decedents with a linear back scar consisted of 27 decedents, nine of whom died as a result of acute intoxication with some substance of abuse. In the matched control group one decedent died of acute intoxication with a substance of abuse. Decedents with a back scar were thirteen times (odds ratio 13.0; 95% confidence interval 1.9-85; p= 0.011) more likely to die of a drug overdose than the controls. In other words, a body with a laminectomy scar is 13 times more likely to die as a result of an overdose than is another body without a laminectomy scar. Given the small p-value, chance is an unlikely explanation for these

results. The confidence interval is large because of the few cases in the study; a larger study will narrow the confidence interval.

**Conclusion:** This study shows that, when found at postmortem examination, the presence of a linear scar in the midline of the back of the sort following laminectomy is a powerful marker for a drug related death. In a practice where toxicology is not routinely performed on all cases, the presence of a laminectomy scar should lead to toxicological analysis for that case.

Back Scar, Drug Overdose, Intoxication

#### G23 The Use of Raman Spectroscopic Imaging in Cases of Ethylene Glycol Toxicity

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After attending this presentation, attendees will have reviewed ethylene glycol toxicity, the pathophysiology and histology of ethylene glycol poisoning, and be introduced to the concept of Raman imaging and how it can be used to identify calcium oxalate crystals in tissues.

This presentation will impact the forensic science community by introducing a new method to identify crystalline deposits in the kidneys when ethylene glycol is suspected.

The American Association of Poison Control Centers reported 6,077 exposures to ethylene glycol in the United States, resulting in 40 deaths in 2002. The State of Maryland had 15 cases of ethylene glycol intoxication from 1996-2009. Ethylene glycol is a colorless, odorless liquid which is the principal component of antifreeze. The toxic dose varies but more than 0.1 ml/kg body weight is the reported toxic dose requiring medical treatment. The primary symptoms are CNS depression followed by a cardiopulmonary stage with eventual renal failure. The lethal dose is approximately 100 ml in an adult. It is metabolized to oxalic acid which binds the calcium in the body forming calcium oxalate crystals that eventually lead to the renal failure. In addition, it does not show up on toxicologic analysis in a routine volatile screen.

Forensic pathologists may be presented with a death without an obvious cause, but crystals may be seen in the kidneys that suggest ethylene glycol poisoning with initial negative toxicology. Four cases involving probable ethylene glycol ingestion and the use of Raman imaging to identify calcium oxalate crystals are presented. The cases presented include three cases of known ethylene glycol toxicity and one case of suspected ethylene glycol toxicity with negative ethylene glycol and glycolic acid blood analyses and crystals in the kidneys.

The case that prompted the use of Raman imaging was that of a 52year-old black male found deceased with vomitus on a pillow next to him in his father's vacant home. The decedent had no known psychiatric or past medical history. At autopsy an anomalous right coronary artery and dull green stomach contents were found. Microscopic examination of the kidneys revealed multiple polarizable crystals consistent with calcium oxalate. This prompted additional police investigation revealing the subject was estranged from his family. No additional medical or social history was gained. Toxicologic analysis of blood for ethylene glycol and oxalic acid was negative. Raman imaging showed that the crystals were indeed calcium oxalate. The cause of death was anomalous right coronary artery complicated by oxalosis and the manner classified as Undetermined. Three other cases of known ethylene glycol toxicity underwent Raman imaging. In all cases, toxicologic analysis of the blood was positive for ethylene glycol, autopsy showed crystals in the kidneys, and the cause of death was ethylene glycol intoxication and manner was undetermined.

Raman molecular imaging is a method used to identify molecular structures. It is a physical phenomenon involving the interaction of light with molecules. This method is based on inelastic (Raman) scattering of monochromatic light from a source such as a visible laser, a near infrared laser, or near ultraviolet laser. The laser interacts with phonons in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy is then related as data concerning the phonons in the system being studied. The unstained aluminum slide is illuminated with a laser beam, light from this spot is collected with a lens, and then sent through a monochromator. Wavelengths similar to the laser are filtered out, and the rest of the light is collected into a detector. A given solid material has characteristic phonon modes that can help to identify it.

Raman molecular imaging was able to characterize the unknown crystals as calcium oxalate in all four cases of suspected ethylene glycol toxicity. The confirmation of the calcium oxalate crystals in the kidneys in the case with negative blood ethylene glycol and oxalic acid was helpful. In this case, the inability of investigation to establish clear social and medical history or the source of the oxalosis left the possibility of primary or secondary hyperoxaluria or an exogenous ingestion. Therefore, the manner was best certified as Undetermined.

This case series demonstrates the utility of Raman imaging to confirm the presence of calcium oxalate crystals in the kidneys. The correlation of these crystals to ethylene glycol intoxication requires complete toxicologic analysis and thorough investigation. Raman imaging could have many broad applications in the forensic pathology community and to the forensic community in general in the identification of unknown substances in tissues of all types.

Raman Imaging, Ethylene Glycol, Calcium Oxalate

#### G24 Death From Severe Anorectal Injury of a Jet Ski Passenger

Dennis Rhee, MD\*, and Lynn A. Salzberger, MD, Southwest Institute of Forensic Sciences, 5230 Southwestern Medical Avenue, Dallas, TX 75235

After attending the presentation, attendees will understand the basic principles of jet ski propulsion and the potential for hydrostatic injury to the perineum.

This presentation will impact the forensic science community by bringing awareness to an unusual mechanism of injury in jet ski accidents. Such knowledge would prevent initial confusion and unnecessary use of resources to investigate other causes. In addition, it would serve to promote better safety practices in the use of jet skis.

This presentation will highlight the unusual case of a young woman who sustained lethal anorectal trauma after falling from the back of a jet ski.

It is recognized that both the popularity of personal watercraft and injuries related to their use have been increasing. Non-lethal lacerations, contusions, sprains, and fractures make up the majority of these injuries. Rare, but more serious injuries include closed head injury and intraabdominal injury involving high velocity and rapid deceleration. The latter types of injury are typically those which result in fatality involving personal watercraft.

In the case presented, the autopsy showed a midline laceration posterior to the vaginal introitus which passed through the anus. The laceration extended to involve the deep soft tissues and the distal rectum was seen to be transected and free in the pelvic cavity. The full extent of her injury became apparent when the laceration was seen to extend within the retroperitoneal space to a level above the kidneys.

Given the extensive injury, initial suspicion surrounded possible impalement by a solid object. Witnesses at the scene as well the operator of the jet ski reported that the decedent fell straight back into unobstructed water. A subsequent review of the accident site revealed no fixed obstructions. The decedent was initially conscious in the water, but became unresponsive shortly after being pulled to shore. Bloody drainage was seen from her perineum, and her wound was extensively packed. Despite this, resuscitation was unsuccessful.

A review of her medical history revealed a recent c-section. Her obstetrician reported that her c-section had been uncomplicated. Autopsy supported this as her gynecologic organs were intact. No other injuries were identified. The combination of history, literature, and autopsy findings indicated that the cause of death in this case was due to severe anorectal trauma from the water thrust of a jet ski.

Risk factors for injury in this case included the fact that the decedent was a passenger and fell straight backward. When a passenger falls from a jet ski, the throttle does not shut off automatically as it would for a driver who fell. Further, the decedent was wearing a bathing suit at the time of the accident. A wetsuit may have provided more protection.

While occasional case reports of similar, non-fatal injuries from jet ski accidents have been documented in the surgical literature, such a case has never been reported in the forensic literature to our knowledge. In the surgical cases reported, patients who sustained vaginal and/or rectal lacerations underwent successful repair with recovery of normal function.

Anorectal, Trauma, Jet Ski

#### G25 Sudden Unexpected Infant Death: Peripheral Retinal Hemorrhages Associated With Accidental Positional Asphyxiation (Wedging)

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After attending this presentation, attendees will learn that peripheral retinal hemorrhages extending to the ora serrata are not specific for abusive head trauma (shaken baby syndrome) and can be associated with accidental positional asphyxia (wedging).

This presentation will impact the forensic science community by emphasizing the importance of routine retinal examinations (postmortem monocular indirect ophthalmoscopy) in infants dying suddenly and unexpectedly.

This presentation will inform attendees of something they do not know–multiple retinal hemorrhages, involving the peripheral retina and extending to the ora serrata in infants, are not specific for abusive head trauma (shaken baby syndrome).

Current data (1999 -2007) from the Centers for Disease Control lists accidental suffocation as the leading cause of unintentional death in infants. Of those deaths 65.7% were due to accidental asphyxiation in bed (all mechanisms). Accidental positional asphyxia from wedging often occurs when an infant becomes entrapped between the mattress and wall, headboard, or bed frame of an adult bed. Despite the number of unintentional wedging deaths in infants, to find published reports of retinal hemorrhages (RHs) associated with accidental positional asphyxia (wedging) were not found. A number of authors have asserted that multiple retinal hemorrhages (RHs) involving the peripheral retina and extending to the ora serrata occur only in abusive head trauma (AHT) or rarely with severe head injuries from motor vehicular

collisions or crush head injuries. This reports two infants who died suddenly and unexpectedly from wedging who had multiple RHs including peripheral RHs extending to the ora serrata.

Case 1: A father had been sleeping in an adult bed with his previously healthy 4-month-old infant son while the mother slept in another room with one of the infant's two older siblings. During the night the father heard the 3-year-old sibling wake up; he got up to check on her, but fell asleep her room. At 8:00 a.m., the mother found the infant unresponsive, wedged head down between the mattress and headboard of the bed. Paramedics pronounced him dead at the scene. At autopsy, he had parallel lines on his forehead corresponding to the mattress edging and ticking. Postmortem monocular indirect ophthalmoscopy (PMIO) revealed multiple RHs in the right fundus mainly over the equatorial region and two RHs in the left fundus at the mid-periphery. Microscopically, the right-sided RHs extended to the ora serrata and primarily involved the nerve fiber layer with focal involvement of the inner and outer nuclear layers. The left eye had one tiny retinal hemorrhage in the inner nuclear layer. No optic nerve sheath hemorrhages were identified grossly or microscopically. The dura mater had remote subdural membranes over the right and left frontal and left parietal regions. The brain had no ischemic or traumatic lesions. His postmortem radiographic skeletal survey revealed no acute or healing fractures.

Case 2: A previously healthy 6-month-old infant was sleeping in bed with her mother and was last seen alive at 2:00 a.m. Her mother found her wedged between the mattress and wall, face down on a stuffed animal, at about 6:00 a.m. She immediately drove her to the local emergency department where resuscitative efforts were unsuccessful. The infant was born at term by cesarean section without complication. At autopsy no scalp or subgaleal hemorrhages, skull fractures, cerebral edema, or epidural, subdural or subarachnoid hemorrhages were present. PMIO revealed bilateral RHs. Ocular examination disclosed 30-50 flame-shaped and dot hemorrhages circumferentially located from the mid equator to the ora serrata on the right side. The left eye displayed two posterior RHs measuring approximately 1/4 disc diameter in size, located inferior and nasal to the fovea at the 3:00 position and 1 disc diameter from the optic nerve head at the 5:00 position, respectively. In addition, from the mid equator to the ora serrata were 40-60 pinpoint to flame-shaped or dot hemorrhages extending to, and focally abutting, the ora serrata in all 4 quadrants. No optic nerve sheath hemorrhages were observed grossly or microscopically. Intradural extravasated blood involved the falx cerebri and the right calvarial dura. The brain had ageappropriate development with no ischemic or traumatic lesions. Her postmortem radiographic skeletal survey revealed no acute or healing fractures.

These two cases with reliable histories of positional asphyxia demonstrate the importance of routine postmortem ocular examination of infants to better appreciate the spectrum of RHs seen in this age group. Multiple retinal hemorrhages in an infant, involving the peripheral retina and extending to the ora serrata, are not specific for AHT.

Sudden Unexpected Infant Death, Retinal Hemorrhages, Accidental Positional Asphyxia (Wedging)

### G26 Antiepileptic Drug Intoxication: Report of One Case and a Forensic Pathologist's Approach

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After attending this presentation, attendees, will learn a better forensic approach in investigating fatal cases where there is suspicion of antiepileptic drug misusage.

This presentation will impact the forensic science community by showing a fatal case of intoxication with valproic acid. In addition, further discussion is made in order to clarify and systematize forensic approaches (crime scene investigation and autopsy procedure) in cases involving suspicion of antiepileptic drug misusage, fatal consequences of antiepileptic drug, particularly valproic acid, including its direct toxic effects, adverse reactions and interactions with others drugs, possible mechanisms, causes and manners of death in these type of cases, and promotion of prevention measures with physicians to avoid fatal cases in patients taking antiepileptic drugs.

Valproic acid is formally an antiepileptic drug but currently it has wider clinical uses, including treatment of some psychiatric disorders, such as bipolar and affective disorders. Since prescription of valproic acid has been growing, it is becoming an increasingly common agent to be used in intentional overdoses. Although considered a relatively safe drug, it is known to cause hepatotoxicity and pancreatitis, amongst other adverse reactions. In patients co-ingesting other medications, specifically, those acting as CNS depressants, side effects and toxicity can become more dangerous and even fatal for the patient.

This study presents a 45-year-old blind female, who was found dead by her husband inside their house. The forensic pathologist called to the scene, found five empty blisters-packs of valproic acid. Previous pathologic history included epilepsy, bipolar disorder, and chronic alcohol abuse with prior suicide threats. At autopsy, external and internal examination didn't reveal significant traumatic lesions. The organs showed generalized congestion, the liver was significantly enlarged, the pancreas showed no macroscopic abnormalities and a whitish substance was present in the stomach.

Histological ancillary investigation confirmed congestion in the lungs and kidneys, and also, mild hepatic steatosis. Toxicological results revealed high concentrations of valproic acid (556.0  $\mu$ g/mL); therapeutic concentrations of other psychiatric drugs (tiapride, mirtazapine, nordiazepam, and oxazepam) and blood ethyl alcohol concentration of 1.34 g/L.

After excluding death due to natural or traumatic causes, a direct toxic effect by valproic acid was considered. Taking into account the autopsy, histopathology and toxicological findings, along with the circumstantial evidence, the cause of death was attributed to suicide by intoxication with valproic acid in association with other CNS depressants.

In conclusion, this case illustrates that is crucial for forensic pathologists to: (1) participate or have detailed information from the crime scene, prior to autopsy; (2) know the deceased' complete medical history and prescribed medication; (3) do a careful postmortem examination to exclude natural and traumatic causes of death; (4) study target organs of valproic acid action by macroscopic and microscopic approach; and, (5) do toxicological studies and exclude other causes of death.

When prescribing multiple CNS depressant drugs to patients with alcohol abuse and suicidal ideation, physicians should always be

particularly aware of the risk of valproic acid toxicity, interactions with other drugs and possible adverse reactions, besides the potential accidental or intentional intoxication.

Forensic Pathology, Antiepileptic Drug Intoxication, Valproic Acid

#### G27 Are Peripapillary Intrascleral Hemorrhages Pathognomonic for Abusive Head Trauma?

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After attending this presentation, attendees will learn that peripapillary intrascleral hemorrhages are not diagnostically specific for abusive head trauma (shaken baby syndrome).

The presentation will impact the forensic science community by stressing the necessity for consistent postmortem ocular examinations of infants and young children to identify all conditions associated with certain ocular findings such as peripapillary intrascleral hemorrhages.

This presentation will inform attendees of something they do not know-that peripapillary intrascleral hemorrhages are not diagnostically specific for abusive head trauma (shaken baby syndrome) and exemplify the need for unbiased consistent ocular examinations, both clinically and at autopsy.

The American Academy of Pediatrics' Committee on Child Abuse and Neglect, Section on Ophthalmology, has acknowledged that searching for retinal hemorrhages (RHs) only in infants suspected of abuse creates a selection bias. However, they have also stated that postmortem eye removal might not be indicated "in children who have clearly died from witnessed severe accidental head trauma or otherwise readily diagnosed systemic medical conditions." Although infrequently described in the child abuse literature, peripapillary intrascleral hemorrhages have been considered "probably pathognomonic" for abusive head trauma (shaken baby syndrome) due to severe repetitive acceleration-deceleration forces with or without blunt head trauma.

**Case 1:** A 2-day-old male neonate had significant blunt force head trauma including bilateral subgaleal hemorrhages, right subscalpular hemorrhage, bilateral parietal skull fractures, diastatic separation of the sutures, subdural and subarachnoid hemorrhages, cerebral edema, and hypoxic ischemic brain injury. Indirect ophthalmoscopy revealed 30-50 flame-shaped and dot RHs involving all four quadrants of the left globe, extending anteriorly from the posterior pole past the equator to the ora serrata. Peripapillary intrascleral hemorrhages were in the left eye in addition to bilateral optic nerve sheath hemorrhages and bilateral periorbital soft tissue hemorrhage. The right globe had an artifactual papillomacular fold but no RHs. Neither eye exhibited papilledema. Hemothoraces and bilateral rib fractures were present. He had been delivered by cesarean section at 36-weeks estimated gestational age.

**Case 2:** A 3-day-old female neonate had severe blunt force head trauma. She had a small contusion on the right side of her forehead plus faint abrasions and ecchymoses overlying the right mastoid process. She had bilateral subgaleal hemorrhages, bilateral parietal skull fractures, and a diastatic fracture of the left lambdoid suture. Subdural and subarachnoid hemorrhages, cerebral cortical contusions, cerebral edema and diffuse hypoxic-ischemic brain injury were identified. Indirect ophthalmoscopy revealed multiple bilateral RHs. Subsequent examination of the orbital structures demonstrated bilateral preretinal, intraretinal, and subretinal hemorrhages, optic nerve sheath hemorrhages, peripapillary intrascleral hemorrhages and perineural extravasated blood. The right fundus had 15-20 flame-shaped RHs radiating from the optic nerve head for a distance of two to four disc

diameters. The largest of these measured approximately three disc diameters and was nearly confluent between the fovea and the superior temporal vascular arcade. No hemorrhages were evident past the equator on the right. On the left, the fundus had 10-15 flame-shaped RHs in all four quadrants, located mainly posteriorly, measuring approximately <sup>1</sup>/<sub>4</sub> disc diameter in size. Two faint RHs at the 7:00 and 8:00 positions were flame-shaped and located 3-4 disc diameters from the ora serrata. Papilledema was not evident on either side. She had been delivered by cesarean section at 38-weeks estimated gestation age.

Both neonates had been delivered by emergency cesarean section following the involvement of their respective mothers in motor vehicle collisions. Both had Apgar scores of 0 at 1, 5, and 10 minutes and required prolonged resuscitation lasting 20 minutes and 14 minutes, respectively, before a heart rate was established. In the first case, the mother, a passenger in the vehicle, was ejected in a single vehicle rollover accident. She suffered only minor injuries. In the second case, the mother was the restrained driver of a van that crossed over the midline and hit an oncoming car. Extraction was prolonged and the mother suffered multiple pelvic fractures but no other serious injuries. In both cases the babies' heads were engaged in the pelvis at the time of the accidents. No uterine or placental injuries were found in either case and the mothers were not in labor at the time of the accidents. Neither neonate had a documented clinical fundal examination while hospitalized in the intensive care unit.

*In-utero* skull fractures with severe brain injury are uncommon but well documented. It is believed, RHs with peripapillary intrascleral hemorrhages have not been previously reported in neonates sustaining *in-utero* skull fractures and traumatic brain injuries. These cases demonstrate that peripapillary intrascleral hemorrhages are not diagnostically specific for abusive head trauma and exemplify the need for consistent, unbiased ocular examinations, both clinically and at autopsy.

Peripapillary Intrascleral Hemorrhages, Accident, Intrauterine Traumatic Brain Injury

#### G28 The Correlation of Serum Stress Hormone Levels With Cause and Circumstance of Death

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The goal of this presentation is to alert forensic professionals that biological markers have the potential to provide significant information concerning the psychological state and stress levels of someone just before death.

This presentation will impact the forensic science community by providing medical examiners and the courts a mechanism to understand the degree of stress someone was going through immediately prior to death.

Interleukin 6 (IL-6) is a major regulator of immune function, and has been shown to increase due to both physical and psychological stress. Knowledge of whether or not an individual was under psychological stress prior to death may be important in many cases. In this study, levels of IL-6 and its soluble receptor (sIL6-r) with an assumed level of psychological stress prior to death was correlated. Postmortem serum samples were obtained from the New Hampshire Medical Examiner's Office and analyzed using ELISA to determine concentration of both sIL-6r and IL-6. The raw data for the soluble receptor could be placed into four groups. However, these groupings were inconsistent with stress levels based on a study of the case histories of the decedents. The data for IL-6 however correlated well with the level of psychological and emotional stress an individual was under prior to death. This study shows that measurement of postmortem serum IL- 6 can be a potentially useful technique for determination of psychological and emotional stress prior to death.

Stress Hormones, Interleukin 6, Circumstance of Death

#### G29 Stippling Mimics — Differentiating Pseudostippling From Stippling: A Report of Four Cases

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After attending this presentation, attendees will be able to recognize factors from scene investigation, history, autopsy, and histology which may help in reliably differentiating stippling from stippling mimics, and understand the role of history and investigation as well as direct observation in differentiating stippling from stippling mimics.

This presentation will impact the forensic science community by assisting forensic pathologists in recognizing patterned injuries which may mimic stippling, and in utilizing history and scene investigation integrated with observation to draw valid conclusions about the origin of apparent stippling.

A valid outcome results from valid input. A forensic pathologist who relies only on observation, whether gross or microscopic, may draw invalid conclusions from what appear to be readily classifiable patterns of injury. Integrating history and scene findings into the decisionmaking process may allow the pathologist to come to reliable and valid conclusions about the source of a patterned injury that appears to be stippling.

A 20-year-old man died in a parking lot from a gunshot wound to the face, less than three weeks after sustaining nonfatal gunshot wound injury. Initial observation of the fatal gunshot wound, which entered the cranium through the tip of the nose, suggested a band or outline of stippling above the eyebrow, consistent with wearing a pair of glasses or sunglasses at the time he was shot. Multiple witnesses reported that the decedent was shot by an assailant from a car across the parking lot, far outside any possible stippling range. Scene re-investigation showed that the marks of pseudostippling matched the gravel in the parking lot. There were no glasses.

A 25-year-old male front-seat passenger in a vehicle, along with the driver was fatally shot by police during a chaotic incident that resulted from a confrontation following a police chase. The driver's body showed typical distant gunshot wounds, but the passenger, who was shot twice, had one distant gunshot wound, and one gunshot wound of the face surrounded by a dense 3" x 3" oval of apparent stippling. History and scene investigation suggested glass fragmentation injury from a bullet which passed through the passenger's window prior to striking him. A similar finding was noted in a homicide a year later when the driver of another vehicle was found dead in the front seat.

A 22-year-old woman was shot by her ex-boyfriend in a homicidesuicide event. The shooting was partially witnessed. The boyfriend shot the victim from a balcony of an outside staircase on which he stood two stories above her. He died immediately afterwards in the same location from a characteristic gunshot wound to the right temple. The decedent appeared to have stippling to the left axilla, and wounds suggestive of blunt to sharp force trauma across the neck, torso, and thigh. Extensive scene investigation was performed and co-ordinated with the autopsy findings to explain the apparent discrepancy between the locations of the shooter and the victim, and the victim's wounds.

These case reports are utilized, along with examples of true stippling for comparison, to demonstrate the dangers of invalid conclusions about patterned injuries when only observation is relied upon.

Stippling, Pseudostippling, Glass Fragmentation Patterns

#### G30 DNA Extraction From Paraffin Blocks: Organ Selection and Pre-Embedding Fixation Times – Practical Implications for Forensic Pathologists

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After attending this presentation, attendees will gain an understanding of which organs or tissues are optimal for paraffin archival for the purposes of subsequent DNA extraction; attendees will recognize the importance of the length of formalin fixation prior to paraffin embedding.

This presentation will impact the forensic science community by helping attendees to be able to direct histologic sampling to one or two tissue types (in decomposed and non-decomposed bodies) for the purposes of DNA sampling. Attendees will understand the critical time parameter for pre-embedding formalin fixation.

Synopsis: In recent years re-examination of old death investigation cases in the form of prosecutorial post-conviction review, defense review (prompted by the Innocence Project and others), law enforcement cold case initiatives (often with federal grant support), and ancestry inquiries is increasingly frequent. These reviews are aided and sometimes initiated by the existence of advanced DNA technology that may not have existed until after conclusion of the original investigation. Therefore the ability to obtain usable DNA evidence from items stored for extended periods is increasingly important. While standard forensic practices vary with regard to storage times for various pathology specimens (formalin fixed wet tissues in particular), forensic centers and hospital pathology services often retain paraffin embedded tissue blocks indefinitely; current National Association of Medical Examiners (NAME) accreditation guidelines require a retention time of at least 10 years. Consequently, paraffin blocks are an available resource for stored DNA evidence. However, forensic pathology practice varies regarding number of organs sampled for paraffin preservation, and even whether or not sections are taken in all types of cases; NAME accreditation guidelines allow discretion as to types of cases in which slides are produced but do recommend a one-year retention of paraffin embedded tissues when slides are not produced. To address the question of which tissue would be optimal for DNA recovery using modern methods, these experiments were designed to determine whether decomposition changes that answer, and whether the length of pre-embedding fixation is a critical variable.

Tissue obtained at autopsy was divided into three groups based on formalin fixation times of 1, 5, and 12 days prior to dehydration and paraffin embedding. Sections of each organ were deparaffinized with heat and DNA was purified from the residual tissue via organic extraction. Subsequent purification was accomplished by one of several different methods. Extracted DNA was quantified then amplified using a PCR amplification kit and separated by 3130xl capillary electrophoresis. The electropherograms were analyzed for DNA profiles.

Tissues compared were heart muscle, skeletal muscle (psoas), liver, spleen, and brain. Cases included both non-decomposed and decomposed bodies. Preliminary results for quantitiative DNA recovery indicate that spleen is superior to liver, heart or skeletal muscle. A striking loss of recoverable DNA is observed between pre-embedding fixation times of 5 to 12 days. The loss of DNA with increasing fixation times is maximum in spleen and skeletal muscle followed by liver and heart, but the differences between organs are small. Studies are ongoing, but preliminary data suggest that although quantitative DNA recovery is

greatest from spleen, the degree of degradation may also be greatest with spleen. Studies comparing brain to the other tissue types and the effects of decomposition are ongoing.

**Impact:** This project was undertaken to better define which tissue types are the best for extraction of DNA from paraffin blocks using modern DNA technology. With this knowledge forensic pathologists will be able to selectively sample organs in order to efficiently preserve DNA evidence while minimizing the expense of embedding multiple tissues and organs from all cases.

**DNA Extraction, Paraffin, Formalin Fixation Time** 

#### G31 The Potential Value of Bone Marrow Analysis for Forensic Purposes: Evaluation of Needle Aspiration and Biopsy Taken From the Sternum

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After attending this presentation, attendees will understand the potential contributions of postmortem investigations of bone marrow (BM) taken from sternum in order to validate the diagnosis of some type of death.

This presentation will impact the forensic science community by emphasizing the potential contributions of postmortem BM evaluation that should become a routine procedure, especially if the forensic pathologist can not detect real cause of death during autopsy.

The importance of studying the bone marrow in clinical practice is well known and techniques such as marrow needle biopsies, smears from aspirate, and imprint preparations, allow the diagnosis of several blood disorders. On the other hand, many studies have explored the involvement of bone marrow also in systemic illnesses, including metastatic involvement with tumors, granulamatous diseases, AIDS, in staging of carcinomas, and for the follow-up evaluation of patients undergoing chemotherapy or transplantation. Other studies have strongly suggested that inflammatory cells originating from the BM contribute to sustain pathophysiological processes, e.g., allergy, sepsis, healing wounds. For example, in allergies, progenitor cells migrate to the site of allergic inflammation via blood, where they differentiate into tissue-dwelling and classic effector cells, such as mast cells and eosinophils. These modifications are probably secondary to the production of various cytokines which either block or stimulate the proliferation of hematopoietic stem cells (growth factors) and their differentiation.

A number of studies has been published in recent years about the use of BM specimens taken from iliac crest and rib as alternative tissue in forensic toxicology, concerning the detection of postmortem alcohol and drug content. Nevertheless, there is a lack of studies regarding an alternative role of the sternum aspiration and needle biopsy which can help to understand pathophysiological changes in response to stress, infection, or other external stimuli.

A study based on BM samples (needle aspiration and biopsy) taken from the sternum which were obtained from 70 autopsy cases performed in the Section of Legal Medicine, Bari University, from subjects died due several causes (cardiovascular diseases, craniocerebral trauma, sepsis, etc) will be presented. The histopathological results will be discussed in the light to underline the potential value of BM analysis for forensic purposes.

Assuming that by using sternum evaluation, the limit of poor samples possibly obtained by iliac aspiration, especially in postmortem work-up, might be avoided. Moreover, cytomorphological evaluation on sternum smears might offer more elements than those obtained by just histopatological examination, because of the less frequent postmortem alterations frequently described in bone marrow biopsy. In fact, BM is surrounded by solid cortical bone, which results in mechanical stability, this makes it more secure than other organs, e.g., against postmortem changes. Finally, sampling from sternum can easily be performed in larger amounts, easily accessible in routine autopsies, without changing the structure of the corpse in a relevant way.

The goal of this preliminary study is to demonstrate the presence of bone marrow postmortem activated cells in various causes of death as well as to analyze, for the first time in the literature, the sternum as the most important site for studying cells of such lymphoid organ in cadavers.

Bone Marrow, Postmortem, Immunology

# G32 2009 H1N1 Fatalities: The New Mexico Experience

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After attending this presentation, attendees will be able to describe the clinical and epidemiologic features associated with H1N1 fatalities, recognize the spectrum of histologic features that can be seen in H1N1 fatalities gain a familiarity with laboratory diagnostic options in cases of suspected H1N1, and recognize the importance of the autopsy in tracking the epidemiology of infectious disease.

This presentation will impact the forensic science community by raising awareness of which subgroups have greater H1N1 influenza mortality risk, and therefore may benefit from early antiviral treatment. It will also illustrate that H1N1 fatalities with a relatively rapid disease course may have far subtler respiratory histologic findings than those of previously published studies.

**Hypothesis:** New Mexico is an ethnically and racially diverse state with a large Native American population, among others. It is hypothesized that this population heterogeneity may predict a similar diversity of clinical and pathologic findings in 2009 New Mexico H1N1 fatalities.

**Methods:** A retrospective review of hospital, laboratory, field investigative, and autopsy reports of all H1N1 positive influenza fatalities reported to the New Mexico Department of Health in 2009 was performed. In those cases in which autopsies were performed, all available microscopic slide sections were independently reviewed by a study pathologist. All respiratory sections were additionally reviewed by a study pathologist with pulmonary pathology expertise.

**Results:** There were 52 H1N1 deaths reported to the New Mexico Department of Health in 2009: of these, 14 were autopsied. In two autopsied cases, H1N1 infection was determined to not be the cause of death. These cases were excluded from further study. In 3 out of 12 autopsied cases, the diagnosis of H1N1 influenza was made via antemortem studies, while in 9 out of 12 cases it was made at autopsy via reverse-transcriptase PCR on nasopharyngeal specimens +/- viral

nasopharyngeal/lung cultures. The most common respiratory histologic findings were alveolar edema (75%), interstitial inflammation (100%), bronchitis/bronchiolitis (83.3%), tracheitis (87.5%), and bronchopneumonia (66.7%). Of the total autopsied and non-autopsied fatalities, race/ethnicity was 42% Hispanic, 36% Caucasian, and 22% Native American. Ages ranged from 2 months – 89 years, with peaks in the 40 (18%) and 50 (26%) year decades.

Conclusions: This study highlights the importance of the autopsy in tracking the epidemiology of infectious disease: in 9/12 (75%) cases, H1N1 influenza was not known to be the cause of death until after autopsy. Most other studies of H1N1 pulmonary histopathology report diffuse alveolar damage (DAD) in the majority of autopsied fatalities (74%-100%). In this series, only 2 out of 12 (16.7%) cases manifested DAD. Also, the majority had a relatively rapid disease course: time from onset of symptoms to death in autopsied cases ranged from 1-12 d (avg 3.5 d) vs. the other largest published series' range of 2-44 d (median 7 d). These findings may indicate that New Mexico H1N1 influenza fatalities generally did not survive long enough to develop the more classic pulmonary manifestations. Native Americans comprised 2 out of 12 (16.7%) of autopsied fatalities and 9out of 38 (23.7%) of non-autopsied fatalities. As the overall New Mexico population is only 9.6% Native American, Native Americans are disproportionately represented among the 2009 NM H1N1 fatalities.

H1N1, Influenza, Autopsy

#### G33 The Potential Use of Aquatic Invertebrate for Postmortem Submersion Interval (PMSI) Determination

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After attending this presentation, attendees will understand that in cases in which a human body is found in aquatic environments, careful aquatic scenes investigation, review of medical records, complete autopsy with skeletal survey, marine biology, and taphonomy testing are required.

This presentation will impact the forensic science community by emphasizing the fact that although estimating postmortem interval in terrestrial environments are standardized and widely accepted in courts of law, estimating immersion interval in aquatic environments are largely unexplored. The Postmortem Submersion Interval (PMSI) in aqueous environments refers to the time period from when the body enters the water to the point of discovery, noting that the body may be totally submerged for all or part of the time period. Understanding the growth phases of aquatic plants and animals that attach themselves to submerged remains is particularly valuable information and can be used to estimate a minimum PMSI.

A case of an adult human body discovered on an Ionian coast (South Italy) in February 2009, whose soft parts were converted into adipocere with partial skeletonization and disarticulation and showed the presence of barnacle stratification on bone surfaces is presented.

Barnacles specimens collected from bone surface consisted of a body divided into two regions: (1) the peduncle (stalk); and, (2) the capitulum. The peduncle is fleshy, large, and long, and it attaches to the substrate using the first antennae. The body is compressed laterally, covered by two folds of mantle, where five thin calcareous plates are attached. The carina is a dorsal unpaired plate, which forms a central keel. Paired scuta are large, and are located at the anterior region of the body. Paired terga are short and are located at the posterior-most region of the body. Six pairs of thoracic, biramous cirri bordered with chaetae are visible through an aperture present in the mantle cavity. In the mantle cavity, there is a short head, a thorax with six thoracic, biramous limbs, a mouth, and a long, setose penis. The length of pedunculated barnacles ranged between 0.7 and 2 cm.

These barnacles belong to the family of Lepadidae, genus Lepas, species Lepas anatifera, order peduncolate barnacles.

The *Lepas anatifera* live in tropical and subtropical waters, and after attachment to the substrate is increased by an average of 1mm/die in seabed with temperatures between 15°C and 30°C. The growth of the barnacle is blocked at temperatures below 15°C or above 30°C.

Therefore stratification found on the surface of long bones of the lower limbs of *Lepas anatifera*, require at least 20-30 days at water temperatures between  $15 \degree C$  to  $30\degree C$  for achieving the maximum size observed in this case (2 cm).

The average temperature estimated in the Ionian Sea in February 2009 was 10.7°C, so it can be assumed that seawater temperature along the Ionian coast drops below 15 degrees for November-March. Hence, in November 2008 the corpse was already skeletonized and already converted into adipocere since the colonization of barnacles was already present on skeletonized limbs. This data suggested the amount of time the body was in standing water was at least six months/one year prior to attachment barnacles (October 2008) and, as a consequence, the range of immersion was identified in a period between October and November 2007 and March/April 2008.

The use of aquatic invertebrate in this case suggests a new avenue of basic research that forensic investigators can apply to cases involving submersed and/or floating human remains. In fact, the study of biology of aquatic invertebrates along with a timeframe of decomposition in the aquatic environments, can provide important clues on the length of soak time, however influenced by a high number of variables can potentially influence this process (e.g., temperature, water depth, currents, tides, season, dissolved oxygen, debris, substrate type, salinity, acidity, interactions between chemical and physical processes, and micro and macrofauna activity).

Adipocere, Marine Biology, Barnacle

#### G34 Epidemic Outbreak of Meningococcal Meningitis in a Nursery: Two Fatal Cases of Waterhouse-Friderichsen Syndrome

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The goal of this presentation is to focus on two fatal cases of undiagnosed meningitis occurring simultaneously in two children from the same nursery. A forensic approach by means of autopsy, microscopic examination, and microbiological studies led to the conclusion that the cause of death in both infants was septic shock due to meningococcal meningitis in association with hemorrhagic adrenalitis.

This presentation will impact the forensic science community by demonstrating how important a thorough forensic investigation is to reach the correct postmortem diagnosis, as well as, by showing how rapidly children can develop a fatal meningococcal infection as well as explaining the importance of an early clinical diagnosis in order to avoid unexpected death and epidemic outbreaks.

Waterhouse-Friderichsen syndrome (WFS), first described in the early 1900s in England and Denmark, is the most severe form of meningococcal septicemia. The infection leads to massive hemorrhage in one or usually both adrenal glands. It is most commonly caused by *Neisseria meningitidis* (NM) but many other species of bacteria and also viruses are associated with WFS.

The onset of a meningococcal infection is non-specific with symptoms of fever, rigor, vomiting, and headache. Soon a rash appears; first macular, then rapidly becoming petechial and purpuric. In most cases the resulting hypotension rapidly leads to septic shock. In WFS, meningitis generally does not occur but if present, many clinical signs can be found such as hypoglycemia with hyponatriaemia and hyperkalemia, thrombocytopenia and typical markers of diffuse intravascular coagulation.

Only microbiological studies can lead to the final diagnosis through culturing of blood or cerebrospinal fluid (CSF).

Fulminate meningococcemia is a medical emergency and needs to be treated with adequate antibiotics as fast as possible, also in order to prevent an epidemic outbreak. The administration of corticosteroids can sometimes reverse the adrenal shock.

**Case 1:** A 21-month-old child, previously in good health, developed high fever (40°C) on a Sunday morning. Paracetamol was administered twice during the day but both times with a low response. Few hours after the onset of the fever, the child began to vomit. In the evening the parents noticed a red\black "purpura" on the abdomen and the back. However, at the time, the child seemed to feel better, ate with a good appetite, and was afterward sleeping normally. Early the next morning the father found him lifeless in his bed.

**Case 2:** A healthy 19-month-old child, later discovered to be taken care of in the same nursery as the previous child, had a very similar clinical history. On the same Sunday afternoon, he developed high fever (39°C) and was treated with paracetamol but with a weak response. The next morning, after a normal night's sleep, he suddenly started to vomit and became cyanotic. The parents immediately called an ambulance but the baby died on the way to the hospital.

Complete postmortem autopsy of both children were performed 24 hours after death. Gross examinations revealed that they were ageaccordingly developed. They were covered with purple petechial spots all over the body but no other remarkable external findings were observed.

Autopsies showed cerebral oedema and venous congestion, diffuse whitish and milky subpial exudation, adrenal glands with massive hemorrhagic infiltration of the parenchyma, and polyvisceral stasis. No other significant abnormalities were found.

The macroscopic appearance led to the suspicion of meningeal infection and hence, CSF, buccal, pharyngeal, and nasal swabs, as well as blood samples were taken for microbiological studies. These showed NM DNA positive for NM serotype B. Furthermore, all samples were culture positive after 72 hours of incubation. No other pathogenic agents were present.

The microscopic histological study, performed by using formalinfixed paraffin embedded tissue sectioned at 4  $\mu$ m and stained with haematoxylin-eosin, revealed subpial and cortical oedema, mild inflammatory infiltration along penetrating, deep brain vessels. The adrenal glands showed massive hemorrhagic infiltration, the lungs focal oedema, and there was polyvisceral stasis.

In conclusion, two infants died one shortly after the other 72 hours after they had last been in the same nursery and 24 hours after the onset of their symptoms. In both cases, a multidisciplinary approach revealed the cause of death to be septic shock due to acute meningococcal infection with hemorrhagic adrenalitis (Waterhouse-Friderichsen Syndrome).

Waterhouse-Friderichsen Syndrome, Epidemic Outbreak, Forensic Diagnosis

#### G35 Postmortem Animal Injuries: A Forensic Pathologist's Perspective

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After attending this presentation, attendees, will become aware of how traumatic injuries, especially those arising after suspicion of assault, could be instead, inflicted by local fauna where the body was found.

This presentation will impact the forensic science community by showing several cases involving postmortem traumatic lesions produced by different animals, which can lead to misdiagnosis, since they may resemble, inflicted and non-inflicted antemortem lesions. Therefore, postmortem animal injuries must always be taken in consideration by forensic pathologists, with the objective of avoiding mistakes in the initial investigation and further autopsy conclusions.

Postmortem animal predation on human death bodies is an important taphonomic phenomenon in forensic pathology, since animals, depending on their size and environment (land, water, or air), can produce a great variety of lesions. These type of animal injuries can be produced during early and/or late postmortem period creating some forensic implications and difficulties, including: (1) mimicking antemortem lesions, particularly when they are produced shortly after death; (2) modifying antemortem injuries, with loss of identifying features, which may lead to interpretation problems; and, (3) mistaken for signs of assault due to differential diagnostic problems.

This study presents several cases of postmortem injuries produced by animals from different environments, including ants, cats, dogs, sea and river fauna with the corpses being found indoors (home) or outdoors (forest, sea marine and river). The postmortem animal lesions didn't show any vital signs and the majority were located in unprotected body extremities, like face, upper and lower limbs. In these cases, autopsy findings allowed to identify both natural and violent causes of death, including two cases of asphyxia by drowning.

In conclusion, cases like those presented, illustrate that forensic pathologists need to have specific and especially attention when postmortem animal injuries may be present. Apart from the crime scene investigation and the autopsy procedure, it is essential that the pathologist: (1) have knowledge of local fauna, in particular from his own geographic area in order to better understand characteristic injury patterns produced by different animals; (2) always make a careful and detailed examination of the traumatic lesions, including morphological features and topographical distribution, so an accurate trauma etiology diagnosis can be done; and, (3) make a correct differential diagnosis which may clarify between antemortem and postmortem lesions.

Forensic Pathology, Postmortem Injuries, Animal Predation

#### G36 Neck Findings in Hanging and Strangulation Cases That Underwent Autopsy in Adana, Turkey

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The goal of this presentation is to demonstrate the neck findings in hanging and strangulation cases.

This presentation will impact the forensic science community by presenting macro-morphological findings in hanging and strangulation cases during the autopsy performance.

Deaths due to the mechanical forces applied to the neck area are frequently encountered in forensic medicine practices. Among these, hanging accounts for the majority of the forensic cases. It is seen in the majority of the studies performed in various regions of the world that hanging is the leading method of suicide. Strangulation cases are also seen though not common. The origin of the event is sometimes suspicious in the case where the crime scene has been altered, or in the case that the corspes had been pulled down or had fallen down because the string had broken.

In these cases, different problems may be in question and the direction of the inquest may change. Regarding the frequently encountered death cases due to the mechanical forces applied to the neck area caused by the above mentioned reasons, assessment and interpretation of the neck findings have important place for the forensic medicine. The consistency of these findings with the inquest and the information about the crime scene, as well as the method used and the presence, type, and characteristic of the traumatic findings in the neck area gain special importance. Fracture in the bone and cartilage tissues of this area and hemorrhage into the soft tissues are of great importance for the diagnosis as well as for the etiology to be exposed. These lesions are considered the indicators of a mechanical force applied to this area. Therefore, forensic medicine specialists carefully examine the neck during autopsies, remove the hyoid bone and thyroid cartilage, and completely evaluate. This present study was conducted in Adana province that is located in South Turkey, has a population of 2.5 million with high rate of unemployed subjects, and is exposed to high rate of internal migration because of extensive land available for agriculture. The records of the autopsies performed at the Group Presidency of Adana Institute of Forensic Medicine (IFM) between the years 2008 and 2009 were retrospectively reviewed. One-hundred and seventy cases (6.2%) that were assessed to have died due to a mechanical force (hanging or strangulation) were included in the study among 2,726 cases. Of the cases, 159 died of hanging and 11 died of strangulation. It was determined that 104 of the cases (61.2%) were male and 66 were female, hanging accounted for 93.5% and strangulation accounted for 6.5%, all of the hangings were suicidal, whereas the strangulations were murder. The age of the cases ranged between 4 and 86 years; the majority of the cases (n=37, 21.7%) were between 21 and 30 years of age, whereas 32 cases were between 11 and 20 years of age. It was determined that two girls between the ages 0 and 10 years died of strangulation. It was observed that 121 of the hanging cases were typical (the node was behind the neck), whereas 38 were atypical. Thyroid cartilage fracture was determined in 43 cases (25.3%) from hanging and strangulation, whereas hyoid bone fracture was determined in 25 cases (14.7%) and both thyroid cartilage fracture and hyoid bone fracture were determined in 11 cases (6.5%). Fracture or dislocation in cervical vertebra was observed in seven cases. A total of 86 cases (50.6%) had thyroid cartilage, hyoid bone, and cervical vertebra findings. It was determined that, 90 of the cases (52.9%) had hemorrhage into the soft tissues either with or without fracture and that 60 cases (35.3%) had no finding other than skin lesions. Both thyroid cartilage and hyoid bone fractures were observed in 2 cases (18.2%) died of strangulation, whereas only hyoid bone fracture was

observed in one case and hemorrhage into soft tissues was observed in five cases. It was determined that the neck findings were higher in hanging cases as compared to the strangulation cases. Toxicological analyses revealed that ethanol was present in 18 cases with a range 34mg/dl to 334mg/dl. This present study was performed to put forward the prevalence of neck findings in the hanging and strangulation cases that are frequently come across by the forensic medicine specialists, as well as to discuss the results with the information in the literature.

Hanging, Hyoid Fracture, Thyroid Fracture

#### G37 Evaluation of Cardiac Conduction System Pathologies in Sudden Deaths in Adana Region

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The goal of this presentation is to encourage the forensic pathologists in evaluating the cardiac conduction tissues in sudden death cases.

This presentation will impact the forensic science community by presenting the microscopical findings of the cardiac conduction tissues in sudden cardiac death cases.

A forensic pathologist is frequently asked to find the cause of death in cases of sudden unexpected deaths in adults. Approximately 50% of all medico-legal deaths are due to natural causes. Approximately 1-5% of all those cases remain as negative autopsies. Sudden cardiac death is usually defined as death from cardiac causes without apparent antecedent symptoms or within the first hour after onset of symptoms. Studies of morbidity and mortality related to cardiac disease estimate that there are between 300,000 and 400,000 sudden cardiac deaths annually in the United States. On the other hand, Turkey does not have a serial study of cardiac diseases as autopsy findings. Therefore, this preliminary study was planned. Examination of the cardiac conduction system is often looked upon as a last resort in the evaluation of a victim of sudden death. It is reasonable to conclude, then, that unfamiliarity with conduction system anatomy and pathology and lack of experience with the examination techniques are the true reasons for this reluctance, which is not surprising because many anatomic pathology and forensic medicine residents complete their training without learning about the cardiac conduction system. This lack of training is symptomatic of the ongoing decline of the autopsy as a teaching tool. Careful case selection for conduction system analysis, coupled with a sensible approach to dissection and histological sampling, will result in an increased yield of diagnostically specific, potentially lethal lesions with only a minimal increase in the expenditure of time or money.

Twenty-seven SUD and four known cause of death forensic cases had been chosen for this study. The autopsies held in the Morgue of the Adana Branch of the Turkish Forensic Medicine Council. The cardiac tissue and coronary artery samples were dissected as described by the CAP and the Cardiac Conduction System examined as already has been described by Cohle et al and Gulino Sam. Harris' H+E, Masson's Trichrome, Verhoeff's elastic Van Gieson and also for amiloidosis, Lieb's Crystal Violet stains had been used histochemicaly.

The 31 autopsy cases differed in age from age of 17 to 78 years with an average 41.7. Fifteen cases had serious atherosclerotic changes in the coronaries. In 13 cases there were infarctions. In this study cardiac conduction tissue pathologies in the 11 was revealed.

Serious fibrotic and remarkable adipose tissue changes in the SA and AV nodes were found. Many of the similar studies show parallel results with this study. The difference in between these serials can be explained by the difference of the countries, socio-cultural specifics, life conditions, environment, nutrition, and genetic variations. Hypoxic changes of the myocardial tissue may also cause conduction system pathologies. Myocardial infarctions were present in four of the SA nodes and two of the AV nodes of all cases. This is an important finding to understand and reveal the conduction system effects of the early and late myocardial infarctions. Amyloidosis was not found case in this serial. Any significant pathologic changes in any of the control cases was not noticed. In some of the SUD cases, the pathology is not morphological yet functional. Yet, still in some cardiac rhythm disturbances cases, some may find cardiac conduction tissue pathology histologicaly. The relationships between cardiac conduction tissue morphological pathologies and cardiac rhythm disturbances will only be demonstrated clearly by clinico-pathologic evaluations with in large serial studies.

Further study is needed of the cardiac conduction tissue on larger SUD serials, and to understand the pathologies and mechanisms of deaths in especially young SUD cases at our region and country. The findings in this model study are very important in demonstrating the young SUD cases and its relation with the conduction tissue pathologies. Therefore, examination of the cardiac conduction system can be a very useful adjunct to the examination of the heart in cases of sudden cardiac death especially in our region. Careful case selection, proper technique, and mindfulness of nonspecific findings or normal variants increase the likelihood of identifying abnormalities that may serve as the morphologic substrate for sudden cardiac death.

Sudden Cardiac Death, Conduction Tissue, Histochemistry

#### G38 A Peculiar Fatal Lightning Strike Inside a Cottage

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After attending this presentation, attendees will learn about a case of instantaneous death due to a peculiar form of lightning storm.

This presentation will impact the forensic science community by stressing the importance of crime scene investigation in order to assess real causes and means of death.

Lightning strike is a fascinating and unpredictable natural phenomenon with potentially devastating effects and represents one of the most common causes of deaths from environmental phenomena. The incidence of fatalities had been estimated in the United States to be around 150-300 cases/year (Duclos PJ et al, 1990), representing a third of all lightning strikes (Sheela SR et al, 2000).

Benjamin Franklin first demonstrated 200 years ago that lightning consists of a gigantic electrical discharge. The physical processes that take place in and around a thundercloud occur at the micro-particle level and at a much larger scale that involves the entire Earth as an electrical circuit. Lightning happens when the difference in voltage between a cloud and the ground or another object exceeds 2 million V/m. Afterwards, an arc occurs and there is the release of a great amount of electrical energy that can cause severe damage to organs, also resulting in high mortality (Copper MA et al, 2001).

The most vulnerable subjects for lightning strike are individuals who work in open fields such as farmers or swimmers; additionally, it is more rare for lightning to strike inside a building as in the case hereby presented. The risk of being struck by lightning is also a function of population density and it comprises terrain features that may protect or not occupants of an area (Ritenour AE et al, 2008).

According to literature review, data appears to be significantly affected by underreporting when comparing Meteorological Offices to medical and death-certificate databases (Cherington M et al, 1999).

Five most common mechanism of injury were described: direct strike, ground current, flash discharge, contact strike, and blunt trauma.

A 53-year-old man started to renovate his own cottage after lunch. In the evening, receiving no answer from him, the family called the police. When they arrived, with the forensic pathologist, the scene investigation revealed the man lifeless lying on his right side between two metal sawhorses. The body was covered by burnt pieces of his working clothes. The man presented diffuse second, third, and fourth degree burns in several areas of the body but especially in the abdominal region, in the root of the thighs, and on his genitals.

There was complete carbonization of beard and hairs all over the body and the scalp. The surrounding environment showed no signs of burning. His working tools were scattered around and there was a generator that had been set up to perform the work. However, the firefighter technical assistant found no damage to the generator or electrical malfunction.

The Meteorological Office reported that in the same area, few hours before, there had been a thunderstorm. Moreover, another person had been simultaneously injured by a lightning strike while crossing a bridge in the same village.

The cottage presented a rudimentary system of walls containment with steel beams pointing from outside to the center of the premises through the roof. The beams might have played a decisive role in the conduction of an electrical atmospheric discharge. This was further facilitated in the room by the presence of metal working tools directed toward the ceiling.

All investigation data suggested that a lightning had entered into the cottage thorough the beams creating an arc in the point where the victim was working.

Necropsy and histological findings confirmed the suspicion of lightning strike.

In conclusion, a detailed analysis of crime scene investigation, environmental, and autopsy data led to the correct determination of the real nature of the suspicious death which could be related to other different causes which may also be not accidental.

Lightning Strike, Fatal Injury, Crime Scene Investigation

#### G39 Enterobacter Cloacae Peritonitis Secondary to Hemorrhagic Cystitis in a Long-Term Substance Abuser

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The goal of this presentation is to illustrate an unusual case of peritonitis caused by hemorrhagic cystitis.

This presentation will impact the forensic science community by illustrating the need for microbilogical cultures and routine histology in cases of peritonitis where an obvious source or rupture site is not identified.

**Introduction**: The major causes of peritonitis are appendicitis, perforations associated with diverticulitis, peptic ulcers, gangrenous

gallbladder, gangrenous obstruction of the small bowel, incarcerated hernia, and volvulus. Peritonitis secondary to cystitis is a rare, life threatening condition due to the unique anatomic characteristics of the urinary tract. Predisposing factors include anatomic anomalies of the urinary tract, vascular impairment, previous surgery, irradiation and high virulence pathogens. Making a diagnosis of peritonitis due to cystitis is difficult and the prognosis is usually poor.

Materials and Methods: This case involves a 47-year-old African-American female with a long standing history of alcohol, heroin, and cocaine abuse, who was found dead in her secure apartment. According to witnesses, she experienced flu-like symptoms for the past few days. The examination of the scene revealed a cluttered and unkempt dwelling with numerous empty and full malt liquor cans. Dark colored stains were noted on the bed and the floor, and a bucket with vomitus was discovered near the deceased.

**Results**: Postmortem examination revealed a poorly nourished African-American female, weighing 102 pounds and measuring 65.5 inches (BMI – 16.5). Signs of prior drug abuse, i.e., multiple remote circular scars ("skin popping" sites), were noted on the upper and lower extremities. At autopsy, the abdominal cavity contained 500 cc of serous fluid. Fibrinous exudate was observed on the dusky red small and large intestines. The urinary bladder contained 20 cc of dark-brown blood and exhibited a markedly thickened, hemorrhagic mucosal surface. No gross perforation was identified.

Microscopic examination revealed severe full thickness acute and chronic inflammation, focal hemorrhage, and necrosis of the urinary bladder. Both kidneys displayed acute tubulointerstitial nephritis.

Peritoneal fluid and urine cultures grew Enterobacter Cloacae. Postmortem toxicology was positive for Ethanol (0.011% in the blood; 0.020% in the vitreous fluid).

No other pathologic abnormalities or trauma were identified during the autopsy.

**Conclusion**: Secondary peritonitis follows contamination of the peritoneum by organisms released from the infected organs or perforated viscera. Peritonitis due to acute cystitis is a rare occurrence with only a handful of reports published in the medical literature. Most of the cases involved gangrenous inflammation of the urinary bladder with or without perforation.

In this case, integrity of the bladder wall was preserved. The significant amount of blood in the bladder cavity and severe acute transmural inflammation with hemorrhage and focal necrosis supported the diagnoses of hemorrhagic cystitis.

Hemorrhagic cystitis results from damage of the transitional epithelium and blood vessels by infection (bacteria, viruses) and non-infection etiologies (drugs, toxins, radiation). In this case, *Enterobacter Cloacae* colonies were isolated from urine and peritoneal fluid. It is worth noting, that in adults Enterobacter affects individuals with underlying physical or structural anomalies, metabolic disorders or immunodeficiency causing complicated urinary tract infections. Enterobacter comprises 1.9% to 9.6% of all UTI pathogens.

The past history of the deceased played an important role in the evolution of what started as an innocent urinary tract infection (UTI) to a fatal condition. A number of studies have shown that drugs of abuse, including cocaine, opiates, and alcohol, alter not only neuropsychological and pathophysiological responses of individuals but also immune functions. This decedent's extensive history of polysubstance abuse and malnutrition (BMI of 16.5; normal 18-24) apparently caused severe debilitation of the immune system with rapidly progressive infection and the resultant grim outcome.

Peritonitis, Hemorrhagic Cystitis, Cocaine User

#### G40 Two Suicidal Deaths From Head Injuries Caused by Unusual Sharp Force Instruments and Review of the Literature

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After attending this presentation, attendees will become familiar with unusual penetrating sharp force wounds of the head, the external wounds and wound types produced, and internal wound trauma created.

This presentation will impact the forensic science community by reporting the first suicidal death by a meat thermometer to the head and by reviewing the literature of other sharp force penetrating suicidal wounds that have resulted in death.

Deaths due to sharp force penetrating wounds of the head are uncommon due to the thickness of the cranium and the difficulty of penetration to the brain. Even with brain penetration, individuals may survive with or without neurological deficits. These types of injuries are most often self-inflicted but homicide must be considered especially if the injury implementing instrument is no longer present in the wound.

Two unusual suicidal deaths due to penetrating head injuries will be presented. One case is that of a 44-year-old man who had previously served time in prison for second degree murder. He was at his residence when law enforcement officers arrived in order to arrest him on new molestation charges. They received no response after knocking at the door. Another resident arrived and entered the residence. The police remained outside of the dwelling. She found the man, unresponsive but still breathing, lying on the bed with a meat thermometer impaled into the right temple area of his head. Survival time was 26 hours but nonsurvivability was determined within the first few hours of the hospital stay following the CT scans. The thermometer was left in place until autopsy. Postmortem radiograph revealed the thermometer traversed the majority of the right side of the skull and brain. Autopsy revealed a 1/8" round puncture/stab wound on the right temple following removal of the thermometer. The right temporal lobe and basal forebrain were lacerated with massive hemorrhage of the basal forebrain with extension into the ventricular system. A laceration of a dural blood vessel, basilar subarachnoid hemorrhage, focal epidural hemorrhage, and cerebral edema at the entrance defect were also noted. Postmortem toxicology for ethanol and drugs was negative.

The second case was that of a 47-year-old man with a history of schizophrenia. He had been to many doctors in the past trying to "get the wires out of his head." After a request was made by his parents for a welfare check, police found him in his secure residence in his bathtub filled halfway with water mixed with blood. The shower curtain was pulled from the wall and located partially beneath the decedent. On the sink was a plugged in electrical drill with an attached 1-1/2" hole saw drill bit with skin and hair in the teeth. On the top of the head was a roughly circular scalp defect and underlying 1-1/2" circular skull injury with central 1/4" drill hole. Blood spatter on the walls indicated the decedent likely stood in front of the mirror at the sink while inserting the drill into his head and prior to collapsing into the tub. Drug paraphernalia was present at the scene and postmortem toxicology was positive for morphine.

In addition to these cases, a review of the literature will evaluate other unusual cases of penetrating injury of the head with special focus on the regions of the brain and skull injured and the survivability of the injuries.

Heat Thermometer, Electric Drill, Suicide

#### G41 Iatrogenic Laceration of a Pulmonary Angiomatoid Lesion: Fatal Complication or Medical Error?

Paolo Fais, MD\*, and Giovanni Cecchetto, MD, Via Falloppio 50, Padova, 35121, ITALY; Guido Viel, MD, University of Padua, Via Falloppio 50, PADOVA, 35121, ITALY; Rossella Snenghi, MD, and Rafael Boscolo-Berto, MD, Via falloppio 50, Padova, 35121, ITALY; Andrea Zuin, MD, Via Giustiniani 2, Padova, ITALY; and Massimo Montisci, PhD, Via Falloppio 50, Padova, 35121, ITALY

After attending this presentation, attendees will understand the diagnosed lesion.

This presentation will impact the forensic science community by stressing the importance of performing histological examination in any iatrogenic deaths to recognize underlying diseases and their eventual causal role in determining the fatality.

Percutaneous tube thoracostomy is a standard therapy for a number of pulmonary disorders. Even if it remains the most widely performed procedure to manage blunt or penetrating chest traumatism, and it is generally considered a simple procedure, this life-saving maneuver can be frequently associated with several complications, such as lung or heart perforations, arterial or venous injuries, neurological dysfunctions, injuries to the oesophagus, phrenic nerve and abdominal organs, bronchocutaneous fistula, and perforations of the mediastinal pleura with subsequent contralateral pneumothorax.

A case of a 76-year-old man, admitted to a peripheral hospital after a car accident, presenting bilateral flail chest, and subcutaneous emphysema is presented. Bilateral chest tubes were placed between the anterior and the mid-axillary lines. On the 3rd and 9th day of hospitalization the patient underwent surgical stabilization of bilateral flail chest with Kirshner wires and metal plates. The postoperative recovery was characterized by multiple recurrences of pneumothorax and subcutaneous emphysema with oxygen desaturation. For these reasons several bilateral drains were inserted with the trocar technique, the last one on the 25th day of hospitalization. A control CT scan showed that the tip of the chest tube, inserted between the anterior and the midaxillary line, was located in the parenchyma of the left lung. Immediately after the withdrawal of the drainage tube the patient became unstable with low blood pressure and tachycardia, and was intubated with a double-lumen tracheal device. A fibrobronchoscopy performed through the tracheal tube revealed profuse hemorrhagic secretions. Because of the severe clinical conditions, the patient was transferred to our hospital where, despite multiple blood transfusions, he arrived pulseless and died after 60 minutes of cardiopulmonary resuscitation.

At autopsy the victim was found to be affected by an extensive hemothorax resulting from the laceration of a dilated vessel on the anterior surface of the inferior lobus of the left lung. Histology revealed that the vessel consisted of an "angiomatoid lesion," the distal component of a plexiform complex, the hallmark of plexogenic pulmonary arteriopathy, an idiopathic disease that may accompany primary pulmonary hypertension.

The risk of lung perforation during tube thoracostomy depends on several factors related to the patient (pulmonary contusion, pleural adhesion, adult respiratory distress syndrome, age above 60, mechanical ventilation) or to the method used for the insertion of the chest tube. Particularly, lung perforations have been reported more frequently with the trocar technique, where the insertion is determined by a metal rod projecting slightly from the tip of the tube, rather than the blunt dissection technique, where the penetration of the tube through the chest wall is prepared with a Kelley clamp.

In the reported case, even if the trocar insertion procedure was performed correctly, the penetration of the metal rod into the lung parenchyma produced a tear of a sub-pleural angiomatoid lesion. Initially the catheter blocked the blood flow through the iatrogenic injury, but its removal generated a profuse and extensive bleeding into the pleural space.

The treatment of choice in such cases is an emergency resuscitative thoracotomy, defined as a thoracotomy performed immediately in the emergency room/department or in the operating room, because it enables a fast identification and suture of the vascular injury. However, when huge and dilated vessels are lacerated with subsequent extensive pleural hemorrhage (as in the reported case), the outcome is very poor. Thus, the most important thing is to prevent similar emergency conditions by choosing the blunt dissection technique instead of the more dangerous trocar insertion method, particularly in patients affected by adult respiratory distress syndrome or pulmonary hypertension that show an increased incidence of peripheral venous ectasias.

It is believed that the case could be of interest for the forensic community not only for the singularity of the reported lesion, but also for underlining once again the importance of performing histological examination in any iatrogenic deaths to recognize underlying diseases and their eventual causal role in determining the fatality.

Forensic Pathology, Angiomatoid Lesion, Iatrogenic Death

#### G42 Pedestrian Fatalities in Maryland: How Many, Who, When, Where, Why, How, and Ways to Prevent Them

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After attending this presentation, attendees will have a clear picture of the characteristics of pedestrian fatalities in Maryland. The presentation includes social, geographic, medical, and traffic related data from the previous five years.

This presentation will impact the forensic science community by discussing how understanding the nature and causes of a problem in its totality (in this case pedestrian deaths in an entire state), is the first step in correcting it. This presentation will suggest implementations for reducing the rate of fatal pedestrian accidents in the State.

**How Many:** In the last five years, a total of 400 pedestrian fatalities were studied at the State of Maryland Office of the Chief Medical Examiner (OCME). The majority of the cases had a complete postmortem examination (97.5 %), with toxicologic analysis (for the presence of volatiles in 99% and drugs screening performed in 92%).

**Who:** The majority of the victims were male (69.5%), aged 1 to 89 years (mean and standard deviation: 43.9 and 19.9 respectively). 179 individuals (44.8%) were African-American, 168 (42%) Caucasian, 36 (9%) Hispanic, 10 (2.5) Asian, and 7 (1.7%) belonged to other racial/ethnic groups. More than half of the victims (54.3%) were transported to the hospital before they were pronounced dead (data is skewed due to a few cases with long survival; median survival of 59 minutes, mean of 34.6 days), and 181 individuals (45.2%) were pronounced at the scene (15.6 minutes after the accident on average; median of 7 minutes). Another individual died at home three and a half days following the accident, and another at a nursing home, three months after the accident.

**How:** Most (more than 90%) events were witnessed and had a single vehicle involved. The impacting vehicle was recorded in 339 cases (85%), 181 (53.4%) were passenger cars, 62 (18.3%) SUVs, 28 (8.3%) pick-up trucks, 25 (7.4%) vans, 22 (6.5%) other trucks, 9 (2.6%) buses, 8 (2.4%) trains, 3 (0.8%) motorcycles and 1 (0.2%) was a bicycle. The manner of death in the majority of the death certificates were listed as accident (98.3%); there were 2 homicides, 3 suicides, and 2 deaths were undetermined. The cause of death was listed as: multiple injuries in 349 cases (87.3%), head or head and neck injuries only in 27 cases (6.8%), and complications of multiple injuries in 14 cases (4%), with a variety of other causes listed in the remaining 10 cases. Ethanol in blood

was positive in 146 cases (36.7%) with a mean concentration of 0.16% (+/- 0.09; range: 0.01 to 0.39%). Toxicologic screening for drugs was positive in 107 cases; 28 individuals (7%) had narcotics in blood (12 morphine, 8 methadone, 5 tramadol, and 3 oxycodone), 21 (5.1%) cocaine or cocaine metabolites, and 6 (1.5%) had PCP.

When: In the five years studied, there was no clear change in the incidence rate. The highest incidence was found in December (12% of all cases), and November (11.75%), and the lowest in January (5.7%) and July (6.5%). Saturday (19.7%) and Friday (17%) had rates up to 1.8 times higher than Thursday (9.3%) or Tuesday. The majority of the accidents occurred at night (70.3%), 6.2% happened at dusk, 4.5% at dawn, and 19% during the day light.

**Where:** Graphical representation of the location of incidents throughout the State is provided. Location was also classified according to road type and presence or absence of traffic signals at intersections.

**Why:** Attempts to determine possible causes for the accident were made. Detailed examination of the incident description, police report, and in some cases complete accident reconstruction specified which was the party at fault (whether the pedestrian or the driver of the motor vehicle), weather conditions, light, etc.

**Conclusion**: Nearly 100 pedestrians die each year in Maryland. Possible ways to prevent or decrease the rate are provided based on the data collected in the prior five years.

Pedestrian Fatalities, Who, Prevention

#### G43 Sudden Cardiac Death in an Athlete: A Case Report

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After attending this presentation, attendees will learn of a case showing unusual cardiac cause of sudden death in an athlete, including arrhythmogenic right ventricular cardiomyopathy (ARVC), and coronary artery disease (CAD) after chronic cocaine use.

This presentation will impact the forensic science community by revealing various histological cardiac lesions observed after a sudden death in a retired doping athlete.

Cardiovascular diseases represent the most frequent causes of sudden death in athletes, including hypertrophic cardiomyopathy, anomalous coronary artery anatomy, arrhythmogenic right ventricular cardiomyopathy, and aortic aneurysm. Disorder of cardiac rhythm and stenosis of the coronary arteries are physiopathological mechanisms that can explain cardiac arrest.

This case involved a 36-year-old man who was a two-time world champion while he was cocaine-dependant. He participated in triathlons after his career as a recreational sport without taking cocaine. He had neither medical history nor cardiovascular risk factor except tobacco. He died suddenly during sleep. A complete postmortem examination was performed. The descendant was 172 cm tall and weighed 77 kg (BMI 26). The autopsy showed several cardiac lesions:

- A cardiomegaly (520g) with a symmetric left ventricular hypertrophy usually expected in elite athlete; there was no dilatation and no architectural disorganization.
- An epicardial coronary stenosis of the left anterior descending artery and the first diagonal branch (80-90%) with recent thrombosis on the surface of an atheromatous plaque; there is no acute myocardial infarction.
- Several areas of fibrosis in left ventricular, resulting from an ancient ischemia.

• Limited right ventricular hypertrophy with replacement of the myocardium by fibrofatty tissue in restricted expanse, which is a feature of ARVC.

Those findings allow the conclusion that rhythm disorder caused death. The association of ARVC and CAD in athlete is really unusual.

Arrhythmogenic right ventricular cardiomyopathy is a myocardial disease characterized by fibrofatty replacement and ventricular arrhythmias. ARVC is a hereditary disease with autosomal dominant transmission in at least 50% of cases. It occurs specifically in athletes and affects predominantly men. The prevalence in the general population varies between 1 in 1,000 to 1 in 5,000. Diagnosis rests on criteria including signs such as severe segmental dilatation of the right ventricle and fibrofatty replacement of myocardium on endomyocardial biopsy for example. This disease leads to sudden death by ventricular arrhythmias.

Atherosclerotic disease is primarily responsible for sudden death in athletes older than 35 years. Traditional markers of CAD are widely known, like hypertension, obesity, smoking, diabetes, and lipid abnormalities. Cardiac effects of cocaine chronic abuse also exist. It is associated with CAD by multiple pathogenic mechanisms: elevation in blood pressure, acceleration of atherosclerosis, increase of thrombosis risk by activating platelets, and vasoconstriction.

To conclude, this case report brings to light unusual arrhythmogenic factor leading to sudden death in athlete.

Sudden Death, Athlete, Arrhythmogenic Right Ventricular Cardiomyopathy

#### G44 Myocarditis With Giant Cells in an Infant: A Case Report and Review of the Literature

Tera A. Jones, MD\*, Douglas County Coroner's Office, 4000 Justice Way, Castle Rock, CO 80109

After attending this presentation, attendees will be able to recognize the various entities associated with myocarditis with giant cells, most notably idiopathic giant cell myocarditis, and its clinico-pathologic features.

This presentation will impact the forensic science community by highlighting a case of an uncommon disease entity which is commonly fatal, and generally affects young, healthy adults, but can also affect the pediatric population.

The subject was a 26-day-old, Asian female infant born at 31 weeks gestation with no complications at birth. While under the care of her parents, she vomited once and then was reported to be feeding poorly. She was taken to her pediatrician's office where she was "sick appearing." In the clinician's office she became unresponsive, was subsequently admitted to the nearest hospital, and died within four hours. Family history included a "head cold" in an older sibling and her mother was believed to suffer from an autoimmune-type disease which was undiagnosed.

At autopsy, the subject's growth parameters were between the 10<sup>th</sup> to 90<sup>th</sup> percentiles when corrected for prematurity, her skin was free of rashes, and her abdomen was distended. Within the abdominal cavity, there was 60 cc of ascites. The lungs were congested and heavy with a combined weight of 53 grams. The heart weighed 16 grams; it was normally formed, and had a probe patent ductus arteriosus. Externally, the epicardium of the heart was mottled pale tan to erythematous. Cut sections of the myocardium were equally mottled. The other major organs were appropriate weights and unremarkable for an infant of her age. No lymphadenopathy was identified. Blood cultures obtained from the hospital and at autopsy were negative. Toxicology and vitreous electrolytes were unremarkable.

Histological sections of the heart revealed patchy myocyte necrosis with mononuclear cells, a prominent collection of eosinophils, and scattered multinucleate giant cells. No granulomas were identified. The intramyocardial vessels and epicardial fat were free of inflammation. Histological sections of the other organs were free of granulomatous inflammation, viral cytopathic effect, or vasculitis.

Myocarditis with giant cells is seen in association with many recognized entities including tuberculosis, fungal infections, rheumatic myocarditis, measles, syphilis, foreign body reaction, Wegener's granulomatosis, hypersensitivity reaction, and sarcoidosis. Idiopathic giant cell myocarditis is as the name implies a myocarditis with giant cells, but of unknown etiology. It is a rare, but commonly fatal form of myocarditis which has been recognized since the beginning of the 20th century. This disease generally affects previously healthy, young adults (mean age 42 years); however, approximately 16 cases have been reported in the pediatric population. The youngest to date was 6-weeksof-age; however, the majority of reported pediatric patients are teenagers. Symptoms generally are due to congestive heart failure, although numerous other symptoms have been reported including sudden death and palpitations. Diagnosis has classically been made at autopsy, although, the disease is being diagnosed by endomyocardial biopsy and following cardiac transplant. Gross identification of the disease ranges from "normal" to serpiginous areas of myocardial Histology demonstrates myocyte necrosis, with necrosis. lymphoplasmacytic inflammation with eosinophils and multinucleate giant cells. While the disease generally affects previously healthy people, approximately 20% of patients have immunologic disorders including inflammatory bowel disease, optic myocytis, thyroid disorders, systemic lupus erythematosus, Takayasu's arteritis, myasthenia gravis as well as others. The most successful treatment consists of cardiac transplantation with immunosuppression. Giant cell myocarditis has, however, been known to recur post-cardiac transplant at a rate of 20-25%. Without treatment, the average survival time from diagnosis to either death or cardiac transplantation is 5.5 months.

Based on the history including no known exposure to any drugs, maternal history of an autoimmune disease, and following review of the histology and other studies, the cause of death of this infant is due to idiopathic giant cell myocarditis. Based on the literature review, this is the youngest reported patient with the disease.

Myocarditis, Heart Failure, Sudden Death in Infants

#### G45 Massive Systemic Fat Embolism Detected by Postmortem Imaging and Biopsy

Patricia M. Flach, MD; Steffen G. Ross, MD; Garyfalia Ampanozi, MD; Stephan A. Boliger, MD; Gary M. Hatch, MD; Michael Thali, MD; and Tanja Germerott, MD, Institue of Forensic Medicine, University of Bern-Virtopsy, Buehlstrasse 20, Bern 3012 SWITZERLAND

After attending this presentation the participants will learn about systemic fat embolism and the characteristic image features of systemic fat embolism on pre-autopsy computed tomography compared to autopsy and histopathological findings.

This presentation will impact the forensic science community by raising awareness of the feasibility to detect systemic fat emoslism on postmortem computed tomography prior to autopsy. Finding on computed tomography were significant and serve as quality improvement to forensic procedures.

**Purpose**: The purpose of our case study is to describe the findings of lethal systemic fat embolism (FE) on postmortem unenhanced computed tomography (PMCT), PMCT-Angiography (PMCTA), and image-guided lung biopsy, with correlation to conventional autopsy and histopathology.

**Materials and methods**: An 89-year-old woman with traumatic femoral neck fracture died due to cardiac arrest during implantation of a cemented total hip prosthesis. The patient was under long-term anticoagulation for atrial arrhythmia. In the course of the hip trauma, anticoagulation had to be stopped and antidote (vitamine K) was

administered. No disorder of lipid metabolism or transport or renal failure was known. The body underwent whole-body PMCT (Somatom Emotion 6, Siemens, Erlangen, Germany) with subsequent cannulation via an unilateral inguinal incision and contrast application by a modified heart-lung machine. PMCTA was then performed with an arterial and venous injection. The body was moved from the supine to prone position to improve filling of nondependant vessels. After PMCT and PMCTA, image-guided biopsy of the lung was obtained. The harvested specimens were stained to detect fat embolism.

**Results**: Unenhanced PMCT revealed a distinct fat level on top of sedimented layers of corpuscular blood particles and serum in the systemic arterial system and the pulmonary trunk. This finding was measured (Hounsfield Unit) and compared to possible small position-dependent air embolism and evaluated as negative. PMCTA showed no clotting suggesting pulmonary thrombembolism. The triple layered intravascular finding was reproducible after PMCTA and after turning of the corpse. Autopsy showed no evidence of patency of the foramen ovale that would account for paradoxical embolism. In addition, there were no autopsy findings other than fatal fat embolism that were relevant to the cause of death. There were no petechial rash or kidney changes visible. There was no evidence for cholesterol embolism, e.g. triggered by anticoagulation. Both image-guided biopsy and histopathological specimens confirmed the findings of PMCT/PMCTA demonstrating severe FE (Grade IV).

**Conclusion:** PMCT/PMCTA established the cause of death as systemic fatal FE. It is believed that this is the first description of these unusual systemic imaging findings in the postmortem setting. Autopsy and histopathological specimens validated imaging and biopsy findings. **Fat Embolism, Postmortem Computed Tomography, CT** 

#### G46 Radiocarbon and Stable Isotope Results of Fingernails of Breastfed Mother-Infant Pairs to Investigate Deviation of Year-of-Birth Determinations Due to Diet

Kaelyn M. Ormsby, BS\*, and Guaciara M. Santos, PhD, KCC-AMS Preparation Laboratory, University of California, Irvine, B321 Croul Hall, Irvine, CA 92697; and Benjamin T. Fuller, PhD, Max Planck Institute for Evolutionary Anthropology, Department of Human Evolution, Deutscher Platz 6, Leipzig, D-04103, GERMANY

The goal of this presentation is to find a possible explanation to justify outliers in <sup>14</sup>C results from a recent pilot experiment measuring human tissues to determine the year-of-birth of deceased individuals of known birth records.

This presentation will impact the forensic science community by exploring new methods and techniques to aid in human identification.

Researchers have investigated the possibility of acquiring the yearof-birth and year-of-death dates by using radiocarbon (14C) measurements from a broad range of human tissues. This is possible due to high concentration of radiocarbon in the earth's atmosphere during the thermonuclear bomb testing carried out between 1953 and 1963, and its propagation into the food chain through photosynthesis. Measuring the magnitude of this <sup>14</sup>C concentration allows year-of-birth determinations for individuals that were born in this period. Recently, Hodgins (2009)<sup>1</sup> studied human tissues of 36 deceased individuals of known birth dates. To estimate the year-of-birth, Hodgins measured <sup>14</sup>C of tooth enamel. More than 50% of his results were consistent with the true birth dates, and uncertainties for most were as good as 1.5 years. However, a significant percentage of the measurements yielded estimated birth dates off as much as 4 years. Since diet life histories of individuals were unknown, Hodgins speculated that a <sup>14</sup>C depleted marine diet may have played a role in explaining some of these date deviations. Since some human non-turnover tissue, such as eye lens crystalline and tooth

enamel, start forming while in utero and stop at approximately age of 3 and 17 years, respectively, this notion raises the question of how much an individual's childhood diet can affect the age determinations. To examine this possibility isotopes d13C, d15N, and <sup>14</sup>C in fingernails collected from breast milk fed infants and their mothers from before birth through the weaning period were measured. In this study, the motherinfant pairs were from the same region in the United States and their protein diet was recorded during the course of sampling. Samples that would most likely show some differences from one another since they were from individuals with different protein dietary preferences were chose, but in this preliminary investigation no significant variability was observed. This may be attributable in part to the fact that the individuals sampled were from the same region, and so a more diverse population would possibly produce more variability. To further investigate the outliers that were observed by Hodgins, and to determine the magnitude of any dietary biases on <sup>14</sup>C measurements to estimate the year-of-birth, future research should be done directly on non-turnover tissue of individuals of varied recorded diets from different locations.

**References:** 

<sup>1.</sup> Hodgins, Greg. "Measuring atomic bomb-derived <sup>14</sup>C levels in human remains to determine Year of Birth and/or Year of Death". 2009. National Institute of Justice Final Report.

**Isotopes, Diet, Fingernails** 

#### G47 The Effect of Cultural Cranial Deformation on Neurological Development: A Beneficial or Disadvantageous Practice?

#### Anna Williams, PhD\*, and Mitzi A. Richards, MSc, Cranfield University, Defence Academy of the UK, Shrivenham, SN6 8LA, UNITED KINGDOM

After attending this presentation, attendees will gain an appreciation of the different methods of cultural cranial deformation and the existing medical conditions that cranial deformation simulates. There are clear similarities between cranial development in individuals with culturallyinduced cranial deformation and individuals with different forms of the congenital condition craniosynostosis. Attendees will understand the implications of cranial deformation for the neurological development of the individual, and these will be compared with those with craniosynostosis. Ethnographic material written through participant observation amongst societies that practice cranial deformation, and medical reports of function in craniosynostotic individuals will be examined to determine whether the practice has a beneficial or disadvantageous affect on individuals' neurological function.

This presentation will impact the forensic science community by detailing the effect of artificial or culturally-induced cranial deformation on the neurological function of the individuals. This is of significance to forensic anthropologists as it is a method of body modification that has implications for the survival of the participants. Neurological conditions are known to affect bone morphology, for example bone atrophy due to paralysis. It also allows an appreciation of cranial growth processes and the interrelationships between the cranial vault, base, and face, as well as the foramina that conduct the cranial nerves. An analysis of the morphological changes to the nerve foramina, coupled with an examination of ethnographic accounts of the physical symptoms exhibited by the individuals has not been attempted before, and constitutes a novel contribution to our understanding of cranial deformation in past and existing societies. The modern condition of craniosynostosis can inhibit neurological development, and can offer the forensic anthropologist insight into the physiological consequences of the social practice. This has implications for investigations of human rights violations and the recognition of how social and cultural practices can dramatically affect human physiology.

This project builds on previous research conducted by Dingwall,<sup>1</sup> Schijmane,<sup>2</sup> and Cheverud *et al*,<sup>3</sup> among others, to determine whether or not artificial cranial deformation practiced by past and extant peoples has an effect on neurological function. It aims to refute or support the hypothesis that cranial deformation must have an effect on the development of the brain and the skull and therefore affect neurological function in an observable way.

Intentional artificial cranial deformation, practiced for a variety of cultural reasons, is of great interest to anthropologists due to its value for reconstructing aspects of past and contemporary social systems, as well as understanding modern medical conditions. Deformations have been carried out for many social and aesthetic purposes, ranging from increasing perceived beauty to encouraging obedience in infants. It is associated with instilling ethnic identity and social stratification. This paper addresses the question of whether artificial cranial deformation of infant skulls, as practiced with boards, pads, stones, or bandages, had any adverse or beneficial consequences for neurological development, and whether these were ignored or embraced by the societies practicing the tradition.

Previous research has not made links between cranial modification and ethnographic evidence of abnormal neurological function (whether impaired or improved); however, papers written comparing the skull morphology of modern pathological specimens and ethnographic examples of artificially deformed specimens have shown that some features appear different to un-modified skulls, for example, the patterns of venous sinuses and meningeal vessels, which may affect neurological function.<sup>4</sup> The resulting consequences of possible neurological change have not been compared to ethnographic data. The paucity of such research may have implications for wider anthropology, as cultural or social phenomena such as tribal demise or proliferation, or shared spiritual experience may be attributed to neurological modification as a result of artificially-induced cranial deformation. Some traits and idiosyncrasies peculiar to distinct peoples may have a neurological foundation.

A study was conducted using two types of artificially deformed crania from the Natural History Museum, London, to examine whether changes in cranial foramina morphology could explain some of the symptoms observed in ethnographic accounts. Cephalic indices and ethnographic accounts of observed effects of artificial cranial deformation were collated, and compared to measurements and documented symptoms and CT scans of individuals exhibiting the medical condition craniosynostosis which appears to express similar morphological changes to the skull. The cephalic indices of artificially deformed skulls were found to be similar to those of skulls with craniosynostosis, which is known to cause an increase in intracranial pressure and precipitate conditions such as strokes and 3rd, 4th, and 6th cranial nerve palsy. This supports the hypothesis that the symptoms exhibited by individuals with artificial cranial deformation would be similar to those with craniosynostosis, and the explanations for the observed symptoms of cranial deformation substantiate the theory that brain function is affected.

This study represents original research that has not been undertaken elsewhere, and constitutes a valuable contribution to anthropological knowledge. It will further the understanding of the nature of cranial deformation, neurological development and pathology, with significant implications for socio-cultural anthropology, forensic anthropology, and medicine.

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- <sup>3.</sup> Cheverud J. Kohn L. Konigsberg L. and Leigh S. (1992) Effects of fronto-occipital artificial vault modification on the cranial base and face. *American Journal of Physical Anthropology*, 88:323-345.
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#### G48 Morphological Identification of Right Ventricular Ischemia Determining Right Heart Failure in Cases of Fatal Pulmonary Thromboembolism

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After attending this presentation, attendees will be aware of the possibility of detecting right ventricular failure due to ischemia in cases of pulmonary thromboembolism

This presentation will impact the forensic science community by making the public aware about the possibility of detecting morphological signs of right ventricular failure due to right heart ischemia.

Pulmonary thromboembolism is a medical emergency that may potentially determine right ventricular failure. Even if the pathophysiology of this phenomenon has been widely investigated, no morphological demonstration of right ventricular ischemic damage determining right ventricular failure in cases of fatal pulmonary embolism has been reported till now.

An immunohistochemical investigation was performed with the antibodies against Fibronectin and C5b-9 in 26 cases of fatal pulmonary thromboembolism (16  $\bigcirc$ , 10  $\bigcirc$ , mean age 56.4 years) as well as in 25 cases of acute myocardial infarction (16 $\bigcirc$ , 9 $\bigcirc$ , mean age 60.8 years) and 20 cases of hanging (3 $\bigcirc$ , 17 $\bigcirc$ , mean age 40.8 years). In each case at least one tissue slide from both cardiac ventricles (wall of the right ventricle, anterior and/or posterior wall of the left ventricle) was available. The reactions were semi-quantitatively classified and the expressions in the groups were compared. In cases of pulmonary thromboembolism the occurrence of positive reactions at the right ventricle was significantly higher than in cases of myocardial infarction and global hypoxia due to hanging. This may indicate the primary ischemic involvement of the right ventricle and be interpreted as morphological sign of right ventricular failure.

Right Ventricular Failure, Acute Pulmonary Hypertension, Immunohistochemistry

#### G49 Autopsy Performance in Transfusion Recipient Fatalities Reported to the United States Food and Drug Administration (FDA) During Fiscal Year 2008

Stephen L. Sgan, MD\*, District 2 Medical Examiner's Office, PO Box 14389, Tallahassee, FL 32317

After attending this presentation, attendees will learn recent updates regarding the classification of fatal transfusion reactions, review recent transfusion-recipient fatality data relevant to forensic practice, especially regarding autopsy performance and medical errors, consider approaches to the investigation of deaths potentially related to transfusion of blood products, and learn how to contribute to the national investigation of transfusion-associated fatalities through the FDA transfusion fatality program.

This presentation will impact the forensic science community by sharing how findings suggest that ME/C systems can provide important contributions to the investigation of transfusion-related fatalities through increased awareness, vigilance, autopsy performance, and reporting.

This presentation will inform attendees of something they do not know: (1) classification of fatal transfusion reactions; and, (2) recent

transfusion-recipient fatality data relevant to forensic practice, especially regarding autopsy performance and medical errors. This presentation will inform attendees of something they do not know/do: (1) how to approach the investigation of deaths potentially related to transfusion of blood products; and, (2) how to contribute to the national investigation of transfusion-associated fatalities by the FDA transfusion fatality program through increased awareness, vigilance, autopsy performance, and reporting.

**Background**: Many deaths investigated by medical examiner/coroner (ME/C) systems are associated with a blood transfusion shortly before death. Complications of transfusion may occur and are occasionally fatal. The transfusion service is required to report fatal complications of transfusions to the FDA Center for Biologics Evaluation and Research (CBER). A CBER Medical Officer (CMO) reviews submitted information and determines to what extent, if any, the transfusion may have contributed to death. CBER publishes an annual summary of the reported fatalities. As part of its investigation, FDA requests the reporting facility to provide information on whether or not an autopsy was performed, but autopsy data have never been published in the annual summary.

**Hypotheses:** (1) Transfusion-associated fatalities reported to the FDA are under-reported to ME/C systems, despite the fact that several of these deaths are due to medical errors and therefore likely be certified as Accidental in manner of death; (2) a significant number of these cases would also otherwise typically fall under ME/C jurisdiction, such as cases involving transfusion for traumatic injuries; (3) there is a very low autopsy rate in these cases; and, (4) for the group of fatalities in which the FDA could not rule out transfusion as contributing to the death, a higher autopsy rate could have potentially helped to determine the cause of death with a higher degree of certainty and therefore allowed more definitive classification of some of these cases as either transfusion-related or not.

**Methods**: After review of the 2008 U.S. FDA Annual Summary report of fatalities following transfusion, the most recent year for which data had been published at the initiation of the project, a Freedom of Information Act request was submitted to CBER for the "Table of Final Conclusions" prepared by a CMO for each of the 72 reported transfusion-recipient deaths. Sixty-nine individual reports with some data redacted were received, as three cases had been withdrawn prior to CMO review. Available documents were mined for data that would address the hypotheses and potentially be of interest to participants of death investigation systems.

**Results**: Of the transfusion-recipient deaths reported (N=69), there were 35 males (51%), 33 females (48%), and 1 sex unspecified. Age ranged from 6 weeks to 97 years (median=66 years). The overall reported autopsy rate was 26% (18/69). Performance of an autopsy was reported in 24% (11/46) of cases in which transfusion was determined by the CMO to have contributed to the death and in 43% (6/14) of cases in which transfusion was determined to be unrelated to the death, but in only 11% (1/9) of cases in which transfusion could not be ruled out or confirmed as contributing to the death. Human errors in pre-transfusion specimen collection, compatibility testing or blood administration accounted for 30% (14/46) of transfusion-related deaths; all of these were due to hemolytic transfusion reactions (HTRs). Ninety percent (90%, 9/10) of the deaths due to ABO incompatibility (ABO HTRs) occurred when Type A donor red blood cells were erroneously transfused to non-A recipients, 89% (8/9) of whom were Type O. Of the deaths due to incompatibility of non-ABO red blood cell antigens (non-ABO HTRs), 71% (5/7) were due to errors that occurred in the blood bank during compatibility testing. Autopsy performance was reported in only 14% (2/14) of the deaths due to human error. Trauma patients accounted for 6% (four cases) of all reported deaths, and for each of these a transfusion complication was determined to contribute to the death (three cases) or could not be ruled out (one case). For five of the eight deaths without an autopsy in which transfusion could not be ruled out or confirmed as a contributing factor, the CMO listed a differential

diagnosis that suggested autopsy findings may have helped with further classification.

**Conclusions**: In this study, a significant number of reported transfusion-related deaths were due to human error. Transfusion complications may cause or contribute to death in cases that would typically otherwise fall under ME/C jurisdiction, including trauma cases. Lack of autopsy findings may impede the determination of whether or not a transfusion contributed to death and thereby prevent definitive classification.

Transfusion, Fatal, Autopsy

#### G50 A Case of Atypical Chronic Subdural Hematoma: A Spontaneous Rupture of Dural Lymphoma Nodule?

Renaud Clement, MD\*, 1 Rue Gaston Veil, Nantes, FRANCE

After attending this presentation, attendees will understand the call for creating an entity of spontaneous chronic subdural hematoma.

This presentation will impact the forensic scientist community by presenting a case report about chronic subdural hematoma (SDH) and the different causes of bleeding beneath the *dura*.

**Introduction:** SDH is usually associated with brain injury following trauma. Hemorrhage resulted from the rupture of the cerebral bridge veins of the meninges, from a tear of superficial cortical arteries or from a focus of intraparenchymal hemorrhage associated with an overlying contusion such as in temporal lobe which ruptures through the contused cortical area. Acute SDH is due to direct impact trauma or sudden acceleration-deceleration of the head without injury of the head. Chronic SDH may be traumatic or may rise spontaneously.

Case Report: A 40-year-old Caucasian woman traveled to an African country. Her medical history included local radiation therapy, several years beforehand, for the treatment of breast cancer. She had been in complete remission for more than a couple of years. This woman's status of health presented no constitutional or acquired hemostasis disorders. She was completely free of medicine. She had no known addictive tendencies. Several days after her arrival, she presented paroxysmal hyperthermia, accompanied a few hours later by photophobia, difficulty in walking and confusion. Neurological state worsened with the appearance of coma (Glasgow score of 6). She was hospitalized and resuscitation measures did not prevent the patient's death. Following cold storage, the victim's body was repatriated to France, where an autopsy was performed to determine the primary cause of death because liability could be assigned against insurance (transfer with delay time between neurological deterioration and hospitalization). On opening the cranial space, a subdural hematoma forming a right hemispheric biconvex lens was discovered. It weighed 90 grams, was wine red in color and consisted of an encased fluid mass. No traumatic lesion was found during external or internal examination of the skull. Histological investigations then uncovered a multi-organ generalized lymphoid infiltration. Examination of the cerebral cortex showed these lymphoid infiltrations as well. A small-cell lymphoid nodule, disrupted by erythrocytes was found in the falx cerebri of the meninges. Following these additional investigations the main cause of death was a chronic right circumferential SDH. This hematoma could originate with the "spontaneous" hemorrhagic rupture of a nodule of lymphoid infiltrate in the meninges of the falx cerebri. This nodule was a dural metastasis of a multi-organ lymphoma.

**Discussion:** Chronic SDH is well known as incidental finding during forensic autopsy. In forensic medicine, the formation of chronic SDH is always linked to trauma. The entity of spontaneous SDH doesn't exist in forensic medicine. Chronic subdural hematomas occur more frequently in men, in the elderly, and in patients using anticoagulant or platelet aggregation inhibiting drugs. The consumption of alcohol is also

a predisposing factor. In these circumstances, the development of a SDH involves necessary the intervention of trauma. It can be minimal such as some encountering in the impacts of everyday life. In the medical literature, several cases of atypical chronic SDH characterized by the presence of pre-existing pathological dural lesions, especially cancerous ones, have been described. These tumors of the dura mater can result from primitive neoplasias of the central nervous system in the meninges or from dural metastases of cancers. In the present case history, several forensic medical elements contributed to the atypical nature of this chronic SDH: no major or minor traumas were identified in this young woman of forty years; she was non-menopausal; and she was not a chronic or acute consumer of alcohol and/or medications that could interfere with hemostasis or coagulation. In some previous published cases, the hypothesis of trauma, even if minimal, leading to displacement of the brain within the cranial space was suggested and could not be excluded. And spontaneous chronic SDH have been described. This presentation will review the possible mechanisms which rupture the lesion and will discuss the fact that if trauma could not be completely excluded, the entity of spontaneous chronic SDH could be created in forensic medicine.

Subdural Hemorrhage, Forensic Medicine, Spontaneous

#### G51 A Comparison of Trauma Associated With Manual and Automated Cardiopulmonary Resuscitation

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After attending this presentation, attendees will be able to associate certain injury distribution patterns with the type of CPR administered.

This presentation will impact the forensic science community by assisting medical examiners in identifying fracture distribution patterns associated with automated CPR specifically ZOLL AutoPulse® Noninvasive Cardiac Support Pump use. Posterior fractures such as those observed with AutoPulse® CPR are generally noticed in cases of inflicted trauma. By understanding the fracture pattern associated with AutoPulse® CPR, a potentially erroneous interpretation of therapeutic injuries as inflicted can be avoided.

This presentation will detail the results of a retrospective study of the effects of therapeutic intervention with manual cardiopulmonary resuscitation (CPR) compared to the effects of automated mechanical CPR device use. After this presentation, attendees will be able to associate certain injury distribution patterns with the type of CPR administered.

In 2007, Houston TX was selected as a test site for the ZOLL AutoPulse<sup>®</sup> Noninvasive Cardiac Support Pump. According to ZOLL, the benefits of this device include continuous CPR without fatigue, relief for EMS personnel who are then free to perform other life-saving tasks, and improved blood flow for patients with cardiac distress. The purpose of this study was to identify the trauma associated with AutoPulse<sup>®</sup> use, particularly how it compares to standard manual CPR. Expanding on previous research that found upper body skin abrasions associated with AutoPulse<sup>®</sup> use, this study also included the occurrence of hard tissue trauma between the two forms of CPR. It is well established that manual CPR can result in rib and sternal fractures. A comparison of the distribution and frequency of manual CPR fractures to AutoPulse<sup>®</sup> fractures as well as abrasion occurrence can potentially help rule out erroneous interpretations of inflicted trauma.

Autopsy records from 137 decedents brought to the Harris County Institute of Forensic Science, Houston TX, between the years 2006 to 2009 were analyzed. According to the sample records, manual CPR was performed on 49 individuals (24 males, 25 females) and AutoPulse® CPR was used on 88 individuals (52 males, 36 females). The median age for the manual CPR group was 48 years and the AutoPulse® CPR group was 54 years. The distribution of rib fractures from the anterior, lateral, and posterior compartments as well as sternal fractures and skin abrasions were recorded. Kruskal-Wallis ANOVA comparisons between fractures from the manual CPR group and the AutoPulse® CPR group demonstrated a statistically significant difference (p<0.05) between the number of anterior fractures, lateral fractures, posterior fractures, sternal fractures, and skin abrasions. In manual CPR, anterior fractures had the highest frequency followed by lateral fractures. Posterior fractures were only found in one case, secondary to body placement during manual CPR. In AutoPulse® CPR, anterior fractures had the highest frequency followed by posterior fractures and lastly, lateral fractures. Sternal fractures were found at a higher frequency in the manual CPR group than the AutoPulse® group. Skin abrasions were more common in the AutoPulse® CPR group, located primarily along the anterior chest, lateral chest, and shoulder. In the few cases that abrasions were observed in the manual CPR group, they were located along the sternum.

The results of this study identify the distribution patterns of fractures associated with manual and automated CPR. When rib fractures are found in the anterior or lateral rib cage in association with sternal fractures, they are consistent with manual CPR. When rib fractures are found in the anterior and posterior compartments with chest skin abrasions, they are consistent with automated CPR resulting from AutoPulse® use (and not other types of devices, which were not included in this study). It should be noted that it is mandatory for Houston EMS personnel to initially administer manual CPR before AutoPulse® use and this combination may account for the anterior rib fractures observed in the AutoPulse® CPR group. During manual CPR, chest compressions are administered for an extended period of time, thus causing sternal fractures. The small number of sternal fractures seen in AutoPulse® CPR is likely due to the short duration of manual CPR. The significance of this study to the forensic community is in the importance of identifying fracture distribution patterns associated with AutoPulse® use. Posterior fractures such as those observed with AutoPulse® CPR are generally noticed in cases of inflicted trauma. By understanding the fracture pattern associated with AutoPulse® CPR, medical examiners can avoid a potentially erroneous interpretation of therapeutic injuries as inflicted.

Trauma, Cardiopulmonary Resuscitation, Fractures

#### G52 Sudden Unexpected Death Associated With Undiagnosed Lymphocytic Thyroiditis: Report of a Case and Literature Review

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After attending this presentation, attendees will have a better understanding of the significance of pathological changes in the thyroid gland in determining the cause of death, since in many patients thyroid dysfunction remains undetected during life, due to the lack of clinical signs and symptoms characterizing some nosographic entities, such as silent (painless) thyroiditis, or the Hashimoto disease.

This presentation will impact the forensic science community by emphasizing the importance of taking all natural diseases into proper account when investigating sudden deaths, even if clinical records are not indicative or when the anamnesis is poor.

In this perspective a careful gross examination and an adequate sampling of the thyroid gland are mandatory. Findings obtained by histology and updated tissue analyses should be always interpreted in relation to complex data coming from a multidisciplinary approach, finally leading *per exclusionem* to the diagnosis of sudden death due to an episode of transient thyrotoxicosis. Silent (painless) thyroiditis is regarded as follicular destruction-induced hyperthyroidism resulting in the release of stored thyroid hormones in the circulation. The above mentioned disease is characterized by a transient hyperthyroidism with spontaneous resolution in two to five months, even though cases of relapse can occur quite often. The thyrotoxic phase of this syndrome is short and requires no, or only symptomatic therapy, but it is assumed that untreated thyrotoxicosis might lead to sudden death by several mechanisms (cardiac arrhythmia, hyperpyrexia, electrolyte disturbances, and epileptic seizures). Macroscopically the thyroid glands are normal sized and non tender; histologically, focal, or diffuse lymphocytic thyroiditis is present. In some cases anti-thyroid antibodies can be detected, indicating an autoimmune pathogenesis and postmortem dosage of thyroid hormones, when interpreted in relation to the histological findings, can provide further information about the functional status during life.

In the present case a previously healthy 18-years-old woman was found dead prone near the entrance of her house, where she was living alone. The crime scene investigation did not offer any significant finding, and external examination of the body showed a single contusion at the forehead, consistent with an accidental fall from the standing position. Clinical history was unremarkable, but information regarding a possible family history of thyroid disease was not available.

Full autopsy was carried out including a detailed macroscopic/microscopic cardiac examination, tissue molecular analyses for viral detection, chemical analyses, and toxicology. At the autopsy all internal organs were unremarkable and the thyroid gland was macroscopically normal. The one relevant pathological finding was a prominent lymphocytic infiltration with follicular disruptions, rare oxyphilic changes and low grade fibrosis. Since the histological picture was consistent with lymphocytic thyroiditis, immunophenotype characterization and lymphocyte clonality analyses were performed in order to rule out the diagnosis of hematologic malignant neoplasm.

In this case the lymphocytic thyroiditis could by exclusion offer a reasonable explanation of the sudden unexpected death occurred during an episode of transient thyrotoxicosis, cardiac arrhythmia being the most likely mechanism of death.

In consideration of the autoptical findings, further investigation into the medical history was carried out, revealing that the deceased a few days before death complained chest pain to the general practitioner; moreover, three months before she required the prescription for psychoactive drugs, due to the recent onset of insomnia and unexplained anxiety; contemporaneously, she was noticed loosing weight.

Since the young woman had one sister and one brother, at the end of the medico-legal investigation a clinical diagnostic protocol on the relatives was recommended to the general practitioner.

In conclusion: the presented case highlights to forensic pathologists the importance of sampling and careful studying the thyroid gland to evaluate the possible role of a thyrotoxic episode related to a silent thyroid disease, as a cause of sudden death in otherwise unexplained fatalities. Review of the literature reported only a few cases of lymphocytic thyroiditis as a possible cause of death, but in such cases a full multidisciplinary approach (with special regard to biomolecular and chemical analyses) was not carried out.

Furthermore, the present case investigation, first aiming to the solution of forensic concerns, also represented the start up for diagnostic protocol on the relatives, at that time still asymptomatic, with final possible positive outcome on their health care.

Silent Thyroiditis, Sudden Death, Thyrotoxicosis

#### G53 The Pattern of Immunoreactivity for von Willebrand Factor in a Variety of Thrombotic States

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After attending this presentation, attendees will understand the role of von Willebrand Factor (vWF) in thrombotic thrombocytopenic purpura (TTP) and recognize variations in patterns of immunohistochemical staining against von Willebrand Factor antigen in thromboemboli from thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, disseminated intravascular coagulation, non-bacterial thrombotic endocarditis, renal allograft vascular rejection, and stasis thrombosis.

This presentation will impact the forensic science community in cases of precipitous deaths with little or no antemortem workup that are characterized by a thrombotic state. Although previous reports have discussed the value of immunohistochemical staining against von Willebrand Factor antigen in deaths where thrombotic thrombocytopenic purpura is suspected, this is the first case report to provide images that demonstrate the staining patterns of several other entities within the differential diagnosis.

Although previous reports have detailed the value of this stain in deaths where TTP is suspected, this is the first case report to provide images that demonstrate the staining patterns of several other entities within the differential diagnosis. The variations appear to reflect the etiology of the thromboemboli and their relative content of vWF. The visual references included here will be especially helpful to the medical examiner in cases of precipitous death when there has been little or no antemortem workup.

TTP is a thrombotic microangiopathy, historically requiring a pentad of symptoms for clinical diagnosis: microangiopathic hemolytic anemia; thrombocytopenia with or without purpura; acute renal insufficiency; fever; and neurologic abnormalities. It is now understood that few patients present with all features; however, the presence of neurologic abnormalities is often helpful in distinguishing TTP from hemolytic uremic syndrome (HUS).

The case of an adult male is presented with microangiopathic hemolytic anemia, thrombocytopenia, and an episode of hematuria two days prior to hospital admission. The patient did not report diarrhea or fever, and did not exhibit neurologic symptoms. Pulseless electrical activity and renal failure were present at the time of admission. The patient had a rapid clinical decline and died before a diagnosis could be made. Autopsy did not reveal significant gross pathology. Histologic sections contained myocardial necrosis with relatively widespread microthrombi in small cardiac vessels and, less frequently, microthrombi within glomeruli and renal arterioles. Vascular lesions also included intimal thickening and disruption, and fragmented red cells.

TTP is currently thought to be driven by a deficiency in ADAMTS-13, a metalloprotease that cleaves vWF to render it ineffective in its role in intravascular platelet aggregation. Deficiencies may be inherited or acquired, and may lead to unchecked formation of vWF-rich thrombi in those vessels subject to shear stress (including arterioles and capillaries). Because vWF is produced in arterial endothelial cells and megakaryocytes, thrombotic lesions in TTP, non-bacterial thrombotic endocarditis (NBTE) and allograft vascular rejection will demonstrate immunoreactivity to vWF antigen. The characteristically fibrin-rich thromboemboli formed in states that are not mediated by vWF will exhibit minimal-to-no immunoreactivity.

Tissue controls in the current report included a single example of each aforementioned disease entity, including a mixed immune-TTP

control case, internal positive controls (arterial endothelium), and internal negative controls (hepatic veins and sinusoids). In TTP, there was dense, relatively homogeneous staining of the entire vWF-rich thrombus. In NBTE, there was variably dense, granular staining of characteristically platelet-rich bland vegetations (both on valves and in embolized material). In renal allograft rejection, there was heterogeneous staining, most dense in areas of vascular damage, with only minimal peripheral staining of the thrombi.

In HUS, there was minimal peripheral staining of thrombi. In disseminated intravascular coagulation (DIC), and in stasis thrombosis (the latter secondary to a myocardial infarct), there was focal dense staining only within the more cellular "layered" regions of organizing thrombi, where platelets may become entrapped.

Tissue from the presented case demonstrated vWF-rich thrombi in cardiac and renal vessels as well as in rare small cerebellar vessels, and looked most similar to the mixed immune-TTP control tissue, supporting myocardial necrosis secondary to TTP as the cause of death.

Overall, this case with its corresponding array of tissue controls represents a spectrum of patterns that correlates well with the pathophysiology of each specific pathologic entity. In conclusion, when interpreted in combination with anatomic findings at autopsy, vWF staining provides support for a diagnosis of TTP even when the clinical history is limited or atypical.

TTP, von Willebrand, Hemolysis

#### G54 Giant Cell Myocarditis as a Cause of Sudden or Unexpected Death: A Report of Two Cases and a Review of the Literature

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After attending this presentation, attendees will have an awareness of giant cell myocarditis as a cause of sudden or unexpected death and its diagnosis.

This presentation will impact the forensic science community by increasing knowledge and awareness of an uncommon cause of sudden or unexpected death with two case presentations.

Giant cell myocarditis (GCM), formerly known as Fiedler's myocarditis, is an inflammatory process of unknown etiology restricted to the heart, typically occuring in young and middle-aged adults. There is no clear gender predilection, but the prevalence of GCM is higher in caucasians than in other races. Because of its isolation to the heart and fulminant clinical course resulting in sudden or unexpected death, GCM is usually diagnosed at autopsy, and therefore may be encountered in a Gross findings at autopsy are variable. forensic setting. Microscopically, there is myocyte necrosis associated with an inflammatory infiltrate composed of histiocytic giant cells, lymphocytes, and scattered eosinophils. The differential diagnoses of GCM include other forms of granulomatous myocarditis, such as sarcoidosis and infectious etiologies. In contrast with sarcoidosis, GCM is typically localized to the heart and has a fulminant clinical course. Infectious etiologies can be excluded with the use of special stains. GCM is a rare cause of sudden or unexpected death with a very low prevalence as reported by other studies.

A search of the records of the Cook County Medical Examiner's Office identified 72 cases in which myocarditis was the principle or contributing factor to death in adults aged 18 and older. The search covered the period from January 1, 2000 through July 15, 2010. Of these 72 cases, only two were cases of giant cell myocarditis. The remaining 70 cases consisted of neutrophilic, lymphocytic, or mixed inflammatory infiltrates.

The first case is a 39-year-old African-American female with a history of hypertension and obesity, who presented to the emergency

room with shortness of breath and sinus tachycardia. Two days prior to this event, she was seen in the emergency room with fever, malaise, and an elevated white blood cell count, and was discharged. Soon after presentation to the emergency room, she developed pulseless ventricular tachycardia despite pharmacologic therapy. Following synchronized cardioversion and a brief period of asystole, she developed sinus bradycardia. Transcutaneous pacing was attempted, but she progressed to ventricular fibrillation, then asystole. She died within two hours of admission. At autopsy, the heart was enlarged, weighing 487 grams. Grossly, there was concentric left ventricular hypertrophy and the myocardium was uniformly red/brown with the exception of the papillary muscles of the left ventricle, which were pale yellow/gray. Microscopic examination of the heart revealed foci of myocyte loss, fibrosis, and chronic inflammation with scattered giant cells predominantly in the papillary muscle. Other findings at autopsy included cerebral edema, splenomegaly, and chronic passive congestion of the liver. Toxicologic studies were negative for ethanol, opiates, or cocaine.

The second case is a 33-year-old African-American female with no prior medical history, who collapsed suddenly at a nightclub. At autopsy, her heart was enlarged, weighing 426 grams. Grossly, there were geographic areas of pallor from base to apex involving the myocardium of the anterior, lateral, and septal walls of the left ventricle. Microscopically, there was extensive fibrosis and inflammation with numerous giant cells and only small islands of preserved myocardium. Other findings at autopsy included pulmonary congestion and an incidental ovarian teratoma. Toxicologic studies were negative for opiates or cocaine.

These cases are reported to demonstrate the variation in clinical presentation and autopsy findings of GCM, as well as to illustrate that GCM remains a rare cause of sudden or unexpected death even in a busy, urban medical examiner's office.

Myocarditis, Sudden or Unexpected Death, Heart Disease

#### G55 Death Due to Atrial Septum Defect Repaired by Transcatheter Closure: Who Failed?

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After attending this presentation, attendees will learn about the role of the forensic pathologist in transcatheter procedures and professional liability.

This presentation will impact the forensic science community by showing a case report regarding a bronchial lesion following transcatheter procedure.

A 13-year-old Caucasian male, with past medical history of ostium secundum atrial septal defect previously treated using septal occlusion device with no success.

After a period of time, a new surgical access procedure was performed on the young man using transcatheter closure procedure. The device should be placed in the atrial septum via catheter introduced into femoral vein. The access was monitored with transesophageal echocardiography. The medical record states that twenty minutes after the start of surgery, a sudden decrease of oxygen saturation, and contemporaneous sub-cutaneous emphysema occurred. In spite of cardio-polmunary resuscitation maneuvers and following placement of the trocar and thoracic drainage system, the young man died, because of a contemporaneous ventricular fibrillation. External examination revealed a drainage located in left hemithorax in the first intercostal space; another one in the right hemithorax in the fifth intercostal space, two needle marks in the left third intercostal space and in the right second intercostal space; cyanosis of finger nails of both hands was present.

Internal examination revealed emphysema in subcutaneous soft tissue of the thoracic and abdominal regions, in greater omentum and in the visceral adipose tissue. Also observed marked mediastinal emphysema, bilateral pneumothorax and reduced volume of the lungs.

The macroscopic examination of the heart showed collapse of the fossa ovalis, redundant, with diameter of 2.5 centimeters and with two perforations: the first one with maximum diameter of 1 centimeter and the second one of 0.5 centimeter, divided by fibro-muscolar biceps. The right ventricle was dilated with thin walls (0.3 centimeter maximum thickness), left ventricle slightly dilated with a free wall of 1.5 centimeters.

The observation of air breath showed in the right intermedium bronchus an "S"-shaped laceration with frayed margins slightly that involved, in the point of the bifurication with medium lobe, in the extraparenchymal intrapleuric tract, half circumference of the bronchus.

The dissection of the lungs revealed congestion and hemorrhagic edema. There was hypoxic ischemic multiorgan damage.

Histologic assays showed massive right endo-bronchial bleeding and the site of the bronchial lesion was characterized by incomplete breakup of a cartilaginous ring in correspondence of one of the extremities; the adjacent pulmonary vein with massive blood infiltration of the nearest soft tissues. The borders of the vascular breakup were irregularly dissected and infiltrated by blood cells; in the context of the vascular wall other breaches were observed with partial tonaca media's dissection. Hemorragic edema was found in pulmonary parenchyma with red cells infiltration of the nearest soft tissues and sub-pleural tissues, in association with emphysematous blebs.

On the macro-microscopic evidences the cause of the death has been attributed to an acute respiratory insufficiency by severe pneumothorax following bronchial breakup; the typology of death is attributable to "therapeutic complication."

Along with the histological assays, the authors have verified the iatrogenic nature of the breakup; besides, using the classic forensic criteria the pathologists have attributed the professional liability to one of the professional figures (echocardiographist, hemodinamist, anesthetist) involved in the management of the young patient.

Transcatheter Closure, Atrial Septum Defect, Bronchial Lesion

# G56 Dissecting Intramural Hematoma of the Esophagus: A Rare Case of Sudden Death

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After attending this presentation, attendees will learn that dissecting intramural hematoma of the esophagus is a rare condition with excellent prognosis when treated conservatively. Spontaneous ruptures of hematoma are rarely described as well as sudden death due to DIHO.

This presentation will impact the forensic science community by presenting the rarity of the fatal events due to DIHO and the autopsy technique performed in visualizing rupture, preserving anatomical relationship between cervic-thoracic organs.

Dissecting intramural hematoma of the oesophagus (DIHO) is a rare condition in which intramural hemorrhage leads to submucosal

dissection of the oesophageal wall. It is usually associated with a rapid increase in intraoesophageal pressure, trauma or a coagulation disorder. The clinical presentation is with chest pain, hematemesis and dysphagia/odynophagia and an accurate history is vital to help distinguish it from other causes of acute chest pain, such as myocardial infarction, aortic dissection or oesophageal perforation. The three different types of acute oesophageal injury are a mucosal tear (Mallory-Weiss syndrome), full-thickness rupture (Boerhaave's syndrome) and dissecting intramural hematoma. Neither the Mallory-Weiss nor the Boerhaave lesions are associated with submucosal hematomas or dissections. In some cases the first event may be hemorrhage into the submucosa with secondary rupture into the lumen. The differential diagnosis includes other causes of central chest pain and it is vital to obtain an accurate history of both gastrointestinal and cardiovascular symptoms. Analysis of the precipitating factors suggests that there are three main subgroups. Firstly, a sudden pressure change in the oesophagus (e.g., swallowing, vomiting) perhaps associated with a temporary disruption in the normal co-ordinated opening mechanism of the upper and lower oesophageal sphincters. Secondly, direct injury following an endoscopic therapeutic intervention (e.g., oesophageal dilatation). Thirdly, about one fifth of patients appear to have a truly spontaneous origin although this may be associated with an underlying predisposition to abnormal pressure changes within the oesophagus (e.g., achalasia) or a bleeding disorder (e.g., due to anti-platelets, anticoagulants or thrombolytics). The pathophysiology is characterized by submucosal hemorrhage that dissects the submucosa and classically occurs in the distal oesophagus because this region is least supported by adjacent structures such as the trachea or heart.

A rare case is presented of sudden death due to spontaneous rupture of DIHO occurred in a 42-year-old woman presented at local emergency department with a 24 hour history of sudden onset severe central chest and interscapular pain associated with dysphagia and odynophagia. There was no history of vomiting, hematemesis or trauma. There was little previous medical history of note and he was not taking any regular medication. On examination, vital signs were: blood pressure, 104/49 mmHg with no differential between arms; pulse, 125 beats/min; respiratory rate, 24 breaths/min; body temperature was normal. There was no abdominal tenderness and no maelena. EKG was unremarkable as well as cardiac enzymes. Clinical conditions suddenly got worse; the woman collapsed and resuscitation maneuvers were unsuccessful. Autopsy was performed the day after death. Massive hemothorax was recorded. Thoracic and abdominal organs were removed en masse according to Letulle technique and fixed in 10% buffered formalin for a detailed macroscopic examination. All other organs examination was unremarkable except for cerebral oedema. Vessels were poor of blood. Lungs were increased in volume and size, with few subpleural hemorrhagic spots. Mild white foam on the main bronchi was also detected. Heart was normal in size and volume, with conical shape. Coronaries examination was unremarkable. A large bluish/red intramural haematoma of the posterior wall of the oesofagus extending from just below the cricopharyngeous to the gastro-oesophageal junction was recorded with a complete rupture of the oesophagus wall in the proximal third. Mild cerebral odema and focal pulmonary oedema were observed at histological examination with standard H&E staining. Histological examination of heart was unremarkable except for few foci of contraction band necrosis. Sample of oesophagus dissection was collected excluding recognizable abnormality in the muscle layers, except for rupture. A complete immunohistochemical panel has been performed on esophagus samples. Genetic investigations had been performed also.

DIHO, Spontaneous rupture, Sudden Death

#### G57 Anaphylactic Shock and Postmortem Exam – A Systematic Approach

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After attending this presentation, attendees will have an insight about the efforts being made by the Portuguese National Institute of Legal Medicine in order to harmonize the methodology of forensic autopsies, since not all the medical forensic experts have the specialty of legal medicine.

This presentation will impact the forensic science community by informing attendees of the attempt to establish specific rules concerning the approach of fatal cases of suspect anaphylactic reactions.

The anaphylactic shock is classified as a type I of hypersensitivity reaction that occurs due to the release of biologically active agents, among them, histamine. It is due to exposure to allergens of different types, like drugs, food, animal sting, animal fur. If not promptly reversed, the outcome can be fatal.

The relevance of available circumstantial information, namely context and symptoms prior to death, previous medical history and possible life support procedures applied will be discussed.

The need for a thorough external examination of the corpse is also addressed, in order to search, for instance, for possible sting marks or hives-like lesions that may help to support the diagnostic.

Overall, postmortem findings, either in the external and internal examination, are usually nonexistent or nonspecific, the forensic expert should collect all body samples that may be needed later on to reach a more accurate diagnosis.

Therefore, besides the routine histology (heart, lungs, liver and kidneys), the collection of samples from other organs with known increased mastocyte cells population is recommended. Toxicological exams should contemplate drugs, abuse drugs and/or pesticides, according to the specificity of the case.

Also highlighted is the relevance of collecting peripheral blood for IgE and tryptase concentration levels and that this task should be undertaken as soon as possible after the judiciary's authorization for the autopsy.

Because of the lack of relevant findings, death by anaphylactic shock is considered a diagnosis of exclusion, that is achieved through the evaluation of the available circumstantial information, the findings (or their nonexistence) in the external and internal examination and results of ancillary investigation, namely, histology, in some cases complemented by immunohistochemical techniques (anti-tryptase and anti-CD117), toxicology and serology (IgE and tryptase).

Based on the most recent scientific knowledge, a comprehensive protocol was designed with the purpose of being applied to this situation and serve as a guideline to forensic autopsies.

Forensic Autopsy, Anaphylaxis, Protocol

#### G58 Spontaneous Pulmonary Arterial Dissection: A Case Report

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The goal of this presentation is to present a fatal case of spontaneous pulmonary arterial dissection with a wide immunohistochemical study about alteration of pulmonary wall.

This presentation will impact the forensic science community for the rarity of the deaths due a spontaneous dissection of the pulmonary trunk. Unlike dissection in systemic arteries, pulmonary artery or main pulmonary branche dissection, is usually lethal. So the diagnosis of this condition is very rarely made during life and most commonly diagnosed at autopsy in cases of sudden and unexpected death. Hemorrhagic pericardial effusion and cardiac tamponade usually follow the outward rupture of the proximal main pulmonary artery.

With regard to pathogenesis, pulmonary artery dissection is strongly associated with primary and much more frequently, secondary pulmonary hypertension. Secondary pulmonary hypertension most often results from congenital cardiac lesions, above all with various forms of left-to-right shunting, most commonly patent ductus arteriosus, or congenital ventricular septal defect. These cardiac conditions predispose individuals to the development of pulmonary artery aneurysm by generating sustained high pulmonary flow rates and pulmonary artery pressure. However, other possible causes are Marfan syndrome and other connective tissue diseases, infectious processes and inflammatory conditions, such as Behcet disease. Anyway pulmonary artery dissection is exceedingly rare in the absence of pulmonary hypertension or other pathologic conditions.

The clinical presentation of pulmonary artery dissection is highly variable and the symptoms are nonspecific, most frequently chest pain, dyspnea, cyanosis, and hemodynamic compromise. Diagnostic instruments for this condition are noninvasive imaging techniques, including echocardiography, CT, and magnetic resonance imaging (MRI).

The vascular histopathologic changes associated with the majority of pulmonary artery dissections involve medial degeneration, with fragmentation of elastic fibers. These changes may represent an intrinsic weakness in the vessel wall which is compounded by the increased hemodynamic shear stresses of pulmonary hypertension, thereby predisposing an intimal tear. The pathogenetic mechanism of dissection in absence of histopathologic alterations remains substantially unclear.

The case presented concerns sudden death due to spontaneous pulmonary artery dissection.

A 72-year-old woman was admitted to the Emergency Department for chest pain, spread to mandible, dyspnea, and jugular tightness, and she referred these symptoms after bleach inhalation during housecleaning.

Physical examination, ECG and CT were unremarkable. Cardiac ultrasonography showed concentric ventricular hypertrophy and ascending thoracic aorta ectasia (50 mm). Laboratory blood values demonstrated neutrophilia, lymphopenia, monocytosis and increased erythrosedimentation rate. Two days later she died.

A postmortem examination was performed and revealed a large hemorrhagic area in left posterior mediastinum and pericardial sac containing approximately 150 ml of blood and 250 g of clotted blood. The source of hemorrhage was readily identified as a 2 cm tear in the wall of the pulmonary trunk and so dissection and rupture of the artery.

Microscopic sections of the pulmonary artery revealed regular morphology of the wall layers. The medial layer showed fragmentation of elastic fibers, marked fibrosis and copious erythrocytes. In a section the intimal tear was identified as initial site of dissection.

The immunohistochemical investigation of the pulmonary artery samples in whole artery wall and in laminar dissection was performed with antibodies anti TGF-beta-1, TGFBR1 (ALK-5) e TGFBR2, ALK-1, fibrillin and endoglin. Fibrillin showed a massive and diffuse positive reaction of the whole pulmonary artery near the dissection, but it showed negative reaction in laminar dissection; Endoglin showed a weak positive reaction in the whole artery and a negative reaction in the laminar dissection; TGF-beta1 revealed a weak positive reaction in the whole pulmonary artery and a strong reaction in the laminar dissection; TGFBR1 and ALK-1 showed a moderate positive reaction in laminar dissection; TGFBR1 and a massive positive reaction in laminar dissection; TGFBR2 revealed a massive positive reaction of the whole pulmonary artery, but it showed moderate reaction in laminar dissection.

A fatal hemopericardium caused by spontaneous pulmonary artery dissection was recorded as the cause of death. The histological investigation of the pulmonary artery samples revealed the absence of hypertensive arterial changes and the immunohistochemical showed the absence of any connective tissue disease of the pulmonary trunk. So the presented case illustrates a very rare cause of sudden death in a spontaneous dissection of a normal pulmonary trunk.

Spontaneous Pulmonary Dissection, Sudden Death, Immunohistochemistry

#### G59 Inherited Cardiac Diseases and Molecular Autopsy: Perspectives and Limitations

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After attending this presentation, attendees will understand the importance of postmortem genetic testing, as well its limitations for the diagnosis of sudden cardiac death in young adult or in sudden infant death sysdrom (SIDS) cases.

This presentation will impact the forensic science community by presenting the practical approach to a new diagnostic tool in cases of sudden death in the forensic context.

Cardiac diseases of genetic origin are often the cause of sudden death, especially in young individuals. Postmortem genetic testing, also known as molecular autopsy, is recommended in cases of sudden cardiac death with a negative autopsy. These deaths are currently considered to be due to sudden arrhythmic death syndrome, and are reported in up to 40% of sudden cardiac deaths of young adults. The studies performed on cases of sudden infant death syndrome (SIDS) indicate that approximately 5–10% of SIDS is due to defective cardiac ion channels.

Rhythm disturbances observed in genetically determined cardiac diseases are not always lethal, but can have dramatic consequences if the individual is driving or swimming, for example.

Several cases of sudden death resulting from different genetically determined cardiac pathologies will be presented. In some cases, a morphological substrate, such as arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) or hypertrophic cardiomyopathy (HCM) was observed at autopsy and confirmed by histological examination. In others cases without any pathology observed during standard autopsy procedures, and after a negative toxicological analysis, mutations in the three genes most frequently implicated in inherited arrhythmias SCN5A, KCNQ1, and KCNH2 were found. In the remaining cases even the molecular autopsy was negative.

The first case is a 33-year-old man who died after losing control of his vehicle. ARVD/C was found at autopsy. No traumatic lesions were observed and it was determined to be a natural death. The second case is an 18-year-old man who died after a football match. The only significant finding at autopsy was the ARVD/C. In this case, an electrocardiogram recorded a few weeks before his death showed pathological patterns pathognomic for the ARVD/C. In one SIDS case, the molecular autopsy showed mutations in the KCNH2 gene and in another SIDS case a genetic variant in the SCN5A gene. Both have been described in long QT-cases. In the last presented SIDS case, molecular autopsy was negative but a positional asphyxia was evoked after scene investigation and a cartilaginous meta-hyperplasia of the cardiac conduction system was observed.

The major limitations of the molecular autopsy in forensic practice are the cost of the analyses, the accessibility of a competent laboratory and the legal aspects of postmortem genetic testing. The interpretation of the results and their transmission to the families can also prove to be problematic. Due to the heritability of genetically determined cardiac disease, the autopsy diagnosis is very important for any living relatives. Collaboration with cardiologists and geneticists allows proposing multidisciplinary consultations to them.

In conclusion, the molecular diagnosis of cardiac arrhythmias represents a very useful and attractive tool in cases of sudden death. However, even if the case is presumed to be related to a hereditary cardiac disease the classical guidelines of autopsy practice should be respected (scene investigation, histological examination, toxicological analyses etc.) to avoid the over interpretation of the results of the molecular autopsy. Moreover, due to the heritability of genetically determined cardiac disease, the potential implications for living relatives must be taken into consideration and genetic counseling should be proposed to the family.

Molecular Autopsy, Sudden Cardiac Death, Channelopathies

# G60 Sudden, Unexpected Death Due to Glioblastoma: Three Fatal Cases

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The goal of this presentation is to present three cases of sudden, unexpected death due to glioblastoma, with different brain localization and expression.

This presentation will impact the forensic science community by disussing how a complete methodological forensic approach by means of autopsy, histological and immunohistochemical examinations let us to conclude for an acute central dysregulation caused by glioblastoma and relative complication with rapid increase of intracranial pressure as cause of death.

Glioblastoma is the most common malignant primary brain neoplasm, representing about 12-20% of all intracranial tumors and accounting for about 50-60% of all astrocytic gliomas. In the most European and North American countries, the incidence is approximately 2-3 new cases per 100,000 people per year. The incidence of sudden death due to undiagnosed primary intracranial tumor is low in forensic autopsy and is an uncommon event. In fact only 12% of all cases of sudden unexpected death due to primary intracranial tumors are due to glioblastomas.

Three cases of sudden unexpected death due to glioblastoma according to WHO grade IV are reported.

**Case 1:** a 43-year-old Polish man was found dead in a slope near the track of the railway. Death scene investigation was unremarkable. A complete autopsy was performed 48 hours after death. The external examination revealed only same abrasions and bruises on the face, and the upper and lower limbs. The internal examination revealed polyvisceral stasis, heavy lungs and reddish colored foam on trachea and the main bronchi. The skull was entire. The examination of the brain (cm 21x16x6, g 1630) after fixation in buffered formalin revealed a cerebral edema and an increase in volume of the left frontal lobe. On coronal sections, the cerebral hemispheres were asymmetrical with deviation of midline structures from left toward right. In the left frontal lobe a spherical mass (cm 3.5x3x1.5), with variegated appearance and contained regions of necrosis and hemorrhage was found. The blood alcohol concentration was 0.8 g/l.

**Case 2:** a 79-year-old Caucasian man, with a history of ischemic heart disease and hypertension, was brought to the hospital in the neurological unit for symptoms such as confusion, slackening, sleepiness, and tremor of the upper limbs start few days before. The brain CT scanner examination shows a large hypodense mass in the left

\* Presenting Author

temporal lobe with massive oedema and compression phenomena on occipital and temporal lobe and midline shift. The patient was then referred for neurosurgical consultation, but the day before surgery he suddenly died. General autopsy performed 48 hours after death was unremarkable. The brain weighed 1600 g and measured (cm 22x16x6.5) showed diffusely swollen cerebral hemispheres and an increase in volume of the left temporal lobe. There was no herniation of the temporal lobe unci or cerebellar tonsils. On coronal section, after fixation, the left temporal lobe showed a large mass lesion, which measured 3x2.5x2.2, hemorrhagic and surrounded by necrotic and oedematous tissue.

Case 3: a 71-year-old-Caucasian man, with a past history of hyposthenia of the right arm, cervical spine surgery, chronic kidney disease, and hepatic steatosis. During his detention, showed headache, confusional state, and difficulty in walking therefore he was transferred to the local hospital. The neurological examination revealed poor general condition, marked weight loss, ataxia and ideomotor slowing, depressive syndrome, apathy, fatigue, and lack of initiative. The laboratory examination of blood and liquor was negative for infectioninflammatory disease. To diagnose a multi-infarct dementia the patient was scheduled for TC and magnetic resonance imaging of the brain and the entire spine, but suddenly died prior to the imaging. At autopsy a moderate pulmonary edema and polyvisceral stasis were observed. The brain weighed 1550 g and showed massive edema. A spherical gelatinous solid mass, measuring 1 cm in diameter was attached in the right medulla. On coronal sections, the right temporal lobe showed a reddish-rusty mass lesion, measuring 1x2 cm and the third ventricle was compressed and dislocated.

The etiopathogenetic definition was outlined by histological examinations performed on brain tissue samples using haematoxylineosin (H&E) and Perl's and revealed the presence of diffuse and marked cytotoxic and vasogenic brain edema, and in samples taken from left frontal lobe (case I), left temporal lobe (case II), right medulla and temporal lobe (case III) foci of central necrosis surrounded by neoplastic cells with nuclear pleomorphism, pseudopalisading, multinucleated cells ("giant cells glioblastomas") and vascular proliferation. Areas of extensive haemorrhage near tumor cells were also observed.

The immunohistochemical examination of the brain specimens revealed a positive reaction for antibodies anti-GFAP (glial fibrillary acidic protein), CD68, vimentin and S-100; NSE (neuron-specific enolase), smooth muscle actin, CD34, cytokeratins MNF 116, EMA (epithelial membrane antigen), synaptophysin, HMB45 (Human Melanoma Black) were negative. The positive reaction for GFAP was confirmed by Western blotting. The other organs showed signs of central dysregulation (pulmonary oedema).

The death was attributed in the first and second case to brain edema and massive hemorrhage into the glioblastoma from arrosion of vessels, with an increase in intracranial pressure and compression of cerebrospinal fluid circulation, whereas in the third case death can be explained by distortion and compression of the medulla by the tumor with consequent acute central dysregulation due to glioblastoma corresponding to WHO grade IV.

Glioblastoma, Sudden Death, Immunohistochemistry Stains and Western Blotting

#### G61 Postmortem Tryptase Levels of Anaphylactic and Non-Anaphylactic Deaths

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After attending this presentation, attendees will understand the importance and some limitations of the analysis of serum tryptase in the postmortem diagnosis of anaphylactic shock.

This presentation will impact the forensic science community by helping the forensic pathologists in the interpretation of postmortem serum tryptase levels. In addition to that, the number of anaphylaxis cases presented here is big, given the rarity of this cause of death.

**Introduction:** Anaphylactic reactions are encountered very rarely as cause of death in forensic practice and the postmortem diagnosis can be difficult, given the unspecific autopsy findings. The diagnosis is usually based on several criteria, including an elevated serum tryptase level. The established clinical normal values for serum tryptase can however not be used in the postmortem setting and need to be adapted for postmortem cases. The interpretation of postmortem serum tryptase levels may be tricky. So it is well known that some conditions other than anaphylaxis can lead to high tryptase levels and also false negative results can be encountered.

**Aims:** The presented study is aimed at describing the diagnostic criteria, including serum tryptase levels that were used to diagnose twelve anaphylactic deaths. Moreover, a postmortem normal value for serum tryptase from controls is computed and compared to the published data in the literature.

**Methods:** Twelve anaphylactic deaths, investigated in the Victorian Institute of Forensic Medicine in Melbourne (AUS), have been retrospectively analyzed concerning the diagnostic criteria, autopsy findings and postmortem serum tryptase levels. The findings and the serum tryptase levels were compared to those of a control group consisting of 33 cases with identified, non-anaphylactic causes of death. To better represent the reality of forensic practice, the control group has been increased by 17 individuals with unascertained causes of death, for a second comparison. The obtained cut-off level was compared to the published data.

**Results:** The postmortem diagnosis of anaphylaxis in the 12 cases was mainly based on the circumstantial information surrounding the death, the medical history, and the exclusion of other causes of death. Laryngeal oedema was found in 83% of the anaphylaxis cases and in 17% of the controls. None of them had a skin rash. The tryptase levels of the controls will be presented with known causes of death and of the increased control group after including unascertained cases. Some cases with surprisingly high or low levels will be discussed.

**Conclusion:** Serum tryptase obtained from peripheral blood is the strongest aid in the diagnosis of anaphylaxis as a cause of death. The majority of anaphylaxis cases have tryptase levels of well above  $100\mu g/l$ , whereas the other causes of death had tryptase levels generally under  $41\mu g/l$ . A grey zone clearly exists, and a number of elements should be present to make the diagnosis of anaphylaxis. Even a strongly positive tryptase result should not automatically lead to the diagnosis of anaphylactic shock. In most cases, other elements can be found to support or reject the diagnosis. Other conditions with elevated tryptase levels exist and should be considered in cases with high levels.

Tryptase, Anaphylaxis, Postmortem

## G62 Murder-Suicide in Fulton County, Georgia: 1992-2006

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After attending this presentation, attendees will understand the characteristics of murder-suicides occurring during a 15-year-period in Fulton County, Georgia, with emphasis on circumstances and relationships of perpetrators and victims.

This presentation will impact forensic science community by detailing the features of murder-suicide events and reviewing relevant literature, with the goal of providing information relevant to prevention strategies.

**Background:** Murder-suicide typically involves one or more homicides followed shortly thereafter (usually at the same time but sometimes later) by suicide of the perpetrator. The Fulton County Medical Examiner's Office previously reported a series of twelve murder-suicide cases which occurred in the years 1988-1991. This report is a follow up study of murder-suicide cases in Fulton County, Georgia, which occurred in the 15-year period of 1992 through 2006. Current data are compared with data from the earlier study and other studies reported in the literature.

**Methods:** The Fulton County Medical Examiner's Office maintains a comprehensive database which includes data items to record companion cases and for the past seven years, indication of whether death was part of a murder-suicide event. The data base was searched to detect murder-suicide events and to collect demographic, cause of death, and circumstantial information for each case. Results are compared with our previous study, the literature, and the incidents are classified in the context of a previously published classification scheme.

**Results:** 40 incidents occurred during the 15-year period. There were 40 suicides and 46 homicides for a total of 86 decedents. The number of incidents per year ranged from 1 to 6 with an average of 2.6, and the number of decedents per year ranged from 2 to 17 with an average of 5.5 per year. There were two decedents in 38 of the incidents, three decedents in one incident, and seven decedents in one incident. There was at least one murder-suicide event each year.

In every case, the perpetrator was male. Fourteen of the perpetrators (34%) tested positive for ethanol, five of the homicide victims (11%) tested positive for ethanol, and in three cases (7%), both the perpetrator and victim were positive for ethanol. In 8 cases (26%), the perpetrator was positive for stimulant drugs such a cocaine or methamphetamine.

In 34 incidents (85%), the perpetrator and victim were both shot. One incident involved sharp force injuries of both decedents, another incident involved thermal burns of both decedents, and in four incidents a combination of methods was used. 27 (66%) incidents occurred in or on the property of the perpetrator's place of residence. The most common circumstance was a boyfriend killing a girlfriend (n=13) or exgirlfriend (n=3). The second most common was a husband killing his wife or ex-wife (n=11). An employee killed a coworker in three incidents. In two incidents, one male killed another male during an argument.

Of the perpetrators (all male), 9 (23%) were White, 6 (15%) were Hispanic/Latino, and 25 (63%) were Black. Both Hispanics and Blacks were overrepresented in comparison with their prevalence (43% and 8% respectively) in the county population while whites were underrepresented (account for about 50% of the population). In 37 incidents (93%), all decedents were of the same race. In three cases, the perpetrator was Hispanic/Latino and the victim was White (non-Hispanic). A male killed one or more females in 34 of the 40 incidents (85% of cases).

The number of days between incidents ranged from 12 to 483 with a median of 125 days and a mean of 142 days. Thus, evidence of short term clustering was minimal except for two incidents in late June and early July of 1999. The same year which had the maximum number of incidents (n=6).

Comparison of the present study to our previous study shows only slight differences in the trends observed, which may relate mainly to the much larger size of the most recent case series.

Using the classification scheme of Marzuk, Tardiff, and Hirsch, the most common type of incident (71% of incidents) was "Spousal or Consortial" (in which we included spouses, former spouses, girlfriends, and ex-girlfriends) and the most common motive (27% of incidents) was "Amorous Jealousy." A similar portion of cases involved some form of argument or relationship problem which was not further clarified. Of note, 12 cases (30% of incidents) involved motives that were not apparent. This finding points out the difficulty in clarifying the motive when the people who might be able to explain what happened are dead and further specific information cannot be determined.

All but three incidents involved circumstances in which the deaths of the victims and perpetrators had a close temporal relationship. In one case, the perpetrator died in the hospital after a two months stay for his self inflicted gunshot wound. In a second and atypical case, the perpetrator committed suicide in jail several months after being arrested for the murder of the victim. In the third case, the homicide victim died about five months after the incident from ongoing complications of her gunshot wounds.

**Conclusions:** Similar to other studies, murder-suicides in Fulton County, Georgia show a low but stable rate of occurrence with a predominance of male perpetrators, female homicide victims, same-race victims, two deceased persons, a victim-perpetrator relationship such a spouse or girlfriend, and causes of death which predominantly involve gunshot wounds.

Murder-Suicide, Homicide-Suicide, Violent Death

#### G63 Homicidal Deaths in the Western Suburbs of Paris: A 15-Year-Study With Special Focus on Survival Time

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After attending this presentation, attendees will understand homicide trends in an area around Paris and the value of Injury Severity Score (ISS) for the estimation of survival time.

This presentation will impact the forensic science community by helping forensic pathologists in homicide case investigations when questions about victim's survival time are raised.

The goal of this study was to analyze the homicide pattern in the western suburbs of Paris and its evolution between 1994 and 2008. ISS was also assessed to see if it was correlated with the survival time of homicide victims.

All autopsy reports regarding homicides from January 1, 1994 to December 31, 2008 were retrospectively reviewed. All autopsies were performed in the Department of Forensic Medicine and Pathology of Garches. Out of 4,842 autopsy cases reviewed, 511 homicide cases were selected. The following data were recorded: assailants' and victims' characteristics, crime scene location, homicide motive, cause of death, postmortem toxicological results, ISS, and estimated survival time.

Homicide rate steadily declined over the period at the exception of the number of homicide-suicide per year which remained constant. Homicide victims remained unidentified after medico-legal investigations in 2% of the cases. Child and elder homicide cases represented respectively 10.7% and 8.2% of the cases. Offenders were male in 88% of the cases. Male and female assailants showed distinct homicide patterns: females were involved more frequently in familial quarrel and child abuse. They never killed a stranger and committed homicide exclusively in a private place with a predominance of sharp weapons. Males in contrast assaulted almost equally a stranger or an acquaintance, often in a public place with a predominance of firearm. The victim knew the assailant(s) in 57% of the cases. Homicides mostly took place at the residence of the assailant or the victim. Homicide motive was clearly determined in 71% of the cases. Argument was the most common motive in 44% of the cases. Sexual assault was rarely found (ten cases). Gunshot wounds were the most common cause of death (37%), followed by stab wounds (27%), blunt trauma (19%) and asphyxia (13%). A decrease of gunshot wounds as a cause of death was found over the studied period. Alcohol was the most common toxic detected in blood victim, in 48.5% of the cases when toxicological results were available. Blood alcohol concentration ranged from 1 to 500 mg/dL with a mean value of 150 mg/dL. Survival time was determined in 162 cases and ranged from 0 minute to 25 days. The mean ISS was different according to the cause of death: 3.4 for deaths by asphyxia, 38.6 for deaths by stab wounds, 39.6 for deaths by blunt trauma and 60 for deaths by gunshot wounds. ISS and survival time showed a significant correlation (r=-0.56; p<0.05) only for short survival time (less than three hours) and after exclusion of deaths by asphyxia (n=58). Correlation was weaker when there was a long time of resuscitation.

In conclusion, this autopsy series research pointed out that homicide pattern strongly differed according to the sex of the victim and of the assailant. ISS could be used to help in estimating the victim's survival time, taking into account the compounding factor of resuscitation.

Homicide, ISS, Survival Time

#### G64 Pattern of Limb Lesions in Suicidal Hanging: A Criteria Tool in the Distinction of Suspicious Cases

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After attending this presentation, attendees will have a better understanding of the usual pattern of lesions in suicidal hangings and will be aware of the pattern of lesions to be considered suspicious.

This presentation will impact the forensic science community by providing tools to improve the screening of suicidal hangings for possible suspicious cases.

**Introduction:** In this era of limited resources, hanging cases are investigated in several jurisdictions by a limited scene analysis and a rapid external examination of the body. Full body autopsies are becoming less and less frequent in these cases, based on the assumption that hangings are virtually always suicide. Homicidal hangings are therefore at serious risk to be missed, particularly since they are difficult to detect. However, to recommend a full body autopsy on all hanging victims is not realistic considering the limited human and financial resources.

In a recent six-year retrospective study on the pattern of limb bruises in hanging conducted in the Province of Quebec, Canada, it was suggested that the presence of bruises on the anterior upper limb and the presence of bruises on both the upper and lower limbs were two criteria that should alert the pathologist to be more cautious. The presence of bruises on the posterior lower limb was initially proposed as an additional criterion of suspicion, but failed to reach statistical significance. The present study aims at evaluating the validity of these criteria on a different population and to further investigate the usual pattern of limb lesions in suicidal hangings.

**Material and Methods:** A total of 214 suicidal hangings, investigated at the Office of the Chief Medical Examiner in Alberta, were reviewed for the presence and localization of bruises, abrasions and lacerations. An age- and gender-matched control group of non-hanging homicidal strangulations, composed of 51 cases, was similarly studied.

**Results:** *Incidence of limb lesions:* Bruises were found in 6% of suicidal hanging victims, abrasions in 5% and lacerations in 1%. Compared to homicidal strangulation victims, suicidal hanging victims are less likely to present bruises ( $\chi^2$ =84.301; p=.000; Phi=.564), abrasions ( $\chi^2$ =75.231; p=.000; Phi=.533) and lacerations ( $\chi^2$ =8.123; p=.023; Phi=.175).

Usual pattern of limb bruises in suicidal hanging: The usual pattern of limb lesions in suicidal hanging victims was confirmed to be the following: bruises and abrasions are mostly found on the posterior part of upper limbs, on the anterior aspect of lower limbs, and on either the upper or lower limb but not to both in a single case. It was also found that bruises are commonly found on the anterior part of upper arms, but not on the anterior part of forearms. The comparative pattern in homicidal non-hanging strangulation does not display this preferential concentration.

Suspicion criteria for limb bruises: Three criteria were statistically confirmed to be in favor of an homicidal strangulation: the presence of bruises and/or abrasions (i) on the anterior forearms (bruises:  $\chi^2=16.500$ ; p=.001; Phi=.250; abrasions:  $\chi^2=16.224$ ; p=.001; Phi=.247), (ii) on the posterior aspect of lower limbs (bruises:  $\chi^2=39.092$ ; p=.000; Phi=.384; abrasions:  $\chi^2=25.642$ ; p=.000; Phi=.312), and (iii) on both upper and lower limbs in a single case (bruises:  $\chi^2=51.043$ ; p=.000; Phi=.439; abrasions:  $\chi^2=24.682$ ; p=.000; Phi=.305).

**Conclusion:** In the evaluation of a given case, the presence of the following distribution of bruises or abrasions should alert the pathologist to be more cautious and to further investigate the case: the presence of bruises or abrasions on the anterior forearms, on the posterior legs, or on both upper and lower limbs in a single case. Of course, the localization of bruises and abrasions is not to be interpreted without all other scene elements and autopsy findings.

Hanging, Bruise, Abrasion

#### G65 Decubitus Ulcers and Ligature Marks as Evidence in a Homicide Case

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After attending this presentation, attendees will better understand the application of medicolegal investigation, specifically the role of forensic pathologist during homicide investigation. In addition, attendees will become familiar with the relatively rare injury patterns in forensic practice.

This presentation will impact the forensic science community by providing knowledge to medicolegal investigators, especially in deciding cause and manner of death in equivocal death investigations. This presentation will increase the competence of the medicolegal examiners and forensic pathologists in examination of complicated cases, when the autopsy findings may become unique evidence in the following legal action and adjudication.

The 30-year-old woman was found dead in the house where she was living with her fiancée and her mother-in-law. At initial inspection, bad condition and exhaustion of the body suggested natural death by malignant disease with no preview of medical history. Some unusual circumstances aroused suspicion. The victim was isolated in the dark room with no possibility to call for help, because her private cell phone was taken away. Crime police found adhesive tapes and linen strap near the bed. Insensitive behavior of the household aroused suspicion and demanded careful pursuit of the following medicolegal investigation.

External examination of the body revealed three different types of injuries: decubitus ulcers (pressure sores), scabs (as ligature marks), and bruises of various ages. The decubitus ulcers stage II and III of the coccyx-sacrum region and on the both sides of the buttocks, ulcers stage II of the left elbow and the left ankle, ulcers stage I of the left trochanter and over the pectoral spine near the left shoulder blade were described. The circular scabs around the neck, and both wrists indicated ligature marks, so as the necrosis of the II-III fingers on the left hand. The bruises of various colors were presented on the left hand and upper arm, as well as along both medial femoral regions. The autopsy findings showed that the sacro-coccigeal ulcers extended into the subcutaneous tissue and secondary resulted in bronchopneumonia with purulent effusion into the left thoracic cavity. The lipofuscin pigmentation of hepatocytes and myocytes as histopathological changes indicated a state of long time deprivation of food. The forensic pathologist pronounced the cause of death violent death by bronchopneumonia caused by infected decubitus ulcers.

It is believed that no similar cases described in the recent literature have been found. Homicide of a young woman by the infliction of decubitus ulcers caused by immobility and fixation of the victim's body with ligature (tapes and strap) including elements of social and physical separation combined with starvation has not yet been described in the criminal records in Croatia.

The forensic psychiatry expert determined the specific relations between the victim and the perpetrators. This study concluded that the perpetrators didn't act alone. The male perpetrator was a drug addict who had permanent schizotypal disorder of personality, with characterization of egocentrism, latent aggression, lower tolerance threshold and emotional coldness. His mother was a person with dominantly narcistic and dissocial personality disorder, with an intention to control the life of her son. The victim was a person with predominantly passive-dependent personality disorder, psychologically and socially predetermined to victimization. Forensic psychiatrist concluded that the perpetrators planned the crime together, carried on by the motive of jealousy.

According to Croatian Penal Code the perpetrators of a criminal offence were convicted of intentional murder to 30 years imprisonment.

The case presented shows the importance of a detailed crime investigation and close cooperation between crime police and forensic pathologist, especially if the presumed course of events is ambiguous. Recognition of the relatively rare injury patterns and understanding the mechanism of death seems to be the most important factor in elucidation of the presented homicide case.

Decubitus Ulcers, Ligature, Homicide

#### G66 Soccer Scams, Search Engines, Scientists, and Slaughter: Investigating a Complex Double Homicide in North-East England

Stuart J. Hamilton, MB ChB\*, 9 Troon Close, Consett, DH8 5XF, UNITED KINGDOM

After attending this presentation, attendees will see the value of using relevant subspecialty expertise in forensic pathology and the benefits of good communication between the various experts and investigating authorities in ensuring that evidence given in court is reliable and supported by validated scientific principles.

This presentation will impact the forensic science community by showing how a complex case can be brought to a satisfactory conclusion by methodical examination of the available evidence.
In 2008, two Chinese nationals who had been students at Newcastle University were found dead in their home. They had clearly been the victims of homicide and a pet had also been killed. There was virtually no evidence at the outset as to when or why the murders had occurred and no suspect existed. Scene examination showed that both victims had been bound and suffered significant blunt force trauma. At autopsy, evidence of incised wounds was identified in one victim and signs of asphyxia in the other.

The brains were submitted to a forensically experienced neuropathologist who provided valuable evidence with respect to the survival period after injury. The meal that one of the victims had eaten when last seen alive had been at the restaurant where she worked. The nature of this meal was known and the gastric contents of this victim were examined by both the pathologist and a scientist to attempt to identify the components of the stomach contents and the degree of digestion.

Although a national appeal was made on television for information, a suspect was developed by an unusual method. A strong case including DNA evidence was made and ultimately this individual was convicted on two counts of murder.

Good communication between the experts and police led to the development of a powerful and reliable case assisted by relevant subspecialist expertise.

Blunt Trauma, Homicide, Organized Crime

#### G67 Death Certification of "Suicide by Cop"

Amber R. Neitzel, BS\*, 550 East Van Buren Street, Phoenix, AZ 85004; and James R. Gill, MD, Office of the Chief Medical Examiner, 520 First Avenue, New York, NY 10016

After attending this presentation, attendees will understand the concept of "suicide by cop" and the criteria used to certify these deaths as suicides.

This presentation will impact the forensic science community by discussing examples of "suicide by cop" and why suicide (and not homicide) is the appropriate manner for these deaths.

A death certification of "suicide by cop" is controversial among some medical examiners and coroners (ME/C). These are often complex investigations and the opinions of the medical examiner must take into consideration all relevant issues. Five such deaths are presented that were certified as suicides and discuss the medicolegal issues involved with these certifications. In order to certify such a death as a suicide, certain criteria should be met. The five criteria used to make this certification include evidence of: (1) suicidal intent; (2) intent to be shot by law enforcement; (3) possession of a lethal weapon or facsimile; (4) intentional escalation of the encounter; and, (5) legal use of force by law enforcement. These legal actions of law enforcement are what distinguish these deaths from other instances of "assisted-suicide" that would be certified as homicides if they involved the illegal actions of another.

All of the decedents were male and their average age was 34 years (range 26 to 43). Both ethanol and cocaine were detected in two of the decedents and the other three had a history of psychiatric illness. All of the decedents possessed weapons or a facsimile of a weapon. There were three handguns, two knives, and one silver cigarette lighter in the form of a gun.

Suicide by cop is a circumstance that involves the completion of intentional acts that may result in dichotomous determinations of the manner of death. In some jurisdictions, these deaths are certified as homicide. Since these deaths occur at the hand of another, the invoked reasoning is that homicide therefore dominates the certification. But what if there was reliable evidence that a person wanted to die and committed an intentional act to further that goal? If the death was the result of an intentional act to do self harm or cause death of one's self, then the manner of death is more appropriately certified as "suicide" in these instances despite the fact that the decedent did not pull the trigger.

For police shootings, the conventional certification remains "homicide" in the absence of other compelling circumstances. But just because these deaths occur at the hand of another, there should not be an automatic homicide determination in all instances. Suicide should be considered in these deaths. Despite the absence of direct self-infliction, there is overwhelming evidence that these five individuals intended to end their own lives. Their use of an unusual method to accomplish this goal may inappropriately result in a reflexive certification of homicide. Instances of suicide by cop and contend that these types of deaths are best certified as suicides will be presented.

Suicide, Police, Manner

#### G68 Study of Lethal and Non-Lethal Filmed Hangings: New Insight Into the Pathophysiology of Hanging

Anny Sauvageau, MD\*, Office of the Chief Medical Examiner, 7007 - 116 Street, Edmonton, AB T6H 5R8, CANADA

After attending this presentation, attendees will have a better understanding of the pathophysiology of hanging, of the effect of the type of suspension and ischemic habituation on the agonal sequence, and on the appropriate scientific answer to the time to die by hanging.

This presentation will impact the forensic science community by providing new insight into the pathophysiology of hanging, based on the ongoing study of the working group on human asphyxia.

**Introduction:** Contemporary understanding of the pathophysiology of hanging is still largely based on old writings and experimentation from the end of the 19<sup>th</sup> century and beginning of the 20<sup>th</sup>. Apart from a few animal studies that gave very limited information on the pathophysiology of hanging in human, there was little new development on this issue until the creation of the Working Group on Human Asphyxia in 2006. Here presented are the newest results from this ongoing study.

**Material and Methods:** Fourteen lethal filmed hangings (nine autoerotic accidents, four suicides, and one homicide) were analyzed, as well as three non-lethal filmed hangings by an autoerotic asphyxia practitioner.

**Results and Discussion:** Lethal filmed hangings: In the fourteen lethal filmed hangings, the following sequence of agonal responses was observed: rapid loss of consciousness in  $10 \text{ s} \pm 3 \text{ s}$ , mild generalized convulsions in  $14 \text{ s} \pm 3 \text{ s}$ , decerebrate rigidity in  $19 \text{ s} \pm 5 \text{ s}$ , beginning of deep rhythmic abdominal respiratory movements in  $19 \text{ s} \pm 5 \text{ s}$ , decorticate rigidity in  $38 \text{ s} \pm 15 \text{ s}$ , loss of muscle tone in  $1 \text{ min } 17 \text{ s} \pm 25 \text{ s}$ , end of deep abdominal respiratory movements in  $1 \text{ min } 51 \text{ s} \pm 30 \text{ s}$ , and last muscle movement in  $4 \text{ min } 12 \text{ s} \pm 2 \text{ min } 29 \text{ s}$ .

*Effect of the type of suspension:* A comparison of time delay for agonal responses in complete suspension and incomplete suspension do not reveal impressive differences. These results suggest that the type of suspension may not be an important factor in the timing of agonal responses and therefore in the time to irreversible damage and death.

*Effect of ischemic habituation:* Considering that autoerotic practitioners might develop over time a certain ischemic habituation over time, it is theoretically possible that these cases present a deceleration of the sequence. On the other hand, since they often play for a longer period with the hanging process before the final hanging, it could be argue that on the contrary, their hanging sequence will be accelerated. Overall, the time delays for the early responses to hanging seem to be relatively similar between both groups, with the exception of an accelerated start of deep abdominal respiratory movements in the autoerotic practitioners. As for the late responses to hanging, they seem to be decelerated in autoerotic practitioners.

*Non-lethal filmed hangings:* In the three non-lethal filmed hangings, a loss of consciousness was observed in 8 to 16 seconds, followed by convulsions in 9 to 26 seconds. Decerebration rigidity was observed in one non-lethal filmed hanging (at 20 seconds). The ligature, which was not tied tightly to the shower rod, then detached from it, causing the fall of the man and the interruption of the hanging. Upon interruption of the hanging, the man quickly regained consciousness and seemed to present a full recovery without any noticeable symptoms.

*Estimation of the time to irreversibility and to die by hanging*: The scientific basis for the generalized assumption that death by hanging occur in three to five minutes will be reviewed. There is no forensic study to sustain this estimate of five minutes to die. In fact, this number seems to be based on three types of studies: a series of near-hanging victims in emergency medicine, studies of carotid endarterectomy, and physiopathological studies of brain ischemia. Though this estimation of the time is certainly precise and accurate enough for the needs of clinicians, it will be demonstrated that scientific evidence are not strong enough to be used in court. So how long does it take to suffer irreversible damage by hanging or by strangulation? The only honest and scientifically valid answer is not known.

Asphyxia, Hanging, Pathophysiology

#### G69 Pitfalls in the Interpretation of the Hyoid and Thyroid Fractures in Strangulation: The Importance of Anatomical Variations

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After attending this presentation, attendees will have a better knowledge of the importance of anatomical variations in the interpretation of fractures of the hyoid bone and thyroid cartilages.

This presentation will impact the forensic science community by developing and increasing awareness of the pitfalls associated with anatomical variations of the hyoid bone and the thyroid cartilage in strangulation cases.

Strangulation is defined as asphyxia by closure of the blood vessels and/or air passages of the neck as a result of external pressure on the neck. Three subtypes are recognized: hanging, ligature strangulation, and manual strangulation.

A proper neck dissection is a key element in the investigation of these deaths. Despite the usefulness of x-ray and computed tomography as ancillary techniques, manual dissection of the neck structures remains the most widely used technique to assess the integrity of neck structures. Considering the relative complexity of the neck dissection, it is important that it is performed by a trained forensic pathologist.

Apart from basic anatomical background and technical skills, forensic pathologists are in general well trained in recognizing postmortem artifacts encountered during the neck dissection. Unfortunately, anatomical variations as pitfalls in the interpretation of fractures of the hyoid bone and thyroid cartilage are however unknown to most. This comes to no surprise considering that forensic textbooks and the forensic literature have failed to pay any attention to these anatomical variations.

The anatomists have described several anatomical variations of the hyoid bone and thyroid cartilage that are of great interest to forensic pathologists. The triticea, a very small cartilage located in the thyroid-hyoid membrane, is encountered in approximately 13 to 16% of individuals. This cartilage can easily be mistaken as a fracture of the superior horns of the thyroid cartilage. Asymmetrical length of the superior horns of the thyroid cartilage, morphological differences between horns, and unilateral absence of one horn are all variations that

also constitute pitfalls in the interpretation of fractures of the thyroid cartilage. In the hyoid bone, the forensic pathologist should be aware of the following possible variations: unusually long great horn, uncommonly long lesser horns, difference in the fusion time of the greater horns to the body, and calcification of the stylohyoid ligament. The consistency of the hyoid bone and thyroid cartilage in relation to the victim's age should also be taken into consideration in the interpretation of autopsy findings.

Forensic pathologists should be aware of the anatomical variations of the hyoid bone and thyroid cartilage and should be trained in recognizing them, in order to avoid erroneous interpretation of autopsy findings. The role of x-ray and computed tomography as ancillary techniques will be discussed, but the importance of a proper manual dissection, with palpation of the fractures, will be reinforced. After removing the viscera from the chest and abdominal cavities and removing the brain (dry neck dissection), it is recommended to dissect *in situ* the muscles layers and then to remove the neck organs from the mouth and cervical column, in order to perform a dissection ex-situ of the hyoid and thyroid cartilage. Ultimately, the hands and eyes of the pathologist constitute an invaluable tool, provided there is proper training and knowledge. The dissection technique to assist in the discrimination of anatomical variations versus fractures of the neck structures will be further described.

Despite the tremendous importance of correct interpretation of anatomical variations in the identification of fractures of the neck structures in strangulation, this issue has not been properly discussed in the forensic literature so far. This presentation is aimed to fulfill this gap. **Hyoid, Thyroid Cartilage, Strangulation** 

#### G70 A Comparison Study of Homicides Between Beijing, China and the State of Maryland, United States

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After attending this presentation, attendees will have learned the epidemiological characteristics of homicides in Beijing, China and the state of Maryland, United States.

This presentation will impact the forensic science community by illustrating the differences in the pattern of homicides between China and the United States, and by discussing the influence of cultural dimensions and socioeconomic development on violent crime.

A retrospective comparison study was conducted on homicides occurring in 2008 comparing Beijing, China and the State of Maryland, United States. Beijing, the capital of China, with a population of 16, 950,000, covering 6.489 square miles, is made up of two suburban counties and 16 urban districts. Maryland, with a population of 5,633,597 and a total area of 12,407 square miles, comprises twenty-three counties and Baltimore City. In 2008, a total of 398 homicides occurred in Beijing. The homicide rate was 2.34 homicides per 100,000 population. Maryland, however, had 536 homicides (9.51 per 100,000 population), which was more than four times as high as the homicide rate in Beijing. Males were much more likely to become homicide victims than females in Maryland (Male: Female = 5.2:1, based on the rate), when compared with homicides in Beijing (Male: Female = 1.4: 1). The age distribution of homicide victims was similar between Beijing and Maryland, with the majority of the victims in their 20's to 40's.

The most common cause of homicide in Beijing was sharp force

injury (52.8%), followed by blunt force injury (24.1%), asphyxia due to suffocation/strangulation (17.8%). Only two deaths were caused by firearm injury combined with sharp force injury. On the other hand, the most common cause of homicide in Maryland was firearm injury (74.8%), followed by sharp force injury (10.3%), blunt force injury (5.0%), and suffocation/strangulation (3.5%). There was a significant difference between Beijing and Maryland regarding the homicide death scene location. More than 71% of the homicide victims in Beijing were found inside of buildings, such as residential houses (44.2%), business/government offices (10.0%), stores (7.5%), night clubs (6.5%), and other facilities (2.8%). However, only 37.9% of the homicide victims in Maryland were found inside of buildings with fewer than 30% of the victims found in residential houses. The majority of Maryland homicide victims were found either on the street (42.9%), or in the park/wood/field (12.9%), or other outside locations. The possible motives of homicide in both regions will also be discussed.

Forensic Science, Homicide, Epidemiology

#### G71 Blood at the Scene of Death Due to Hanging: Artifact or Antemortem

## Surendra K. Kumar, MD\*, Army College of Medical Sciences, Delhi Cantonment, New Delhi, 110010, INDIA

After attending this presentation, attendees will understand the distinctive difference between antemortem and postmortem collection of blood, the dynamics of blood collecting at the scene, principles of artifacts, importance of determining that the blood at the scene of crime was not antemortem but was a postmortem phenomenon, correlating the blood with the injuries, and example of dubious presentation of suicidal and homicidal deaths due to asphyxia.

This presentation will impact the forensic science community by showing how suspicion and or allegations of not reporting injuries or reporting incorrectly are related to the evidence of blood at the scene of crime. Blood oozing out of injuries sustained during medical treatment needs to be differentiated from those injuries that were inflicted after the death of an individual. Misinterpretations can be reduced and scope and diagnostic accuracy could be enhanced by the exclusion of antemortem nature of blood at the scene of occurrence.

In India, those who do autopsies are generally not supposed to visit the crime scene. Autopsy opinions about cause and manner of death are sometimes in conflict with the opinion of those who had observed blood at the scene. In order to set aside an autopsy opinion of "suicidal hanging" and to believe that of "ligature strangulation" in three different cases, proving how blood at the scene could be postmortem was a big challenge.

Manner in which the blood at the scene had been perceived during the investigation or even some time after the occurrence and investigation was significant. Such a perception formed the basis to confront the autopsy opinion in three controversial cases. Baring the truth that blood at the scene was not that had oozed out of the injuries sustained during life in these cases makes an interesting case. In the first case of suicidal hanging, bleeding was from the injury that was inflicted after the death by the tip of a scissors used to cut ligature material around the neck. The second case relates to a probe into the reinvestigation of a suicidal death of a hanged victim who had been discovered dead on the fifth day. Earlier investigation and autopsy opinion of hanging were considered botched. The contention was that the victim had injuries; these injuries were not reported and had been missed deliberately both by the investigators and in the autopsy. The blood at the scene was the result of collection from constant dribbling due to postmortem hypostasis. It was not as was being presumed to have collected at the scene from some missed injury on the front of the body of hanged victim. The third case was of a lady found dead in her own house. Co-existence of a thread like ligature mark around her neck with the fresh bleeding cut throat injury and bleeding sharp weapon injuries on both the wrists remained a mystery. One of these or both of these were the cause of deaths remained the issue. Death was due to ligature strangulation became the diagnosis that blood had come out of the injuries which were caused by shaving blade after the death.

Antemortem looking postmortem blood raised issues to challenge the credibility of the autopsy opinion and or police investigation in three cases. A case study to show how misinterpretations of blood at the scene discredited family version as well as autopsy opinion of hanging in the case of two suicidal deaths will be presented. Inference indicating ligature strangulation appeared weak and ineffective so long as antemortem appearing injuries were found to be postmortem cuts in the third case. It is recommended that death investigators be familiar with the sensitivity to exclude the probability of blood at the scene being postmortem and unrelated to the actual mechanism of death.

Antemortem, Postmortem, Blood

#### G72 The Influence of the Meteorological Factors on Occurrence of the Suicide Cases

Zalina Muzafarova\*, Main Bureau of Forensic Medicine, Mirakhmedov Street, 143, Tashkent, UZBEKISTAN

The goal of this presentation is to demonstrate the correlation of the dependency between meteorological factors in defining the reasoning in suicides for forming training for prevention and alertness.

This presentation will impact the forensic science community by assisting in increasing the understanding of suicide.

During the investigation of suicide cases, the focus is ordinarily on the location of the event, the type of death, personal information and additional documents but it is also important to study other factors influencing the death, factors like meteorological conditions. Full– fledged suicide has many affects on society and many scientists pay very close attention to it, but most of the significance is that suicide is a social and medical problem.

Meteorological and heliophysics factors as well as socio–economic events have a great influence on the emotional state of an individual, and may also be factors in the occurrence of several diseases. Moreover, they may also increase the occurrence of suicide.

Factors such as atmospheric pressure, air temperature, humidity, and solar radiation have been fully analyzed with respect to the influence on suicide. Reports from suicide cases with conclusions of medical examiners for a five-year period were provided by the Main Bureau of Forensic Medicine in Tashkent, Uzbekistan. Information about temperature, humidity, atmospheric pressure, rain, and magnetic storm indices in Tashkent city were obtained from Uzhydromet, the Hydrometeorological Services Center in Tashkent.

Study results were analyzed for significance using the Student t-test method.

The results of the analysis of both suicide cases and meteorological factors have been combined and adequate statistics had been created. After certain procedures it had been highlighted that the correlation between two types of factors is indirect. This correlation was worked out by Health Institute of the Ministry of Health of the Republic of Uzbekistan for early prognosis of suicide occurrences affected by many factors.

This study of the effects of meteorological factors on suicide rates showed a strong correlation only with atmospheric pressure and air temperature. The method of direct correlative relationship (2, 3) worked out by the Republican Information Analytical Center was applied to this data to study the effect of many meteorological factors on suicide rates. The results of this study allowed for the creation of a formula for predicting the number of suicides cases as a function of air temperature and atmospheric pressure:  $X = 0.014198 \{ T (^{\circ}C) \} - 0.0000708 \{ P (GPa) \} + 0.65990;$  where, X= number of expected suicides per day; T= expected air temperature; P= expected atmospheric pressure.

According to given equation, the average fluctuation of the number of expected occurrences is  $\pm 0$ , 05, at a confidence of 95%.

The above study shows that there is a certain relationship between meteorological factors such as air temperature and atmospheric pressure with the occurrence of suicide cases. The developed formula based on the combination of meteorological factors makes it possible to predict the expected suicide states and take preventive measures.

Forensic Pathology, Suicide, Meteorological Factor

#### G73 Complex Suicide: An Unusual Case With Six Methods Applied

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After attending this presentation, attendees will be acquainted with complex suicide, potential methods applied, determination of maindominant cause of death, and will be provided with more details about traumatic brain injury caused by screwdriver.

This presentation will impact the forensic science community describing six methods in committing complex suicide that should be of great interest in common forensic practice.

Complex suicides are committed by using more than one method. They account for 1.5% to 5% of all suicides. Depending on the time delay between the employed suicidal mechanisms they can be defined as "primary complex suicides" if the mechanisms are applied simultaneously and "secondary complex suicides" if the mechanisms are applied in quick chronological sequence.

The report presents a case of complex suicide of a 44-year-old male, found dead in the vicinity of his car, in a deserted frozen field few kilometers away from the nearest town. The doors of the car were opened, and victim's head and clothes were soaked in blood. Blood spots were found on both front car seats. Neither weapons nor any tools were found around his body outside the car. A farewell letter was handwritten on two sheets of paper and was found on the dashboard of the car. The motive for committing suicide was not given in this letter. On the right front car seat there were blood spots, a screwdriver handle, an automobile crane, one razor blade, one cell phone, car keys, a pencil, and a woolen hat. In front of this seat there were two half-emptied red plastic bottles with hydrochloric acid, and one almost empty transparent plastic bottle with traces of liquid of unknown origin. A single receipt from the nearby town supermarket was found in the car. One screwdriver, pack of razor blades, and two bottles of concentrated hydrochloric acid were listed in the receipt. Police investigation excluded homicide and no medical data confirmed mental illnesses.

The autopsy revealed wrist cuts, neck cuts, acid burns in the GI tract, multiple stab wounds to the head by screwdriver, and several uncertain signs of hypothermia. In the parietal region, along the midline, there was an epidural hematoma measuring 5x1 cm in diameter. The brain was swollen, with flattened gyri and narrowed sulci, measuring 1.64 kg. Marked indentations on the ventral surface of cerebellum, indicating tonsillar herniation were found. The brain tissue along all wound tracts was destructed, but there were no diffuse injuries in white matter, nor were concentric zones of necrosis around the tracts noticed. Toxicology analysis (GC-MS) of gastric and intestinal content revealed Cypermethrin (synthetic pyrethroid used as an insecticide in commercial agricultural applications), while analysis of blood, urine and bile was negative. The same substance was identified in liquid from transparent plastic bottle, taken from the scene.

The stab wounds to the head were determined to be the cause of death, while external hemorrhage and hypothermia were contributing factors. This is the first case of complex suicide reviewed in literature where six suicide methods were applied. This particular case is interesting because the victim used a screwdriver as a tool for inflicting stab wounds to the head, which is a rare suicidal method.

**Complex Suicide, Traumatic Brain Injury, Screwdriver** 

#### G74 Bitten by Snake Shot: Attempted Homicide and Successful Suicide With Multiple Gunshot Wounds

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The goal of this presentation is to illustrate an unusual case of an attempted homicide and successful suicide with a revolver and multiple snake shot cartridges.

This presentation will impact the forensic science community by illustrating the need for close collaboration between the forensic pathologist and the firearm examiner when dealing with cases of unusual gunshot wounds.

**Introduction:** Suicide is one of the most important public health issues in the United States. Suicide represents the eleventh leading cause of death in the United States. Suicides comprise approximately 12% of the caseload of the Allegheny County Medical Examiner's Office in Pittsburgh, Pennsylvania. Suicide rates for this country have been relatively stable over the past decade averaging approximately ten per 100,000 populations. The most common method of suicide in the United States is the use of a firearm.

Homicide-followed-by-suicide (referred to as "homicide-suicide") incidents are rare events but can have a profound impact on families and communities. The National Violent Death Reporting System based on 2003-2005 data, revealed 408 homicide-suicide incidents in 17 participating states. Most incidents were committed with a firearm (88.2%) and perpetrated by males (91.4%), those over 19 years of age (97.6%), and those of white race (77.0%). Over 55% of male homicide-suicide perpetrators had prior intimate partner conflicts.

**Materials and Methods:** The case involved a 53-year-old Caucasian male and his wife with a long standing history of domestic violence. The couple began to argue when suddenly the decedent pulled out a revolver and shot his wife in the face several times. The wife ran downstairs, exited the house, and ran to the neighbor's house for help. She was transported to a local hospital, treated for three gunshot wounds to her face and then subsequently released weeks later.

The decedent was found in the upstairs bedroom in bed with a revolver lying on his left leg. Two wounds were noted to the left and right sides of his head.

**Results:** The external examination revealed a Caucasian male with two contact penetrating gunshot wounds to both sides of his head. Dense soot deposits were present on the skin and within the wound tracks. Faint muzzle abrasions with micro-stretch lacerations were identified surrounding the entrance wounds. Gunpowder residue was grossly visible on both the left and right hands. Radiographs of the head revealed two separate aggregates of pellets. Autopsy revealed a gunshot wound of entrance just lateral and slightly superior to the right orbit. The path of the pellets was leftward through the orbital rim, posterior, and inferior to the orbital globe and came to rest in the sphenoid sinus. The second entrance wound entered superior and anterior to the left ear. The path of the pellets was rightward through the frontal bone and frontal lobe of the brain where they were recovered. The firearm used by the decedent was a revolver made in West Germany chambered in 22 long rifle. The ammunition in the revolver consisted of five spent rounds and one live cartridge of .22 caliber long rifle shotshells loaded with number (size) 12 shot.

**Conclusions:** Collaboration between the pathologist and firearms examiner concluded that the decedent shot himself near his right orbit first and then changed hands and shot the lethal round into the left frontal region of his head. This is supported by gunshot residue on both hands and autopsy evidence that the path of the pellets on the right side of the head did no major damage. A literature review revealed only two published papers pertaining to the use of snake shot or shot shells. This case report offers to further build upon the knowledge of terminal ballistics of handgun shot shells.

Snake Shot, Handgun, Suicide

#### G75 Survived Strangulation: A Case Report

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The goal of this presentation is to analyze and discuss the injury pattern potentially associated with situations of survived strangulation and the various consequences that may result from them, based on real cases. This presentation will also attempt to determine, if findings and symptoms of victims can be intensity of the assault and the severity of strangulation and if general evaluation criteria can be established on the basis of objective findings.

This presentation will impact the forensic science community by presenting severe survived strangulation cases researched, and introducing other cases described in literature. Difficulties usually exist in clinical forensic medicine regarding the interpretation of the findings in reference to the intensity and duration of the assault and, ultimately, the threat it represented for victim's life. This assessment is particularly important when conclusions must be drawn in the context of penal law, in order to allow the court to decide about the life risk involved in situations of aggression.

**Cases Report:** Several cases are presented involving different situations, from victims of assault to incidences of accidental self-inflected strangulation. The majority the cases presented are of severe life-threatening strangulation that is cases with petechial bleedings on conjunctivae, mucosal surfaces and facial skin, as well as otorrhea, loss of consciousness, loss of urine, vomiting, etc.

Conclusions: The interpretation and significance of the injury pattern is discussed as well has the contribution that this pattern may give to a differential diagnosis between assault and self-inflected strangulation and to the evaluation of the severity of the situation and the threat to life. Also stressed is the fact that forensic assessment must be as detailed as possible, due to the fact of a rapid change of the lesions pattern, with the risk of becoming impossible a correct interpretation of the facts. Finally, the transitory physical consequences of these situations and of the permanent results that may result from them, as well as of their contribution to an appreciation of the severity of the aggression will be discussed. The analysis of these cases also stress the importance, as previously stated by Plattner et al (2005), of a clinical and radiological examination in addition to the forensic examination. It also shows that applicability in forensic practice of the classification in three different degrees of severity of these situations, proposed by Plattner et al (2001).

Strangulation, Injury Pattern, Survival

#### G76 Cause of Sudden Death Due to Cardiac Rhabdomyoma in an 11-Month-Old Baby

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After attending this presentation, attendees will become familiar with the possibility that a completely asymptomatic benign cardiac tumor may induce sudden death in a previously healthy infant.

This presentation will impact the forensic science community by making attendees aware of the insidious development of benign cardiac tumors also in infants and children, focusing the possible responsible mechanisms of sudden death in such cases and providing a reference for additional study on these subjects.

Neoplasms of the heart can be characterized as primary and secondary. Primary cardiac neoplasms occur infrequently in both adult and pediatric age groups. In the general population, their incidence ranges between 0.17% and 0.19% in unselected autopsy series. In infants and children, cardiac tumors were reported at a frequency of 0.027%. Approximately 75% of primary cardiac tumors are benign, and 25% are malignant, in the general population. Benign lesions usually predominate, making up more than 90% of all pediatric tumors. Approximately 50% of the benign tumors are myxomas, and about 75% of the malignant tumors are sarcomas.

Rhabdomyoma is the most frequently occurring cardiac tumor in children. It usually presents during the first few days after birth. It is associated strongly with tuberous sclerosis, a hereditary disorder characterized by hamartomas in various organs, epilepsy, mental deficiency, and sebaceous adenomas. Fifty percent of patients with tuberous sclerosis have rhabdomyoma, but more than 50% of patients with rhabdomyoma have or will develop tuberous sclerosis. The exceptional patient is one with a solitary, single rhabdomyoma who does not have or develop tuberous sclerosis.

Over 90% of rhabdomyomas are multiple and occur with approximately equal frequency in both ventricles. The atrium is involved in fewer than 30% of patients. Pathologically, these tumors are firm, gray, and nodular and tend to project into the ventricular cavity. Micrographs show myocytes of twice normal size filled with glycogen and containing hyperchromatic nuclei and eosinophilic-staining cytoplasmic granules. Scattered bundles of myofibrils can be seen within cells by electron microscopy.

The most common presentation is heart failure caused by tumor obstruction of cardiac chambers or valvular orifice flow. Clinical findings may mimic valvular or subvalvular stenosis. Arrhythmias, particularly ventricular tachycardia and sudden death, may be a presenting symptom. Atrial tumors may produce atrial arrhythmias. The diagnosis is suggested by clinical features of tuberous sclerosis and is made by echocardiography.

Benign cardiac tumors in childhood have an excellent prognosis when completely excised and appear to have a good short-term prognosis even when excision is incomplete. Symptomatic tumors often are both multiple and extensive, particularly in patients with tuberous sclerosis, who unfortunately, have a dismal long-term outlook. In such circumstances, surgery offers little benefit.

**Case Report**: A mother was bathing her 11-month-old baby. Suddenly the infant showed a worsening dyspnoea. Parents accompanied the baby to the emergency room immediately, but despite the reanimation manoeuvres, the doctor could only pronounce the death. The infant had a negative obstetric, remote and recent pathological anamnesis, except for a documented fall two days before. Also the familiar history was negative for sudden death.

A complete postmortem examination was performed within 48 hours after death. The body was that of a regularly developed 11-monthold infant. External examination was insignificant, except for the presence of a little and superficial wound on the sternal region.

The internal examination revealed a peduncolated mass at the cardiac apex, a second superficial subepicardial neoformation at the posterior wall of the left ventricle and a third transmural nodule of the posterior wall of the left ventricle. A polyvisceral congestion, cerebral and pulmonary oedema, with a massive increase in lung weight were also evident.

The histological examination of cardiac specimens, stained with haematoxylin–eosin, showed a demarcation and separation of the three masses from the surrounding regular parenchyma. The striated muscle cells appeared diffusely vacuolized, enlarged, with round to oval slightly irregular nuclei and variable cytoplasmatic clearing. There were occasional spider cells; muscular tissue residues were also visible. The immunohistochemical studies documented a positive expression of myoglobin, Actin, Vimentin, Desmin, CD34. The result with antibodies Anti-Ki67, -S100 was negative. This microscopic examination was consistent with rhabdomyoma.

Cultural tests and toxicological screening resulted negative. There were no signs of sclerosi tuberosa.

It was concluded that the infant had three cardiac lesions consistent with a primary cardiac tumor, the rhabdomyoma, which caused the sudden death. In particular one tumoral mass occupied almost the whole posterior wall of the left ventricle, rising from the apex to the valvular level, so compromising the regular contraction of the left ventricle. The neoplasms probably had caused two days before a near syncopal episode that the parents erroneously referred as a fall.

Sudden Infant Death, Cardiac Rhabdomyoma, Benign Cardiac Tumors

# G77 Suicide by Table Saw — A Slice of Interpretation

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The goal of this presentation is to illustrate an unusual means of suicide with a home radial arm circular table saw.

This presentation will impact the forensic science community by illustrating the need for close collaboration between the forensic pathologist and the scene investigators (in combination with careful photography) to elucidate the sequence of events and to rule out the involvement of other individuals in unusual cases involving violent, nonfirearm related, means.

**Introduction**: Suicide represents a common manner of death in the United States. A painful means (with or without the expectation of a rapid loss of consciousness) are uncommon. The use of a electrical saws for suicidal purposes is documented in the literature, but remains a very rare event. The use of violent means of suicide is even more uncommon in individuals with no significant prior history of psychological disorder(s).

**Case Presentation**: The subject of this case is a 50-year-old Caucasian male who became recently depressed due to debt problems. The man and his wife had discussed the possibility of dying together in a murder-suicide. It is in question whether the wife reported a domestic assault on the same morning which she managed to escape from her husband, before calling the police. The decedent was found dead in his garage shortly thereafter on the morning of November 24, 2009.

The investigation of the scene revealed a plugged in table saw. The body was lying on a large pool of blood about five feet away from the table saw. The walls of the garage were extensively covered with blood spatter in a pattern of arterial spray. The table saw itself had been overturned and had blood on both the saw blade, and on the upper and lower surfaces of the saw.

The autopsy on the decedent noted an oblique, Y-shaped incised wound in the right side of his neck. The wound was located on the superior anterolateral aspect of the neck, beneath the right mandible and measured 14 cm in total length, 0.4 cm in width, and 3.5 cm in maximal depth. The edges of the wound are abraded along the superior margin and smooth along the inferior margin. The wound transected the right jugular vein and the right external carotid artery and penetrates into the right sternocleiodmastoid muscle. The cause of death was exsanguinations due to the incised wound of the neck. Postmortem toxicology study was negative.

**Discussion**: Investigation was emphasis on ruled out the wife or others might have been involved in the death of her husband due to the initial findings at the scene, the absence of a suicide note and the unusual means used. Further interview of the wife revealed that decedent and his wife had a discussion of ending their own lives with a murder-suicide fashion. The 6-14 inch table circular saw used in this case had double protective features to prevent self injury. Further study indicated there is the possibility that the saw can be used by self to produce the similar injury as the decedent sustained. It can also be explained that people keep consciousness and moving their bodies within short of time after sudden loss of large amount of blood.

**Conclusions:** Suicide itself is much more common in individuals with long standing psychological problems, including bipolar mood disorders, depression, and schizophrenia. Violent means of suicide have been more closely associated with bipolar disorders with tendency towards self mutilating behavior. A differential thought process must be considered to interpret the pattern of events surrounding the scene of a death by violent means. Cooperation between the forensic pathologists, the police and crime scene investigators made it possible to reconstruct the unusual situation and to exonerate a third party.

Suicide, Forensic Pathology, Table Saw

### G78 The Significance of Gross Adrenal Hemorrhage — Undiagnosed Waterhouse-Friderichsen Syndrome: A Case Series

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After attending this presentation, attendees will understand the importance of finding gross adrenal hemorrhage at autopsy, and that further laboratory studies and clinical-pathologic correlation is warranted to identify the signs and symptoms of pre mortem adrenal dysfunction.

This presentation will impact the forensic science community by addressing how bilateral adrenal hemorrhage can complicate severe sepsis of various origins, not only severe meningococcemia. Clinical suspicion of sepsis and septic shock warrants clinical studies to diagnose adrenal hemorrhage and insufficiency. Undiagnosed adrenal hemorrhage will result in an unfavorable outcome despite adequate treatment.

Adrenal hemorrhage and clinical adrenal insufficiency is classically associated with meningococcemia as part of the Waterhouse-Friderichsen syndrome. It is proposed that non-traumatic adrenal hemorrhage in cases of sudden unexplained death are associated with bacterial sepsis of various etiologies and that undiagnosed adrenal insufficiency may contribute to the fulminant clinical course.

Adrenal hemorrhage and resultant clinical adrenal insufficiency has been reported in literature as an uncommon complication of bacterial sepsis and is generally associated with an unfavorable outcome in the majority of cases. Other causes named in the literature include stress, anticoagulation therapy, and hypotensive events. Therefore, the finding of adrenal hemorrhage at autopsy is not necessarily associated with sepsis and premortem functional adrenal insufficiency, as is seen in Waterhouse-Friderichsen syndrome. Four cases of non-traumatic gross adrenal hemorrhage are identified in 800 consecutive forensic autopsies and are described and analyzed, with particular attention paid to the patient's signs and symptoms possibly secondary to adrenal failure and the clinical course. It was found that patients with this grossly identifiable adrenal hemorrhage die suddenly as a consequence of acute illness of several days duration. All subjects were males, of different ethnicities, and with ages ranging from 2 to 47. All subjects have a clinical history suggestive of sepsis. At autopsy the most relevant findings are in the lungs, where findings range from heavy, congested lungs to gross findings of necrotizing pneumonia with abscess formation and empyema. Postmortem cultures yielded positive results in three out of four cases, with Staphylococcus aureus, Streptocoocus pneumonia, and Pseudomonas aeruginosa determined to be the definitive agent and the underlying cause of death in each case respectively. The fourth case had a positive culture with yeast and a coagulase negative staphylococcus. No cases had a positive culture for Neisseria meningitidis. In each case, signs and symptoms compatible with premortem adrenal insufficiency were reported; in no instance was the adrenal hemorrhage clinically identified. The precise mechanism(s) of adrenal hemorrhage in sepsis or other initiating condition(s) is unclear. However, once adrenal hemorrhage ensues, significant morbidity and mortality may result from adrenal crisis including shock and death. The pediatric population is statistically at increased risk for this complication. In light of the clinical information and autopsy findings, a component of adrenal failure may have contributed to the grave consequences of infection. Herein, the causes and potential consequences are discussed of adrenal hemorrhage by reviewing a series of four cases in light of the available published literature and conclude that additional autopsy and clinical studies may be warranted to determine the clinico-pathologic correlation of this postmortem finding.

Adrenal Hemorrhage, Waterhouse-Friderichsen, Sepsis

# G79 Metastatic Calcification of AV-Node as a Cause of Complete Heart Block and Death

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After attending this presentation, attendees will have a better understanding of the mechanism, and the common and uncommon complications associated with dialysis-associated myocardial calcification.

This presentation will impact the forensic science community by increasing awareness of some of the possible complications of renal failure, such as myocardial calcification and conduction abnormalities, in individuals on dialysis.

Metastatic calcium deposition into soft tissue is a well documented phenomenon that occurs rarely in people treated with dialysis. The pattern of calcification and the organs involved is highly variable and thus the symptoms are myriad. A case is presented of a 37-year-old woman on nightly ambulatory peritoneal dialysis for 20 months. She had stage 4 renal failure due to uncontrolled hypertension. She presented to the hospital complaining of shortness of breath and cough of two days' duration. She had not been feeling well enough at home to perform her dialysis for the past two days. At admission she had a GFR of 2, was hyperkalemic (6.3 mEq/L), anemic with thrombocytosis and leukocytosis (23.5 K/uL) and a left shift. She had developed a new third-degree heart block with a ventricular rate in the 30's. Her troponin-I was elevated at 1.56 ng/mL. Her total calcium was 9.4 mg/dL and her phosphorus was also elevated at 18.6 mg/dL for a calcium x phosphorus product of 174.8 mg<sup>2</sup>/dL<sup>2</sup>. She was transferred to the ICU where she became asystolic for 5-6 seconds, but had a spontaneous return of circulation. A transvenous pacemaker was placed emergently with good capture and effective right ventricular pacing. However, she quickly became hypotensive, lost conciousness, and became pulseless. After 35 minutes of unsuccessful resuscitative efforts she was pronounced dead. The case was referred to the Office of the Medical Examiner due to her sudden and somewhat unexpected clinical decline.

At autopsy, the left ventricle demonstrated a uniform mottled pailyellow process. The coronary arteries had thin, pliable vascular walls with widely patent lumina. Microscopic exam revealed widespread calcium deposits in the myocardium including the conduction system. There was also evidence of acute myocardial ischemia. Cardiovascular complications are the leading cause of death in patients with end-stagerenal-disease (ESRD). Derangements of calcium and phosphate metabolism are known to lead to soft tissue calcification. The calcification of the coronary arteries in patients with ESRD is a common cause of morbidity and mortality. The National Kidney Foundation recommends that the calcium-phosphate product be maintained below 55 mg<sup>2</sup>/dL<sup>2</sup> to minimize the risk of metastatic calcification of soft tissue and vasculature. In patients with a severely elevated calcium-phosphate product the deposition of calcium can be rapid. If the deposition occurs in the cardiac conduction system sudden cardiac death can occur without the presence of coronary artery calcification.

Pathologists should be aware of this potential complication of ESRD in cases of sudden death in patients with elevated calcium and phosphate or in cases in which the values were not obtained near the time of death or known at the time of autopsy.

Dialysis, Calcification, AV-Node

#### G80 Postmortem Interval and Cardiac Troponin Effect

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The goal of this presentation is to show the cardaic troponin effects in PMI and its value in daily forensic use.

This presentation will impact the forensic science community by demonstrating the statistical data of the cardiac troponins experimentally and the estimation of PMI accordingly and also the daily use of it.

In clinical practices, cardiac troponins (cardiac myofibrilspecificproteins) are specific markers of myocardial damage. In addition, measurements of cardiac isoform of troponin are recognized as important tests in the postmortem diagnosis of myocardial necrosis when such a lesion is suspected but cannot be established. Previous studies have suggested the possible application of these markers in the postmortem diagnosis of acute myocardial infarction. However, some reports showed that elevated postmortem cardiac troponin I (cTnT) levels in cardiac tissue and pericardial fluid may reflect postmortem interval. Postmortem interval may provide valuable information for evaluation cases in both criminal and civil law pursuits, for their elucidation as well. Time-since-death markers have lagged behind the progress in technology of the past years. Since the earlier attempts, failed to meet the definite postmortem interval, for variable reasons with much success, the postmortem biochemical changes in various body fluids and tissue have been tried for the estimation of time of death. The degradation of cardiac Troponin I in myocardial tissue and pericardial fluid has been investigated. The goal of this study is to investigate the potential use of myocardial tissue and pericardial fluid cTnI level as an estimator of postmortem interval. Cases selected from routine necropsies performed in the Council of Forensic Medicine, Istanbul. Samples were obtained from 98 deceased, where exact postmortem interval was known. Isolation of cTnI from heart tissue and pericardial fluid was chosen because it is found in a highly protected internal location.

The findings were elucidated according to patient records, scene of death, autopsy, and complementary toxicological and histological studies, depending on the probable intensity of myocardial damage and cause of death. No statistically significant difference was found between cause of death and titration alterations of cTnI in cardiac tissue and pericardial fluid speciments (p>0.05). On the other hand, alteration in the level of cTnI in the pericardial fluid dependent on the period of time after death showed statistically significant positive correlation (r=0.523 p<0.0001). Especially differentiation between period of first 12 hours after death and interval beyond could be established within confidency interval of 95% using the estimation of pericardial fluid cTnI level. Meaningful statistical correlation in between the pericardial effusion and cardiac tissue cTnl titrations(r=0.427 p<0.0001) was noticed. This result shows us the protein degrading effect of the PM autolysis to the pericardial effusion. This is a similar finding with the similar studies and it is very valuable to show the autolytic degradation instead of the reflection of the tissue necrosis. The positive correlation between the level of pericardial fluid cTnI and the postmortem interval and discriminative properly of this marker for estimation of the postmortem interval should provide a superior tool for this purpose. The data presented demonstrates that this technique represents a major advance in time since death determination providing reliable quantitative biochemical markers from a protected organ versus estimates such as those based on direct temperature measurements.

Furthermore, it could be shown that cardiac tissue is not influenced by autolytic changes in the postmortem interval to a considerable extent. Although previous forensic pathological studies have suggested the possible application of cardiac troponins in the diagnosis of myocardial infarction, there appears to be insufficient data with regard to its influence of postmortem interval. These results suggest that immune enzymatic studies concerning postmortem differential diagnosis of myocardial infarction may provide considerably reliable data with probability of false positive results on a negligible level. In forensic medicine, there is a need for more sensitive biochemical markers for estimation of postmortem interval and diagnosis of myocardial injury. A study of the distribution of biochemical markers in different fluids is of great significance in postmortem diagnosis, because their distribution depends on the location of tissue damage and release kinetics. Further studies are required to compare these results and create the possibility for new conclusions.

Postmortem Interval, Cardiac Troponin I, Forensic Autopsy

#### G81 Ante- and Postmortem In-Human Cocaine Packs Detected by Computed Tomography

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After attending the presentation, attendees will understand how to detect intra-corporal cocaine packs on CT. The differences of body packing, stuffing, or pushing will be elaborated and the varying appearance of the packs in CT will be demonstrated. Further, the necessity of a tight collaboration of the custody ward, the forensic institute, and the radiology department will be shown.

This presentation will impact the forensic science community by raising awareness of the difficulties and pitfalls on CT imaging of drug mules, understanding the variety of drug containers, and the upcoming medicolegal issues.

**Purpose:** The goal of this presentation is to depict the findings on computed tomography (CT) in detection of concealed cocaine – filled packs in the alimentary tract of living and dead human transporters.

**Materials and Methods:** The study population consisted of 15 antemortem and one postmortem CT exams with detected intra-corporal cocaine containers. The images were assessed retrospectively by investigators with special training and experience in reading images of drug carriers. Radiological findings were compared with listed evidence in the feces or alimentary tract of each detained suspect or deceased victim.

**Results**: Cocaine-filled containers were detected by CT in each case. The appearance and morphologic shape were compared to the evidence secured on a custody ward or during autopsy. Window leveling from abdominal window to lung window of the CT images was crucial and allowed for correct diagnoses.

**Conclusion:** Reading CT images of drug mules needs special knowledge of the appearance of the various drug containers and of the important window leveling in order to detect even hypodense or tiny packs within the alimentary tract. A reliable and fast method such as CT is needed due to the limited space at custody wards to triage holding, discharge or transfer to regional prison. During the last years, forensic and medical issues have lead to an increasing number of if needed, judicially warranted CT examinations. Pre-autopsy postmortem scans allow for exact localization of incidental or suspected findings of foreign bodies such as in-human drug containers. Obviously, the radiologist needs to be well schooled in the appearance of the drug containers in order to diagnose those correctly – therefore a tight collaboration with the custody ward, the associated forensic institute and the radiology department is desirable.

Body Packer, Cocaine, CT

### **G82** Decomposition in a Closed Vehicle Environment in Southern Ontario

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After attending this presentation, attendees will understand the importance of chemical and entomological evidence associated with decomposing remains concealed in a closed vehicle environment. This

presentation will impact the forensic community by providing new information regarding the decomposition process in a vehicle trunk in southern Ontario and providing more accurate estimates of time since death in this unique environment.

A body placed in a closed vehicle environment will undergo a distinctly different process and rate of decomposition than a body placed in an outdoor environment. A closed vehicle has the potential to significantly affect decomposition processes by reducing entomological access to the body, increasing ambient temperatures, and promoting desiccation and mummification of the remains. In Canada, there is currently no published literature which has studied the effect of a closed vehicle environment on entomological activity and the chemical processes which occur during soft tissue decomposition. This information would be valuable to forensic pathologists and coroners when estimating time since death in forensic investigations involving decomposed remains recovered from a vehicle.

The goal of this study was to investigate the chemical process of soft tissue decomposition and the entomological evidence associated with a body placed in the trunk of a vehicle. The study was conducted in the southern region of Ontario, Canada during the summer months of June and July. Two pig carcasses of similar biomass were used in the study. The experimental carcass was placed in the trunk of a darkcolored vehicle and sealed. The control carcass was placed on the soil surface approximately ten meters from the vehicle. A data logger was placed in the vehicle to record temperature and humidity. A weather station was placed near the control carcass to record ambient temperature, humidity, and rainfall. Soft tissue samples were collected from the upper and lower torso region of the carcasses. Entomological evidence was collected directly from the carcasses and from pitfall traps surrounding the carcasses. Samples were collected at regular intervals until the carcasses reached the skeletonization or dry remains stage.

Decomposed soft tissue was analyzed using gas chromatographymass spectrometry to determine the lipid degradation process and resulting fatty acid content within the samples. Unsaturated and saturated long-chain fatty acids were identified at all stages of the decomposition process. Variations in the lipid degradation pathways were evident between the experimental and control carcasses. Adult and immature insects were collected from the carcasses in order to determine the succession throughout decomposition. An important delay of insect colonization was observed in the vehicle as well as a significant decrease in species composition.

Observational measurements confirmed that the decomposition process was distinctly different in the closed trunk of the vehicle when compared to the decomposition process on the soil surface. The chemical, entomological, and environmental data provided additional confirmation of the distinct process in which a decomposing body will undergo in a closed vehicle environment. This information will be valuable to law enforcements agencies and forensic pathologists and may aid in providing more accurate estimations of time since death.

Forensic Entomology, Decomposition Chemistry, Closed Vehicle Environment

#### G83 Child Abusive Head Trauma in Portugal

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The goal of this presentation is to better understand how child Abusive Head Trauma (AHT) is managed in Portugal.

This presentation will impact the forensic science community by filling the void of epidemiology background concerning fatal AHT in Portugal, and will allow comprehensive knowledge of the Portuguese experience on this issue. Only then will it be possible to develop assertive and coordinated policies and strategies for action in this field in order to minimize the number of cases as well as its consequences. Until now, in Portugal, only isolated and non-articulated studies have been made on this issue, reflecting only loco-regional incidences or even only the numbers of one organization. For these reasons, it is not possible to infer what is really going on at a national level.

The absence of a systematic approach on child abuse (CA) makes it impossible to have the real knowledge of the prevalence and incidence of this problem in the Portuguese population. Currently there are no more than estimates of certain entities, certainly underestimating, from the Ministry of Justice, the *National Commission for the Protection of Children and Young People at Risk* (CNCJR), the *National Institute of Legal Medicine* (INML), the criminal police forces, the victims' support associations, or the health care services. Furthermore, the most important problem is that the referred data represents only a portion of incidences because frequently cases are not reported. However, gathering such sort of data is vital in order to find the proper and most suitable approach to these situations in Portugal.

In AHT cases, it was verified that in the last years there has been an important increase in the number of published reports in the international medical literature describing AHT (Hymel KP, 2002) and considering that it represents the most frequent cause of death due to CA, the main cause of traumatic death in children under 1-year-old (Duhaime AC e col, 1999) and the cause of significant morbidity, with up to 50% of survivors having permanent neurologic sequelae (Duhaime AC e col, 1996). Between 24% and 33% of traumatic brain injuries (TBI) hospital admissions in children under 2-years-of-age are from AHT (Duhaime AC e col, 1987; Dashti SR e col, 1999). The incidence of severe inflicted TBI in Scotland is 24.6 infants per 100 000 person-years which is comparable with epidemiologic data in the United States, where this incidence is 29.7 infants per 100 000 person years (Barlow KM, Minns RA, 2003; Keenan HT e col, 2003). In fact, it is known that for AHT up to 30% of the cases are difficult to be initially recognized (Jenny C e col, 1999), mainly because victims, almost under 2-years-of-age, cannot describe the abuse (Bechtel K e col, 2004), and because many cases are of mild or moderate severity (Berger RP e col, 2004).

However, in spite of all these difficulties, the various forms of AHT (including shaken baby syndrome (SBS)) are now well recognized and diagnosed in most countries (Barlow KM e col, 2005) which cases are being identified and reported; however, doesn't seem to be the case in Portugal. The intervention in a situation of CA can be initiated by multiple kinds of professionals of different institutions. Among those professionals teachers, childhood educators, physicians (mostly pediatricians, practitioners, and gynecologists), nurses, psychologists, policemen/women, and social workers are highlighted. According to the Portuguese Criminal Law these professionals are mandatory reporters

and according to the Protective Law and the Deontological Code (in what physicians concern), they have the duty to report suspected cases. The problem is that there are numerous initial referral sources, multiple professionals (with different formations and awareness, presenting possible "prejudices" and non-official instructions and forms for reporting cases.

In Portugal there are no published data concerning non-fatal AHT, and this work represents the first national approach regarding fatal cases due to this kind of abuse. Analyzing the fatal cases of suspected CA observed in the medico-legal services of Portugal, between 2005 and 2008, it has been verified that AHT represented only 12.5% of the suspected CA causes of death (1.2 infants per 100,000 person years), despite in the literature, AHT represents the most frequent cause of death due to CA. The single diagnosed case of SBS will be presented.

These Portuguese results must be carefully analyzed and compared with other foreign studies, which are completely different. According to the facts, it is presumed that an important number of cases of AHT is still undiagnosed or underdiagnosed in Portugal (being diagnosed only when specifically looked for), or remain unreported or underreported by the health professionals.

Child Abuse, Abusive Head Trauma, Shaken Baby Syndrome

#### G84 Gravesoil Microbial Community Structure During Carcass Decomposition

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After attending this presentation, attendees will understand that there is potential for the use of fatty acids to characterize gravesoil microbial community with the ultimate goal of estimating postmortem interval (PMI).

This presentation will impact the forensic science community by the development of an additional method to determine extended PMI. This additional method can be used in conjunction with other methods to estimate PMI, such as forensic entomology.

Estimating PMI is important for every death investigation. It allows for the acceptance or rejection of alibis as well as helping to identify victims. At present forensic entomology is arguably the most reliable means to accurately estimate PMI at outdoor death scenes. However, active blowfly larvae, which are critical to insect based estimates of PMI, can leave a body as early as ten days postmortem. When active blowfly larvae are not present at a death scene, forensic science is often ill equipped to estimate PMI accurately.

A controlled laboratory experiment was conducted to determine if soil microbial ecology has the potential to be used as an estimator of PMI. To do this incubation units were constructed that comprised petri dishes (150 mm x 25 mm) filled with 360 grams (g) of washed sand inoculated with 40 g of Pawnee clay loam soil. Soil was collected from Nine Mile Prairie, a natural tall-grass prairie ecosystem, which is located approximately nine miles northwest of Lincoln, Nebraska. Soil of the Pawnee series is a fine, montmorillonitic, mesic Aquic Argiudoll (Mollisol). These incubation units were calibrated to a water holding capacity of 55% and left to equilibrate for seven days in plastic containers (20 cm x 34 cm x 11 cm) that contained methanol washed pea gravel and distilled water (100 ml) to regulate humidity.

After seven days, a mouse carcass (killed with carbon dioxide) was placed on its left side on the inoculated sand within 30 minutes of death. Nylon mesh (0.1 mm x 0.1 mm) was then used to cover the plastic container to prevent insect colonization. The temperature was kept at approximately  $20^{\circ}$ C during the experimental period and the water

content of the inoculated sand was maintained at 55% every 3-4 days by adding distilled water. Carcass decomposition was monitored every 24 hours for 35 days using a decomposition scoring system. In addition, carcass mass loss was measured at 7, 14, 21, 28, and 35 days postmortem. A destructive harvest design was used to avoid the influence of carcass disturbance on the rate of decomposition. Following carcass harvest, inoculated sand was collected and analyzed for lipid phosphorus, fatty acid methyl esters, pH, total nitrogen, and total carbon. This experiment was replicated four times and controls (inoculated sand with no carcass) were used. Results and discussion will be presented to demonstrate the effectiveness of soil microbial ecology to act as an estimator of PMI.

Forensic Taphonomy, Extended Postmortem Interval, Ecology

#### G85 Laceration of the Inferior Vena Cava Following Blunt Abdominal Trauma in a Case of Child Abuse

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The goal of this presentation is to describe and discuss a child abuse case with multiple blunt injuries that include blunt abdominal trauma with laceration of the inferior vena cava (IVC).

This presentation will impact the forensic science community by demonstrating an uncommon finding resulting from blunt trauma in a child abuse case.

The majority of injuries to the IVC are due to penetrating trauma. Only 10% of these injuries will be caused by blunt trauma. This may be due to the fact that the IVC is a retroperitoneal organ and is therefore relatively protected from injury. Injury as a result of blunt trauma would only result from a force of great magnitude.

This case involved a 22-month-old, Hispanic, male infant who arrived dead to the emergency room. The stepfather stated that he witnessed the infant falling to the ground while walking and hitting his head against the adjacent wall. The stepfather tried to resuscitate the child but he continued to lose consciousness. He waited for the infant's mother to arrive home and they took the child to the emergency room. His social history revealed that he lived with his mother and the stepfather and there was no family history of child protective services involvement. At the time of the event, he was under the stepfathers' care. The stepfather denied any physical abuse against the child.

At autopsy the body corresponded to a well-developed and wellnourished male infant. He was 33 inches tall and weighed 31 pounds. External examination of his face and head showed multiple recent contusions and abrasions over the face and scalp. Multiple foci of subgaleal hemorrhage were present over the skull. The brain had mild subarachnoid hemorrhage over the left parietal and occipital lobes. Examination of the brain disclosed no other trauma. The head had no fractures. The torso also revealed multiple recent contusions. The abdomen was moderately distended. After entering the peritoneal cavity, 150 mL of liquid blood and 35 grams of blood clots were noted. There was a laceration to the proximal suprahepatic segment of IVC with presence of blood clots adjacent to the laceration. Moderate hemorrhagic infiltrate was present in the subintimal layer of the IVC along the supradiaphragmatic segment of the vein extending to the right atrium of the heart. Gross examination of the abdominal viscera found no other source of bleeding. The right pleural space had 40 mL of liquid blood. The right and left lungs had multiple contusions. Small lacerations were present next to the hilum of the right lung. Examination

of the extremities showed multiple recent contusions and no fractures. Toxicological evaluation was negative for alcohol, cocaine, opioids, and canabinoids. The cause of death was blunt force injuries and the manner of death was ruled a homicide.

Intra-abdominal hemorrhage is most commonly associated with a clear history of trauma. In young children, the liver and spleen are the most common abdominal viscera to sustain a traumatic injury. Lacerations of the inferior vena cava resulting from blunt trauma are relatively rare, but extremely serious with a high mortality and may be difficult to repair. The majority of injuries of the IVC are due to penetrating trauma and only a small percentage is caused by blunt trauma. Lacerations to the IVC are uncommon injuries in the pediatric population. Lacerations of this vessel indicate a force of great magnitude with a profound level of injury. The presented case has evidence of blunt trauma in multiple regions of the body. The abdomen and thorax were the most severely affected regions. The unique feature of this case is the finding of IVC laceration with no other abdominal viscera involvement. In this case intr-athoracic and intra-abdominal tensional forces produced by blunt trauma to the torso could explain the lacerations of the IVC and hilar area of the right lung.

Laceration of Inferior Vena Cava, Blunt Trauma, Child Abuse

#### G86 Inferior Vena Cava Compression: A Possible Mechanism for Arrest Related Death

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After attending this presentation, attendees will understand a potential pathophysiologic mechanism of arrest related death (ARD) not previously studied or discussed in the literature.

This presentation will impact the forensic science community by providing a possible explanation for sudden, arrest-related death that is not yet established in the literature.

The physiology of sudden ARDs proximal to restraint has not been elucidated. Prior work has not suggested a relationship between position, restraint, or thorax compression up to 50 lbs with regard to clinical impact on respiration. However, the impact of these variables on Central Venous Return (CVR) has not been studied. Decreased CVR is a theoretical concern in a subject with tachycardia from resistive exertion, mental excitement, or sympathomimetic ingestions. A sudden change in CVR could cause an acute decrease of cardiac preload leading to possible decreased coronary artery perfusion pressure and ischemia or the induction of a maladaptive neuro-cardiogenic reflex. This, in turn, could lead to a brady-asystolic cardiac arrest. This study used ultrasound to measure the size of the Inferior Vena Cava (IVC) as a surrogate marker of CVR when positional change and thoracic compression occurs.

This was a prospective study of human volunteers. Subjects had ultrasounds of their IVC in transverse and longitudinal planes performed in four positions. Maximum and minimum measurement values were obtained in each position after accounting for respiratory variability. The four positions were: (1) standing; (2) lying prone; (3) lying prone with 100 lbs of weight applied uniformly to the upper back; and, (4) lying prone with 147 lbs of weight applied uniformly to the upper back. The weight was meant to simulate thoracic compression during a restraint procedure. A custom table and weight mechanism was used to allow access to visualize the IVC in the prone position and to apply the weight uniformly to all subjects. IVC values were measured with a handheld ultrasound with a phased array (5-2MHz) transducer, operated by an RDMS sonographer. Data were analyzed using descriptive statistics and k sample for equality of medians test.

There were 24 subjects that completed the study protocol. The median (interquartile range) IVC measurements for all positions are as follows:

- Longitudinal maximum was 1.86 cm standing (1.57-2.16), 1.67 cm prone (1.05-2.26), 1.205 cm with 100 lbs compression (0.83-1.58), and 0.805 cm with147 lbs compression (0.46-1.29), (p < 0.0001).</li>
- Longitudinal minimum was 1.21 cm standing (1.01-1.51), 1.14 cm prone (0.64-1.61), 0.70 cm with 100 lbs compression (0.45-1.02), and 0.28 cm with 147 lbs compression (0.0-0.79), (p<0.0001).
- Transverse maximum was 1.63 cm standing (1.43-1.93), 1.45 cm prone (1.17-2.02), 1.12 cm with 100 lbs compression (0.76-1.65), and 0.74 cm with 147 lbs compression (0.46-1.13), (p<0.0001).</li>
- Transverse minimum was 1.18 cm standing (0.93-1.39), 1.01 cm prone (0.77-1.47), 0.38 cm with 100 lbs compression (0.0-1.15), 0.31 cm with 147 lbs compression (0.0-0.52), (p<0.0001).</li>

There was significant difference between the IVC size in the longitudinal and transverse planes at maximum and minimum between all positions. The IVC size was greatest while standing. It became sequentially smaller with prone positioning and application of weight force. It was smallest while lying prone with 147 lbs of thorax compression. These findings support a possible pathophysiologic mechanism of ARDs that has not previously been reported. Further study in this area is recommended.

Arrest Related Death, In-Custody Death, Sudden Death

#### G87 Intra-Abdominal Hemorrhage Associated to an Intrapartum Rupture of the Umbilical Cord: A Case Report

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The goal of this presentation is to describe and discuss a case of an intrapartum rupture of the umbilical cord leading to an intra-abdominal hemorrhage and newborn death.

This presentation will impact the forensic science community by demonstrating an uncommon case of intra-abdominal hemorrhage and death of the newborn as a complication of the rupture of the umbilical cord in a precipitous delivery.

Intra-abdominal hemorrhage in the newborn is uncommon. Bleeding from umbilical vessels in the cord can occur in the perinatal period, the predisposing factors being a short cord, varices, velamentous insertion of the cord, or true knot of the cord. Even more uncommon is bleeding secondary to rupture of the umbilical cord.

A 25-year-old gravida 6, para 4, came in active labor at 36 6/7 weeks of gestation. The mother had a late prenatal care and vaginal infection. The case was complicated at delivery due to violent expulsion of the baby girl who was caught by the physician attending the delivery. The umbilical cord ruptured causing hemorrhage to the baby and the mother. Apgar scores were 1, 4, and 6 at 1, 5, and 10 minutes, respectively. The baby was in respiratory distress, pale with poor response to bag-mask ventilation. Neonatologist intubated the baby in delivery room and she was transferred to Neonatal Intensive Care Unit (NICU). Initial work up showed blood gases results as follows: PH 7.35, pCO2 32 mmHg, and pO2 54 mmHg. Hemoglobin was 15.7 and

Hematocrit 44.1%. Skeletal survey was negative and Head Ultrasound showed mild left ventricle dilation and no evidence of intraventricular hemorrhage. The next day the baby continued with respiratory distress with significant anemia, Hb was 10.9 and Hct 30.4%. Despite blood transfusions and other therapeutic measures the baby remained critically ill with marked hypoxia and poor perfusion. On the 3<sup>rd</sup> and 4<sup>th</sup> days at NICU, a tense abdomen was noted with paleness below the level of the diaphragm and plethoric upward, suggestive of compartment syndrome secondary to aortic compression due to hemoperitoneum. A Penrose drain was placed for abdominal decompression but patient did not improved and died.

At autopsy the body corresponded to a female preterm baby. She was 18.7 inches tall and weighed 6.2 pounds. External examination did not show signs of trauma. Among the medical intervention there was an umbilical arterial catheter in place without disruption of the artery. After entering the peritoneal cavity, 30 ml of liquid blood was noted and some blood clots in the right subdiaphragmatic area. As the peritoneal cavity was entered, it was noted that the umbilical vein and falciform ligament were disrupted. A hematoma was noted at the site of disruption adjacent to the peritoneal surface. The liver had a non-ruptured subcapsular hematoma at the anterior and superior surfaces of the left lobe without lacerations of the parenchyma. The rest of the thoracic and abdominal organs had no signs of trauma. The brain had no hemorrhages or lesions. The placenta weighted 509 grams with a centrally inserted umbilical trivascular cord that measured 11 x 1.3 cm. On microscopic examination revealed acute chorioamnionitis. Toxicological evaluation was negative for alcohol, cocaine, heroin and canabinoids.

The normal umbilical cord resists trauma, the forces of normal delivery, and does not bleed. However, in dysmature infants the cord is thin and weak and liable to rupture. In precipitous delivery, a rapid increase in cord tension can rupture the fetal aspect of the cord. Short or entangled cords may rupture, as may abnormal cords, such as those with velamentous insertion on the placenta. Although birth trauma involving intra-abdominal organs is also uncommon, it must be suspected in the newborn with pallor, abdominal distension, anemia, and shock without evidence of external blood loss, intracranial hemorrhage, or gastrointestinal bleeding. The size of the infant and the presentation at delivery are important risk factors for abdominal trauma. The liver is the abdominal organ most commonly injured in the birth process. Subcapsular hematomas rather than hepatic lacerations are more apt to occur.

In this case, several recognizable factors increased the risk of umbilical cord rupture, such as prematurity of the infant combined with a precipitous delivery. Disruption of the umbilical vein represented the source of intra-abdominal bleeding. The subcapsular hematoma could be attributed to the abdominal birth trauma or be part of the tensional injury secondary to the rupture of the umbilical cord.

Umbilical Cord Rupture, Intra-Abdominal Hemorrhage, Subcapsular Hematoma

### G88 Aortic Aneurysm Rupture Into the Lung With Formation of Pseudoaneurysm

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The goal of this presentation is to describe and discuss a case of aortic aneurysm rupture into the lung parenchyma with formation of pseudoaneurysm.

This presentation will impact the forensic science community by demonstrating a rare complication of a thoracic aortic aneurysm.

Rupture of thoracic aneurysm into the lung with formation of pseudoaneurysm is rare. There are few reported cases discussing the diagnostic approach and management of this complication. In the researched literature there are no reports of this complication as an autopsy finding.

This case involved 72-year-old, black Hispanic male with history of poorly controlled arterial hypertension and two cerebrovascular accidents. He was a heavy smoker and occasional alcohol drinker. He was found lying supine on the street. The paramedics pronounced him dead at scene after evaluation. There were no signs of violence or foul play at scene.

At autopsy the body corresponded to a well-developed and wellnourished adult male. He was 66-inches tall and weighed 152 pounds. External examination showed no significant evidence of trauma. Reflection of the skin over the anterior thorax showed no significant hemorrhagic infiltrates or fractures. On internal examination the left thoracic cavity contained 700 grams of clotted blood and 600 ml of liquid blood. Examination of the thoracic organs revealed that the source of bleeding was a ruptured aneurysm of the middle third of the descending thoracic aorta. The aortic aneurysm ruptured into the parenchyma of the lower lobe of the left lung forming a pseudoaneurismatic structure that contained a fusiform mural organized thrombus that measured 16.5 x 6.5 x 5.0 cm. Cut sections of the affected pulmonary parenchyma demonstrated that the cavitary lesion was surrounded by a well formed and circumscribed wall. Sections of the thrombus showed a surface with a multilayered arrangement. Focal areas of hemorrhage were present in the pulmonary parenchyma surrounding the cavity. The aorta showed severe atherosclerosis with calcification and focal ulceration of the atherosclerotic plaques. Histopathologically the aorta had no evidence of inflammation; however, degenerative changes were recognized near the possible rupture site. The heart weighed 300 grams and had mild left ventricular hypertrophy. The rest of the thoracic and abdominal organs had no remarkable macroscopic pathology. Postmortem toxicological evaluation was negative for alcohol, cocaine, opioids, and cannabinoids. Serological test for syphilis was negative.

Reports of patients with aortic aneurysm rupturing into the lung with formation of pseudoaneurysm are few. There are no reported cases in the researched literature describing the presence of this condition as an autopsy finding. An aortic aneurysm or dissection that ruptures into the lung parenchyma or erodes into a bronchus can lead to acute, massive hemoptysis, hemothorax and death. This case is particular because the aneurysm ruptured into the visceral pleura and lung parenchyma forming a pseudoaneurysmatic structure where the blood lodged. Two factors appeared to combine and contribute in the formation of this pseudoaneurysmatic structure, delaying the free extravasation of blood to the pleural cavity and imminent death. First is the anatomic location of the aortic aneurysm. In this case the aneurysm was located in the mid portion of the descending segment, adjacent to the medial aspect of the lower lobe of the left lung. The second factor is the elasticity of the lung parenchyma that cushioned the aortic aneurysm wall, allowing a slow passage of blood with formation of the cavity. Rupture and extravasation of blood to the pleural cavity occurred when the intracavitary pressure exceeded the elastic capacity of the tissues surrounding the pseudoaneurysmatic structure. Fibrous tissue attachment between the lung and aorta could have also played a role, but it was not clearly demonstrated at autopsy.

Aortic Aneurysm, Pseudoaneurysm, Lung Parenchyma

#### G89 Is DNA Purified From Forensic Autopsy Material Suitable for Molecular Biological Studies?

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After attending this presentation, attendees will understand more about the suitability of DNA, purified from forensic autopsy samples for advanced molecular research.

This presentation will impact the forensic science community by providing information about how decomposition and time from death to autopsy affects the usability of DNA for molecular studies. Knowledge about the degree of DNA fragmentation and degradation is an important tool for planning of future molecular biological studies.

The quality of molecular-biological studies obviously depends on the tissue in which the markers must be investigated. At forensic laboratories, a large number of frozen, biological samples are stored (collected at the autopsies), which can be used as templates for molecular biological studies. These samples are extremely valuable for all types of molecular biological studies in both diagnostic and research purposes. The decomposition and thereby the following changes in quality of DNA occur shortly after death. Degradation and fragmentation of DNA purified from autopsy material depends on several factors, such as time since death to autopsy, the degree of postmortem changes, the keeping of the corpse, external and environmental influences, storage of samples, and the addition of the chemicals to blood samples and other tissues for storage. It is believed there are no studies on this issue. The current study is a pilot for a major project, which is to define the molecular biological markers for sudden unexpected death. The suitability of purified DNA from tissues taken at autopsies including frozen blood with or without additional chemicals and paraffin embedded and frozen tissue is validated, as template for molecular biological studies in order to define the main risk factors for DNA fragmentation and degradation. By using PCR primer sets that amplify DNA fragments of varying length and DNA extracted from tissue samples with different degree of postmortem decomposition. Using the internal autopsy database the study group is defined consisting of tissue samples without signs of decomposition of tissue, with moderate decomposition of tissue and with severe decomposition. Frozen tissue samples of the detected cases (blood samples and muscle tissue) are available as well as frozen blood samples with the addition of potassium fluoride. DNA from tissue samples were purified using commercially available kits. Ten different PCR primer sets were designed to amplify 100 to 1000 basepair long fragments of human genomic DNA. PCR products were analyzed by agarose gel electrophoresis and ethidium bromide DNA staining.

Preliminary results suggest that the degree of fragmentation and degradation of DNA after death increases corresponding to grade of decomposition of tissue. The lengths of DNA fragments in samples with high grade of decomposition are significantly shorter than in samples without decomposition of tissue. It was possible to generate DNA fragments of at least 1,000 basepair lengths from samples taken from individuals that died within one week before autopsy was performed. On the other hand DNA samples from individuals that died at least two weeks before autopsies only could generate PCR product up to 600 basepair long.

Validation, DNA Fragmentation, Tissue Decomposition

#### G90 Evaluation of a New Approach for Estimating the Postmortem Interval Based on the Direct Skin Surface Analysis Using FTIR Spectroscopy

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The goal of this presentation is to determine with accurate methods the Postmortem Interval (PMI).

This presentation will impact the forensic science community by facing one of the main issues in forensic sciences, the estimation of time since death (postmortem interval). Most methods currently employed have considerable inaccuracy. To be able to determine PMI is one challenge that can change a forensic investigation, and give answers, that until now were not properly supported in court.

The estimation of postmortem interval is a main issue in forensic sciences. Most methods currently employed have considerable inaccuracy.

Most of these methods are based on medical knowledge. With this work we intend to solve a forensic problem with the help of other areas of science not usually involved in medical studies.

The interaction of infrared (IR) electromagnetic radiation with the matter is a widely established technique to probe the chemical composition of materials.

The IR spectrum is divided into three zones; near- (14000-4000 cm-1), mid- (4000-400 cm-1) and far-infrared (400-10 cm-1). The midinfrared (MIR) region is used to analyze the fundamental vibrations of molecules and is strongly absorbed so materials have to be analyzed as thin films or in small path length cells (e.g., milk analysis).

Near-infrared (NIR) spectroscopy is based on molecular overtone and combination vibrations, which are forbidden by the selection rules of quantum mechanics. This means that NIR can penetrate much further into materials than MIR. This makes NIR very useful in probing bulk material with little or no sample preparation.

Because NIR probes the overtone and combination bands the spectra are usually very complex. Individual bands can not be assigned to specific features as with MIR. This means multiple wavelength (multivariate) calibration techniques are used to extract structural information. The design of powerful software packages, such as PLSplus/IQ, allows users with minimal chemometric experience the opportunity to generate and maintain their own calibration models without relying on general models from a third party that are not specific to their materials.

The increased processing power of computers has allowed the introduction of Fourier Transform (FT) infrared analyzers. Prior to this technology instruments either had to either use filters to look at the absorption of specific wavelengths or use diffraction gratings to scan through the wavelengths and measure the changing absorptions. FT technology uses interferometers that allow all the information at all wavelengths to be collected simultaneously. This means much more information can be collected in a shorter time.

Fourier transform near-infrared (FT-NIR) spectroscopy is an analytical technique that has gained great popularity in recent years. It is an effective tool for investigating chemical changes at molecular level and its major strengths include fast and easy equipment operation, good accuracy and precision, and the potential to perform nondestructive analyses. In its reflectance mode, FT-NIR spectroscopy is widely used to study, for example, the human skin and other tissues. And in the last few years, using fiber-optic technology, the direct real-time in situ analysis became possible.

The utilization of FT-NIIR spectroscopy is being studied here to directly test the human skin in order to, in combination with chemometric data analysis (PCA – principal component analysis; PLS – partial least-squares models), look for possible surface chemical changes occurring after death that may correlate with PMI. Studies performed to date (20 cases) showed promising results. Figure 1A shows typical spectra obtained from six corpses in the 48 hours postmortem period and Figure 1B shows the correlation between the predicted PMI *versus* the known (real) time since dead.



This study shows the usefulness of coupled with chemometric data analysis for estimating PMI, and the importance of the interaction between different areas of knowledge.

Postmortem Interval, FTIR Spectroscopy, Accuracy

#### G91 Unusual Style Cut Throat Injury: A Case Report

Suresh K. Shetty, MD\*, Kasturba Medical College, Light House Hill Road, Mangalore, 575001, INDIA

After attending this presentation, attendees will understand the circumstances and possibilities of injuries in a rare case of self-inflicted cut throat injury.

This presentation will impact the forensic community by helping officials responsible for the maintenance of law and order to administer justice.

Suicide is one of the leading causes of death in the world. The incidence and pattern of suicide vary from country to country where cultural, religious, and social values play a vital role. Hanging, poisoning, drowning are the common methods of committing suicide. Suicide by incising one's own throat without hesitation marks remains a rare, and only few cases have been reported in forensic literature. An unusual and rare case of self-inflicted cut throat injury of a 45-year-old ex-military man without tentative cuts over the neck, which has resulted from a curved sharp weapon is presented.

A case report of self-inflicted cut throat injury without tentative cuts, a rare event is presented. Such cases are rare to be reported in forensic literature. It is recommended that medico legal death investigators be aware and familiar with such injuries in a detailed autopsy, which may ultimately prove or disprove the case, which may be of significant value to the investigating authority.

Self-Inflicted, Cut Throat Injury, Hesitation Marks

#### G92 Mass Fatality Management: A Multi-National Perspective

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After attending this presentation, attendees will understand the challenges faced a multi-national recovery and identification effort.

This presentation will impact the forensic science community by presenting lessons learned from the field in a multi-national mass fatalities incident, which can be applied to a future incidence response.

In response to the Haiti Earthquake, several international organizations responded to assist in fatality management and repatriation of non-Haitian human remains. Each of the fatality management and response organizations was dispatched by a governmental agency, but with little coordination between the organizations. Initially, the prohibitive conditions of an entire Haitian infrastructure in disarray and the extreme difficulty of providing for the logistical requirements of supporting a deployable morgue unit without local support was the primary factor limiting human identification efforts. However, difficulty in determining jurisdiction and logistics of repatriation of multi-national citizens became one of the primary difficulties in the response effort. A major complexity in the recovery and victim identification of foreign nationals was the number of independent countries on the ground attempting to identify their own citizens for repatriation. Another major factor was the actual recovery of the victims that were buried under tons of rubble, which were a safety hazard for recovery personnel.

Not only was the logistical aspect of this operation complex, but the recovery and initial identification of the multi-national victims was extremely difficult. This is the first global mass fatality incident where an attempt had to be made to determine the nationality of the victims prior to recovery to ensure accurate identification, repatriation and disposition of the remains. The coordination of antemortem biological information was crucial to this effort and it took a great deal of coordination between countries.

This presentation will discuss how to more effectively coordinate a mass fatality response in the event of future disasters involving multinational populations from multiple countries with varying capabilities for fatality response as well as the complexity of victim identification in this scenario.

Fatality Management, Mass Disaster, Mortuary Operations

#### **G93** Improving Evidence and Victim Recovery Protocols at the Mass Fatality Incident

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After attending this presentation, attendees will understand problems faced by coroner and medical examiners relative to their responsibility to properly recover and identify plane crash victims. Attendees will be presented with effective strategies and protocols for dealing with these scenes.

This presentation will impact the forensic science community by describing efficient and effective evidence and victim recovery protocols applicable to large-scale, widely-dispersed mass fatality scenes

The crash of a major airliner results in an extremely chaotic situation. After the first responders have dealt with survivors and fire resulting from the crash, the *processing* of the scene can commence. The primary goals of this processing effort are: (1) determining the cause of

the crash; (2) comprehensively recovering the victims and their personal effects; (3) determining the identity of all of the biological tissue; and, (4) removing all of the debris from the site.

With respect to the first goal, in the United States; if the cause is likely accidental (vehicular malfunction or human error), the National Transportation Safety Board (NTSB) will be in charge of the investigation. If it is instead determined that criminal intent may have been involved, the Federal Bureau of Investigation (FBI) will take custody of the scene.

The efforts of both the NTSB and FBI are focused on the nonhuman evidence at the scene. The recovery, identification and interpretation of the human remains (Goal 2) are the province of the Medical Examiner/Coroner (ME/C). While nearly all ME/C offices can deal with the morgue component of victim identification on their own or, they can request the services of federal groups such as DMORT, most offices do not possess the training, expertise, experience, or protocols to deal with a large scale scene containing the highly fragmented and commingled remains of large numbers of victims. This presentation will demonstrate that the best approach to the processing of outdoor crime scenes, especially large-scale scenes such as a plane crash, is to employ forensic archaeological methods. A new set of protocols for the processing of large-scale disaster scene will be presented.

The new protocols are based on the Weldon Spring protocols developed during the past decade (Dirkmaat and Hochrein 2000). The Weldon Spring Protocols are based on a systematic sequence of search, documentation, and recovery methods that is intended to result in the most efficient and effective scene processing effort. By effectiveness we refer to the proportion of physical and contextual evidence identified, documented, and recovered at the scene, while efficiency relates to the time and personnel required for effective recovery completion under a particular protocol. The goal of the present study was to optimize these two factors through the logistic and technological enhancement of the Weldon Spring protocols. To attain this, different technological configurations, affecting all the components of the protocol, from evidence location to data acquisition and recordation, were developed and tested in terms of their efficiency and effectiveness at real forensic cases and realistic mock scenes. Comprehensive guidelines for needs assessment and decision-making, targeting the identification and resolution of trade-offs related to technology availability and amortization, budgetary and personnel constraints, and training were also developed, in an attempt to offer different configuration alternatives to fit the needs and resources of a wide array of agencies without significant effectiveness losses.

The technological enhancements include high-resolution GPS units for the quick recordation of precise spatial recording, bar code scanning for data entry and sharing, and the utilization of wireless networks at the scene. The combination of these elements resulted in a reduction of recording times from minutes to just a few seconds, higher data integrity, with a standardization of evidence codes and the virtual elimination of any risk of reference duplication. This translates into an almost automatic coordination of all the recovery teams involved, in a manner that not only reduces the amount of time required by each team to locate, map, document and recover each evidentiary item, but also allows more teams to be deployed simultaneously at the scene.

Mass Fatality Incident, Forensic Archaeology, Victim Recovery

#### G94 Telluric Movements of Death: The Cemetery of Gargano's Mafia Inside the Ravine of Zazzano (Foggia, Italy)

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The goal of this presentation is to offer a multidisciplinary approach in forensic investigation that presents identification of victims as belonging to victims of local criminal organization. Skeletal remains of four different cadavers were recovered in a ravine, a surprising movie of this recovery completes the peculiarity of the case.

This presentation will impact the forensic science community by discussing how skeletal remains recovery requires a multidisciplinary approach in forensic activity, and identification of missing represents the principal aim.

The Gargano, also known as Spur of Italy is a subregion of Italy which coincides with the headland stretching in the North of Puglia and corresponds to the East of the Province of Foggia. It is entirely surrounded by the Adriatic Sea except in the West, bordering the Tavoliere. The frequent and constant carsic erosions in this area produced cavities that, in time, due to telluric movements created grottoes, dolins, and ravines hundreds meters underground. A ravine is a small valley-almost like a canyon but narrower-which is often the product of stream cutting erosion. Ravines are typically classified as larger in scale than gullies, although smaller than valleys. A ravine is generally a slope landform of relatively steep (cross-sectional) sides, on the order of twenty to seventy percent in gradient. Ravines may or may not have active streams flowing along the down slope channel which originally formed them; moreover, often they are characterized by intermittent streams, since their geographic scale may not be sufficiently large to support a perennial watercourse. The ravine of Zazzano is located on the Gargano area. The ravine is a 30 meter large ravine, 107 meters deep underground which was used in the past as abusive rubbish dump. Old and wrecked cars were also put down the ravines, stacking on each other in column. During a cleaning operation, local authorities, a team of speleologists found human skeletal remains and activities were interrupted. A prosecutor was immediately alerted and forensic pathologists were called for scene investigation, skeletal remains recovery, and identification. In a wrecked car, one completely skeletonized cadaver was found with its clothes; a reddish rope still tied to the arms bones and the head found inside a plastic bag. A second completely skeletonized cadaver was found later in another wrecked car some meters down the previous one. Bones of a third cadaver were recovered on the ground of the ravine, partially covered by mud. The cadaver lying in a prone position; head, thorax, and upper arms were found inside a jute bag, a reddish rope still tied lower arms. A fourth cadaver, completely buried under the mud was found, lying in supine position; the head was found inside a plastic bag. Recovery activities were completed in three days. A video recording of recovery was performed and is presented. Local authorities identified the owners of recovered wrecked cars. Forensic activities involved radiological investigation by means of standard approach and total body multislice TC scan contributing in determining causes of death: suffocation, gunshot wounds and efforts of mutilation, variously combined. Anthropological investigation determined sex and race of skeletons; dental records and dental casts were performed by forensic odontologists. DNA profile has been developed for identification. At the end of forensic examination all the fourth cadavers were identified as

belonging to men suddenly missed in the nineties, suspected as victims of the local criminal organization. The gravine is now famous with the name of cemetery of the Gargano's mafia.

**Skeletal Remains, Ravine, Homicide** 

#### G95 Use of Multidetector Computed Tomography (MDCT) in the Evaluation of Gunshot Wounds

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After attending this presentation, attendees will understand the process used by the Armed Forces Medical Examiner System (AFMES) to integrate MDCT into the evaluation of gunshot wounds. Attendees will be able to describe the advantages and limitations of utilizing MDCT in the evaluation of gunshot wounds.

This presentation will impact the forensic science community by detailing a novel approach to overcome the limitation of the visualizing entrance and exit gunshot wounds with MDCT.

Postmortem forensic imaging is a critical tool in the evaluation of gunshot wounds. Traditionally, fluoroscopy and digital/plan film x-rays have been utilized to document and locate bullets and bullet fragments in cases of gunshot wounds. In the last several years, traditional imaging techniques in conjunction with postmortem MDCT has made it possible to obtain precise three-dimensional localization of bullets and bullet fragments. In addition, this technique has been shown to be an effective method for aiding in the documentation of gunshot wound paths and evaluation of internal organ injury prior to autopsy.

One of the main limitations of utilizing MDCT in the evaluation of gunshot wound paths is the inability of MDCT to precisely locate the surface entry and exit wounds. Although the presence of gas in the soft tissue and disruption of tissue surfaces may be helpful in the precise location of these wounds, the collapse of the temporary cavities, compression of soft tissue defects and the position of the of the body on the scanning table can limit the detection of the entry and exit wounds.

In order to overcome this limitation, a novel technique was developed utilizing radio-opaque markers. Briefly, the body is first imaged by digital x-rays to identify any bullets or bullet fragments in the body or clothing. Next, digital photographs of the body and gunshot wounds are taken and the locations of the gunshot wounds are marked with a 1.5 millimeter radiopaque marker. The body is then imaged with MDCT. The resulting images are processed with imaging software to produce a three-dimensional image of the body with the precise location of the entry and exit wounds on the skin surface. Reconstructed images are manipulated to obtain any desired orientation of the body and wound pathway. These images can then be used to demonstrate the gunshot wound pathways in medicolegal proceedings. It must be noted that this technique does not overcome the limitation of MDCT in distinguishing entrance gunshot wounds form exit gunshot wounds. This distinction is made by combining the postmortem forensic imaging with the findings from the external inspection and internal dissection of the body.

Computed Tomography, Gunshot Wounds, Virtual Autopsy

#### G96 Gunshot Wounds Covered by Different Textiles: Determination of GSR Through Micro-CT Analysis

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After attending this presentation, attendees will have novel information on the role of Micro-CT analysis of gunshot wounds for estimating the firing range.

This presentation will impact the forensic science community by adding new data on the estimation of the firing distance of intermediaterange gunshot wounds in clothed victims, through a micro-CT analysis of the gunshot residue.

Estimation of the firing range is often critical for reconstructing gunshot fatalities, where the main measurable evidence consists of the gunshot residue (GSR). Several techniques and methods have already been used for characterizing GSR, such as Atomic Absorption Spectroscopy, Neutron Activation Analysis, Autoradiography, Routine-CT, Scanning Electron Microscopy, and Histochemistry. Recently, a novel approach, based on the use of Micro-CT, proved to be an objective, reliable, rapid, and inexpensive tool for estimating the firing range in intermediate-range shots.

Aim of the Study: It is well known that the presence of clothes covering the body heavily affects the distribution of GSR on the entrance wound, hindering the estimation of the firing range on the basis of the sole macroscopic inspection.

The goal of the present study was to evaluate the differential distribution of GSR, with regard to the different kinds of textiles covering the skin, by means of micro-CT analysis, with the final purpose of reconstructing the firing distance.

**Materials and methods:** Human legs, surgically amputated, were cleaned of dried blood and any other contaminants, and cut into sections of approximately 6 cm in length.

A total of 60 sections were selected; each section was covered with a single type of textile, chosen among cotton fabric (n = 15), jeans (n = 15), leather (n = 15), and waterproof synthetic fabric (n = 15). Bare skin sections were used as controls (n = 15).

Firing was carried out perpendicularly at distances of 5, 15, and 30 cm, using a .32 pistol loaded with full-jacketed bullets. A total of 75 shots were performed (five replicates for each distance). After each firing test, the gunshot wounds were photographed and formalin fixed.

The skin specimens, comprising the epidermis, dermis, and subcutaneous fat, were cut into parallelepipeds (height 1 cm, side 1 cm) with a lancet. Samples were scanned following standard processing procedures, using a high resolution scanner.

The acquired raw data were reconstructed with reconcilliation software, which uses the back-projection algorithm to reconstruct axial subsequent images saved as bitmap format. The bitmap images were analyzed by a CT analysis software: the selected volume of interest (VOI side of 1 cm and height of 3.8 mm) was focused in the centre of the specimen in order to have the entire entry wound positioned in the middle. All the samples were binarized using the same parameters.

The percentage of GSR deposit was calculated analyzing all particles with a density higher than 1000 Hu (particles with a density lower than 1000 Hu were excluded to reduce iron artefacts). The 3D images were reconstructed through a Ct-Vox Software.

**Results:** The visual inspection of the skin did not allow the estimation of the firing distance for the covered gunshot-wounds; the

morphological features of the entrance wounds (blackening and tattooing) were, indeed, not discernible.

The micro-CT analysis revealed that:

- GSR particles were less represented in cases compared to controls;
- In cases GSR particles were distributed inside the cavity and the fatty tissue of the entrance wound, while in controls they were present mainly on the skin around the hole; and,
- Increasing the firing range, the radiological detection of GSR progressively decreased in both cases and controls, allowing a good discrimination of the firing distances tested in the present study.

**Conclusions:** Micro-CT analysis might be useful for the forensic assessment of the firing range, particularly when the morphological features of intermediate-range wounds are not visually discernible (i.e., black people or clothed victims).

Forensic Pathology, Gunshot Wounds, Firing Range

#### G97 Child Abuse vs. Cachexia: Do Healing and Acute Rib Fractures Trump a Diagnosis of Probable Cardiac Dysrhythmia Due to Electrolyte Abnormalities

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After attending this presentation, attendees will gain an awareness of potential differential diagnoses between forensic anthropologists and medical examiners/coroners on child deaths becomes evident.

This presentation will impact the forensic science community by informing attendees of the difficulties that accompany medical/anthropological diagnoses of child abuse, and the complications that arise when specialists attempt to contribute to cause and manner of death in infants.

One of the most difficult tasks confronted by forensic pathologists is the determination of cause and manner of death in suspected child abuse cases. In the last 15 years, forensic anthropology has demonstrated a potential for contributing to the cause of death, by systematically examining questionable skeletal areas after processing the skeletal elements free of soft tissue for a close look. Certainly, an accurate analysis of acute and healing fractures contributes immensely to a final diagnosis of infant's deaths. But do the two professions, with different approaches and diverse responsibilities, ever conflict in diagnoses? Of course they do. Below is a case where such a conflict arises.

An unembalmed body of a well developed, poorly-nourished female was examined and autopsied. The body appears younger than the reported three-months. Inanition is evidenced. The pale skin shows no acute injuries, or scars, nor were there any indications of trauma from the external exam. A V-shaped incision was performed previously by a tissue harvest team to remove the heart and proximal aorta. The clavicle and first rib on the right side were sectioned for this procedure. The first indication of skeletal injuries is first discovered during the internal examination of the ribs, where hemorrhage, acute, and possible healing rib fractures are visible.

Pathologic diagnoses documents small body size, where height and weight are diagnosed as in the 3<sup>rd</sup> percentile for age. Morgue examination weight is 7 lbs 6 oz, where birth weight was 6 lbs 3 oz. This presents neglect or failure to thrive. Also noticed is documented dehydration and small organ weights. Finally, blunt force skeletal injuries are present, with acute, chronic, and acute-on-chronic rib fractures. History indicates that aunt called 911 at 15:30 after last seeing the child alive at 8:30 that morning. The aunt is the legal guardian.

The anthropologist was called in at the first recognition of skeletal trauma. At that point it was decided to remove all ribs, both clavicles, and vertebrae C-7 through L-4 after extensive photographic documentation. These were processed free of obvious soft tissue, but preserved in anatomical position to give a better idea of three dimensional relationships of the complicated trauma to bone.

Dry bone examination combined with faxitron radiographs indicate numerous rib fractures as listed in Table 1.

Table 1. Summary of rib fractures in 3-month-old infant.

RIB FRAC	TURES			
<u>Acute</u>	<u>Chronic</u>	Stable Chronic	Acute On Chronic	Other Procedure
15*	6	10 (3 questionable)	2	1 (tissue bank)

\*All rib head apex tears

As one would guess, the anthropological report documents and describes the 33 insults to bone that clearly point to non-accidental trauma, with the history as reported. However, from a medical examiner point of view, this case was everything but a clear case of child abuse.

It is ruled the death of this 3-month-old as attributed to probable cardiac dysrhythmia due to electrolyte abnormalities. Postmortem testing for calcium and vitreous sodium yielded abnormally low levels. Multiple blunt force injuries in the form of acute and chronic rib fractures were also noted at autopsy. No external signs of trauma are seen on the body. Differential diagnoses of the infant's abnormalities include natural and non-natural causes. Neglect and child abuse cannot be ruled out, however, nor can a natural cause such as a metabolic disorder be eliminated. To complicate issues, the infant had been taken to the pediatrician regularly and they were treating the low body weight. The last physician visit was 16 days before death. In view of these issues, the manner of death is best certified as "Undetermined."

Maybe the question in this case should be formulated, "Do diagnoses of probable cardiac dysrhythmia due to electrolyte abnormalities *trump* healing and acute rib fractures?" To the anthropologist perspective, this is an unsettling thought. To the medical examiner/coroner, while still unsettling, their responsibilities are medical interpretations of cause and manner of death, not simply biomechanic interpretations of bone fracture. The repercussions of a homicide ruling without a traumatic cause of death are immense. Thus, the debate goes on.

Child Abuse, Cachexia, Healing Rib Fractures

### G98 The Identification of French Victims in the Massive Earthquake on January 12, 2010 in Haiti

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The goal of this presentation is to give attendees a clear understanding of France's structure and procedures in terms of identification of its nationals in the event of a major natural disaster and to demonstrate that the international response is as efficient and effective as it is at a national level.

This presentation will impact the forensic science community by showing the successful collaboration between a forensic scientist and a first response rescue team. To illustrate this, the French national team of identification was followed on site at Port-Au-Prince from January 13, 2010 until April 1, 2010. It also demonstrates that an early intervention is key to optimizing the effectiveness of the identification process and to achieving the overall success of the operation.

The earthquake that struck Haiti's capital Port-au-Prince caused more than 200,000 deaths. The major contributing factors to such high casualties were primarily its incredible strength and secondly the instability of the existing infrastructure. The challenges that had to be overcome were the significant increase of sanitary requirements and the issue of corpses' management. Identifying such a high number of victims proved extremely difficult firstly because of such a large volume of corpses and secondly because of their high levels of depreciation. The success of this operation was only made possible due to a thorough preparation combined with a structured and systematic approach.

In response to similar events, France has a national DVI team (Unité Nationale d'Identification des Victimes de Catastrophes – UNIVC) since 1992. It was established by the Criminal Research Institute of the National Gendarmerie (IRCGN). The team is made up of specialists from the Criminal Identification Department who are able to be deployed on site very quickly and are trained to be adaptable and responsive to any given situation.

Since 2006, authorities based in the French island of Martinique, in the West Indies, have been focusing on contingency and emergency plans due to the island's major exposure to natural risks and its remote location. These revised response procedures were put into practice for the first time in Haiti in 2009 to such great effect that it has subsequently brought about modifications of the national strategy (plan ORSEC). This strategy encompasses a forensic scientist, with expertise in mass deceased management, as part of the initial first aid response team.

The first evaluation reported at least 70,000 deceased in PORT-AU-PRINCE 24 hours after the earthquake. The police's chief of the United Nations and the Haitian Prime Minister, who were actually themselves survivors of the disaster, were immediately contacted to organize the clearance and logistic requirements, as well as the coordination of the mass burial of corpses in common graves commencing 36 hours after the earthquake.

Communication was a key factor in this operation not only with the population, to dispel the myth that corpses in a disaster can cause the rapid spread of diseases, but also with the international nursing staff to reiterate that sanitary precautions in the movement of bodies.

The second major objective was to perform an accurate census of the deceased French nationals by locating and collating their position at the time for the purposes of identification and repatriation to families. This was achieved by setting up an *"antemortem"* unit at the French embassy for the registration of missing persons and reported fatalities.

Between 72 and 96 hours, 30 names were indexed. Survivors were then contacted and advised on the best practices for the storage of the deceased prior to burial. These instructions also gave details on how to preserve vital evidence i.e. ensuring personal effects of the corpses were not removed, drawing up an accurate map to locate corpses, collecting local and national coordinate.

As a result, all French deceased nationals (33 of approximately 1,200 present) were identified by the end of March 2010 and placed in a temporary mortuary at the French embassy with thanks to the close cooperation of the American and Canadian DVI.

Identification, Mass Disaster, Earthquake

#### G99 Comparison of Methods for Measuring Decomposition of Submerged Carrion in Fresh Water

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The goal of this presentation is to determine a method for measuring submerged decomposition experimentally that limits contamination to the carrion.

This presentation will impact the forensic science community by discussing how, currently, there is no standard for measuring submerged decomposition *in situ*. This study compared current proposed methods for experimentally measuring the amount of decomposition undertaken by carrion underwater, and hopefully, the results may guide future underwater decomposition research, using more standardized techniques that limit contamination of the decomposition process.

Continuous monitoring of decomposition and calculations of the postmortem submersion interval (PMSI) of carrion at depth can be problematic for forensic investigators due to risk contamination of the carrion caused by the extraction from the experimental environment and weighing processes. Underwater photography and evaluation utilizing the Heaton et al. total aquatic decomposition (TAD) score at depth was compared to weighing the carrion before and after submersion, as well as full forensic necropsies. The actual time of submersion was known for each carrion. Perinatal piglets were used as human analogues for experimental purposes. This study suggests that weighing the piglets after they have been submerged in fresh (stagnant) water yields inconsistent results due to the unpredictability of algae growth in water ecosystems with high algal contamination. In addition, while underwater photography does reveal some evidence of decomposition in situ, usefulness is limited by required training, expensive equipment, and further algal growth issues which can obscure the visual data. The results of this study indicate that in order to objectively measure decomposition over time, the carrion should be examined either at depth using the TAD scoring system, or a set of piglets should be submerged with one piglet harvested from the experimental environment over set periods. This piglet should then undergo a pathological examination (with histological sampling and TAD scoring, as was done in this study), rather than relying on underwater photography. This allows for normalization between piglets and excludes weight and algal growth issues, thereby showing the amount of decomposition over time. The acquired TAD score can then be used with the calculated Accumulated Degree-Days (ADD) to determine an approximate PMSI. These results may not be generalizable to other submersion conditions in water ecosystems with different salinity, temperature, degree of algae growth, and amount of other animal activity.

Underwater Decomposition, Postmortem Submersion Interval, Visual Scoring System

#### G100 Fatal Sexual Violence Against Women: Normative, Baseline Studies of Postmortem Genital Anatomy — What Can We Say About Normal?

#### Sharon R. Crowley, MN\*, FCNS, 122 Emeline Avenue, Santa Cruz, CA 95060

After attending this presentation, attendees will better understand the nature and appearance of the postmortem anogenital tissues; be able to discuss findings from control groups of normative, baseline studies for comparison with cases of fatal sexual violence; and discuss taxonomy, examination adjuncts, and database variables useful in the postmortem sexual assault evaluation.

This presentation will impact the forensic science community by improving the diagnostic acumen of the forensic examiner, helping avoid ambiguity of interpretation of clinical findings in postmortem genital examinations, and improving knowledge about fatal sexual violence against women.

Until recently, a paucity of data existed on the "normal" appearance of the genital anatomy during the postmortem interval. There is a lack of data from scrutiny and photodocumentation of the postmortem anogenital tissues. The use of colposcopy is well established for both adult and child *living* victims. During the autopsy, gross visualization alone may not allow the detection of the more subtle findings that usually constitute genital trauma in sexual assault (Crowley-AAFS: 2003). Comparisons to either living sexual assault victims or postmortem cases of non-sexual etiology were extremely difficult.

This presentation proposes to describe ongoing research on postmortem genital anatomy. These cases constitute the first normative, baseline data on the anogenital tissues during the postmortem interval. The focus of the present discussion is to describe the findings from two normative, baseline control groups, with a total sample of 48 female cases.

**Group I** consists of 30 female cases drawn from the Body Donation Program, at the University of California, Davis, California. Most donors are received  $\leq 24$  hours of death. All cases selected for this baseline study are fresh, or fresh-frozen, vs. embalmed. Cases are examined based upon availability, i.e., female gender and received by the program in a time frame compatible with access by the primary investigator.

*Group II* consists of 18 coroners' cases, from another jurisdiction. These were also examined using the mobile colposcopy and examination system described by Crowley (JFS: 2004). The manner of death was accidental in seven cases and natural in 11.

**Materials and Methods:** This research project is an observational study, with a cross-sectional design. The examination methodology employs photocolposcopy at 7.5X, 15X magnification, or both, plus 35 mm photography via the colposcope. In most cases, additional photographs are taken with a 35mm single lens reflex (SLR) manual or digital camera, for comparison to colposcopy. Inspection and photodocumentation of specific anogenital sites is employed, prior to manipulation of the genital tissues. On select cases, concomitant application of a 1% solution of toluidine blue dye has also been incorporated, in order to evaluate the reliability of this general nuclear stain as an adjunct to the postmortem examination. The same 12 anatomic sites are visualized, inspected, and photographed in both controls and sexual homicide cases. These include the labia majora, peri-clitoral area, peri-urethral area, labia minora, hymen, vagina, cervix, perineum, fossa navicularis, posterior fourchette, anus, and rectum.

There are some core data elements germane to both control and sexual homicide groups. These include age and reproductive status, (pre-pubertal, reproductive age, peri-menopausal, and post-menopausal) and genital examination techniques. Other common variables include the unique case identifier, date and time of the examination, interval from death to arrival in forensic science morgue, general condition of

\* Presenting Author

body, race and ethnicity per CDC definitions, cause and manner of death, and contributory and/or concomitant medical and gynecological conditions, especially those presenting lesions.

The 30 female cases from Group I range in age from 60-99 years. The mean age is 83.1 years old. This is a largely homogeneous group; 93% of the sample is Non-Hispanic/White. The majority of Group I presented to the forensic science morgue within 24 hours. Postmortem mucosal autolysis was present at a minimum of one out of the 12 anatomic sites in 80% of the sample. Postmortem skin slip in the anogenital area was present in 16.6%. A 1% Toluidine blue dye solution was applied and decolorized with a dilute acetic acid solution in 21 of the 30 body donor cases. There was a false positive uptake in 100% of the cases. This was true regardless of the anatomic site of dye application.

The age range for the 18 cases in Group II was 32 months to 89 years of age. The mean age was 47.87 in this Group. The ethnicity and race distribution was as follows: Non-Hispanic/White (66.6%); NonHispanic/Asian Pacific Islander (5.5%); Hispanic/White (11%), and African American (16.6%). The postmortem interval to arrival at the forensic science morgue was  $\leq 24$  hours in 88.8%, 96 hours in 5.5%, and  $\geq 5$  days (active decay) in 5.5%. Postmortem mucosal autolysis was present in greater than 50% of Group II. Toluidine blue dye was not applied to any in this sample.

**Discussion:** The postmortem arena superimposes a unique set of factors. Many were not previously studied or documented in the literature. Analysis of results from baseline studies allows eventual comparison to genital injuries sustained by both sexual homicide victims and living sexual assault victims. A relational database was described (Crowley, AAFS: 2010) as a method to simplify and quantify data for interpretation, analysis, and linkage to other cases.

Taxonomy germane to the postmortem arena should incorporate salient terms that will be consistent and universally applicable and acceptable within the forensic community (Crowley & Peterson: AAFS, 2004). Postmortem artifact, such as *mucosal autolysis* and *skin slip*, visualized in the anogenital tissues, is documented for each anatomic site. Inclusion into case documentation permits aggregate summaries of individual and population-based summaries. Appropriate taxonomy and correct identification of "normal" will help improve our diagnostic acumen and increase the reliability of our methodology.

The significantly false positive results obtained from application of Toluidine Blue dye on the postmortem anogenital tissues should preclude any recommendation for its use in the postmortem sexual assault examination. It appears to be consistently picked up by the shedding tissues that comprise part of the normal artifact. The inexperienced examiner might misconstrue this for a significant finding.

It is certainly true that in equivocal cases, the forensic pathologist can simply remove en bloc, for dissection and microscopic evaluation, the tissues germane to genital findings. However, it may prove to be beneficial to have an initial *in situ* examination of the anogenital anatomy, via colposcopy. The ultimate goal of this research is to improve our understanding of what is normal, and what is not, during the postmortem interval for the anogenital tissues. In this manner, the capacity and understanding of fatal sexual violence against women will continue to grow.

Fatal Sexual Violence Against Women, Body Donation Program, Colposcopy

#### G101 The Effects of Household Chemicals on Blow Fly Oviposition and Development Using Human Cadavers

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After attending this presentation, attendees will have a better understanding of the effects that household chemicals have on the insect's role in the decomposition of the human body. This research was inspired from a murder that occurred in Lafayette, Indiana 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 postmortem interval (PMI).

This presentation will impact the forensic science community by exploring the hypothesis that bodies treated with the chemical ammonia would not significantly vary from bodies with no treatment and bodies treated with Raid<sup>®</sup> would significantly vary. This hypothesis was based on the results of previous research conducted on swine.

Six human bodies, (four male and two female), were obtained for use in this study and frozen prior to placement in the field. This research was conducted at the Anthropological Research Facility at the University of Tennessee in Knoxville. The field research started on July 18, 2010 and concluded on August 6, 2010. The bodies were placed in the field in sealed body bags and allowed to thaw for a period of 48 hours prior to treatment with chemicals. After the bodies were removed from the bags they were checked for any evidence of insect activity and none was observed. Two bodies (male) were not treated with chemicals and served as controls. Two bodies (one male, one female) were coated with 1275 g (3 cans) of Raid for Flying Insects, (active ingredients 0.05% permethrin, 17.5% tetramethrin, 0.05% d-cis/trans allethrin). Coating involved spraying the bodies with Raid until runoff occurred. Two bodies (one male, one female) were coated with 9.45 L (5 bottles) of Great Value brand household ammonia. The ammonia was poured onto the body until runoff occurred. Treatments were randomly assigned using a random number generator. The bodies were monitored and photographed twice daily and notes were taken to document blow fly activity. The following major stages of insect activity were noted: adult flies, fly eggs, fly larvae, migrating fly larvae, presence of beetles, and the end of maggot migration (characterized by the absence of observable larvae on the body). This allowed researchers to document differences in development time as well as the initial onset of blow fly life stages. Adults and larvae were collected following standard operating procedures outlined in Haskell and Williams (2008) each day to document any differences in species composition or development among treatments. Larvae were collected in KAA (composed of 95% Ethanol (77%), Acetic Acid (15%) and Kerosene (8%)) and adults in 70% EtOH.

The research was still in progress during the writing of this abstract, but the results and conclusions of the study will be discussed during the presentation.

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, then the estimation of the 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.

Forensic Entomology, Chemicals, PMI

### G102 Mass Disaster Procedures: Forensic Pathology and Genetic Techniques Used to Locate Nine Missing People in the Calabria Region (Southern Italy)

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After attending this presentation, attendees will understand the importance of a unified and well organized multidisciplinary approach resulting in a set of guidelines useful during a mass exhumation.

This presentation will impact the forensic science community by documenting the management of the first mass exhumation and DNA identification of one hundred corpses. This procedure will be useful in the future following an actual mass disaster event.

The Public Prosecutor's Office of the city of Paola (CS, Calabria Region, Southern Italy) opened an investigation into a number of criminal offenses that have repeatedly occurred in aged care facilities where people with physical and psychological handicaps are managed.

The aged care facility manager was a suspect and due to his irregular management was arrested.

In different periods over recent years nine residents had gone missing from the house. The resident's families reported this to the legal authorities about their missing relatives to the legal authorities. Moreover the families expressed concern that during the last meeting when they saw their relatives, they were all injured.

An investigation was organized in order to find the missing patients. This was concentrated in the local cemetery where the police found unmarked graves without any identification.

The public prosecutor gave permission to the Legal Medicine Department of Magna Graecia University of Catanzaro to exhume the unmarked graves and examine each corpse so they could be identified.

In a period of 15 days, 101 corpses were exhumed for identification using DNA and to determine if any trauma had occurred either antemortem or perimortem.

The medico-legal task force which was multi-disciplinary consisted of three phases: a planning phase; a pre-analytical phase; and, an analytical phase.

**Planning Phase:** During this phase mass disaster operative procedures were enacted. A medico-legal camp was built close to the cemetery area. Briefing meetings were organized to coordinate the schedule of pathologists, molecular biologists, entomologists, mechanical engineers, computer scientists, physics, and law enforcement agents (Arma dei Carabinieri and fire brigade).

**Pre-Analytical Phase:** Under the direction of the coordinating pathologist, pneumatic tents were positioned courtesy of the fire brigade. Each tent had its own purpose (external inspection, x-ray, autopsy, biological laboratory). The camp site was powered by portable electric generators. Moreover, the camp set up was established in order to prevent the infestation of insects attracted by human remains which would complicate or invalidate the medico-legal analyses. Approximately 40 people worked each day in the camp.

Analytical phase: This phase was developed in the Genetic Laboratory of the RIS, Carabinieri Messina. The methodology was previously validated at the Forensic Genetic Laboratory of Magna Graecia University where techniques had been previously established to extract DNA from old and badly preserved bones. The main difficulty of this work was the DNA extraction from corpses in colliquative, corification, and scheletonization stages.

A piece of femur diaphysis (4 cm length) was sampled from each corpse and fixed in alcohol. Subsequently all muscle tissue and internal trabeculae (if present), was removed from the bone. The bone was then washed in water-alcohol-ether, then pulverized and demineralized.

The pulverization of 0.5 g of bone was achieved by impact and friction using 30 Hz frequency and spun together with a single tungsten carbide ball inside a cylindrical steel container.

The bone powder demineralization was obtained using an EDTA solution 0.5 M pH 8,0.

After chloroform extraction, the DNA purification was completed using silica gel columns.

The quantity of obtained DNA was determined by REAL-TIME PCR technique.

The DNA personal profiles were obtained with multiplex amplification and subsequent separation in capillary electrophoresis.

The results obtained allowed the DNA identification of the corpses regardless of their stage of decay. The obtained DNA profiles were compared with relatives to confirm the identity of the exhumed bodies. Mass Disasters, Forensic Pathology, Forensic Genetics

#### G103 Variance in Growth Rates of *Calliphora vomitoria* on Different Tissue Types: Mass Raised vs. Single Raised

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After attending this presentation, attendees will have a better understanding of the variances in growth rates for *Calliphora vomitoria* that occur not only when raised on differing tissue types, but also when raised in mass versus single.

This presentation will impact the forensic science community by demonstrating the need for further understanding of larvae activity and growth rates on differing tissue types.

Without a more thorough understanding of larvae species and their possible species specific growth rates and interactions with tissue types, the use of them in determining postmortem intervals (PMI) is suspect.

Recent studies show that there are significant differences in maggot growth rates depending on what type of tissue they consume. While previous studies have determined that the structure of the tissue didn't make a difference, what exactly causes the variation in growth rates has not yet been discovered. Since most comparison studies have been conducted using lab raised larvae (commonly grown on cow liver or pig liver), there may be significant problems with using such larvae to determine PMI in real cases. To better understand the implications of the variation in growth and development, development rates of larvae raised on various tissue types need to be explored more completely. This raises the question of whether the specific tissue consumed, or a change within the mass's activity, causes a change in growth rate.

A comparison between single raised larvae and mass raised larvae was used to judge the possible connection between being raised in mass and differences in growth rates on varying tissue types. If the difference in growth rates between different tissue types was not related to being mass raised, then single raised larvae and mass raised larvae from the same tissue type would show the same variations in growth rates. This experiment examined the growth rates of single versus group raised maggots (N=100) on various tissue types. Growth rate differences were measured in two ways: mean maggot size, and instar stage. Larvae were procured from eggs laid in the lab by a mixture of wild caught and lab raised *C. vomitoria*. Larvae were laid on kidney, liver, heart, lung, or brain before being transferred within an hour of hatching to the tissue on which they were to be raised. All larvae were transported from the tissue on which they were laid to either a piece of the same tissue type or a different tissue type. By this means, it could be seen if a variation in origin tissue and sustenance tissue for a larvae during the first instar stage had an effect on the rate at which it matured.

Each tissue type had three replicates of masses being born and raised on the same tissue type and three replicates of larvae being born and raised on differing tissue types, for six replicates in total. Single raised larvae were replicated in groups of twenty for each origin tissue, resulting in batches of forty replicates total per a raising tissue type. All larvae were killed approximately six days after hatching and were measured by length from mouth to instar markings to the nearest .01  $\mu$ m.

Whether the tissue type on which a larva was born was switched during first instar was not statistically significant. However, the relationship between size, tissue type, and whether a larva was mass grown or single grown was significant. In PMI studies larvae are used to determine PMI based on length at time of death, or time needed to grow them to adulthood in a laboratory setting combined with accumulated degree days. The variations in growth seen in this study differ from those seen with previously studied species, signifying that tissue based variations in growth rates are species specific. The wider variance in lengths noted in mass raised larvae as compared to single raised larvae indicates that size is not the best determinant of age as previously presumed. These results could mean a significant change in the way fly larvae are viewed and utilized in the field of taphonomy and forensic entomology for determining PMI.

Development Rate, Forensic Entomology, Postmortem Interval

#### G104 Identification of Two Homicide Victims and Linking of Separate Crimes Solved by Radiographic Discovery of a Healed Bullet Wound.

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The goal of this presentation is to provide details on the investigation, and forensic examination of two separate homicide cases which were solved based on the radiographic discovery of an old healed bullet wound. A primary point of discussion of this presentation will be how the smallest and least suspected piece of evidence can be utilized to solve a difficult case or cases. The importance of detailed forensic anthropological examination in skeletonized or badly decomposed cases will be noted during the presentation.

This presentation will impact the forensic science community by providing present and future forensic investigations insight as to the forensic analysis of decomposed and skeletonized remains so as determine the identity of deceased as well as the possible cause and manner of death. The forensic audience attending the presentation will become more aware of the importance of old healed injuries in the identification process as well as possibly identifying past activities or linking criminal activity.

In the summer of 2006 near Warren, Ohio skeletonized remains of an adult individual were discovered in a heavily vegetated area near a water treatment facility. The remains of the deceased were noted to be without any associated clothing or foot ware. Forensic anthropological examination of the remains found them to be consistent with that of a Negroid male who was in his fifth decade of life at death. Based on the environmental conditions at the scene and the decompositional state of the remains a postmortem PMI of approximately six to eight months was assigned. The lack of soft tissues, absence of skeletal trauma in addition to the absence of clothing and location of the remains were noted as extremely suspicious by the coroner's pathologist.

Approximately two years passed while the remains of the deceased remained unidentified. In June of 2008, another set of skeletonized remains were discovered near Warren, Ohio. The remains discovered were recovered from several plastic garbage bags that were located along a highway. Each of the garbage bags recovered contained various portions of a highly decomposed body that had been dismembered. Forensic anthropological examination of the dismembered remains revealed them to represent a single individual that of a Negroid male who was in his mid to late twenties at death.

Detailed examination of the remains revealed that the dismemberment had been accomplished with a saw. The sawing dismemberment was noted as unusual as the cuts were made along the longitudinal/saggital plane of the body. A major portion of the middle and lower vertebral column had been cut longitudinally including a near perfect halving of the sacrum. A single gunshot wound was noted on the skull and considered to be the probable cause of death.

While conducting an examination of the innominates for aging and sexing purposes a very slight and almost unnoticeable small depression was noted along the inferior side of one of the innominates. The slight depression which was adjacent to the inferior side of the sciatic notch at first appeared to represent a simple skeletal anomaly attributed to human skeletal variation. Radiographic examination of the innominate revealed the presence of a fairly intact nine millimeter bullet. The bullet was removed from the innominate utilizing a small craft saw and submitted as evidence of an old healed bullet wound.

The discovery of the old healed bullet wound served as a key lead to the identification of the homicide victim. Identification of the victim led police on an investigation that in turn resulted in the identification of the prior unknown remains discovered near the water treatment facility. Further investigation of this case revealed that the earlier death was also a homicide and led to the arrest of the killer.

Anthropology, Ballistics, Human Identification

### G105 Utilizing Multi-Detector Computerized Tomography to Evaluate Concrete-Encased Human Remains

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After attending this presentation, attendees will recognize the value of postmortem multi-detector computerized tomography (MDCT) evaluation of an unusual case of human dismemberment followed by encasement in concrete; appreciate the value of MDCT osseous examination and documentation; learn methods to utilize MDCT analysis of concealed biologic remains and review procedures utilized for orientation and extraction of human remains from concrete.

This presentation will impact the forensic science community by expanding the scenarios in which the use of MDCT may be utilized to improve postmortem evaluation and will demonstrate opportunities to utilize MDCT in medical examiner/coroner facilities where MDCT is reasonably available.

The Mesa County Coroner's Office is situated in rural Western Colorado at Community Hospital in Grand Junction, Colorado. Because the morgue is located within the hospital, state of the art radiographic equipment and ancillary staff are available.

Case Report: the decedent is a 41-year-old male homicide victim whose dismembered remains were concealed with concrete in two fivegallon plastic buckets. According to police reports, the decedent was killed by his son who later confessed and led police to his father's remains which had been stored in a shed for approximately two months following the homicide and dismemberment. Unable to determine the veracity of the reported circumstances, the presence of human remains was confirmed using MDCT. The remains were limited to the decedent's head with cervical spine, hands, feet, and heart. The imaging also served to establish preliminary forensic findings, namely the presence of a bullet in the left orbit. In addition, fractures of the left and right orbital plates were noted, while the remainder of the calvarium was intact. Other osseous findings include a fracture of the left distal second metacarpal and left distal first phalange, as well as a metal plate in the left orbit. In order to remove the remains from the buckets with minimal damage, the outside of the buckets were marked to indicate the orientation of the remains. A circular saw with a concrete cutting blade was used to cut into the concrete along predetermined planes of predetermined depth. The properly oriented concrete incisions allowed for coronal separation around the head providing anterior and posterior intact concrete mold halves. External examination of the head revealed that the skin and portions of soft tissue had been removed prior to encasement. The ears and eyelids were missing, and the eyes were sunken and softened due to decomposition. A small caliber, slightly deformed bullet was recovered from the left frontal sinus/superior orbital ridge. Due to the intentional removal of the decedent's facial skin and postmortem change, the entry wound was not visible and range of fire could not be determined; however, absence of soot from sections around the remaining soft tissue likely exclude a contact gunshot wound. In the absence of postmortem MDCT or conventional radiographs, it is entirely possible that the presence of a gunshot wound could have been overlooked. While the extent of brain decomposition precluded its examination, the MDCT and gross examination findings indicate that the bullet did not penetrate the cranial cavity. Examination of the outer table of the left orbital ridge of the calvarium revealed hemorrhage in the soft tissue. Neck and throat examination indicate the unlikelihood of strangulation based on the presence of an intact hyoid bone, thyroid cartilage, and thyroid cornu and absence of hemorrhage of the laryngeal mucosa. Because of the limited amount of remains available for examination, trauma to the remainder of the decedent's body could not be evaluated and therefore the cause of death was classified as undetermined. The manner of death was classified as homicide. Positive identification of the remains was established by comparison with antemortem dental records and confirmation of an orthopedic metal plate in the left brow.

In summary due to the location of our morgue facility, MDCT is readily available and was utilized to confirm the presence of human remains concealed in concrete. Furthermore, MDCT permitted orientation of the remains for optimal removal, documented orthopedic devices to augment identification, and assisted in the evaluation of injury. This defendant pled guilty and the case did not appear in court. If court proceedings had ensued, the use of a three dimensional volumetric MDCT rendering would have been utilized to present information to the jury. It is believed, a three dimensional volumetric rendering provides objective detailed visual imagery without the graphic, frequently repulsive appearance of wound photographs, and MDCT is useful in the evaluation of selected postmortem examinations.

Multi-Detector Computerized Tomography, Gunshot Wound, Homicide

#### G106 Use of Multidetector Computed Tomography (MDCT) in the Medicolegal Investigation of Human Remains After a Natural Disaster

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After attending this presentation, attendees will understand the process used by the Armed Forces Medical Examiner System (AFMES) to integrate multi-detector computerized tomography (MDCT) in the handling of human remains recovered from the natural disaster in Haiti. Attendees will be able to describe strengths and limitations of the process model employed.

This presentation will impact the forensic science community by offering one alternative for processing human remains following a natural disaster or other mass casualty event.

A disaster mortuary is established both to identify victims and determine cause and manner of death. Conventional radiography has been routinely used to screen for foreign bodies, personal effects and anatomic, dental, or surgical findings. MDCT has proved to be a useful technique in support of forensic examination in military and civilian mortuaries. The disaster in Haiti provided the AFMES the opportunity to utilize MDCT in the processing of victims of that event.

The three step processing model used employed: (1) digital radiography and whole body MDCT; (2) visual external inspection of the body; and, (3) forensic autopsy if steps one and two did not establish reasonable explanation for cause and manner of death or produced findings that required internal examination (e.g., ballistic fragments, external wounds).

There were 28 cases received and 27 processed using the model (one case did not have MDCT). In 20 cases MDCT and visual inspection showed evidence of blunt force injury and no suspicious findings. The medical examiner did not perform an autopsy and cause/manner of death was "blunt force injury/accident." In 19 of 20 non-autopsied cases MDCT gave more information than digital radiology, the exception being a case where disarticulated bones were received. Key findings were skeletal injuries to the head/neck, spine, thorax, and pelvis. In seven cases MDCT and visual inspection was judged inconclusive and complete autopsy was performed. These cases were signed out as "probable positional asphyxia/accident" (2), "cardiac arrhythmia/natural (2), blunt force injury/accident" (2) and "complications of a natural disaster/accident" (1). None of the 27 cases showed internal metallic fragments or suspicious external wounds. In 23 of 27 cases, moderate to severe decomposition was present and our prior forensic experience was helpful in distinguishing changes related to postmortem decomposition, recovery and handling from acute injury sustained during the event.

In conclusion, the use of MDCT together with external visual inspection by a medical examiner provided sufficient information to establish cause and manner of death in 74% of the cases sent to the AFMES during recovery operations in Haiti. This related directly to the ability of MDCT to determine findings consistent with blunt force injury not apparent on digital radiographs. This model using MDCT and visual inspection offers a rapid alternative for investigating human remains recovered after a natural disaster. It is believed that MDCT alone without external visual inspection by a medical examiner would not be adequate. It is also recognized that a medical examiner may deem a full autopsy to be required for a variety of other reasons (e.g., statutes, policy directives).

Computed Tomography, Natural Disaster, Virtual Autopsy

#### G107 Evaluation of the Randox Whole Blood Drugs of Abuse (DOA) Microchip Arrays for Use With Alternative Postmortem Samples as a Rapid Near-Body Screen

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The goal of this presentation is to illustrate a rapid and simple tissue preparation method which allows drugs of abuse (DOA) to be screened using the Randox whole blood DOA microchip arrays.

This presentation will impact the forensic science community as the entire process can be undertaken and results obtained in the mortuary whilst the postmortem is taking place. Also the quantity of sample needed to screen may obviate the need to remove large tissue samples for laboratory analysis, saving time and costs, especially in negative cases.

A procedure is described that allows small aliquots of postmortem samples of blood, urine, vitreous humor, liver, and psoas major muscle to be analyzed for the following drugs, simultaneously: acetaminophen, amphetamine, barbiturates, benzodiazepines, benzoylecgonine, buprenorphine, cannabinoids, fentanyl, ketamine, lysergic acid diethylamide (LSD), methadone, methaqualone, methylamfetamine, methylenedioxymethamfetamine (MDMA), opioids, phencyclidine (PCP), propoxyphene, tricyclic antidepressants, zaleplon, zolpidem, and zopiclone.

Femoral blood, urine, vitreous humor, liver, and psoas muscle were obtained from forensic autopsies, ranging from suicides to natural causes. Tissue samples were cut into 1 centimeter cubes and homogenised with 1 millilitre SPE diluent. The homogenates were centrifuged for ten minutes at 3000 rpm and 70 microlitres of supernatant transferred to Eppendorf tubes. The samples were then diluted 1:3 with SPE diluent. Femoral blood, urine and vitreous humour were prepared and applied to the assay following the manufacturer's protocol for whole blood. Femoral blood from each case subsequently underwent confirmatory analysis using high performance liquid chromatography with diode array detection (HPLC-DAD) and liquid chromatography tandem mass spectrometry (LC-MS/MS).

Over 100 postmortems were screened for a combination of the previously mentioned drugs of abuse. A good agreement was obtained between the Randox assays and HPLC-DAD and LC-MS/MS analyses. Of the positive cases, urine and liver samples had a greater percentage agreement with confirmatory analyses than femoral blood, vitreous humor, and psoas muscle. The discrepancies between assay screening and confirmatory analysis may reflect differences in drug distribution between tissues as well as confirmatory analyses detecting concentrations below the assay's cut-offs.

In conclusion, the Randox whole blood DOA arrays can be used to alternative postmortem samples rapidly and simply. The simple procedure will benefit the forensic community as the entire process can be undertaken and results obtained in the mortuary while the postmortem is taking place. Also the quantity of sample needed to screen may obviate the need to remove large tissue samples for laboratory analysis, saving time and costs, especially in negative cases.

Drug Sceening, Postmortem, Alternative Samples

#### G108 Postmortem Vitreous Beta-Hydroxybutyrate: Interpretation in a Forensic Setting

#### Michael Heninger, MD\*, Fulton County Medical Examiner's Office, 430 Pryor Street, Atlanta, GA 30312

After attending this presentation, attendees will be aware of the range and frequency of postmortem vitreous beta-hydroxybutyrate (BHB) levels likely to be encountered in a forensic setting.

This presentation will impact the forensic science community by providing a more thorough basis for interpreting vitreous BHB levels.

Beta-hydroxybutyrate is one of three ketone-related substances commonly measured in the clinical laboratory and is also useful in postmortem testing. Ketones increase when the primary metabolic fuel source switches from glucose to fatty acids. Ketones are most useful as a marker for diabetic ketoacidosis and are also increased in alcoholic ketoacidosis, starvation states, and severe infectious disease processes. They can be measured in many body fluids including blood, urine, and vitreous fluid during postmortem investigations.

The medical examiner is often faced with an elevated vitreous BHB level that appears to have little or no bearing on the case. When can elevated vitreous ketones be safely ignored? This retrospective study was undertaken in order to gain a better understanding of the frequency and usefulness of postmortem vitreous BHB levels in the forensic setting. Moderately elevated levels were common and were not often related to the cause of death. More severely elevated levels of BHB were related to the cause of death with increasing frequency as the levels increased. Markedly elevated vitreous BHB coupled with elevated vitreous glucose usually indicated diabetic ketoacidosis. When vitreous BHB was elevated and the vitreous glucose was low, an alcohol related death was common.

A computer database was searched for postmortem vitreous beta-Hydroxybutyrate (BHB) levels measured in 1,795 forensic cases over a six year period (2003 to 2009) in the normal course of death investigation. Levels ranged from 0 to 22.7 mmol/L and averaged 1.2 mmol/L. 562 (31.3%) were less than 0.4mmol/L . 637 (35.5%) were between 0.4 and 1.2 mmol/L. 439 (24.5%) were between 1.2 and 2 mmol/L. 105 (5.85%) were between 2 and 6 mmol/L. 52 (2.9%) were greater than 6 mmol/L. Comparison of vitreous BHB with vitreous glucose levels in 1,781 cases showed moderately good correlation r=0731. Comparison with blood alcohol levels in 1,561 cases showed no correlation r= -0.053. Diabetic ketoacidosis was diagnosed in 76.9% of the cases with vitreous BHB above 6 mmol/L; 37.5% to 13.5% of cases with decreasing BHB levels from six to two mmol/L and less than 2% of cases with BHB less than 2.0 mmol/L. Alcoholic ketoacidosis appeared in only 4 cases. Conditions thought to be ketogenic (diabetes, alcoholism, severe infections) were found in over 92% of the cases with BHB above 6 mmol/L and a third of the cases with BHB levels below 2.0, 1.2, and 0.4 mmol/L. Cases of sudden violent death, age 20-40 and less than 90 minutes from incident to pronouncement time, and with no obvious reason for elevated BHB amounted to 11 cases and showed vitreous BHB levels closer to normal with an average of 0.57 mmol/L. The BHB level was elevated (0.4 - 1.72 mmol/L) in 32 of 34 SIDS-like cases included in the study.

Beta-Hydroxybutyrate, Death Investigation, Sudden Infant Death Syndrome

### G109 The Rising Trend of Ecstasy and Mimic Drugs Among Teenagers in Santa Clara County, California — A Guide and Awareness for Medical Examiners, Coroners, and Toxicologists

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The goal of this presentation is to educate the forensic community about the rise of ecstasy use and mimic drugs in California.

This presentation will impact the forensic science community by presenting a comprehensive report detailing the current growing trend of illicit pill ingestion among teenagers in Santa Clara County to include ecstasy, as well as, other mimic drugs (phenylpiperazines) which may escape detection in a basic toxicologic analysis. At the end of the presentation, attendees will be introduced and will be able to appreciate the new trend of "thizzin" and "popping" by teenagers in this county as well as nationwide. This presentation will also reiterate the importance of complete toxicologic screens on fatal overdoses to help identify common and not so common drugs of abuse and/or cutting agents.

According to 2001 data collected by the Substance Abuse and Mental Health Services Administration (SAMHSA), there have been approximately 100 deaths resulting from ecstasy overdoses in the United States. Nationwide, ecstasy abuse seems to outweigh methamphetamine abuse and only lags behind alcohol, marijuana, and cocaine as a popular drug of abuse among 12th graders. What is most disturbing is the indiscriminate ingestion of different colored and shaped pills ("thizzin") advertised to teenagers as ecstasy, when in reality, some of the ecstasy pills may be cut with cocaine, heroin, phenylpiperazines, and talc, just to mention a few agents. In Santa Clara County, a rise is being seen in teenagers abusing ecstasy and ingesting pills which may be composed of various drugs, some which may evade the basic toxicology screen. In the past year, Santa Clara County has seen two deaths resulting from ecstasy intoxication or complications thereof. Recently, a male who attended a rave party in a surrounding county died of an acute ecstasy overdose.

A retrospective search over the past nine years at the Santa Clara County Medical Examiner/Coroner Office disclosed a total of five ecstasy overdoses, two within the past year. In April 2009, a 22-year-old female was found deceased in a motel room after partying all night and ingesting ecstasy. During the course of the evening, she complained of headaches and began vomiting. Her friends left her alone in the motel room only to find her deceased hours later. The autopsy examination revealed a morbidly obese woman with an unremarkable internal examination except for marked brain swelling and pulmonary edema. Toxicology revealed the peripheral blood was positive for MDMA at 140 ng/mL (non-lethal) and its metabolite MDA at 20 ng/mL. Vitreous electrolytes revealed a profound electrolyte abnormality that consisted of hyponatremia. The cause of death was attributed to ecstasy-associated hyponatremia.

In January 2010, a 16-year-old adolescent was ingesting ecstasy with friends and had ingested a total of four tablets along with energy drinks when she became unresponsive shortly thereafter. Autopsy examination revealed that both coronary ostia arose from the same semilunar cusp but the coronary arteries pursued a normal anatomic course, along with findings of marked pulmonary and cerebral edema. Toxicology revealed the peripheral blood was positive for MDMA at 3200 ng/mL (fatalities arise at 1000 ng/mL) and its metabolite MDA at 140 ng/mL. Death was attributed to an ecstasy overdose.

After this period of recent fatalities, a survey of 1,852 students was conducted at two large comprehensive high schools and two continuation high schools located in Santa Clara County. A one-page anonymous questionnaire to identify use of various mind altering substances was administered from March 24, 2010 to May 12, 2010 to students ranging from ages 14-19.

In all, 25.32% of all respondents admitted to "ever use" of ecstasy (469 out of 1852) which is 390% higher than the teens responding to the national 2009 Monitoring the Future Survey (MTF) and nearly twice the level from the national 2009 Parents and Teens Attitude Tracking Study Report (Partnership for a Drug Free America) (PATS). Additionally, 8.48% of the teen respondents admitted to the use of ecstasy in the past 30 days; 471% higher than the MTF results and 41% higher than the PATS results. Students who had taken ecstasy admitted to maximum doses ranging from one to ten tablets with an average of four tablets. Results from the review of pictures of pills from www.ecstasydata.org by a smaller subgroup of 30 students confirmed that 70% contained phenylpiperazines

For at least local teen populations and perhaps growing geographical regions, it is hypothesized that the drug's ease of availability, reduction in its cost, limited awareness of the risks and risk of death, growing teen permissive attitudes and enabling behaviors from their social subculture, appear to have pushed this drug to their third most frequently used drug surpassed only by marijuana and alcohol. Complete toxicological screens are suggested in this population given their extreme dosing behaviors and the apparent frequent presence of phenylpiperazines.

Ecstasy, Mimic Drugs, Rise in Use

### G110 Laboratory Variation and Postmortem Redistribution in the Interpretation of Postmortem Fentanyl Levels

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After attending this presentation, attendees will understand the principles of postmortem redistribution and interlaboratory variation and how to best utilize those concepts when evaluating postmortem fentanyl levels in central and peripheral samples.

This presentation will impact the forensic science community by providing an understanding the importance of using caution when interpreting very small, quantified fentanyl levels in postmortem samples.

The concept of postmortem redistribution has been extensively studied in some drugs, such as amitriptyline. The concept has also been looked at in regards to fentanyl, which due to its transdermal delivery mechanism, has interesting and unique pharmacokinetics and likely undergoes postmortem redistribution. It is hypothesized that fentanyl levels drawn from peripheral samples in the field, hours before autopsy, would be significantly lower than fentanyl levels in peripheral and central blood samples drawn at autopsy.

For this study, ten cases had fentanyl levels drawn in the field by investigators. The fentanyl level in this sample was compared to the level of fentanyl in peripheral and central samples taken at autopsy 15-24 hours later. Fentanyl levels are measured in very small quantities, ng/ml. At these very small amounts, the standard laboratory error could also greatly impact the values reported by the laboratory. In the process of comparing field and autopsy specimens and autopsy peripheral and central samples, we also sent most samples to a second accredited forensic toxicology lab. The ratios between the field and autopsy specimens and the heart and femoral blood levels were compared, and the interlaboratory variation was evaluated as well. The spearman correlation coefficient was similar (0.41) for the field and autopsy specimens from as single case analyzed at laboratory #1 as the coefficient for a single heart blood sample run at laboratory #1 and laboratory #2 (0.62) and a single autopsy peripheral blood sample run at laboratory #1 and laboratory #2 (0.57). Thus, the variation in values was similar between the same specimen analyzed at two different laboratories and between samples drawn from different sites and at different times. Other evaluations of the heart:femoral blood ratio of fentanyl and measurements of correlation and variation will be discussed.

Fentanyl, Postmortem Redistribution, Interlaboratory Variation

### G111 Toxicology and Pathology of 149-Methadone-Related Deaths

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After attending this presentation, attendees will understand that careful interpretation of methadone related deaths in the presence of concomitant drug intake and pathological changes is very important.

This presentation will impact the forensic science community by illustrating the difficulty of interpreting postmortem methadone blood levels due to the possible interaction with others drugs acting on the QT-interval or on the cytochrome P450, as well as the presence of pathological changes.

Methadone-related deaths are often difficult to interpret, especially in the presence history of chronic drug use, concomitant intoxications and if pathological changes are observed. Historically, the presence of methadone was often considered to be an incidental finding of the postmortem examination, unrelated to the cause of death. It was recently reported that methadone may prolong the QT interval, resulting in torsade de pointes. Sudden deaths with therapeutic levels of this synthetic opioid have been reported. Moreover, clinicians are increasingly aware of interactions between methadone and others drugs that prolong the QT interval or decrease the elimination rate of methadone.

The goal of this study was to evaluate methadone related deaths by dividing them into three groups according to the peripheral blood level of methadone: lower than 200  $\mu$ g/L, 200 to 1000  $\mu$ g/L, and higher than 1000  $\mu$ g/L. The primary purpose of the study was to determine whether differences exist between the presence of illicit drugs, drugs acting on QT interval and drugs metabolised by cytochrome P450. This study also aimed to determine whether there are differences between the cardiac, hepatic and pulmonary pathology of the three groups.

**Materials and Methods:** Methadone-related cases were reviewed retrospectively. The age of the victims ranged between 17 and 65 years. Most of the cases were male (109 cases). For all cases the complete autopsy, including histological examination and a full toxicological screening, was performed.

**Results:** The methadone blood levels were lower than 200  $\mu$ g/L in 37 cases; between 200 and 1000  $\mu$ g/L in 89 cases; and higher than 1000  $\mu$ g/L in 23 cases. In the last group methadone was detected in hair for all victims. Hair analysis was performed in 61 cases: 49 cases tested positive for methadone (80.3%) and 39 cases were positive for cocaine. Higher methadone blood levels were observed in men (p-value 0.052) and did not differ significantly by age.

Only in five cases methadone was alone, in 90 cases other drugs metabolised by cytochrome P450 were found, without significant

differences between the three group (p-value 0.81). Illicit drugs were found in 62 cases (p-value 0.29), drugs acting on QT interval in 79 cases (p-value 0.07) and respiratory depressant drugs, mostly benzodiazepines, in 139 cases (p-value 0.38).

Different pathological changes (cardiac, pulmonary, hepatic) were observed in 97 cases (*p*-value 0.24). Coronary disease was observed in 60.6% of chronic methadone or cocaine abusers.

**Discussion:** This study illustrates the difficulty of interpreting postmortem methadone blood levels due to the possible interaction with others drugs acting the QT-interval or on the cytochrome P450, as well as the presence of pathological changes. The various interactions between drugs remain unclear and do not appear to be related to the methadone blood level. Genetic variability may exist in the response of sub-group of individuals to the drug and its metabolism, making them more susceptible to an overdose. More postmortem studies should be performed in order to further understand and prevent fatalities which are mostly often observed during substitutive programmes or during illicit intake.

Methadone, QT Interval, Overdose

### G112 Deaths in Unlicensed Alcohol Rehabilitation Facilities

Lawrence Nguyen, MD, Los Angeles County Department of Coroner, 1104 North Mission Road, Los Angeles, CA 90033; and Christopher B. Rogers, MD\*, Los Angeles County Medical Examiner's Office, 1104 North Mission Road, Los Angeles, CA 90033

After attending this presentation, attendees will be aware of the risk of death in unlicensed alcohol rehabilitation facilities.

This presentation will impact the forensic science community by making pathologists aware of a series of deaths of alcohol abusers in unlicensed facilities, and making the public aware of the risks of alcohol withdrawal without medical intervention.

A series of 17 deaths in alcohol rehabilitation facilities occurred in Los Angeles County between 1996 and 2010. In each an intoxicated Spanish-speaking alcoholic man was dropped off at an alcohol rehabilitation group by his family. The individuals remained at the facilities for varying periods of time and underwent several procedures for detoxification

Reported treatment methods have included use of restraints, forcing the victim to drink ethanol or isopropanol, restraint with forced ingestion of alcohol, application of onions to the feet, and inserting a spoon in the victim's mouth.

In three cases there was a history of restraint use. Two additional decedents had both ethanol and isopropanol in the blood, although it was unclear whether the isopropanol was given at the facility. The causes of death included alcohol overdose, alcohol withdrawal, hemoperitoneum due to cirrhosis and a ruptured splenic vein, and diabetic ketoacidosis related to chronic pancreatitis. Most cases were closed as accident or natural, although three cases involving restraint were moded homicide. In some cases, other members of the group were charged with involuntary manslaughter and false imprisonment.

The police are familiar with these groups and are able to close the facilities. However, new groups often form at the same addresses, requiring additional police action. Los Angeles County has disseminated a public health warning about these centers and has published a list of 57 alcohol treatment centers using non-medical methods of detoxification. However, it has been difficult to eradicate these groups.

It is recommended that medicolegal death investigators be aware of this information, as similar groups may exist in other areas.

#### Alcoholism, Investigation, Detoxification

#### G113 Was This Drug Overdose Due to Intravenous Injection or Oral Ingestion of Heroin — Can You Tell?

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After attending this presentation, attendees will be alerted to and understand potential pitfalls associated with interpreting opioid levels in various body fluids and other matrices. This will be illustrated by presentation of a recent case where a question requiring an answer was whether heroin had been taken intravenously or orally. Research data will be presented followed by an explanation of the various mechanisms thought to cause these apparently anomalous findings.

This presentation will impact the forensic science community by informing attendees of the pharmacokinetics of opioids in the gastrointestinal system, and alerting them to the dangers of not fully understanding the behavior of these drugs in the body.

Death due to heroin overdose is almost always the result of intravenous injection of the drug in Australia. A case is described where a heroin overdose was initially thought to be the result of oral ingestion of the drug, primarily as a result of higher concentrations of morphine in stomach contents than in blood. During the subsequent criminal trial and investigation, however, the issue of the entero-hepatic circulation of morphine was raised as a possible reason for the presence of morphine in the stomach contents.

For many drugs and poisons, a simple way of making the distinction between oral and parenteral administration is to analyze the stomach contents and compare the levels of the drug in the stomach with those in blood; a higher stomach contents concentration of the drug would generally be strong supportive evidence for the assertion that the drug or poison was administered orally. Morphine; however, in common with a range of other drugs, undergoes entero-hepatic circulation as part of the metabolism and elimination of the drug. The entero-hepatic circulation is a complex mechanism whereby chemicals that have undergone conjugation reactions in the liver, such as morphine, once in the gastrointestinal tract, may be subject to passive re-uptake, entering the circulation via the hepatic portal vein, returning to the liver where the chemical can be biotransformed again and then re-eliminated. Morphine may undergo several cycles of entero-hepatic circulation resulting in a significant increase in the retention time and its consequent duration of action. Further, both during life and in the perimortem and postmortem period, the pyloric sphincter offers at best a partial barrier to reflux of morphine-containing gastrointestinal contents from the duodenum to the stomach.

These mechanisms would explain the presence of significant concentrations of morphine in the stomach contents of intravenous heroin users and we hypothesised that such physiological mechanisms can result in higher concentrations of morphine in stomach contents than in blood, despite the drug having been administered intravenously.

This study reports on the distribution of opioids in blood, stomach contents, urine, liver and bile in 29 deaths due to intravenous heroin overdose. The mean total and free blood morphine concentrations were 0.60 mg/L and 0.32 mg/L, respectively, and the mean stomach contents total morphine concentration was 1.16 mg/kg. All cases had detectable morphine in the stomach contents, and 24 of 29 cases had higher concentrations of total morphine in stomach contents than in blood. The mean total morphine concentration in bile was approximately 100 times that in blood, and the liver total morphine concentration averaged twice that of blood levels.

Morphine was detected in the stomach contents in all cases in this study, and in 83% of cases the stomach morphine level was higher than that in blood. This would indicate that the entero-hepatic circulation materially affects morphine levels in the body, and that reflux of morphine from the duodenum into the stomach appears to be the norm, at least after death. Furthermore, even if the gall bladder had been removed surgically at some prior time, stomach morphine concentrations can still be higher than the blood total morphine levels, as illustrated in one case.

It's concluded that the current study demonstrated that stomach morphine levels cannot be relied upon to determine whether heroin had been orally or intravenously administered. Given the large number of drugs and poisons which undergo entero-hepatic circulation, it would appear prudent to not make comment on route of administration of such drugs unless definite evidence of oral ingestion of the drug can be obtained, for example through visualization of appropriate pill fragments.

Heroin Overdose, Illicit Drug Use, Pharmacokinetics

#### G114 First Reported Case of Bromo-Dragonfly Fatality in the United States, San Jose, California, County of Santa Clara

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The goal of this presentation is to educate the forensic community of overdoses with rare chemicals used by young adults.

This presentation will impact the forensic science community by illustrating and emphasizing the importance of a thorough scene investigation, keeping an open mind to the curiosity of young adults and drug experimentation, and good communication with the consulting laboratory.

Like LSD, Bromo-DragonFLY is a psychedelic hallucinogenic drug that is extremely potent. In 1998, Matthew Parker synthesized Bromo-DragonFLY and because the molecule's structure resembled a fly, it was nicknamed FLY. Bromo-DragonFLY is a psychedelic phenethylamine and a non-subtype selective 5-HT2 (serotonin) agonist, considered less potent than LSD, but exhibits a longer duration of action and can last for up to two to three days.

The drug is ingested and the mechanism of drug toxicity is unclear, but based on numerous reports from individuals experimenting with this particular drug and their side effects, the mechanism of action appears to involve a severe peripheral vasoconstriction. Delayed onset of seizures, gangrenous extremity involvement, and extremely bad trips have been reported with one drug trip being described as "It was like being dragged to hell and back again many times. It is the most evil thing I've ever tried. It lasted an eternity."

In September 2009, the Santa Clara County Medical Examiner Office was called to the scene involving the sudden death of an otherwise healthy 18-year-old white male. According to the investigation, he was experimenting with and ingesting a new drug called 2C-B-FLY, which had been purchased through the internet, with his brother and his brother's girlfriend. The decedent's brother stated that the decedent ingested the least amount of the drug. Over the next two to three hours, the decedent appeared to be having a "difficult trip" then underwent seizure-like activity and became unresponsive. The decedent could not be resuscitated by emergency services and expired. The autopsy examination revealed an unkempt white male whose autopsy was unremarkable except for marked pulmonary edema, a non-specific finding seen in drug overdose cases and a thymic cyst. Because the medical examiner had no experience with 2C-B-FLY, additional insight to the drug and its effects was gained by searching a online library, a website suggested by the decedent's father. During this time, the comprehensive toxicology screen on the peripheral blood reported the presence of phentermine at a concentration consistent with therapeutic levels, marijuana, nicotine and cotinine, and atropine, most likely due to resuscitative attempts. The medical examiner remained suspicious that this death represented an acute drug overdose, especially in light of the circumstances surrounding the death, and consultation with a toxicologist was pursued.

Further information gathered later in the course of the investigation revealed that in October 2009, a batch of Bromo-DragonFLY, purchased from Denmark, was distributed as the less active compound 2C-B-FLY, with a packaging label of "batch b1," one of which was purchased by the decedent's brother. Toxicological analysis specifically for 2C-B-FLY and Bromo-DragonFLY was undertaken. The analytical technique used for this work was gas chromatography/mass spectrometry (GC/MS). 2C-B-FLY was not detected in any of the specimens. Only Bromo-DragonFLY was detected in each of the specimens at the following concentrations: in peripheral blood 22 nanog/mL; in gastric fluid 38 nanog/mL; in urine 28 nanog/mL; and in bile 350 nanog/mL. Bromo-DragonFLY levels in beta-glucuronidase treated urine and bile were 49 ng/mL and 470 ng/mL, respectively. Review of the literature revealed one paper from Denmark in 2009 of an 18-year-old woman who died of a fatal Bromo-DragonFLY overdose and the reported femoral blood concentration was 4.7 ng/mL (MF Andreasen et al., 2009).

Since October 2009, rare lethal overdoses were reported from the distributed batch and to our knowledge this case represents the only United States fatality resulting from Bromo-DragonFLY. The decedent's brother and his girlfriend were admitted to the hospital for observation, and luckily recovered from their drug trip, although both were experiencing effects of the drug hours later. Both parties reported the drug trip was long lasting and not a comfortable experience.

In summary, this case illustrates the combined efforts of the medical examiner-coroner office and the toxicologist to identify the substance which led to the sudden death of a young adult experimenting with a purchased, non-controlled drug from overseas. Although our case represents the only reported fatality from Bromo-DragonFLY in the United States, it serves to illustrate and emphasize the importance of the combined efforts of different agencies to help render a cause and manner of death.

Bromo-Dragonfly, Overdose, Drug Expermientation

#### G115 Sudden Unexpected Infant Death: Lymphocytic Meningoencephalitis With Multiple Retinal Hemorrhages

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After attending this presentation, attendees will learn how multiple retinal hemorrhages extending to the ora serrata are not diagnostically specific for abusive head trauma (shaken baby syndrome).

The presentation will impact the forensic science community by expanding the list of conditions in infants associated with multiple retinal hemorrhages that extend to the periphery of the retina.

This presentation will inform attendees of something they do not know–multiple retinal hemorrhages, involving the peripheral retina and extending to the ora serrata in infants, are not specific for abusive head trauma (shaken baby syndrome). Numerous authors contend that specific ophthalmological findings in infants and young children with abusive head trauma (AHT) include numerous retinal hemorrhages that extend far into the periphery of the retina. Listed non-traumatic causes of retinal hemorrhages coagulopathy, sepsis, meningitis, vasculopathies, increased intracranial pressure, and cardiopulmonary resuscitation—reportedly do not result in the multiplicity and peripheral distribution of the hemorrhages associated with AHT.

This case presents a 7<sup>3</sup>/<sub>4</sub>-month-old male infant with multiple retinal hemorrhages extending to the ora serrata who died suddenly and unexpectedly from severe, diffuse lymphocytic meningoencephalitis. He had had rhinorrhea and upper respiratory congestion for about two weeks. His mother had been giving him acetaminophen every four hours. Otherwise, he had been healthy. He was placed down for a nap around 2:30 p.m. and was found unresponsive at about 3:00 p.m. Resuscitative efforts were begun immediately. A call was made to 911 at 3:05 p.m. and EMS arrived at 3:13 p.m. He was transported to the emergency department (ED) and arrived at 3:35 p.m. He was pronounced dead at 4:07 p.m. following 32 minutes of resuscitative efforts in the ED.

The medicolegal autopsy was performed 17 hours after he was pronounced dead. There was no evidence of trauma, skull fractures, intracranial hemorrhages or injury of the brain or spinal cord. Microbiological cultures of blood, trachea and lung were noncontributory. A skeletal survey did not reveal any evidence of acute or healing fractures. Subsequent toxicological analysis did not detect any licit or illicit drugs that caused or contributed to his death.

Postmortem monocular indirect ophthalmoscopy detected multiple retinal hemorrhages. The fundal hemorrhages in the left eye were over the posterior pole extending past the equator and abutting the ora serrata in all four quadrants; three small retinal hemorrhages were in the right globe.

His calvarial *dura* was smooth with areas of hyperemia and congestion of dural vessels, but no subdural extravasated blood or membranes were present. The dural venous sinuses were patent and the leptomeninges had no areas of extravasated blood. The cerebrum had a well-defined grey-white junction with no lesions in the cortex, white matter, or subcortical nuclei. The cerebral ventricles were of normal caliber and the ependymal lining of the ventricles appeared normal for age. The brainstem was normally developed with no gross abnormalities. The cerebellum exhibited normal folia, white matter, and dentate nuclei. The spinal cord had no areas of hemorrhage or exudates.

Microscopically, the cerebrum, brainstem, and cerebellum showed a multifocal lymphocytic infiltrate with numerous microglial nodules and neuronophagia. The inflammatory process involved the cerebral grey and white matter (including the basal ganglia), brainstem grey matter, and cerebellar white matter. The brainstem involvement was diffuse, with inflammatory foci in the midbrain, pons, and medulla; the spinal cord was not involved. No viral inclusions or areas of necrosis were seen. There was lymphocytic involvement of the cerebral leptomeninges and small perivascular lymphocytic collections were just deep to the ependyma. No significant ventriculitis was present. Immunohistochemical (IHC) stains for CD4 and CD8 showed a multifocal T-cell inflammatory infiltrate within the cerebral parenchyma and around blood vessels. An IHC stain for CD20 highlighted a smaller number of B-cells around blood vessels and within the parenchyma. The ICH stain for CD68 highlighted the microglial nodules and IHC stains for CMV, HSV-1, and HSV-2 were negative. The Centers for Disease Control performed IHC testing for panentero viruses and EV71 plus polymerase chain reaction (PCR) for enterovirus and parechovirus-all were negative.

Meningoencephalitis is a rare complication of common infantile viral infections. Most viral infections with central nervous system manifestations cause either meningeal involvement, namely aseptic meningitis, or a mild clinical syndrome of meningoencephalitis rather than a fatal form of encephalitis. The causative agent in this case was not apparent despite IHC and PCR testing for enteroviruses and parechoviruses. Of particular interest, this infant had numerous retinal hemorrhages in the left globe distributed posteriorly, equatorially, and peripherally—a finding considered by many authors unique to AHT and indicative of repetitive acceleration-deceleration injury (shaken baby syndrome). It is imperative that forensic pathologists not equate multiple retinal hemorrhages with a peripheral distribution exclusively with AHT. Postmortem ocular findings must not be interpreted in isolation, but correlated with the circumstances of the death plus the anatomic and histopathologic findings.

Retinal Hemorrhages, Lymphocytic Meningoencephalitis, Abusive Head Trauma

### G116 Parietal Pseudofracture in Children Suggesting Non-Accidental Trauma: A Report of Two Cases and Review of the Literature

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After attending this presentation, attendees will learn the characteristics of variation in sutures of the pediatric skull that may make it difficult to distinguish from real fractures based on imaging criteria alone.

This presentation will impact the forensic science community by emphasizing the legal complications caused by mistaking normal variation of the pediatric skull for fracture and by aiding in better understanding of these pseudofractures of the skull in infants.

**Introduction**: Two cases of infants who died at home are reported. Both were previously healthy and had no history of trauma according to the parents. As in all suspected cases of SIDS, a complete autopsy was performed. For both cases, radiographic or computed tomography (CT) scan findings were initially interpreted as parietal fractures and raised the possibility of non accidental trauma.

Case reports: The first case was a 3-month-old female child who was found dead at home by her mother. An autopsy was performed the same day. A skeletal radiographic survey showed two linear radiolucencies in the parietal region mimicking a fracture of the right parietal bone. At autopsy there was an H-shaped abnormality of the right parietal bone with no associated soft tissue swelling. The brain was normal. There were severe pulmonary lesions and a test for the respiratory syncytial viral antigen was positive. Histological sections of the parietal bone showed two vertically unossified membranous strips linked by a horizontal membranous strip. Death was attributed to pulmonary infection. The second case was a 6-month-old male child who was found dead at home by his mother. An autopsy was performed. A bone window CT scan showed a linear defect in the left parietal bone. At autopsy, no scalp swelling or bruising was noted. The rest of the autopsy was normal. Microscopic sections of the decalcified parietal bone demonstrated neither inflammatory infiltrate nor periosteal reaction. The findings were consistent with an unossified membranous strip. Cause of death was not identified.

**Discussion**: According to the literature, the parietal bone is the most common fracture site in children, in both accidental and non-accidental trauma. However, an extensive study of the embryogenesis of the parietal bone was made by an author who discovered a variety of anomalous parietal suture, described as failure of ossification of a strip of membranous parietal tissue. These normal variations or pseudofractures are rare and may simulate skull fractures, especially in live infants when histological examination is not available. Overlooking

a fracture of the pediatric skull is a serious situation, but to mistake normal variation for a skull fracture may cause legal complications as well. Awareness of differential diagnosis such as vascular markings, sutures, and artifacts that may masquerade radiographically as skull fractures in infants is essential for the forensic pathologist.

Membranous Unossified Strip, Skull Fracture, Non-Accidental Trauma

#### G117 Methadone and Cocaine Related Death in A Young Boy: A Case Report

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After attending this presentation, attendees will learn of the possibility of cases where a synergic effect of cocaine and methadone could be assumed as the cause of death.

This presentation will impact the forensic science community by the discussion regarding pharmacokinetic and pharmacodynamic drug interactions between cocaine and methadone.

**Background and Learning Objective:** In recent years, a significant increase in the number of fatal intoxications with methadone has been reported in Italy. The abuse of methadone is most frequently seen in conjunction with the abuse of other drugs. Cocaine and methadone are rarely co-intoxicants in cases of combined drug toxicity. The interpretation of blood methadone concentrations alone or combined with other psychoactive drugs requires careful and accurate consideration of the subject's potential chronic use of and tolerance to the drug. Moreover, determining the cause of death in methadone and cocaine positive cases requires a strong correlation with autopsy results and investigative findings. The goal of this study is to discuss the possible mechanisms and eventually the synergic effect of cocaine and methadone in causing the death of a young boy.

**Case Report:** A 15-year-old young man was found dead during the early morning in his bed at home. Police investigations ordered by the public prosecutor revealed that the young boy, the night before, had used cocaine and methadone for the first time.

**Results:** At the autopsy, lungs were edematous and congested with absence of major diseases. Main findings at the histological investigation were widespread myocardial interstitial edema and focal vascular congestion. Toxicological analysis detected cocaine, methadone, and related metabolites at the following concentrations. **Blood:** *benzoylecgonine* = 50 nanograms/ml; *cocaine*=40 nanograms/ml; *methadone*=274 nanograms/ml; EDDP = 166 nanograms/ml. **Urine**: *benzoylecgonine* = 9000 nanograms / ml, *cocaine* = 153 nanograms / ml *methadone* = 300 nanograms/ ml; *EDDP* = 200 nanograms/ml. Traces of cocaine were also found in the nasal mucosa.

**Conclusions:** It is well known in forensic field that it can be very difficult to determine what mechanism(s) are responsible for drug interactions especially in cases as such, where the deceased cannot be considered as habitual drug-user. Moreover, it should be taken into account that the presence of methadone is often an incidental finding during postmortem examination which is unrelated to the cause of death and that postmortem measurements of methadone or its metabolite cannot be used in isolation to identify which deaths are associated with methadone toxicity. Very little information is available from the literature regarding methadone-cocaine co-intoxications. In our case we can only hypothesize an interaction between these drugs on different organs, such as heart and central nervous system. Pharmacokinetic and

pharmacodynamic drug interactions mechanism and the possible explanation in determining the cause of death in this case will be discussed.

Methadone, Cocaine, Synergic Effect

# G118 Sudden Death Due to Dengue Fever in an 8-Month-Old Baby

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The goal of this presentation is to present a case of postmortem diagnosis of dengue related death in a suspect sudden infant death syndrome.

This presentation will impact the forensic science community for the postmortem diagnosis of dengue fever like cause of death in a suspect case of SIDS.

Dengue virus (DENV) infection is caused by one of four antigenically distinct but related single stranded, positive-sense RNA viruses in the family Flaviviridae. This virus is transmitted by mosquito vectors, primarily Aedes aegypti. Four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) circulate worldwide. Dengue fever is one of the most significant re-emerging tropical diseases; it is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific. South-East Asia and the Western Pacific are the most seriously affected. Dengue causes a severe flu-like illness and sometimes a potentially lethal complication called dengue hemorrhagic fever (DHF). Dengue hemorrhagic fever (DHF) is a potentially deadly complication that is characterized by high fever, often with enlargement of the liver, and in severe cases circulatory failure. The illness often begins with a sudden rise in temperature accompanied by facial flush, and other flu-like symptoms. The fever usually continues for two to seven days and can be as high as 41°C, possibly with convulsions and other complications. Frequently fatal cases of dengue death occur in the hospital. The clinical features of dengue fever vary according to the age of the patient. Infants and young children may have a fever with rash. Older children and adults may have either a mild fever or the classical incapacitating disease with abrupt onset and high fever, severe headache, pain behind the eyes, muscle and joint pains, and rash. Some cases develop much milder symptoms which can be misdiagnosed as influenza or other viral infection when no rash or retro-orbital pain is present. When dengue infections proceed to DHF symptoms, DHF causes vascular leak syndrome which includes fluid in the blood vessels leaking through the skin and into spaces around the lungs and belly. This fluid loss and severe bleeding can cause blood pressure to fall, then Dengue Shock Syndrome (DSS) sets in, which has a high mortality rate. In babies a pauci-symptomatic fatal case could be confused with a SIDS or a homicide. The case presented concerns an 8month-old male infant was found unresponsive during a nap in his nursery school. The baby was quickly taken by ambulance but was declared dead on arrival at the hospital. Body was cold. The police took information by the nursery school teacher: three hours prior to death, the child was given plain water through a bottle before being put to sleep on a mattress on the floor, the baby frequently slept in prone position. The infant had been cared for by the nursery school since the age of three months. There was a history of mild fever illness for the previous weeks before the death and he was being treated with antipyretic drugs. The prosecutor began an investigation of the nursery school, arranged the autopsy on the body to clarify the exact mechanism of death: SIDS, accident, or homicide? The autopsy was performed six hours after death. The infant was well hydrated and well nourished, with body length of

68 cm and weighed 6920 g. He was pale with mild peripheral cyanosis

noted. Faint lividity was still noticeable at front part of body. There was blood-stained fluid oozing out of the nostrils and mouth on turning the body. There wasn't a rash or petechial hemorrhages on the skin. Fundal ophthalmoscopic examination didn't show retinal hemorrhage. No signs of external injury were detected, with the exception of puncture marks at the dorsum of both hands in a temptative of resuscitation. All the internal organs were congested. Pleural cavities contained 12 cc of yellow fluid. The lungs were edematous with areas of hemorrhage mainly seen on the right side. The upper airway was filled with froth and admixed with blood-stained fluid. Pericardial cavity contained 2 cc of yellow fluid. The heart showed few epicardial petechiae. Abdominal cavity contained 15 cc of yellow fluid. Stomach was empty. Liver was congested and had beefy appearance on cut sections. Other organs were unremarkable except of edema. Histopathologic examination showed in heart samples wide foci of early contraction bands necrosis, colliquative myocytolysis grade II, perivascular and interstitial infiltration of lymphocytes, monocytes and plasmacells. Lungs present alveolar septa mildly thickened by edema and capillary congestion, alveolar edema; lymphocytes, monocytes and plasma cells infiltrates septa and bronchial walls. In some fields, also numerous endoalveolar erythrocytes were observed. In liver, kidney, and spleen samples, there were perivascular mononuclear cells infiltration. An immunohistochemical study using antibody anti CD 3, CD4, CD 8, CD 20 and CD68 for the tipization of leucocytes infiltrations was performed. Serological dengue screening using captured ELISA was positive for IgM but negative for IgG. The case showed that dengue infection may be asymptomatic or paucisyntomatic before a sudden death, so dengue fever should be included in the differential when a forensic pathologist must discern between a SIDS a homicide or a death related-dengue, particularly in endemic areas for dengue, like Malaysia.

Dengue Fever, Histological Findings, Postmortem Diagnosis

#### G119 Sudden Death in the First Year of Life: The Importance of Pancreatic Histomorphological Analysis

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After attending this presentation, attendees will be able to better understand the importance of pancreatic histomorphological examination after a complete autopsy in sudden infant death, to discriminate between the explained natural sudden death and natural idiopathic sudden death (SIDS).

This presentation will impact the forensic science community by discussing the role of endocrine/metabolic pancreas disease as cause of sudden infant death, providing valid evaluation parameters to the diagnosis.

**Material and Method:** From 1990 to 2009, 175 autopsies (137 males and 43 females) were performed on infants suddenly dying of natural causes within the first year of life. The diagnosis of sudden natural death has been established through a complete autopsy and investigation including scene examination, review of family, social and medical history and toxicology studies. The age ranged between 2 and 273 days (median 42 days).

In 19 cases, death was unexplained (SIDS) while in 156 cases, it was due to congenital or acquired diseases (explained sudden infant death), primarily involving different systems: cardiovascular (121

cases); respiratory (15 cases); endocrine/ metabolic (12 cases); gastrointestinal (4 cases); central nervous system (4 cases).

Autopsy protocol were based on Perinatal Autopsy Manual. Washington, D.C.: Armed Forces Institute of Pathology, 1983, and on Paediatric Autopsy Techniques - Enid Gilbert-Barness and Diane E. Debich-Spicer in Handbook of Pediatric Autopsy Pathology, Humana Press pp.7-74, 2005. From a histological point of view, according to 2,875 fetal and pediatric autopsies, the following morphological pancreatic parameters were examined: (1) lobular architecture; (2) interstitial thickness; (3) number, branch and volume of ductular-acinar units; (4) number, size and cytology arrangement of islet (quantitative relation between  $\alpha$ ,  $\beta$ , and  $\delta$  cells); (5) inflammatory infiltrates; and, (6) heterotopic erythropoiesis.

This analysis was performed on serial sections stained with hematoxylin-eosin, Alcian-PAS, Mallory's trichrome and Giemsa and partly investigated immunohistochemistry using antibodies anti-insulin and anti-glucagon.

**Results**: In the context of explained sudden natural death in the first year of life, pancreatic histological examination has allowed us to identify 11 cases related to endocrine/metabolic disease, of which, in nine cases, were interested the islets of Langerhans (endocrinous pancreas), and in two cases ductular-acinar units (exocrine pancreas).

The endocrine/metabolic diseases involving endocrinous pancreas were: glycogenosis (type 1b and 2)(five cases); maternal diabetes (2 cases); nesidioblastosis (2 cases (1 case in monochorionic twin)). The endocrine/metabolic diseases involving the exocrine pancreas were: cystic fibrosis (2 cases) macronesia and polynesia were observed in pancreas of both patients with glycogenosis and in children of diabetic mothers, these aspects were due to hyperplasia of the  $\alpha$ -cells in patients with glycogenosis, and  $\beta$ -cell hyperplasia in children of diabetic mothers.

In these cases also present were cytoatipism of  $\beta$ -cell and eosinophilic granulocyte infiltration of the islets. In subjects with nesidioblastosis there was only a diffuse polynesia neoformation of islet from duct epithelium.

The cystic ectasia of the ductular-acinar structures associated with pink inspissated secretion was observed in cystic fibrosis.

**Discussion and Conclusion:** A complete autopsy is essential to establish the causes of sudden explained death in the first year of life. This approach allows to sample, for histomorphological examination, organs such as the pancreas that are almost always free of macroscopically visible changes.

The results of the study show that, in addition to the consolidated sampling of pancreas in autopsy, a complete and focused histomorphological study as suggested, allowing the identification of endocrine-metabolic anomalies, such as glycogenosis, nesidioblastosis, and cystic fibrosis, only rarely reported in the literature as a cause of sudden death in the first year of life.

This research demonstrated that, glucose postmortem levels in plasma and vitreous are not reliable for identifying potential endocrinemetabolic diseases, certainly the histomorphological data of the pancreas is the most reliable.

Glycogenosis, Nesidioblastosis, Sudden Infant Death

#### G120 Isolated Coronary Anomalies and Sudden Death in the Young

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After attending this presentation, attendees will be able to better understand the importance of coronary examination (origin, course, and lumen canalization) during autopsy of young people suddenly dead, to identify coronary artery anomalies.

This presentation will impact the forensic science community by improving knowledge of natural causes of juvenile sudden death (SD).

**Background:** Coronary artery anomalies (CAAs) had been some of the most confusing, neglected topics in cardiology. In the 1990s, the subject of CAAs underwent to profound evolution related to substantive methodological changes regarding the definition, incidence, morphology, clinical presentation, diagnostic work-up, prognosis, and treatment. CAAs are most frequently found in association with congenital hearth diseases (great arteries transposition, tetralogy of Fallot, common artery trunk) and hypertrophic or dilated cardiomyopathy, but can also occur in the absence of other cardiovascular diseases ("*isolated coronary artery anomalies*"). Isolated CAAs are rare, found in: 0.2-2.2% of autopsies of all ages; 0.5% of pediatric autopsies; 0.6-1.3% of coronary angiograms in adults. CAAs represent common causes of exercise-related sudden death (SD) in young people ( $\leq$  35 years of age). The mechanism of SD is believed to be episodic myocardial ischemia.

**Objectives:** The goal of the current study is to detect the frequency, type, and possible pathophysiology due to "isolated" CAAs in an autopsy population of the young, suddenly dead for cardiovascular diseases (CSD).

**Methods:** In the time interval from January 1990 to December 2009, 236 consecutive cases were collected of cardiovascular sudden death in young people. In all of the cases, the analysis of death circumstance (*most of the deaths analyzed were testified*), the complete autopsy and the toxicological essays let us establish that death was: natural, violent, sudden. The juvenile CSD was defined as unexpected death as a result of natural cardiovascular causes within one hour of initial symptoms in persons  $\leq$  35 years of age.

Results: Forty nine sudden deaths in young people were reported, identified solely at autopsy and due to: right coronary artery from the left sinus (n = 15); right coronary artery above anterior commissure (n = 11); left coronary artery from right coronary sinus (n = 3); intramyocardial course (n = 10); obstructive valve-like ridge in the Valsalva's sinus and intra-right coronary ostium (n = 9); left anterior descending artery from right coronary sinus (n = 1). The CAAs was either isolated (n = 43,87.8%) and associated to hypertrophic cardiomyopathy (n = 6, 12.2\%). In all patients (43 males and 6 female, age ranging from 13 months to 35 years; median, 22.6), sudden death was the first manifestation of the disease and familial history was negative. The fatal outcome occurred after physical effort (n = 27, 55%), at rest (n = 16, 32.6%), or after emotional stress (n = 6, 12.4%). Unquestionable ischemic damage within the related myocardium, in the absence of obstructive coronary atherosclerosis or other cardiac diseases, was observed in all cases: acute myocardial infarction (n = 29, 59.2%), healing myocardial infarction (n = 4, 8.2%), healed myocardial infarction (n = 16, 32.6%). In this study of Juvenile CSD, death was precipitated by isolated CAAs in 21% of cases.

**Conclusion:** Data from this collection confirms that isolated CAAs may account for juvenile CSD and that fatal event is frequently the first

manifestation of the disease, it is precipitated by effort and depends on ischemic damage within the related myocardium. Recognition during life of these anomalies, by the use of non-invasive procedures, is mandatory to prevent the risk of SD and to plan the screening in competitive athletes.

Coronary Artery Anomalies, Juvenile Sudden Death, Forensic Pathology

### G121 Pathologic and Anthropologic Manifestations of Documented Repetitive Blunt Trauma in a Child Abuse Case

Pramod Gumpeni, MD\*, Jason M. Wiersema, PhD, and Luis A. Sanchez, MD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will see the pathologic and anthropologic manifestations of repetitive blunt trauma to the ribs of child.

This presentation will impact the forensic science community by illustrating the utility of a collaborative effort in the interpretation of repeated injury.

The child had been in the care of the birth mother for the first six years of his life and had reached all appropriate mental and physical developmental milestones. The mother placed the decedent in the care of the decedent's father (with whom the child had no prior contact) fifteen days prior to his death in the interest of fostering a paternal relationship.

The decedent presented to the Harris County Institute of Forensic Sciences (HCIFS) following his demise at a local hospital. The terminal history, provided to the HCIFS investigator by the father's girlfriend was that the decedent had been repeatedly beaten about the chest by the father for an approximate 8-hour-period. The beating was apparently precipitated by the child's refusal to go to sleep. The father reported that the child began exhibiting seizure-like activity after which emergency medical services were contacted. The unresponsive decedent was transported to the hospital, where he was pronounced six minutes after arrival.

The birth father and his female acquaintance ultimately confessed that the child was beaten in a similar manner for the duration of the two week period during which he was in their custody. They stated that the decedent would be made to sit on his knees, with his arms held up while the father would repeatedly punch him in the left axilla and chest. After several days, the child exhibited pain, and the father began punching the decedent in the right axilla and back. In addition, the female acquaintance admitted to the use of a belt to strike the decedent on the back. The father ultimately stated that he pushed the decedent forcibly into the shower, striking the back of the decedent's head against the wall immediately prior to the onset of his seizure-like symptoms.

Autopsy of the child showed numerous confluent contusions over the decedent's torso, predominantly in the left and right axilla extending down to the flank, the upper chest, and over the mid and lower back. There were numerous abrasions over the extremities. Several contusions were present over the scalp, with brain contusions identified on internal examination. A large fibrous mass lesion consisting of resolving hematoma, granulation tissue, and early callous formation was found in the left upper axilla involving the anterior bodies of left ribs 2-4. There were also bilateral pleural effusions, with 550 cc of serosanguinous fluid in the left pleural cavity, and lacerations of the liver and right adrenal, with marked retroperitoneal hemorrhage. Initial x-rays of the chest showed a heterogeneous mass lesion in the left upper chest.

Per HCIFS protocol, the decedent underwent a complete skeletal examination in search of skeletal injury. This process involved resection of the muscle and periosteum overlying the long bones, ribs, and scapulae. Skeletal trauma was limited to the ribs, and the rib cage was recovered for anthropological analysis. There were multiple series of serial rib fractures displaying morphology consistent with direct impact(s) at the site(s) of the fractures. The fractures were in varied stages of healing, ranging from acute fractures with sharp margins and no visible callus formation to the presence of large, disorganized soft calluses overlying complete transverse fractures. The array of skeletal injuries was consistent with repeated impacts to the anterior and lateral chest.

This case provides a unique view of the effects of repetitive blunt injury directed to specific regions of the body over the span of two weeks, and the physiological consequences of such trauma to both bone and soft tissue. It also illustrates the utility of a detailed terminal history in the interpretation of blunt force injury.

**Repetitive Injury, Blunt Trauma, Child Abuse** 

#### G122 Pregnancy, Caesarean, and Pheochromocytoma: A Case Report With a Fatal Outcome

Isabelle Plu, PhD\*, Denis Barrès, MD, and Dominique Lecomte, PhD, Institut Médico-Légal, 2 place Mazas, Paris, 75012, FRANCE

After attending this presentation, attendees will be aware of the clinical, physiological, diagnostic, and therapeutic features of pheochromocytoma during pregnancy.

This presentation will impact the forensic science community by explaining how pheochromocytoma can cause sudden death (maternal and fetal death) in pregnant women if the condition is undiagnosed and left untreated. From this case report, attendees will be able to identify the clinical milestones which can indicate medical malpractice and which can determine whether the fatal outcome was predictable.

The subject of this presentation is a 43-year-old full term pregnant woman who was scheduled for a cesarean delivery in October 2009. At the start of the C-section, she developed a sudden and malignant high blood pressure with hemoptysis, sweat, and tachycardia. The C-section delivered a dead newborn who was successfully resuscitated. The mother died after persistent cardiac arrest.

A judicial autopsy was requested. It revealed an acute pulmonary edema which explained the death and a voluminous tumor of the left adrenal gland which was necrotic and hemorrhagic suggesting a pheochromocytoma. The diagnosis of pheochromocytoma was confirmed by pathological analyses.

The magistrate requested the obstetrical records of the patient be studied. The patient's first pregnancy was in 2005-2006 and her last pregnancy was in 2009. According to these medical records, the woman had a no serious medical history:

- During her first pregnancy (2005-2006), the patient developed gestational diabetes mellitus which was successfully treated by controlling diet. Throughout the follow-up during the pregnancy, blood pressure and cardiac rhythm were stable. At 31 weeks of pregnancy + 5 days, the patient had an episode of malaise during a fetal ultrasound, which resolved spontaneously.
- The baby was delivered by C-section before labor in January 2006. C-section was performed because of low fetal heart rate and failure to induce labor. The newborn was healthy and the patient's diabetes mellitus disappeared after the delivery.
- During her second pregnancy (2009), the patient developed gestational diabetes mellitus which was treated by controlling her diet and insulin (16 weeks of pregnancy). In July 2009 (at 29 weeks of pregnancy + 6 days), the patient had a drop in blood pressure with hypoglycemia and a low fetal heart rate was detected. The patient was admitted to hospital for further

investigations and medical supervision for three days. All medical investigations were normal and all abnormalities disappeared spontaneously. A delivery by C-section was scheduled at 37 weeks of pregnancy because of previously scared uterus and gestational diabetes mellitus. The woman was admitted to the hospital one day before. The C-section delivered a dead newborn who was successfully resuscitated. The mother died after resistant cardiac arrest.

This case is interesting from both a medical and medico-legal point of view.

Pheochromocytoma is a rare tumor of the adrenal glands which secretes catecholamine. It can be diagnosed by the classic triad of symptoms —headache, sweating, and tachycardia— which result from arterial hypertension, paroxysmal high blood pressure, acute pulmonary edema, and fatal cardio-pulmonary failure. In pregnant women, the incidence of pheochromocytoma is very low, and its symptoms can mimic gestational hypertension, preeclampsia, or eclampsia. Diabetes mellitus can be due to pheochromocytoma in pregnancy, but is seldom the only symptom. Because of the low incidence of pheochromocytoma in pregnancy, any systematic/mass screening by urinary catecholamine measurement is not requested in pregnant women, except in cases of refractory hypertension.

From a medico legal point of view, we can presume, with hindsight, that the gestational diabetes mellitus was a symptom of the pheochromocytoma, as was the malaise and the low blood pressure which happened during the gestations. However these features are not specific to pheochromocytoma, and are frequent in pregnancy, which explains the difficulty in diagnosis.

**Conclusion:** This case is unusual. First, it led to maternal death; and, second, the diagnosis of the tumor was postmortem, being unnoticed during the management of pregnancy. It can also presumed that the pheochromocytoma was asymptomatic between the two pregnancies of the patient since no medical history was reported in her medical records.

Pheochromocytoma, Cesarean, Maternal Death

# G123 Infant Death Evaluation: What is the Constellation of Abusive Injuries?

M.G.F. Gilliland, MD\*, Brody School of Medicine at East Carolina University, Pathology & Lab Medicine, Brody 7S-10, Greenville, NC 27858-4354

After attending this presentation, attendees will be able to identify components of a constellation of abusive injuries that can be used to reliably identify a subset of abusive injuries.

This presentation will impact the forensic science community by providing knowledge of components of a constellation of abusive injuries that can be used to reliably identify a subset of abusive injuries allowing them to more competently perform determinations of cause and manner of death.

**Hypothesis:** No single finding is pathognomonic of abusive injuries to infants and children. Findings suggestive of abusive injuries must be used in conjunction with other information to reliably determine that a death is the result of abusive injuries. Additional investigative information about the reliability or number of histories provided by caregivers has been described as useful in this regard. Investigative information about delays in seeking medical attention has been seen more commonly in abusive injuries.

**Materials:** Information about the circumstances surrounding collapse or death, medical treatment, past medical history, law enforcement investigation, and social service information (when available) was used in a prospective study of 169 child deaths with autopsy and postmortem ocular examinations to make cause and manner determinations. The patterns of ocular and systemic injuries in children

dying as the result of non-accidental injury were compared with those found in injuries from motor vehicle accidents, falls, asphyxia and in natural disease. The immediate causes of death included: 76 (45%) intentional injuries, 36 (21%) inadvertent injuries, 47 (28%) natural causes, and 10 (6%) undetermined causes.

**Results:** The triad of findings of subdural hemorrhage, brain edema, and retinal hemorrhages was seen in 47 of the total 76 (62%) non-accidental injury deaths and in eight inadvertent injury deaths of the total 36 (22%). The triad was not seen in any of the 46 natural deaths or any of the ten classified as undetermined deaths. Treating these three findings (the "triad") as a "laboratory test" to identify abusive injuries did not meet criteria for reliability of diagnosis. The sensitivity of the presence of the triad was only 62% in detecting non-accidental injuries. The specificity of the absence of the triad in inadvertent injuries was 78%.

Histories of the circumstances of change of status have been important in identifying abusive injury. The original recognition of the "battered baby" followed inquiry into the phenomenon of absent or changing histories in the presence of subdural hemorrhages and extremity fractures. In this population, the sensitivity of finding inconsistent histories with the presence of the triad was 80%. The negative predictive value of finding a consistent history when the triad was absent was 88%. The relative risk of the triad being found with an inconsistent history was 4.56 with confidence limits of 2.53-8.20 and a-value << 0.01.

Delay in seeking treatment has also been identified as a marker of abusive injuries. In this population information was available to identify the interval between onset of symptoms and presentation for medical attention in 127 deaths. This information was then used to look at the deaths with the triad of retinal hemorrhages, subdural bleeding, and brain swelling.

Triad	< 24 hours	24-72 hours	> 72 hours	Total
No triad	55	14	4	73
Triad	38	13	3	54
Total	93	27	7	127

Additional investigative information was used to determine the cause and manner of death to distinguish abusive injuries from accidental injuries. Among children having the triad, delay in seeking treatment was only seen with abusive injuries.

Manner	< 24 hours	24-72 hours	> 72 hours	Total
Abusive	30	13	3	46
Non-abusive	8	0	0	8
Total	38	13	3	54

**Summary:** Deaths with an inconsistent history, delay in seeking medical attention, and autopsy findings including the triad of subdural hemorrhage, brain edema, and retinal hemorrhages can be reliably used to identify deaths which are more likely to be the result of inflicted injury. Thorough investigation and complete autopsy findings must be used to establish whether or not a particular child's death was caused by inflicted injuries.

Review of the findings and investigative information in this study allows identification of a constellation of reliable markers of abusive injuries, and components of the constellation needed to avoid wrongful accusations of injury. The components include: triad of retinal hemorrhages, subdural bleeding, and brain swelling; inconsistent or multiple histories; and delay in seeking medical attention.

Abusive Injuries, Wrongful Accusation, Child Deaths

#### G124 Utility of Whole Body Postmortem Computed Tomography Imaging in Detection of Elder Abuse: Comparison With and Potential Substitution for Standard Autopsy

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After attending this presentation, attendees will be familiar with the value of CT Imaging in the detection or exclusion of injuries associated with elder abuse.

This presentation will impact the forensic science community by demonstrating how whole body CT imaging can be an efficient triage tool that assists the medical examiner's decision on whether to proceed to conventional autopsy in suspected cases of elder abuse.

Elder abuse (EA) contributing to death is a crime that is difficult to exclude without a full conventional autopsy (CA), even where allegations of abuse are limited to non-physical issues. The potential for use of whole-body postmortem CT (PMCT) as a triage tool to determine the need for CA based on detection of injuries suggestive of physical abuse was investigated.

**Method and Materials**: Fifty-two decedents (12 M, 40 F; mean age 76 y, range 52-93 y) with associated allegations of EA had PMCT and subsequent CA by state medical examiners within 24 hours of death. PMCT scans were interpreted by radiologists experienced in forensic imaging. Sensitivity of PMCT for injuries suspicious for abuse and other major findings were determined with CA as the standard of reference.

**Results:** PMCT was concordant with CA for evidence or absence of elder abuse in all cases. PMCT demonstrated multiple previously unreported fractures of varying age consistent with EA in only 1/52 cases. Recent fractures consistent with cardiac resuscitation (CPR) or typical accidental trauma were noted on PMCT in 18/52 (35%) and 5/52 (10%), respectively, but at CA were undetected in 7/18 (39%) and 4/5 (80%), respectively. PMCT misinterpreted an undisplaced cervical fracture in the setting of severe degenerative disease. Cause of death was determined by PMCT in 24/52(46%) and by CA in 50/52(96%) cases.

**Conclusion:** PMCT is reliable for the detection or exclusion of skeletal injuries suspicious for elder abuse and may be used in correlation with history and external examination to determine the need for CA where allegations or suspicion of abuse are raised. Acute upper anterior bilateral rib fractures were noted in all decedents who underwent full CPR, likely related to osteopenia/osteoporosis. PMCT was not reliable for determination of specific cause of death in this decedent group.

Elder Abuse, Computed Tomography, Autopsy

#### G125 Findings of the Examinations of Suspected Animal Cruelty Cases Submitted to the Birmingham Jefferson County Animal Control

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After attending this presentation, attendees will understand some of the basic principles of the elements necessary in the investigation of animal cruelty, including characteristic injury patterns and examples of the practical application of comparative forensic pathology.

This presentation will impact the forensic science community by shedding new light on an old technique by showing how both macroscopic and microscopic injuries are a key aspect of an animal cruelty or animal abuse investigation. The techniques developed in this project have the potential to be applied in crime profiling to track animal abuse, which may be a predictor for child abuse or domestic violence.

The objective of this project was to establish routine performance of medical examinations, necropsies, and histopathology as the first step in establishing a solid case of animal abuse.

Comparing the changes in the morphology of the lesions observed in this study with the ones that are in current forensic pathology provided a unique opportunity to record the differences between human pathology and animal pathology. The increase in knowledge in the field of forensic veterinary medicine gives this study merit, because those differences are currently underdeveloped in veterinary science.

In general pathology, it is assumed that humans and animals often exhibit similar physiopathology. For example, in gunshot cases, there are many similarities in the entrance and exit wounds in humans and animals. However, differences occur because of the animal's fur, which can hide a wound, and the structure of the blood vessels, which can change the bleeding patterns. In the case of a dog with an embedded collar, there will be edema in the cervical area above the collar. By combining the principles of general pathology with special veterinary pathology, animal abuse can be accurately documented.

As part of this project, medical examinations, necropsies, and histopathology were performed on more than 50 animals at Birmingham Jefferson County Animal Control during the summer of 2010.

Animal Abuse, Comparative Pathology, Necropsy

#### G126 Fatal Tiger Attack on a Zoo Patron: Patterns and Types of Injuries in Large Predatory Cats

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After attending this presentation, attendees will gain a better understanding of fatal attacks on humans by large predatory cats. This presentation will impact the forensic science community by emphasizing the expected wound locations and patterns inflicted by large members of the cat and dog families.

On December 25, 2007, a four-year-old Siberian female tiger escaped its enclosure in the San Francisco Zoo and focused its attack on two young males. One of the males was fatally mauled while his companion was injured. Responding San Francisco Police Department officers came in contact with the escaped tiger and used their service pistols to subdue it.

The victim was autopsied at the San Francisco Medical Examiner's Office and showed evidence of large animal bites and crushing trauma typical for a large cat attack including biting of the posterior upper body/neck, fracturing of the neck, and "abrasion rings" surrounding the incisor wounds. Toxicology was positive for cannabinoids and ethanol.

A necropsy of the tiger was done by the San Francisco Zoo veterinarian, with pertinent body parts (head, paws, stomach contents, and tail) delivered to the San Francisco Medical Examiner's Office. The stomach contents contained no body parts, and the back claws showed evidence of shredding of the nails which most likely occurred as the tiger propelled itself up the textured cement wall of the enclosure. It was determined that the tiger was struck three times by gunfire (once in the left frontal sinus, twice in the thoracic cavity with one of the shots being the fatal strike to the heart and lung). The bullet in the frontal sinus was recovered at the San Francisco Medical Examiner's Office before impressions were taken of both the maxillary and mandibular jaws. Plaster casts of the jaws were made for metric comparison to the patterned injury of the deceased young man.

Large feline attacks on humans are quite rare, with the most common attacks by mountain lions in the western United States. This presentation will demonstrate features typical of large predatory cat attack, as well as techniques used in taking impressions of the tiger's jaws to fabricate study models. Also briefly compared will be large cat attacks to large dog attacks.

This is the first known attack by a captive large cat at a zoo in the United States on a zoo patron not in the animal's enclosure. A second documented large cat fatal mauling occurred in 2008 at the Denver Zoo on a trained keeper by a jaguar.

Fatal, Tiger, Attack

# G127 The Utility of Skeletal Examination in Recognition of Occult Skeletal Injury

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The goal of this presentation is to illustrate the effectiveness of the skeletal examination method at locating otherwise obscure fractures in children.

This presentation will impact the forensic science community by illustrating particularly to forensic pathologists the effectiveness of the skeletal examination method in the recognition otherwise occult fractures in children.

The Harris County Institute of Forensic Sciences (HCIFS) has been conducting skeletal examination, an autopsy method for recognizing skeletal fractures in children, described by Love and Sanchez in 2009, since March of 2007. The method involves incising and reflecting the skeletal muscle and periosteum overlying the long bones, scapulae, and ribs of infants and children with medical history and/or soft tissue injuries that are suspicious for inflicted trauma. The current study is a retrospective analysis of the utility of this method in the recognition of subadult skeletal injury.

The method is intended to expose occult fractures typically not recognized during standard radiograph surveys and autopsy (Love and Sanchez 2009). The traditional autopsy protocol provides good visibility of the skull and thoracic skeleton but not the appendicular skeleton. For this reason, an increase in the number of long bone fractures, particularly classic metaphyseal lesions, was expected in cases that have undergone this method, relative to those that predate it. However, a significant increase in the number of either skull or rib fractures was not expected.

Following HCIFS protocol, a skeletal examination is performed on all children with a history, or autopsy findings suspicious for inflicted injury aged three years and younger. Fifty-four total cases have undergone the procedure since March of 2007. This presentation considers only those cases that were ultimately classified as homicides with blunt injury included in the cause of death. HCIFS anthropologists have completed 41 such cases (experimental sample). These cases were compared to the same number of consecutive cases with the same criteria (41) that predate adoption of the method (control sample). All cases in the control sample underwent complete autopsies as defined in the National Association of Medical Examiners standards, but were not subjected to anthropological skeletal examinations.

Of the 41 cases that have undergone skeletal examination, skull fractures were noted in 22 (53%), compared to 20 (49%) in the control sample. Seventeen (41%) of the 41 cases in the experimental sample had thoracic fractures (ribs, vertebrae, clavicles or scapulae), compared to 16 (39%) of the 41 in the control sample. Both of these numbers are expectedly similar between the control and experimental samples. However, the number of long bone fractures recognized per case since the method was adopted has increased significantly. Long bone fractures were noted in 17 of the 41 (41%) cases in the experimental sample, as compared to 6 of the 41 (14%) in the control sample. Worth noting is the fact that the fractures noted in four of the six cases in the control sample were complete fractures mentioned in the medical records prior to autopsy. The fractures recognized in the experimental sample are predominantly classic metaphyseal lesions. In addition, both the number of cases with multiple fractures and the number of fractures per case is appreciably higher in the experimental sample than in the control sample. The average number of rib fractures identified in cases with multiple rib fractures is 13 for the control sample and 22 for the experimental sample. The average number of long bone fractures identified in cases with at least one long bone fracture is one for the control sample and four for the experimental sample.

These preliminary results indicate that skeletal examination affords the anthropologist or pathologist the means to better recognize fractures that are difficult to appreciate on radiographs, and has increased the degree to which fractures in infants and young children, particularly of the long bones can be recognized, described, and interpreted. An additional benefit of skeletal examination is that it also facilitates interpretation of the degree of healing and the forces associated with individual fractures.

Skeletal Examination, Child Abuse, Fractures

### G128 Interpretation of Anogenital Findings in Forensic Autopsy: Problems and Challenges

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The goal of this presentation is to highlight the difficulties in anogenital examination during autopsy and in interpretation of sexual violence signs.

This presentation will impact the forensic science community by raising awareness of the difficulties in establishing the medical legal diagnosis of sexual crimes on deceased victims, and to outline the importance of scientific research in postmortem genital and anal changes. Sexual violence is a current topic that has been thoroughly studied, leading to numerous publications. However, these papers deal, almost exclusively, with the study of living victims. The few publications about postmortem anogenital examination and related findings advocate that this expertise and related injuries interpretation should be similar to the one performed in living victims. However, in daily practice, the major difficulty for medical legal experts in the interpretation of sexual violence injuries, lies in the fact that currently there are no published studies allowing us to obtain a rigorous differential diagnosis between these injuries and anogenital tissues appearance in the postmortem interval caused by postmortem phenomena, like, cadaveric lividity, dehydration, and putrefaction which could lead to over or misinterpretation of macroscopic sexual violence signs in anogenital area.

Autopsy case reports of five female homicide victims, performed in the North Branch of the Portuguese National Institute of Legal Medicine between June 2009 and June 2010 are reported.

The victims' age ranged from 9 to 89 years old. In all of the cases, anogenital injuries with multiple severity degrees, from bruises to vaginal and uterus perforation, were found. Depending on the type and severity of the injuries:

- a) Two different postmortem technical approaches were performed: macroscopic anogenital examination (four cases); and abdominopelvic amputation (one case). The colposcope was not used in any of the cases and blue toluidine coloration was performed in one of the cases;
- b) Several complementary procedures were performed: toxicological, in five cases; genetic, in five cases; and histological, in three cases.
- Photographic documentation was performed in all cases.

Complementary procedures results revealed drugs intoxication in two cases, a male profile in three cases and uterus and vaginal vital laceration in one case.

In autopsy daily practice, medical legal doctors have many difficulties, especially in technical, methodological, and interpretation areas.

Postmortem phenomena, as rigor mortis often make the cadaver manipulation and positioning difficult, not allowing adequate anogenital view. Cadaveric lividity, dehydration and putrefaction phenomena could mimic sexual violence injuries, as abrasions, bruises, hematomas, among others or even hide them, leading to over or misinterpretation of macroscopic sexual violence signs.

To overcome these difficulties, autopsy should be performed as soon as possible, before washing the corpse by a medical legal doctor with expertise in sexual violence in order to prevent loss of biological evidence.

The forensic examination must follow the methodology for the same type of examination in living victims, through: the use of suitable materials, such as the speculum and anoscope; techniques for image magnification macroscopic, such as colposcopy; staining techniques, such as blue toluidine, and photographic documentation.

There must be collected histological and biological samples, in order to exclude various disorders that can mimic signs of inflicted genital trauma or sexually transmitted infection and rule out postmortem artifact and to search for heterologous biological material (DNA profile).

If evidence of trauma is found, special dissection is necessary so that the rectum, anus and perianal tissues are removed *en bloc* with the perineum, uterus, vagina and vulva being included in the female.

Given the paucity information on the nature and appearance of the anogenital tissues in the postmortem interval, the opinion here is that scientific research is essential to improve knowledge about genital anatomy and variants, sexual violence physical indicators and their lesional mechanisms, differential diagnosis and, above all, interference of postmortem phenomena in these anatomical areas.

Sexual Violence, Medical Legal Autopsy, Postmortem Interval

#### G129 When Lightning Strikes: 17 Fatal Lightning Strikes in New Mexico

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The goals of this presentation are to describe the prevalence of fatal lightning strikes, familiarize attendees with the most frequent decedent and scene demographics identified in fatal lightning strike scenes and autopsies, and have attendees recognize the importance of thorough scene investigation and full autopsy examination with histology and specific examinations in lighting strike fatalities.

This presentation will impact the forensic science community by providing data identified in lighting strikes fatalities and suggesting investigative steps to provide the most thorough scene and autopsy examination.

**Hypothesis:** There are specific demographics with lightning fatalities in New Mexico that may help identify risk factors and target a population or region for preventative measures.

**Methods:** A retrospective review of all fatal lightning strikes in New Mexico between January 1979 and December 2009 was performed using an electronic database searching the key words "lightning" and "electrocution." Cases of electrocution that were not from lightning were eliminated. Demographics evaluated included county of strike, underlying health conditions, month and time of day of strike, activities performed, toxicology, exam findings and the age, sex, and race of the decedent.

**Results:** During this 30-year time period, 17 lightning strike fatalities were identified. Fill autopsies were performed on 14 cases and three were external only examinations. The cases were distributed over 14 different counties; with the highest number of cases in a single county being two. All (17/17) of the cases were male. The majority of cases (52%) of the cases fell between 31-50 years. 52.9% (9/17) of the cases had underlying health conditions. 56% percent of the cases occurred between 2:00 p.m. and 6:00 p.m. 62% of occurred in opens spaces, roadways and parking lots. Exam findings included the classic arborizing Lichtenberg figures (35%), burns and singed hair (50%), and blunt force injuries (24%). Only four of the 14 full autopsies documented examination of the tympanic membranes. On cases where toxicology was performed (70.5%), no drugs of abuse or ethanol were detected.

**Conclusions:** Full scene investigation including weather reports, location of strike, time of day, month, activities performed, equipment used during the strike, and a thorough medical history should be collected when evaluating a fatal lightning strike. A full autopsy should include not only documentation of all external and internal injuries with evaluation of the tympanic membranes but identify natural disease. Intoxication does not appear to be a factor in the lightning deaths reviewed. To prevent lightning deaths, public service announcements in New Mexico for lighting warnings should be targeted towards males during spring and summer and emphasize the avoidance of open spaces.

The goal of this review was to compare the epidemiology of lightning strike fatalities in New Mexico to those previously described in national studies, and provide suggestions for the standardization of autopsy evaluation of lighting strike fatalities so that data may be used for prevention strategies.

Lightning, Autopsy, Prevention

### G130 Drag Racing of Snowmobiles on Asphalt: A Novel Cause for Sudden Violent Death

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After attending this presentation, attendees will learn about a summer acivity, a variation on what was previously known to done exclusively during winter, and about the fatal consequences that it lead to.

This presentation will impact the forensic science community by providing insights on a topic about which there is almost no information in the forensic literature, as this case report details the sudden violent death of a participant in timed trials of a snowmobile which was used to race on asphalt.

Snowmobiles were initially developed to move people and supplies in regions where heavy snow prohibited the use of more conventional vehicles. Today snow-mobiling is a popular wintertime recreational lifestyle activity in several parts of the world. There are millions of registered snowmobiles users and the recreation/manufacturing complex generates billions of winter tourism dollars for the snow belt areas of North America.<sup>1</sup> More than 50% of snowmobile owners surveyed consider use of the vehicle as a family sport.<sup>1</sup> With the increasing popularity of this recreational activity, there has been an increasing incidence of injuries and deaths, the inevitable consequence of human interaction with high performance vehicles. Until the tail end of the last millennium, the snowmobile remained a stationary fixture in one's garage or side of the lawn during the months of May to November. A fact that unfortunately changed in the early 1990's with the introduction of asphalt drag strip racing for snowmobiles.

Drag racing traditionally has been an acceleration contest between two car drivers, to determine which vehicle has the better speed related performance. The vehicles start from a stationary position and takeoff usually after a signal from a set of "christmas tree lights," and race on a track 1/8 or a 1/4 mile long. Drag racing started in the 1930s, when competitors raced along deserted stretches of roads to see who's vehicle was faster. The National Straightline Snowmobiling Racing (NSSR) is an organization started in 1986, to verify and certify results of dragstrip snowmobile races. In 1993, it included asphalt drag racing as one of the competitive classes that it arbitrated on.

The Empire State Timing Association has operated a Safety Park Dragstrip in the Central New York since the 1960s. It is a 1/4 mile racing strip, with a long stretch of road to allow cars to slow down. The timeslip booth provides the participants data about how long it took to get to various points down the track as well show data on how fast the vehicle was traveling at the half way point (1/8 mile) and at the finish line (1/4 mile) as well as who won the race, if it was a competition.

This case report is about a 24-year-old man who was operating a custom made asphalt snowmobile on a timed trial on the race track. He reportedly had been asphalt racing for the last four years. It was his first day of using his new sled, and he started his third trial run down the track. He was clocked at 161 mph at the  $1/4^{th}$  mile point. He unfortunately lost control of the vehicle, which struck a guardrail. He was ejected off the sled and his body came to rest in a wooded area approximately 300 feet from the initial collision point. The sled, after multiple ongoing collisions with the guardrails on either side, finally came to rest approximately 500 feet from where the body rested. The external examination, with full body x-rays, revealed devastating head injuries, despite the use of a helmet, traumatic avulsion of left forearm, open fractures of left proximal humerus, open fracture dislocation of the right ankle and closed dislocation of the left knee.

A search of the literature revealed no published information on this type of a sudden violent death.
#### **References:**

<sup>1</sup> Pierz JL: Snowmobile injuries in North America; Clinical Orthopedics and Related Research; 2003; 409; p29 – 36.

Snowmobile Accidents, Drag Racing on Asphalt, Sudden Death

#### G131 Blast Overpressure After Tire Explosion: A Fatal Case

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The goal of this presentation is to present a rare case of fatal tire explosion, the rarity of the event and the typical histopathological findings make the case peculiar.

This presentation will impact the forensic science community by presenting an integrated study in association with engineers helping to investigate damaging effects of blast overpressure, in a world where tireblast injuries are not so common and the injuries of the nature described are quite rare and are hardly reported in forensic literature.

Blast Overpressure (BOP) is defined as the increased pressure over atmospheric pressure which is associated with a blast from explosives or weapons. BOP may cause primary, secondary, tertiary, and quaternary injuries. Primary blast injury occurs from an interaction of the pressure wave and the body. Secondary blast injury results from other object invested by the pressure wave impact against the body surface. Tertiary blast injuries occur when the body is accelerated from the blast wave at first and is then abruptly decelerated on rigid objects. Quaternary blast injuries are defined as those injuries of victims of explosions that due to the collapse of a building where the explosion took place. Tire-blast injuries are not so common and the injuries of the nature described are quite rare and are hardly reported in forensic literature. A case of a fatal tire explosion occurred after tire repair and inflation will be presented. Explosion occurred suddenly, strictly close the man who was put five meters far from his site. Rescue maneuvers were unsuccessful and death was declared. Forensic pathologist's crew investigated the crime scene: a 44 cm in length tear was recorded on tire. Blood stains on the ground and environmental structures were also detected. A complete postmortem examination was performed the day after death. Large scalp laceration was detected at external examination. Bruises on face and chest were also recorded. At gross examination, cranium vault was unremarkable except for a mild hemorrhagic infiltration of the galea capitis. Brain was normal in size and volume with mild cerebral edema. Diffuse subarachnoid hemorrhage was observed in both hemispheres. Linear fracture of the skull base was detected. Multiple bilateral rib fractures with mild hemothorax were recorded. Lungs were increased in size and volume, reddish colored, both; hemorrhagic foam was detected on the main bronchi. Hemoperitoneum was also recorded; multiple lacerations of liver, kidney, and spleen, deeping into the parenchyma of the viscera were detected. At histological examination with H&E subarachnoid and intraparenchimal hemorrhage were detected on brain. Acute emphysema, pulmonary edema, and hemorrhages were also described with intense congestion of the septum vessel. Venous air embolism was also recorded and investigated by means of immunohistochemistry (antibodies anti-CD 61 and fibrinogen). Capsular multiple tears and subcapsular and intraparenchimal hemorrhages were also detected at liver, spleen and kidneys microscopic examination. Polivisceral stasis was recorded. Intoxication of alcohol or drugs of abuse was excluded at toxicological investigation. Acute respiratory failure was indicated as the cause of death. А

multidisciplinary approach with engineers let us to establish blast overpressure after tire explosion. After that, impact of blast wave on the thorax produced chest and pulmonary injuries (primary blast injuries). Also abdominal viscera injuries were also attributed to blast overpressure effect. The displacement of the body on the ground and environmental structures after explosion produced scalp laceration, subarachnoid hemorrhage, and cranium fractures (tertiary blast injuries). **Blast Injuries, Tire Explosion, Overpressure** 

#### G132 TASER<sup>®</sup> XREP<sup>TM</sup>: A Case Study

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After attending this presentation, attendees will become familiar with the TASER $^{\mbox{\ensuremath{\mathbb{R}}}}$  XREPTM device, a new less lethal tool for law enforcement.

This presentation will impact the forensic science community by alerting the community to the existence of a new TASER® weapon, and the need for caution in handling it.

TASER<sup>®</sup> XREP (eXternal Range Electronic Projectile) is a selfcontained wireless electronic control device that deploys from a 12gauge pump action shotgun. It delivers a neuromuscular incapacitation (NMI) bio-effect similar to that of the handheld TASER<sup>®</sup> X26<sup>TM</sup>. The TASER<sup>®</sup> XREP<sup>TM</sup> can be delivered to a maximum effective rang approaching 100 feet. The battery supply is incorporated into the main chassis and provides the power to propel the XREP projectile.

The TASER<sup>®</sup> XREP<sup>TM</sup> device consists of a triple-redundant incapacitation system, each part of the system generating a highly refined NMI waveform that incapacitates the subject. The system completes the circuit when the front probes make skin contact combined with the cholla electrodes, conductive hand trap, or rear facing barbs. The system is composed mainly of the following:

- a. Nose Assembly the entire detachable nose section which includes the nose frame, frontal probes, fracture pins and reflex engagement electrodes
- b. Conductive Hand Trap Wire connects the engine to the frontal probes (the insulated wire is wound with the conductive wire)
- c. Cholla Electrodes electrodes attached to the chassis. The electrodes are constrained beneath the sheath during flight, extend after impact, and are conductive.

The nose assembly contains four forward-facing barbed electrodes. When the TASER® device is deployed, the nose assembly impacts the subject and the frontal probes make contact with the skin and are stuck to the body. The energy from the impact breaks a series of fracture pins that release the main chassis of the XREP projectile, which remains connected to the nose by a nonconductive tether. A conductive hand trap wire also connects the frontal probes to the TASER® XREPTM engine and has capacity to deliver NMI. The projectile autonomously generates NMI for 20 continuous seconds. As the chassis falls away, six cholla electrodes automatically deploy to deliver the NMI effect over a greater body mass. The subject instantly loses muscular control of the body and cannot perform coordinated action. The subject usually falls to the ground. After the signal stops, the subject typically regains all muscle control. Whereas other less-lethal weapons rely on pain compliance to stop the subject, with neuromuscular incapacitating weapons pain may be short-lived and may aggravate the subject even further or cause serious long-lasting injuries.

This case is that of an actor suspected of fatally stabbing a former coworker and wounding two others during a violent rampage about a week earlier. On discovering that he was a wanted suspect, the police tried to apprehend him. He fled to a nearby hill and stood on the cliff wielding a samurai sword. A 20-page handwritten suicide note was discovered in the abandoned vehicle of the suspect. In the note he indicated sunset as the time he would end his life. A daylong standoff with police ensued with extensive news media coverage. Helicopters hovered over the scene most of the time. Crisis negotiators were called in. They joined in the efforts to get the suspect to surrender. For about eight hours (9:30 a.m. to 5:00 p.m.) he stayed on the edge of the cliff with his sword, taunting and threatening the police. With the approach of dusk, a decision was made to subdue him with a less than lethal weapon. The TASER<sup>®</sup> XREP<sup>TM</sup> device was deployed. He was hit and he plunged off the edge of the cliff to his death about 45 feet below.

Autopsy findings, mode, and health and safety issues will be reviewed.

Electronic Control Device, Neuromuscular Incapacitation, Police

#### G133 3D in Forensics: TIM Synthetic MRI and Virtobot – Forensic Imaging Workflow of the Future

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After attending this presentation, attendees will know the basic of virtual autopsy and the development in forensic 3D imaging of human corspes in the future.

This presentation will impact the forensic science community by covering new validated practical knowledge, and the professional practice gap in the area of virtual autopsy (CME/ACCME criteria).

Imaging has changed the world and greatly influenced modern medicine.

In the 2009 National Academy of Sciences Report, "Medical Examiners and Coroners Systems: Current and Future Needs" modern imaging technologies (Virtual autopsy, Virtopsy) was suggested as having a great potential to detect forensic relevant findings.

The advent of high resolution multi-detector row CT scanners and fast MRI scanners in the last decade has allowed the development of imaging techniques that have greatly enhanced the diagnostic potential of these two imaging modalities. While conventional radiographs have played a valuable role in forensic diagnosis and practice for over a century, recent investigations with both CT and MRI suggest that these imaging tools are capable of much greater contributions. A major innovation is the ability to display imaging findings in 2D and 3D planes that closely replicate the findings at conventional autopsy and make the interpretation of the studies more easily understood by non-radiologists. CT and MRI may be used to supplement traditional autopsy techniques, to provide a complete anatomic assessment prior to limited autopsy, or in certain circumstances to replace it, such as in blunt accidental trauma, or drowning deaths. These studies may also provide options in the setting of religious and cultural objections to conventional autopsy.

While CT has the advantage of providing rapid whole body imaging of great anatomic detail in a short time, the superior contrast resolution of MR provides soft tissue characterization that is not achievable by CT. MRI is less widely available and more time consuming but may be applied to the postmortem evaluation of specific body parts to aid in the diagnosis of specific causes of death that may be characterized by subtle soft tissue changes. Both CT and MRI provide a permanent pictorial record of anatomic findings that may be retained and analyzed for medical and legal purposes postmortem and offer advantages in quality assurance that may be difficult to replicate with conventional autopsy.

The forensic science and medical examiner communities have shown interest in the use of CT and MR autopsy imaging. However, while CT and MR imaging are widely available in the clinical care of the living, forensic facilities face problems of access to autopsy imaging due to financial, technical, transportation, interpretation, and related difficulties.

For the past 15 years, the Forensic Institute of the University of Bern has been concerned with imaging problems in forensics. In 2009 the robot-supported automated system integration of 3D surface scanning and multislice CT with postmortem biopsies was successful as a "Virtobot" developed. After what is now five years, the over 100 postmortem angiographies show impressive results from the research activities at the University Bern. In the early part of 2010, our Total Imaging Total Matrix TIM-MRI system that has been in operation since 2009 could be extended with the so-called synthetic MRI software. The advantage of this TIM synthetic MRI system lies in the fact that in one examination step various MRI sequences (such as T1-T2-PD, etc.) could be performed from tip to toe without any change of the surface traces. In the daily forensic service applications it has become evident that through applying this approach a increase in quality and a improvement in the forensic diagnostics can be achieved and the examination results based on the imaging are often quicker and, thanks to a more visual 3D reconstruction, can be displayed in a way that lay persons can understand and comprehend. Momentarily, in terms of workflow and process, this Virtopsy-system integration is the only forensic examination track in a forensic institute that has brought together all the modalities and technologies in this form for daily use and research. With "Axon Shadow," the interdepartmental forensic IT structure, now being developed at our Institute, which comprises the functionalities of "ERP," "LIMS" and document management, the forensic processes of all the IFM departments are displayed and supported in a workfloworiented manner.

Virtopsy, Virtual Autopsy, CT and MRI

#### G134 Transition to Digital in the Forensic Morgue: Lessons Learned on the Pathway to Greater Efficiency

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After attending this presentation, attendees will have a better understanding of the advantages of and obstacles to the transition to digital technology in the forensic morgue.

This presentation will impact the forensic science community by illustrating the advantages and elucidating the difficulties of the transition to digital fingerprint, radiograph, and photographic technology.

Maximizing efficiency in the postmortem examination process is critical to the daily operations of a busy medical examiner's office, and is also of particular importance to effective mass fatality preparedness planning. The Harris County Institute of Forensic Sciences (HCIFS) uses technology to maximize the efficiency and accuracy with which it can complete the autopsy process. These technological advancements include the acquisition of a digital radiograph system, a digital fingerprinting system, and digital photography. This presentation will detail the advantages of each of these technologies as well as the obstacles that complicated the transition to each. Generally, the most significant advantages of these technologies are increased efficiency, less waste, greater security, and enhanced user benefit. The most significant obstacles involve adaptation to the specific constraints and requirements of the medical examiner/morgue setting. There is little precedent for the use of some of these technologies in the medical examiners context, and this was reflected in our effort to adopt them.

The HCIFS completed its transition from conventional film radiography to digital computed radiography in December 2009. The digital system includes a central x-ray generator and digital processor, a dedicated server, and a web-based viewing software package that is accessible from each of seven autopsy suites, and from the doctors (pathologists and anthropologists) office and laboratory computers. The advantages of the system include: greater image quality, more functional user interface (with contrast, brightness and annotation capabilities) multi-image split screen viewing, enhanced archival security, and the elimination of the expense, waste, and space requirements associated with film radiography. The most significant obstacles to the transition to digital x-ray technology were: the lack of an existing system that was appropriately configured for medical examiner use, the consequent development of user workflow, an unfamiliar user interface and image format, and training necessary for use of the software by nonradiologists\radiographers. Additionally, the HCIFS developed a means to copy, and label the images in a format that can be accessed by the Harris County District Attorney's Office.

Digital fingerprint technology has enhanced the efficiency of the decedent identification process. The HCIFS system is essentially an extension of the Harris County Sheriff's Office (HCSO) AFIS network, and includes six AFIS stations, each comprised of two types of fingerprint scanners and a 37" all-in-one touch screen computer. The fingerprints are transferred directly to the HCSO server rather than being stored at HCIFS, and HCIFS Investigations and Morgue staff utilize a custom web-based software interface to receive and search fingerprint results. The advantages of the digital fingerprint system include: increased print quality relative to the previous method; more efficient transfer of prints and receipt of results (five minute average turnaround); infinite upgrade-ability; and more secure archiving. The primary obstacles to the transition to digital fingerprint technology were: ensuring compatibility between the HCIFS system and the databases with which it communicates; lack of an existing system that is appropriately configured for medical examiner use, and; configuring and using a system that has not yet been tested elsewhere. The HCIFS is currently incorporating satellite based scanners into the system to facilitate use of the system by HCIFS Investigators in the field.

The HCIFS transitioned to exclusive use of digital photography at the both the scene and in the morgue in 2005. Conversion from film to digital photography has increased quality control and accessibility, while reducing processing and duplication costs. The system required the acquisition of digital cameras, a dedicated photo server and the infrastructure necessary to make use of the images in a variety of settings (the morgue, daily case triage meetings, case review sessions, and pretrials). Secure remote access was provided to the district attorney's office eliminating time and supplies required for duplication. The most significant difficulty with the digital photography system is the ever increasing need for storage, and constant oversight is needed to ensure image security and reduce unnecessary image duplication.

The conversion to these digital systems has increased the efficiency of HCIFS daily operations and has resulted in a concomitant increase in its capacity to accommodate mass fatality investigations. Each of the systems was funded by preparedness grants awarded by the United States Department of Homeland Security and the transition process can serve as a template for other medical examiner jurisdictions.

Digital Fingerprint System, Digital Radiograph System, Digital Photography

## G135 Postmortem Computed Tomography as a Valuable Tool for Diagnosing Trauma Prior to Medicolegal Autopsy

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After attending this presentation, attendees will understand the importance of computed tomography, which provide the detailed picture of trauma before a medicolegal autopsy in traumatic deaths.

This presentation will impact the forensic science community by increasing knowledge about the benefit of the combination of whole body postmortem computed tomography with a medicolegal autopsy. This procedure gives high quality and specificity in diagnosing fractures and other traumas in a deceased. The 3D reconstruction helps to assess the extent of damage and trauma mechanisms. It is also shown how a medico-legal autopsy on a deceased with many traumas is best performed with an ancillary computed tomography. The computed tomography without medico-legal autopsy cannot provide the sufficient diagnostic information.

A case of a traffic accident with two victims is presented. A car with four people was hit from the left side by a fast-moving car with two people inside. Driver and passenger sitting in the front of the car with four people were killed instantly. Whole-body computed tomography was performed before medico-legal autopsies were performed in the Department of Forensic Medicine, Aarhus. The driver suffered fatal traumas in the form of multiple fractures on the left side of thorax, laceration of diaphragm, fractures in cervical column, contusions in the left side of the brain, pelvic fractures, and fractures in the left ankle. The passenger on his right side suffered traumas in his thorax, in column and fractures of the pelvis. The two other passengers sitting in the back got minor traumas. Driver and passenger in the other car were practically without traumas.

With this case are shown photos of the two cars involved in this accident, 3D reconstructions made from the computed tomography scanning results and the subsequent clinical photos from the medicolegal autopsies. It is shown that with these documents the trauma mechanisms can be evaluated with high reliability. Some of the diagnosis of traumas in this case could have been lost without a postmortem computed tomography scanning. Also a computed tomography scanning before a medico-legal autopsy saves time and resources for the forensic examiner and the dissection of the deceased is not necessarily as comprehensive as it can be without an ancillary scanning.

This case will be presented as an example to highlight how with a whole-body postmortem computed tomography, it is possible to achieve comprehensive information about traumas and the trauma mechanisms. It improves the quality of a medico-legal autopsy and is recommended to be used in cases of fatal traumas.

Postmortem, Computed Tomography, Trauma

#### G136 Traumatic Injuries in Fatal Tire Explosions

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After attending this presentation, attendees will appreciate the patterns of injuries from tire explosions and the circumstances where fatal tire explosions can cause danger.

The presentation will impact the forensic science community by illustrating the nature and extent of explosion injuries that may result from burst tires.

Tire explosions during servicing may cause severe trauma. The severity of injury depends on the tire size, air pressure, and distance from the blast. The blast injury has been compared to that of a grenade or land mine, but without the chemical or thermal effects. Overall mortality is significant (19-29%), mostly attributed to head injuries. Two cases of truck tire blasts in which fatal injuries were sustained are reported.

**Case 1:** A 29-year-old male was inflating a large truck tire which was lying flat on the ground. He was leaning over the tire when it ruptured under his chest. He was projected against a garage wall four feet away with his shoulder striking the wall nine feet from the ground. He was pronounced dead at the scene.

At autopsy, there were multiple stippled abrasions and bruises on the face, trunk, and upper and lower extremities, typical of blast injury. The right arm was almost amputated. The rib cage and sternum were extensively fractured. Contusions were seen on the lungs and the left diaphragm was ruptured. The  $3^{rd}$  and  $4^{th}$  cervical vertebrae were dislocated. At the base of the skull, there was a hinge fracture with cerebellum protruding through the fracture site. The brain stem was transected in two places.

**Case 2**: A 28-year-old male was testing a large truck that had reports of a faulty speedometer. A jack was placed under the third axle of the vehicle and the engine was accelerated. At 40 mph, one of the rear tires exploded. The victim's proximity to the tire blast was not witnessed but he was ambulatory briefly before collapsing. In hospital, a lacerated spleen with hemoperitoneum was managed surgically. After hemodynamic stabilization, the patient suffered cardiovascular collapse. Resuscitation was not successful.

The autopsy revealed primarily left sided trauma with left elbow, left hip, and rib cage fractures. Bilateral hemothoraces were documented. The left lower lobe of the lung was contused and the left hemidiaphragm was bruised. The left ventricle epicardial surface was bruised and traumatic rupture of the anterior papillary muscle had occurred. Subsequent examination of the spleen post-splenectomy confirmed the presence of lacerations. Lacerations were seen in the left kidney with bleeding into the perinephric fat.

**Conclusion:** The cause of death in both cases was attributed to multiple injuries. In case one, the brain stem transection would have been immediately fatal. In case two, acute papillary muscle rupture led to cardiovascular collapse. Tire explosions show similar injuries to bomb blasts with typical blast injuries seen. Fatalities are common; however, postmortem findings are infrequently reported in the literature. **Tire, Explosion, Fatal** 



## **PHYSICAL ANTHROPOLOGY**



### H1 Monitoring the Long-Term Applicability of Ground-Penetrating Radar Using Proxy Cadavers

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After attending this presentation, attendees will have a better understanding of the potential benefits of ground-penetrating radar (GPR) and its possible limitations in the search for clandestine graves.

This presentation will impact the forensic science community by providing guidelines concerning investigations utilizing GPR in searches for clandestine bodies interred over a year.

The goal of this presentation is to demonstrate the applicability of GPR in grave detection of cadavers that have been buried for a significant period of time (up to 24 months). By using GPR to monitor controlled graves with multiple burial scenarios, questions can be answered concerning the usefulness of this tool in the search for cadavers that have been interred underground longer than a year. Initial guidelines are offered for the forensic community concerning investigation utilizing GPR for clandestine body searches.

Controlled research using pig carcasses as human proxies has demonstrated that GPR is the best geophysical tool to employ when searching for clandestine bodies. Ground-penetrating radar provides the best resolution for subsurface imaging of all geophysical tools used on land. Additionally, the results are displayed in real-time, and information about depth and size of target can be inferred. This presentation continues the second phase of a larger research project involving the monitoring of controlled graves for a two year period and will focus on year two of data collection using a 250-MHz antenna.

The GPR unit used was a MALA RAMAC X3 M with a 250-MHz antenna. The data was processed using REFLEXW and GPR-SLICE computer programs. REFLEXW was used to display the transect data as reflection profiles, while GPR-SLICE was used to display the grid data as horizontal slices (plan view). These data were collected from a permanent grid measuring 11 m by 22 m containing eight graves total, buried in a spodic (Spodosol) soil. A total of eight graves were created: six represented different burial scenarios and containing a single pig carcass (Sus scrofa) each; the last two represented empty control graves. The eight graves were arranged in two rows with four graves in each row. Burial scenarios included a shallow empty control hole (dug at 0.5 m), a deep empty control (dug at 1.0 m), a shallow pig grave (0.5 m depth), a deep pig grave (1.0 m), a pig carcass buried underneath a layer of lime, a pig carcasses buried underneath a layer of gravel, a pig carcasses wrapped in a blanket, and a pig carcasses wrapped in a tarpaulin. The final four scenarios were buried at a depth of 1.0 m. Data were collected following both a west-to-east transect direction and a north-to-south transect direction with a transect interval of 0.25 m.

Over the first year of grave monitoring, salient grave reflections were observed for all of the scenarios containing a pig carcass. Conversely, the second year of grave monitoring showed decreased responses from the decomposing carcasses. By month 15, a number of burial scenarios had become difficult to detect; the shallow and deep carcasses, buried without additional grave items, exhibited the poorest resolutions. The graves with the best resolution were those with the carcasses either wrapped or covered. The scenario of the carcass covered with gravel exhibited the best resolution of all the scenarios. Of the wrapped carcasses, the tarpaulin exhibited greater resolution than the carcass wrapped in the blanket. The two empty control graves were important for the research by showing the difference between an anomaly produced by disturbed soil and an anomaly produced by an actual carcass. While the deep control grave exhibited a consistent response, it was at a lower level of the grave shaft, compared to the carcass anomalies, and was consistent with the location of the grave floor. Though the horizontal slices provided a grid view of the burials, less graves were detected compared to the resolution exhibited by the reflection profiles. It is therefore recommended that when performing clandestine body searches with GPR both imagery options should be utilized and the data should be processed in the lab before making any definitive conclusions concerning the location of potential targets. This project was supported by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Ground-Penetrating Radar, Controlled Graves, Geophysical Search Methods

## H2 Monitoring the Applicability of Ground-Penetrating Radar on Detecting Shallow Graves Using Proxy Cadavers

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After attending this presentation, attendees will have a better understanding of the benefits and limitations associated with the use of ground penetrating radar (GPR) in the search for clandestine graves, specifically in cases involving small bodies and shallow graves.

This presentation will impact the forensic science community by illustrating the ability of GPR, using a 250-MHz and 500-MHz antenna, to locate bodies in shallow graves in various burial scenarios.

The goal of this presentation is to demonstrate the ability of GPR to detect small cadavers buried in shallow graves over a period of six months. By using GPR to monitor controlled graves with multiple burial scenarios, questions can be answered concerning its applicability in the search for small cadavers in shallow graves. Burial scenarios also help distinguish which component or components of the grave, the disturbed soil, the body, or the additional material added to the grave, is producing the geophysical response once the GPR detects the grave.

The use of remote sensing geophysical techniques in the search and detection of clandestine graves in a forensic context has many advantages, particularly as it is non-invasive and can highlight smaller areas for more detailed searching. Controlled research has demonstrated that GPR is the most accurate geophysical tool in forensic investigations. Ground-penetrating radar is time efficient, results are displayed in real-time in the field, it provides the best subsurface-imaging resolution, and can be used in different scenarios, such as over the concrete of a house foundation or on a forested landscape. This presentation will focus on the first six months of data collection for a project evaluating the ability of GPR, using a 250-MHz and 500-MHz antenna, to locate shallow graves containing small pig cadavers in various burial scenarios.

The ground-penetrating radar unit used for this research was a MALA RAMAC X3 M with a 250-MHz and 500-MHz antenna. The

GPR data was processed using the REFLEXW computer program to display the data in a reflection profile, showing one transect at a time. These grid data were collected from a permanent grid, measuring 9 m by 15 m, containing six graves total, five with a single pig (Sus scrofa) carcass, and one control grave. Multiple-burial scenarios were incorporated into the project: a pig carcass buried under a layer of lime; a pig carcass buried under a layer of rocks; a pig carcass wrapped in a fleece blanket, a pig carcass wrapped in a tarpaulin; and a pig carcass without additional material. The final grave was an empty control grave to measure the response of soil disturbance only versus graves containing bodies. Each grave was 0.5 m deep, and the pig cadavers weighed an average of 25.8 kg. The soil at the research site is classified as Spodosol. However, due to the shallow depths of the graves, they were only buried in sandy horizons. The six graves were arranged in two rows with three graves in each row. Data was collected following both a north-to-south transect direction and an east-to-west transect direction with a transect spacing of 0.25 m.

Over the first six months of monitoring, all graves were detected in reflection profiles, although some had better resolution than others. While all of the graves containing a pig carcass produced prominent reflections for this monitoring period, the graves containing items (rocks and lime) placed over the pig carcass resulted in slightly better resolution. Conversely, the grave containing only the pig carcass produced the lowest resolution, but was easily detected. Throughout the first few months of data collection, a minimal response was exhibited by the empty control grave; however, after several months of soil compaction within the grave shaft, there was no longer a response from this grave. These results for the control hole were important in demonstrating that the reflections produced within the graves containing the pig carcasses were the result of the bodies and items added to the graves and not the disturbed soil. In terms of antenna performance, the 250-MHz data initially provided a better resolution within the first few months. However, over time the higher detail provided by the 500-MHz data consistently resulted in easily discernable reflections. While either antenna would be a good option when searching for shallow clandestine graves, the 500-MHz may be a better option depending on soil conditions.

Ground-Penetrating Radar, Controlled Graves, Geophysical Shallow Burial Searches

### H3 Taphonomy of a Mass Grave in Mid-Michigan: The Case of the Missing Cattle

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Forensic anthropology skills can be applied to a variety of medicolegal situations. The goal of this presentation is to discuss a unique instance of a mass grave site in Mid-Michigan in order to provide future investigators with information about the decomposition, taphonomy, and recovery of deeply buried remains.

This presentation will impact the forensic science community by providing an example of how forensic anthropology expertise, including knowledge of decomposition, skeletal anatomy, and recovery techniques, can aid law enforcement in what may not be a "typical" human remains case.

In August of 2009, the Michigan State University Forensic Anthropology Laboratory (MSU FAL) was called to assist the Livingston County Sheriff's Department with an ongoing legal dispute between two parties over the disappearance of approximately 160 head of beef cattle. The plaintiff in this case claimed the defendant had sold the cattle for profit, while the defendant claimed that these animals had died approximately two years prior and were buried on his farm. In order to settle this dispute, the defendant was required to provide evidence of the buried animals. The excavation was monitored determine how many animals were present, estimate the time since death, and to interpret the stratigraphy of the burial pits.

This case was atypical in a number of ways: the individuals were cows and steers; they were buried in a mass grave; due to the legal issues surrounding this case, the defendant was responsible for exhuming the animals; and there was little scientific control over the operation of the backhoe during the excavation. Regardless, this unique situation provided important information about the decomposition and taphonomy of a mass grave excavated with a backhoe that could aid future researchers.

The mechanical action of the backhoe dispersed and broke up the cattle remains during the excavation. Some skeletal elements survived this process better than others. During the excavation, skeletal elements were organized by element in order to determine a minimum number of individuals (MNI). This process revealed that skulls and innominates were recovered less often than long bones such as femora, tibia, or humeri. This may be due to the fact that, quite often, skulls were crushed while long bones were more durable. In addition, the animals recovered were young, growing, feeder cattle, and many innominates were still separated into their smaller elements which may have made them more difficult to recover. The MNI was eventually determined by the recovery of 23 left tibias.

The cattle were buried in a large pit where some animals were very close to the surface and others were buried quite deep – up to 3 to 4.5 meters. Time since death estimates were based on the degree of decomposition, taking into account the burial depth where the animals were recovered. Cattle recovered near the ground surface were skeletonized, mainly dry, and had some mummified skin and tendons. Deeply-buried animal bones were wet with black decomposing sludge and had adhering skin, fur, cartilage, and tendons. This would be an expected pattern of differential decomposition due to different burial depths. Age-at-death estimates concluded the animals had most likely died between 2 to 7 years based on these observations.

One of the important questions regarding this case when it went to trial was whether there were any additional animals in the pit when the defendant had finished digging. Decomposition staining of the pit walls was noticeably black, where it made a strong contrast with the surrounding lightly-colored soil. It served as a good indicator of where additional animals were located as the defendant excavated the burial pit. At the conclusion of the excavations, no additional animals were in the pit due to the lack of decomposition staining.

This atypical case is one example of how knowledge of skeletal anatomy and field recovery techniques can assist with a variety of investigations. This mass cattle grave excavated with a backhoe presented special challenges to the interpretation of time since death and MNI.

Taphonomy, Decomposition, Recovery

#### H4 The Fromelles Project: Organizational and Operational Structures of a Large Scale Mass Grave Excavation and On-Site Anthropological Analysis

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After attending this presentation, attendees will have an increased understanding of the organizational and operational aspects of a project that includes the recovery, processing, anthropologically analyses, and documentation of 250 sets of human remains and over 6,000 artifacts in a task-specific, on-site laboratory with high security demands in a fixed timeframe.

This presentation will impact the forensic science community by demonstrating how organizational and operational planning can lead to maximizing quality and efficiency, and ensure delivery of results within given time and budget constraints.

Between July 19 and 20, 1916, British and Australian forces fought a hopeless battle against German forces while trying to draw attention away from the Somme. The outcome of this battle was the catastrophic loss of over 7,000 soldiers in less than 48 hours. The Australians reported 5,533 killed, wounded, and missing and the British 1,547.

In February 2009, Oxford Archaeology (OA) was awarded the contract to carry out the recovery of eight mass graves near the village of Fromelles in Northern France. Within less than two months, the project planning was finalized and a team of OA staff and external consultants assembled, including forensic archaeologists and anthropologists, osteoarchaeologists, finds experts, crime scene investigators, anatomical pathology technologists, radiographers, IT experts, and many more.

The process was divided into excavation, x-ray, processing, drying, skeletal and artifact analysis, storage, and DNA. Each section had one or two section heads. These section heads and project managers, assisted by specialist, arrived early onsite to ensure that the entire operation was setup according to their needs. The laboratory and excavation site were secured through fencing, CCTV, and 24-hour guards. Tool-storage, office, and facilities for the excavation team were kept within an inner cordon that could only be entered when team members changed into work clothing and put on full personal protective equipment, including paper suits, hair nets, face masks, and surgical gloves.

The laboratory, store rooms, changing rooms, and office space were set up in April 2009 using connectable office containers. This layout guaranteed a secure and efficient workflow as well the dignified and respectful treatment of the human remains. The anthropological analysis began in late May and had to be completed by the end of November. Final analysis of artifacts and finalizing of reports went on throughout the winter and the first soldiers were reburied in January.

Sets of remains and associated artifacts were transferred from the excavation to the anthropological laboratory using a documented handover procedure witnessed by a crime scene investigator to order to guarantee the continuity and integrity of all evidence. The mortuary manager took charge of the remains and constantly monitored progress throughout the different mortuary stages. Remains and artifacts were first x-rayed using a direct-digital x-ray unit, operated by an experienced radiographer, who also holds a degree in forensic anthropology. All images were stored digitally and moved onto the secure database to give access to the anthropologists.

Remains and artifacts were then separated for processing. Human remains were carefully cleaned to prepare them for anthropological analysis. To ensure the highest quality processing, only staff with experience in osteoarchaeology or anthropology were employed at this stage. After processing and drying, the remains were handed over to one of the anthropologists. All anthropologists had their own workstation, consisting of a fixed table, a digital SLR camera permanently fixed to the ceiling above the table, a PC workstation connected both to the camera and the database server and all necessary measurement equipment and reference material. All laboratory space was adequately air-conditioned to guarantee optimum conditions for both remains and artifacts. All rooms and equipment was completely cleaned daily using hospital mortuary protocols.

It was the efficient and effective work flow and data movement that ensured high quality results within a limited timeframe.

Forensic Anthropology, Forensic Archaeology, DNA Sampling

## H5 Blast Injury in Skeletal Remains: The Case of a Soldier From WWI

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After attending this presentation, attendees can expect to gain an understanding of the utility of unusual occupation pathologies as an individuating characteristic in historical missing person's cases. Attendee will also learn about the patterns of blast trauma injury that can be identified in cases of suspected blast injury, as well as, be able to discuss the potential of the combination of blast trauma analysis and forensic anthropological techniques for historical cases such as the one presented.

This presentation will impact the forensic science community by demonstrating the need and potential for the application of blast trauma identification and analysis in forensic anthropology. This presentation demonstrates that forensic anthropologists should be familiar with this type of trauma as it can be identified in a variety of contexts in which the forensic anthropologist may be called to contribute. The lack of knowledge in the field is stressed to outline the importance of undertaking research on this type of trauma due to its relevance in many forensic and anthropological situations.

Recent years have seen increasing attention given to the analysis of many types of skeletal trauma; however, injuries to the skeleton caused by explosions remain poorly understood. The results of a project with dual objectives aimed at both identification of a specific individual killed during the Great War (1914-1918) and understanding the cause of their death are presented. Assisting in the identification of remains excavated in 2008 from Plugstreet, Belgium, the remains were located buried *in situ* in proximity of the location of the German front lines during the Great War at St. Yvon, north of the Ultimo crater. The remains were fully clothed and found buried under soil in a trench. The remains were accompanied by artifacts such as personal effects, munitions, medical supplies, and a souvenir Pickelhaube. These artifacts clearly indicated that this was not a proper burial and the individual was killed at that location.

Anthropological analyses were performed to determine the age, sex, stature, and pathology of the remains. A number of individuating characteristics were identified regarding age and physical type as well as occupationally-related pathological changes. The biological profile indicated that the individual was a male with a stature ranging between 5' 7" and 5' 10", aged between 30 and 40, which narrowed down the potential casualties due to the older age of the individual, a characteristic which was at odds with the typical enrollment age of soldiers of the time. The skeletal remains also indicated a bone robusticity that suggesting that the individual participated in a physically-demanding occupation during his life. This observation was further supported by the extensive occupationally-related pathologies observed on the vertebrae, illustrated by arthritic changes and prominent Schmorl's Nodes, and arthritic joints of the legs. These pathologies are unusual for an individual of this age

and can provide interesting information to add to the identification. Collectively these observations permitted the exclusion of all possible identities with the exception for a small number of individuals. The anthropological analysis was combined with a stable isotope analysis and a subsequent DNA match to identify Private Alan James Mather, a grazier or rancher from Inverell in New South Wales, missing-in-action from Messines, Belgium since June 1917.

Of further interest was a range of evidence for severe skeletal trauma consistent with the individual being hit by a mortar. Laid out anatomically, distinct injuries were located on the upper left side of the body and torso. Path of injury could be accurately located and followed through the absent humerus and sternum, and the extensively fragmented ribs. Elements in close association to these, such as the manubrium and right arm bones, were completely intact, indicating a very specific path of injury, ending in the torso. Fragments of mortar shell were found embedded in the left temporal bone and left scapula. A large fragment, with an intact driving band (typical of German rifled mortars), was found in the associated grave fill, along the left side of the skeleton, which had been included in a bag of skeletal elements from the upper arm. Subsequently examined historical and archaeological information supports the evidence examined and contributes to the positive identification. Historical records confirm that on June 8, 1917, Private Mather's 33 Battalion was hit by mortar fire. This exemplifies the application of blast trauma analysis by a forensic anthropologist to a historical case. This case illustrates the potential for additional work in this area to further expand understanding of this class of skeletal injury which remains equally relevant in modern contexts.

Forensic Anthropology, Blast Trauma, Occupational Markers

#### H6 Peri-Mortem Fracture Patterns in South-Central Texas: A Preliminary Investigation Into the Peri-Mortem Interval

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After attending this presentation, attendees will have an understanding of peri- and postmortem decompositional changes in bone, and how these alterations are associated with changes in blunt force trauma fracture patterns. Additionally, attendees will learn which features proved most diagnostic in placing these fractures in an appropriate sequence during the peri-mortem interval.

This presentation will impact the forensic science community by providing tools to estimate the postmortem interval from long bone fractures, which will help to bring blunt force trauma analysis in line with the *Daubert* (2003) criteria for expert witnesses.

There have been several studies investigating long bone fracture characteristics during the peri-morteminterval (PMI), but none have been undertaken in the unique climate of southwest Texas. Additionally, the definition of the term "peri-mortem" as it applies to human remains is not unanimously agreed upon. Estimates vary regarding how long the peri-mortem interval lasts. Janjua and Roberts' (2008) research in Ontario indicates that it takes bone approximately 200 days to reach a stage of "advanced decomposition," which they measured based primarily on weathering and color change. Conversely, Bell et al. (1996) claim that buried bones may remain in the ground for five years or more before they begin showing any sort of postmortem change.

Issues arise because bone decomposition is a continuous process; however, anthropologists typically rely on non-quantifiable indicators to establish largely arbitrary divisions separating these three timeframes. To improve understanding of peri-mortem bone changes, 50 pig femora were allowed to weather at the Texas State University Forensic Anthropology Research Facility at Freeman Ranch, in San Marcos, TX for up to 18 weeks (PMI=126 days). A portion of the sample was fractured at regular 2-week intervals by the mechanical application of a known dynamic force, and the resulting fracture outlines, angles, and edges, were methodically examined and documented. Also examined were the number and size of fragments produced.

A jagged fracture surface proved to be the feature most strongly indicative of postmortem drying in the short term, appearing approximately a month after death and appearing at consistently high rates in all subsequent tests. A significant change in the frequency of curvilinear versus transverse fracture outlines separates the first two months of the experiment from the following period. Fracture angle proved to have poor predictive powers, as obtuse and acute-angled fractures, indicative of fresh bone, occurred through the final test at PMI=126 days, though right-angled fractures did begin to appear at PMI=28 days.

There are essentially two "peaks of activity" when it comes to timing peri-mortem fractures in south-central Texas. The first peak occurs around 28 days, and is characterized by the first appearance of a jagged fracture surface, the first appearance of longitudinal cracking, and the beginning of a transition from curvilinear to intermediate fracture outlines. The second peak occurs around 70 days, and is distinguished by the absence of any smooth fracture surface after that point. Statistical tests indicated that different features may be diagnostic over a short period (e.g., 2-week intervals) than those over a longer period (e.g., 8week intervals).

No one feature proved to have extraordinarily high diagnostic value, but fracture characteristics analyzed in conjunction with one another have the potential to time the occurrence of a fracture with some accuracy. The results of this experiment highlight the need to develop a shared knowledge base regarding the interpretation of blunt force trauma, backed by statistically supportable research. This replicable experimental design and method of quantitative analysis will help to bring blunt force trauma interpretation in line with the *Daubert* (1993) ruling, as well as aid in standardizing trauma analysis criteria.

Peri-Mortem Interval, Blunt Force Trauma, Forensic Taphonomy

#### H7 Analysis of Primary Blast Rib Fractures

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After attending this presentation, attendees will understand the results of an analysis of rib fractures associated with primary blast trauma.

This presentation will impact the forensic science community by providing a more comprehensive understanding of the mechanisms and affects of blast trauma, specifically those involving the ribs, resulting in more accurate interpretations of skeletal trauma.

Worldwide, the prevalence of terrorist attacks employing the use of explosive devices has served to shift counterterrorism focus from widescale weapons of mass destruction to conventional explosive attacks. In 2008, bombings alone accounted for more than one-third of all terrorist attacks, with explosives, vehicle bombs, and improvised-explosive devices resulting in the majority of injuries. Forensic anthropologists have become increasingly involved in the identification of blast victims as well as the interpretation of skeletal trauma caused by exploding ordinance. Understanding rib fracture patterns associated with such explosive events would provide significant medical and forensic lead information. This study investigates the rib fractures associated with primary blast trauma (i.e., resulting from the blast wave).

Rib fractures are associated with the majority of traumatic thoracic events and can be important indicators of soft tissue and organ injury. Despite this, rib fractures have historically received little attention in medical and anthropological literature. The relatively small amount of rib fracture research could be due to the habit of viewing ribs as individual bones rather than a protective system for the thoracic cavity, the cumbersome nature of processing the torso, and the medical practice of often overlooking rib injuries due to the potential for more severe injury to the vital thoracic organs. The majority of literature on the broader topic of blast trauma is in medical journals and focus on treatment of injuries rather than conducting controlled, empirical studies. Some researchers have examined the mechanisms of rib fracture in order to understand their structural failure during different traumatic thoracic events, but have not specifically considered blast forces.

A bone's reaction to stress is affected by factors such as force and the mechanical properties of the bone. The morphology of ribs, specifically their cross-sectional shape and degree of curvature along their length, sets them apart from other human bones and suggests that they should be expected to respond uniquely to applied forces. A recent study by Christensen *et al.*<sup>1</sup> examined primary and secondary skeletal blast trauma and reported the presence of numerous butterfly fractures in ribs that were most likely caused by ventrally applied blast force. Building on these findings, the present study involves further analysis of the previous observations, as well as additional simulated (and more controlled) primary blast event forces.

Results indicate that in response to blast and blast-type forces, ribs tend to fracture in the head, neck, and shaft in a manner consistent with compression, shearing and bending forces. Butterfly fractures, which are the result of bending, tensile and compression forces, were frequently observed. This is unsurprising considering that these forces are typically associated with blast events. Rib fracture patterns differed from those normally associated with other types of trauma events such as blunt force (including deceleration), projectile, and sharp force.

These results contribute to a more comprehensive understanding of the effect of blast forces on ribs and the interpretation of rib fractures in forensic contexts and may allow forensic anthropologists to differentiate between blast trauma and trauma resulting from some other cause. Practitioners should bear in mind; however, that blast traumas involve a number of complicated variables. If blast injury is suspected, consideration should be given to bone type, injury location, and all available contextual and investigative information including the amount of explosives utilized, the placement of the explosives in relation to the victim and the presence of potential projectiles.

#### **Reference:**

<sup>1.</sup> Christensen AM, Ramos V, Shegogue CW, Smith VA, Whitworth WM. Primary and Secondary Skeletal Blast Trauma. Proceedings of the 62<sup>nd</sup> American Academy of Forensic Sciences 62<sup>nd</sup> Annual Scientific Meeting 2010 February 22-27; Seattle, WA.

Forensic Anthropology, Blast Trauma, Rib Fractures

#### H8 Pattern and Distribution of Fractures in the William M. Bass and Hamann-Todd Osteological Collections

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After attending this presentation, attendees will understand whether aspects of modern life predispose individuals to different patterns of trauma than earlier, historical populations. The specific patterns and susceptibilities to injury may be unique to individual populations and provide a reference in order to gauge quality of life and health status for the populations under study.

This presentation will impact the forensic science community by providing information that can be used to determine lifestyle factors that predisposed modern, as well as earlier historical populations, to injury. This may allow for predictions to be made as to what types of injuries will be represented in modern medical institutions, based on the lifestyle of the populations feeding into those systems. It is possible that modern activities can predispose certain populations to different risks, and therefore different injury patterns. Few studies take into account the effects of ancestry, age, and sex on frequency and location of these fractures. The goal of this study is to determine whether aspects of modern life predispose individuals to different patterns of trauma than earlier populations, as well as whether differences exist across demographic parameters.

Lifestyle choices, as well as biological and environmental factors, can predispose different individuals to fracture. Habitual daily activities combined with poor health characterize the risk factors for many populations experiencing high fracture rates. These can include a sedentary lifestyle, tobacco smoking, alcoholism, and poor diet. An individual's age, deteriorating senses, osteoporosis, hormonal changes, poor health, and/or inactivity all contribute to biological predispositions to fracture. In addition, several non-biological factors can increase an individual's rate of fracture, including geographic location, climate, technology, occupation, and participation in sporting activities. Modern ways of life have introduced longer lives that are on average less laborious than earlier time periods, as well as city crowding, violent crime, automobiles accidents, and accidents attributable to urban architecture. All of these factors interplay to form an individual's unique susceptibility to fracture.

The present investigation was conducted using the Hamann-Todd Osteological Collection and the William M. Bass Donated Collection. The analysis of both collections was conducted macroscopically without the aid of radiographs. Only complete, adult specimens were used to allow for greater statistical power. Each element of the skeleton, except for hands and feet, was visually inspected for the presence or absence of fractures. Demographic information was recorded for each individual and includes cause of death, age, sex, and ancestry. Statistical analyses were performed using two statistical analysis software programs. The frequency data generated by the two collections in this study were analyzed using cross-tabulations with Chi-square tests, to determine if any differences occurred between the earlier and later populations, as well as between age, sex, and ancestry groups.

Among the significant (i.e., Chi-squared test significant) cranial bones we see several patterns emerge, the first being that white males tend to have more fractures than expected. In contrast, white females tended to have fewer fractures than expected in both collections. In the post-crania, there appears to be higher fracture counts than expected only for the Bass collection. There seems to be a predisposition toward more post-cranial fractures in the more modern sample. The highest fracture counts were attributed to the ribs and nasals with some individuals experiencing more than one fracture. This has been found in other studies, since these areas are often susceptible to not only violence, but traumatic injuries from falls and accidents.

Overall, the results indicate that differences exist across the demographic categories. The variation inherent in the sample may be attributed to the fact that the Hamann-Todd collection was created from a more socio-economically disadvantaged population, as compared to the Bass-donated collection. Overall, there is significant variation found between the demographic groups included in this study, which helps garner a further understanding of modern injury patterns.

Fractures, Age Differences / Sex Differences, Patterns of Injury

<sup>\*</sup> Presenting Author

#### H9 No Country for Young Pigs: Identifying the Use of Captive Bolt Pistols in Non-Natural Death Occurrences

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After attending this presentation, attendees will understand the similarities and differences in trauma to the skull resulting from gunshot wounds and wounds inflicted by a tool used in the slaughtering of livestock, the captive bolt pistol (CBP).

This presentation will impact the forensic science community by presenting the criteria for differentiating defects produced by handguns and the captive bolt pistol.

Case studies from Germany, Italy, and Serbia have shown that different varieties of the CBP have been used in suicides and homicides. While gunshot wounds (GSW) are much more common forensically in the United States, the wide availability of captive bolt pistols, found in commercial livestock slaughterhouses and many family farms, means these tools could be used as a weapon in a homicide.

In this study, skeletal evidence of gunshot wounds in the skulls of humans was compared to captive bolt pistol wounds in the skulls of domestic pigs (Sus scrofa) (n=6). A seventh pig (n=1) was observed for a month separately from the first group in an effort to determine if the wound sizes changes over time in an uncontrolled environment, exposed to the elements. The captive bolt pistol (CBP) produces a characteristic round, sharp-edged entrance wound with internal beveling that resembles a GSW entrance defect. The CBP entrance wounds were measured in an effort to identify the caliber of the weapon used (Ross 1996). While they were classified within the range of a large-caliber weapon, the CBP mean value of the minimum diameter (13.05 mm) was found to be greater than the large caliber GSW mean (11.004 mm). The values obtained when plugging into the Ross (1996) equation was 9.51, classifying it as large caliber. The individual measurements of the CBP entrance sites are all larger in diameter than the mean diameters for the selected small and large caliber weapons (.22, .25, .32, .38) found in Ross (1996). The size of the CBP bolt used (11.9 mm), is slightly larger than bullets from common-caliber handguns: 9 mm, .357 Magnum (9.07 mm), .38 Special & ACP (9.65 mm), .40 S&W (10.2 mm), .44 Magnum (10.9 mm), and .45 ACP & GAP (11.5 mm). While a large caliber value may indicate a captive bolt pistol, other characteristics that aid in differentiating the CBP wound from a gunshot wound include: (1) the absence of radiating fractures from the area of trauma impact; and, (2) the lack of an exit wound, as the CBP bolt does not travel through and exit the skull. Interestingly, previous research discovered that the wound size was equal to or slightly less than the diameter of the bolt (Simic et al. 2002). However, in this study, it was found that the wound defect sizes of bolted pigs were observed to be slightly larger than the 11.9 mm diameter of the bolt itself (mean diameter=13.05 mm). At this point, the resulting difference in size is still unaccounted for as the observed taphonomic processes do not appear to be actively modifying the cranial defects. More research will need to be conducted before a cause can be more conclusively determined.

Captive Bolt Pistol, Gunshot Wounds, Pigs

## H10 Defining Intimate Partner Violence: New Case Studies in IPV

Chelsey Juarez, MA\*, University of California - Santa Cruz, Social Science 1, Department of Anthropology, 1156 High Street, Santa Cruz, CA 95064; and Cris E. Hughes, MA, 2306 East Delaware Avenue, Urbana, IL 61802

After attending this presentation, attendees will be familiar with the most common trends of fracture associated with intimate partner violence (IPV), in particular the hierarchy of facial fractures and their types that are indicative of IPV.

This presentation will impact the forensic science community by presenting the most current data on patterns of skeletal injury common in cases of IPV and by illustrating the range, and characteristics of these injuries in three cases studies.

Women are approximately 4 to 5 times more likely to be victims of intimate partner homicide (IPH) than their male counterparts (Campbell, et al. 2007).<sup>1</sup> The major risk factor for IPH, regardless of whether the male or female partner is killed, is the presence of prior domestic violence. When analyzing human remains for evidence of chronic physical abuse, forensic anthropologists rely on a temporal range of trauma and the presence of specific types of skeletal injuries (Cook, et al. 1997; Galloway 1999; Marks et al. 2009).<sup>2,3,4</sup> Likewise, in the majority of cases, physical IPV occurs over a long period of time resulting in a documentable history of soft tissue and/or skeletal injuries (Campbell and Glass 2009).<sup>5</sup> Identification of IPV from skeletal trauma is critical, because knowing such information increases accurate reporting of IPVrelated deaths, helps to mitigate abuse of future partners and children, and may assist in the identification of perpetrators. As first-incident IPV female homicides increase, it is critical that forensic anthropologists become aware of the patterns of injury, populations at risk, limitations of assessment, and their role in the identification of IPV during analysis of skeletal trauma. The following fracture guidelines may identify IPV (Juarez and Hughes, in press; Arosarena et al. 2009):6,7

1) Most victims are female.

- 2) Most victims are involved in ongoing abuse, which may present as antemortem trauma to the skeleton.
- 3) IPV correlates statistically with peri-orbital fractures and intracranial injury.
- 4) Fracturing to the nasal bones is not unique to IPV and has been correlated with motor vehicle accidents, falls, and assaults by unknown or unidentified assailants.
- 5) Fracturing to the mandible and zygomatic complex is not unique to IPV and has been correlated with assaults by unknown or unidentified assailants.

Three known victims of IPH are examined for evidence of identifiable trauma associated with IPV. In two cases, clear evidence of antemortem trauma, both post-cranial and cranial exist, and in both instances this trauma is consistent with past and recent IPV. However, in the third case IPV-related trauma was only present perimortem. At the time of case analysis, the forensic anthropologists were not aware of the skeletal traumatic patterns often associated with IPV and, therefore, no suggestion for such a case was made.

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Domestic Violence, Facial Fracture, Intimate Partner Violence

#### H11 Skeletal Trauma Patterns in a Vietnam-Era Aircraft Loss: Part I - Lower Extremities

Matthew Rhode, PhD\*, JPAC-CIL, 310 Worchester Avenue, Building 45, Hickam, AFB, HI 96853

After attending this presentation, attendees will gain a broader understanding of the types of skeletal trauma exhibited in the lower extremities of multiple passengers of the same Vietnam era aircraft loss. In addition, based on consistencies in the patterns of fractures observed, a model is posited that delimits the types of lower extremity trauma expected in this type of aircraft loss.

This presentation will impact the forensic science community by serving as a baseline from which future investigators can compare and contrast skeletal trauma seen in casework. Specifically, knowledge of expected trauma patterns for certain aircraft mishaps and military loss incidents will enhance the interpretation of skeletal trauma throughout the field.

One feature that is often lacking from scientific literature reviewing trauma caused in aircraft losses, falls from heights, and automobile accidents, is specific detail regarding the types of fractures sustained. This lack of detail limits the interpretation and analysis forensic scientists can perform on such remains. The paucity of information on skeletal trauma may be related to an autopsy-based, or soft-tissue, perspective as well as a lack of sufficient case material covering more than a few individuals. This project seeks to fill this information gap by presenting a unique opportunity to describe the skeletal trauma exhibited across multiple individuals involved in the same aircraft loss incident. Specifically, this project, involves the analysis of lower extremity trauma (femur, tibia, and fibula) in a series of skeletal remains recovered from the 1965 loss of a C-123 cargo plane with 84 passengers on board. However, due to an incomplete recovery, nine years later in 1974, there are only approximately 30 individuals represented in the sample.

Review of the remains reveals a combination of peri-mortem fracturing and postmortem damage but an estimated 80% or more of the observed fractures are peri-mortemin origin. The nine-year lapse between the loss and recovery did result in some taphonomic damage; however, this did not significantly impede the observation of fracture surfaces and trauma patterns. For each element in the lower extremity, summary and descriptive statistics describing fracture types and orientations are presented for the proximal, middle, and distal thirds. When possible, specific information is given on fracture types in the femoral head and neck, knee, and ankle.

There is a consistency in fracture patterns across the sample, indicating a similar array of forces during the crash. Viewed as a group

assemblage, all individuals on the aircraft experienced dynamic, sudden deceleration, blunt force trauma during the crash resulting in nearly every bone in the lower extremity exhibiting one, if not multiple, fractures. Accepting some variability, a majority of the observed fractures are oblique in orientation, suggesting a predominance of bending (tension and compression) forces acting on the lower extremities.

Drawing this information together, a model describing the types of fractures is posited for this type of aircraft and loss incident. This model is presented to the greater scientific community as tool for comparison to other cases. A basic comparison of trauma patterns in other aircraft losses does indicate similarities, while differences are noted in cases with published data on falls from heights.

Trauma, Skeletal, Lower Extremity

#### H12 The Central Identification Unit (CIU) During the Korean War

Kathleen M. Loyd, MA\*, Joint POW-MIA Accounting Command Central Identification Laboratory, 310 Worchester Avenue, Building 45, Hickam AFB, HI 96853

The goal of this presentation is to provide historical insight into the operations of the United States Army Central Identification Unit (CIU) during the return of United States (U.S.) deceased from the Korean War, spanning from 1951 until 1956. Brief descriptions of forensic anthropologists working at the CIU, Standard Operating Procedures (SOPs), and insight into the analysis of unknown Korean War deceased will be examined through historical documents, anecdotes, and period photographs.

This presentation will impact the forensic science community by contributing to the historical understanding of the CIU during the return of deceased U.S. Korean War servicemen. Attendees will understand the purpose of the CIU, process of analysis and identification, and the historical legacy of the identifications made by the CIU. In addition, a brief synopsis will demonstrate how historical documents about the CIU have been used to develop name associations for Korean War cases previously categorized as unknowns by investigators at the CIU.

On January 2, 1951, the United States Army (USA) opened a forensic identification laboratory at Camp Kokura, Japan to analyze, identify, and return deceased U.S. Korean War servicemen to their families. The CIU employed a staff of mortuary technicians, forensic specialists, and forensic anthropologists to analyze and identify the remains of thousands of U.S. servicemen who lost their lives during the Korean War. Utilizing developments in forensic anthropology refined by forensic anthropologists working on the identification of war deceased from World War II, a small handful of anthropologists at the CIU handled caseloads of over 100 remains per day. In light of such demands, stringent SOPs were followed in order to ensure the integrity of identifications. Official SOPs and CIU documents detail the procedures for receiving, storing, analyzing, preserving, and identifying remains. These documents will be captured through summary and copied examples to demonstrate how remains were recovered, analyzed, identified, and returned by the CIU.

Candid anecdotes from Dr. Kazuro Hanihara's book, "Bones Reveal the Identities of Human Bodies: Scientific Procedures for Identification" provide insight into the working conditions at the CIU and challenges faced by anthropologists working on war deceased.

United States Army Signal Corps silent film recordings of the CIU offer visual reference to the procedures of analyzing remains recovered from the conflict. Though there is no commentary, the film captures how the SOP was employed by staff at the CIU, and shows the laboratory as used by the forensic anthropologists and technicians.

Unknown Korean War deceased presented unique challenges to investigators at the CIU. Various investigative methods of anthropologic analysis and historical research were utilized to examine unknown remains. When an investigation was unable to result in identification, these remains were classified as Unknown "X" cases, and were eventually buried in the National Memorial Cemetery of the Pacific (Punchbowl) in Honolulu, Hawaii. The Joint POW-MIA Accounting Command Central Identification Laboratory (JPAC CIL) has continued to research the unknown cases in an attempt to associate the unknowns with unaccounted-for servicemen. A brief summary of the JPAC research process through a case study will demonstrate the critical importance of historical documents from CIU.

Korean War, Forensic Anthropology, History

#### H13 Introducing COFFA: An International Consortium of Forensic Anthropology Programs

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After attending this presentation, attendees will understand the challenges faced by young researchers and practitioners in academia.

This presentation will impact the forensic science community by introducing a consortium with a mission to provide support for faculty in forensic anthropology in the realms of promotion and tenure and curriculum development. Additionally, membership information in the consortium will be presented.

In 2010, the International Consortium of Forensic Anthropology Programs (COFFA) was founded as a consortium to provide support for faculty and departments who teach forensic anthropology (http://www.coffa.usf.edu). The mission is to support the development and success of fundamental education and training for students, faculty, and practitioners of forensic anthropology.

In the past decade, many new educational programs have started at universities where there was not a tradition of teaching or practicing forensic anthropology. In part, this is the result of the high demand by students due to the so-called "*CSI effect*." For a fresh forensic anthropology PhD, it can be challenging to navigate through the standard roles of university responsibilities, when time is divided among teaching, administration, research, and consulting practice. Even more taxing, can be negotiating the unique roles and challenges forensic anthropologists face trying to incorporate case work into an academic model (i.e., the unique relationship between casework as research and teaching opportunities for students).

A recent 2009 survey presented by the physical anthropology section of the AAFS showed that there were 32 academic programs suited for forensic anthropology training at the time the survey was conducted (http://aafs.org/sites/default/files/pdf/PAEmploymentTrends.pdf). The criteria for making it on the list included an AAFS member on faculty that could mentor students, a graduate level forensic anthropology course, and a graduate program in anthropology.

However, the number of resources and professional networking outlets are lacking for forensic anthropologists. Therefore, similar in model to the Consortium for Practicing and Applied Anthropology Programs, COFFA started with eleven initial members including: Hamline University, Department of Anthropology; LSU, Department of Geography and Anthropology; NCSU, Department of Sociology and Anthropology; Mercyhurst College, Department of Anthropology; MSU, Department of Anthropology; Texas State University – San Marcos, Department of Anthropology; University of Coimbra, Research Centre for Anthropology and Health; UGA, Department of Anthropology; UCF, Department of Anthropology; UF, Department of Anthropology; USF, Department of Anthropology. Most higher learning institutions have specific guidelines for attaining promotion and tenure, which are evaluated according to the realms of teaching, research, and service/engagement. Most institutions incorporate Boyer's (1996, p. 32) "Scholarship of Engagement," which stressed the importance of faculty and universities applying their expertise to "our most pressing social, civic and ethical problems." Although engagement is an evaluation criterion and is outlined in most university guidelines, most traditional academic departments still do not count the applied or engaged scholarship, which defines forensic anthropology during the tenure and promotion process.

COFFA was established to provide faculty support in: (1) tenure and promotion recommendations for programming in forensic anthropology; (2) best practices and lessons learned in teaching forensic anthropology; and, (3) guidelines for training practicing forensic anthropologists. Over the next year, COFFA will develop a set of recommendations on: (a) how to develop meaningful ways of defining, documenting, evaluating, and promoting diverse forms of scholarship in forensic anthropology; and, (b) how to raise awareness and recognition for practical applied work in forensic anthropology among department chairs, deans, and members of tenure and promotion committees (e.g., scholarship of engagement). Additionally, COFFA members plan to develop documents that provide models and suggestions for undergraduate and graduate level curriculum development in forensic anthropology. These documents will provide COFFA members opportunities to share resources and to learn from each others' experiences in developing guidelines for the design and administration of degree-granting graduate training programs for practicing forensic anthropologists.

Forensic Anthropology, Education, Promotion and Tenure

### H14 The American Board of Forensic Anthropology: Historical Trends in Research and Training

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After attending this presentation, attendees will learn about historical trends in research and training of Diplomates certified by the American Board of Forensic Anthropology (ABFA).

This presentation will impact the forensic science community by providing an historical overview of the ABFA and its Diplomates. Moreover, this presentation adds to the growing body of literature describing the development of forensic anthropology in the United States.

While forensic anthropology continues to advance both theoretically and methodologically during the twenty-first century, numerous workers have contributed to the discipline by tracing the historical developments in the field.<sup>1-8</sup> These careful analyses have demonstrated that the craft of forensic anthropology has grown from the peripheral application of physical anthropology in medico-legal contexts to a legitimate, full-time discipline and profession. While numerous scholars indicate that 1972 marked a turning point for the discipline, with the founding of the Physical Anthropology Section of the American Academy of Forensic Sciences (AAFS), 1977 was also a watershed year, as the American Board of Forensic Anthropology, Inc. (ABFA) was founded.<sup>1-8</sup>

The ABFA was originally established by seasoned practitioners interested in creating a board certification process for forensic anthropologists. Since its inception in 1977, 85 individuals have been certified as Diplomates, with 63 active individuals as of 2010. The first two cohorts of Diplomates were automatically granted Diplomate status; however, since 1979 individuals wishing board certification have had to pass a rigorous written examination and laboratory practicum.

This project traces the academic histories of all 85 Diplomates and examines trends in research and training. Dissertation titles were used to decipher broad research patterns and academic institutions were tracked for the purpose of indicating trends in training. In addition, the number of years between graduation and board certification was calculated and each Diplomate's major professor was noted.

All but one of the 85 Diplomates received the PhD degree. Dan Morse (now deceased) graduated from Western Reserve Medical School in 1932 and was certified as a Diplomate 45 years later. Of the remaining Diplomates, dissertation research topics are diverse and variable. Broadly, topical research interests can be classified into six categories: (1) skeletal biological studies and bioarchaeology; (2) forensic anthropology; (3) zooarchaeology; (4) paleoanthropology; (5) primatology or paleoprimatology; and (6) human biology, human variation, or dermatoglyphics. Of these, 56.4% fall into the skeletal biology or bioarchaeology category with 18.8% of dissertations related to forensic anthropology. These data indicate that board-certified forensic anthropologists have far-reaching interests that are not solely devoted to the profession, as over 80% wrote dissertations outside of the forensic purview.

Regarding institutional training, a total of 36 institutions were attended for the terminal degree. These schools are geographically diverse, are found all over the United States, and include both public and private institutions. Eight Diplomates received their degrees from institutions outside the US (Canada, United Kingdom, and South Africa). The number of Diplomates trained at one institution varies with The University of Tennessee granting the most number of degrees (n=17). The mean year difference between completing requirements for the terminal degree and board-certification is 9.4 years and ranges from 2 to 45 years.

This project has demonstrated that board-certified forensic anthropologists are a broadly trained group of professionals and that the ABFA represents a diverse group of practitioners with far-reaching anthropological interests and expertise. Additionally, this historical analysis has demonstrated that several pioneering individuals have had far-reaching influence on the field of forensic anthropology and the development of successful training programs.

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Forensic Anthropology, American Board of Forensic Anthropology, History

#### H15 The Scientific Working Group for Forensic Anthropology: An Update

Thomas D. Holland, PhD\*, DoD JPAC, Central ID Lab, 310 Worchester Avenue, Hickam AFB, HI 96853

After attending this presentation, attendees will be familiar with the recent activities of the Scientific Working Group for Forensic Anthropology (SWGANTH).

This presentation will impact the forensic science community by raising awareness of the SWGANTH's work to establish, identify, and publish "Best Practices" within the forensic anthropology discipline.

In late 2007, the U.S. Department of Defense Central Identification Laboratory (DOD CIL) and the Federal Bureau of Investigation (FBI) cosponsored the creation of the Scientific Working Group for Forensic Anthropology, or SWGANTH. The group's by-laws were adopted at its first formal meeting on January 8, 2008. The 20-member Executive Board, comprised of professionals from the forensic anthropological community, represent a broad cross-section of expertise and jurisdictional involvement. To this end, the permanent members of the Executive Board were specifically selected to represent large, medium, and small graduate-level academic programs, large and small medical examiner offices, the museum and cultural resource communities, as well as federal, state, and local government agencies. As with other "Scientific Working Groups," the SWGANTH does not function as a regulatory body and lacks any sort of direct coercive authority. Rather, the purpose of the SWGANTH is to identify and recommend current "best practice" within the forensic anthropology discipline, to chart a path into the future, and to bring about voluntary compliance through education and peer involvement. This is being accomplished primarily through the work of approximately 20 sub-committees, each chaired by two or more members of the Executive Board, but populated by forensic anthropologists from around the United States and world. Ultimately, the success of the SWGANTH will be directly proportional to the interest and involvement of the larger forensic anthropology community.

The SWGANTH benefits from having co-sponsors in that it is relatively well funded. The group's Executive Board meets twice annually, in the National Capitol Region in the spring and in Hawaii in the winter. At the June 2010 meeting, hosted by the National transportation Safety Board. the SWGANTH Executive Board reviewed and evaluated the work of the sub-committees, ultimately approving ten "Best Practice" guidelines for promulgation. These were then posted on the SWGANTH website for public dissemination. At the most recent meeting, January 2011, the Board voted on eight additional subcommittee recommendations, bringing the total number of approved "Best Practice" guidelines to 18. These are:

- 1. Code of Ethics and Conduct
- 2. [Individual] Qualifications
- 3. [Forensic Anthropology] Laboratory Management and Quality Assurance
- 4. Determination of Medicolegal Significance
- 5. Sex Assessment
- 6. Pathological Conditions and Anomalies
- 7. Facial Approximation8. Age Estimation
- 9. Skeletal Sampling and Preparation
- 10. Personal Identification
- 11. Resolving Commingled Remains
- 12. Stature Estimation
- 13. Trauma Analysis
- 14. Statistical Methods
- 15. Ancestry Estimation
- 16. Taphonomy
- 17. Documentation and Reporting
- 18. Detection and Recovery of Remains

The SWGANTH Executive Board also created three new subcommittees that have been charged with identifying some of the basic elements common to a well-rounded forensic anthropology educational program, isolating "gaps" in the underlying practice of our discipline, and creating a "self assessment" that will aid forensic anthropologists in evaluating their performance relative to the larger community. As with previous guidelines, drafts of these documents will be posted for public comment for at least 45 days prior to a final evaluation and decision by the SWGANTH Executive Board. No specific timetable was established for these sub-committees to issue their recommendations.

**Best Practices, SWGANTH, Guidelines** 

#### H16 Involvement of Forensic Anthropologists in the National Unidentified and Missing Persons System (NamUs)

Bruce E. Anderson, PhD, Forensic Science Center, Office of the Medical Examiner, 2825 East District Street, Tucson, AZ 85714; Elizabeth A. Murray, PhD\*, College of Mount Saint Joseph, Department of Biology, 5701 Delhi Road, Cincinnati, OH 45233-1670; Susan M.T. Myster, PhD, Hamline University, MB 196, 1536 Hewitt Avenue, Saint Paul, MN 55104; and Jennifer C. Love, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will better understand the ways in which forensic anthropologists can engage with NamUs, and learn more about the current scope of the unidentified persons problem in the United States, particularly as it relates to the field of forensic anthropology.

This presentation will impact the forensic science community by communicating to forensic anthropology professionals the ways in which they can become involved with NamUs, reporting on unidentified skeletal remains of forensic interest in various regions of the country that have not been entered into NamUs; and demonstrating basic NamUs case entry for unidentified persons.

Throughout the past decade, forensic professionals have increasingly become aware of the need to create a system that retains and integrates records of unidentified persons and missing persons throughout the United States. Out of these and other realizations, and under the auspices of the Department of Justice, National Institute of Justice, evolved the "President's DNA Initiative" in 2003, and the "Identifying the Missing Summit" in 2005. Later, in 2007, the initial phases of what was to become the National Missing and Unidentified Persons System (NamUs) developed. The scope of the missing and unidentified problem is enormous and has been referred to "the nation's silent mass disaster." Based on records collected in 2004 by the Office of Justice Programs, it was estimated that as many as 40,000 unidentified dead may exist in the United States today. Some of these unidentified persons currently exist only as records; other unidentified decedents lie buried in public cemeteries throughout the United States without benefit of tissue sampling for DNA analysis. Additional sets of unidentified skeletal remains sit in boxes on shelves in police property rooms, county morgues, museums, and anthropology departments. Many of these skeletons, though forensically significant, have dates of discovery that preceded modern anthropological identification methods, and certainly predated the use of DNA technology.

The advent of the NamUs system, and the resources and technology it provides, allows the medicolegal system a new and dynamic way to pursue identification of unknown persons. The responsibility of forensic anthropologists, in collaboration with the coroners and/or medical examiners with whom they consult, is to ensure that skeletal remains within the regions in which they practice are afforded this new technology, including the fully-funded DNA analyses associated with this national identification effort. Those who have been entrusted with remains by coroners and medical examiners are empowered to actively participate in the identification process of the individuals in their charge. To this end, it is essential that forensic professionals become familiar with the NamUs system, use it in their casework, encourage the use of the NamUs system by their colleagues, and that those involved in forensic anthropology education begin to introduce this tool to their students.

This presentation will provide an overview of the NamUs system, with special emphasis on its application to the identification of unknown skeletal remains. Case data entry will be demonstrated, means of obtaining free DNA analyses will be outlined, and ways that forensic anthropologists can become more involved with NamUs will be discussed.

NamUs, Unidentified Persons, Forensic Anthropology

#### H17 Diversification: Evolving Professional Roles for the Forensic Anthropologist in the Medicolegal System

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After attending this presentation, attendees understand the different professional roles forensic anthropologists fill in a medical examiner/coroner office. In light of the current economic climate, the trend to diversify professional duties is expected to rise.

This presentation will impact the forensic science community by highlighting the additional professional roles forensic anthropologists currently serve in the medicolegal system. This trend presents unique opportunities for cross-training in other forensic specialty areas thereby increasing overall marketability for the forensic anthropologist.

The role of a Forensic Anthropologist (FA) in the Medical Examiner/Coroner (ME/C) Office is an important one, but for many offices, the work load of such a position is not enough to justify a fulltime FA staff member. In the past, a FA was used as an external consultant providing services on an "as needed" basis to the ME/C. This proved to be problematic in some cases due to chain of custody-related issues and the long amount of time required for analysis and report completion if the consultant was not local. Over the last 15 years, this mode of operation has evolved with many larger offices bringing a FA on staff to provide case analysis as needed, while also filling an additional role(s) in the office. This arrangement has proven advantageous for the ME/C in that they have a specialist on their staff that is available at any time for FA consults – to include trauma consults with the pathologist during the autopsy examination. In addition, the FA is also trained in office policy/procedures (especially those related to the handling of evidence). The broad educational background of a FA lends itself to other roles in the office to include, but not limited to, medicolegal death investigator, DNA coordinator, identification coordinator, mass disaster planning management, director of photography, trace/evidence/latent print examiner, autopsy technician and forensic database administrator. In this way the FA also builds important working relationships with the entire ME/C staff, police and crime scene personnel, and other investigative agencies. These relationships also lead to greater education and understanding of forensic anthropology for outside agencies. As part of the ME/C staff, the FA is immediately available to assist with the case from the point of the scene investigation/recovery. In the majority of cases, this involvement directly leads to the greatest recovery of skeletal and trace evidence from the scene which is typically correlated with successful case resolution.

This presentation will discuss the current, diversified roles filled by FAs in the medicolegal system and the advantages this provides for

professional enrichment, new training opportunities, certification options and diverse employment opportunities. Stresses associated with the overall unpredictability of the operational movement at an ME/C Office and the demanding workload requirements diversification of duties presents will also be explored. The roles and responsibilities of several FAs currently serving in ME/C offices will be presented and discussed. In addition, the roles of several FAs currently employed in professional forensic positions outside of the ME/C system will also be presented to illustrate the range of opportunities available to the FA.

Diversification of professional duties is a trend that is on the rise, in part, due to the current economic climate. This trend should not be looked on as a negative in that professional focus is being split, but as a positive opportunity to learn and apply additional forensic skills. As will be shown, this trend presents the FA with unique opportunities to diversify their forensic proficiency into other areas and increase overall marketability.

Forensic Anthropology, Medicolegal System, Professional Roles

#### H18 Forensic Anthropology and Virtual Human Remains: Ethics in Uncharted Territory

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After attending this presentation, attendees will have an understanding of some of the ethical considerations that forensic anthropology as a discipline may be facing as it begins to increase the use of virtual human remains and imaging technologies.

This presentation will impact the forensic science community by opening a dialogue in the anthropological community regarding the use of virtual human remains so as to establish ethical guidelines for the future.

The use of virtual human remains in forensic anthropology has been steadily increasing as the technology to capture and view them becomes more affordable and accessible. Within the next decade, it is anticipated that medical imaging tools such as multislice computed tomography (MSCT) and magnetic resonance imaging (MRI) scanners and software, along with other data capture capabilities such as laser scanning will become a routine component of the anthropologist's toolkit in much the same way as radiographs and photographs. Recent work (Decker et al, in press; Decker et al, 2008)<sup>1,2</sup> and others (Thali et al, 2003)<sup>3</sup> demonstrate the potential for virtual remains for the non-invasive examination of remains, as well as the ability to use imaging as a permanent record of an individual. It is now possible to scan an entire human body, whether living or dead, and create a 3D virtual model of it in minutes. This digital human can be explored in a multitude of unprecedented and heretofore unimagined ways, both for crime-solving and research purposes. It has not yet been decided whether these new types of medical images will be considered a simple increase in sophistication from existing tools, or if the differences are so marked that they will be subject to a new set of rules that has yet to be defined.

The discipline must consider the potential contentiousness surrounding the retention and future use of virtual skeletal remains. The big question that has yet to be asked – or answered – is "Are virtual remains governed by same ethics as actual remains?" There are three main areas in which these issues likely will be encountered: forensic cases (involving identified and unidentified individuals), use in education, and use in research. There is potential for a vast amount of knowledge to emerge from such specimens, but issues must be considered that may arise surrounding cultural and religious values of the deceased and the survivors - this is a daunting task in uncharted territory.

Recent reports and investigations by the National Academy of Sciences and the United States Congress have made this an even more pressing issue that must be addressed as the discipline pushes towards standardization of the forensic sciences.

As a field, there must be transparency in our practices and consider the values and viewpoints of the public as part of the discipline's responsibility. In the modern climate, public dissemination is necessary. It may be assumed that the treatment of virtual human remains will be similar to other types of digital evidence in a forensic case. However, real human remains are often handled differently than other types of evidence. Also, due to the nature of the work, human remains handled by a forensic anthropologist are not always part of an investigation so the circumstances of such remains are different. In this presentation, these issues are discussed as well as the different issues surrounding the use of remains in teaching and research.

This presentation will examine current attitudes toward the treatment and use of virtual human remains and explore the pathways that the profession can take to ensure that ethical practices continue to evolve along with laboratory practices. **References:** 

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- <sup>2</sup> Decker SJ, Hilbelink DR, Hoegstrom E, Ford J (2008) Virtual Skull Anatomy: 3 Dimensional Computer Modeling and Measurement of Human Cranial Anatomy. *Proceedings of the American Academy of Forensic Sciences 2008*, Washington DC.
- <sup>3</sup> Thali, MJ, Yen K, Schweitzer W, Vock P, Boesch C, Ozdoba C, Schroth G, Ith M, Sonnenschein M, Doernhoefer T, Scheurer E, Plattner T, Dirnhofer R (2003). Virtopsy, a new imaging horizon in forensic pathology: virtual autopsy by postmortem multislice computed tomography (MSCT) and magnetic resonance imaging (MRI)—a feasibility study. *Journal of Forensic Sciences* 48: 386–403.

Virtual Anthropology, Medical Imaging, Ethics

#### H19 Femmes Fatales: Why Do Women Dominate the Discipline of Forensic Anthropology?

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After attending this presentation, attendees will be able to recognize the global phenomenon showing more women than men are actively engaged in forensic anthropology education and professional practice in the United Kingdom, United States, and Europe; and understand the reasons for this sweeping trend. It is especially obvious that more women than men are applying for and attending higher education courses in the United Kingdom and United States. It is anticipated that the attendees will have observed this trend in their own university courses, whether as tutors or students, and will have their own views on the phenomenon and explanations for it. This presentation will explore the different reasons for the trend, perhaps controversially. Attendees will gain insight into the determining factors that make more women choose to study forensic anthropology, remain in the discipline, and prosper with successful careers, as well as, discover if there are disincentives for men. It is hoped that this presentation will raise questions that will stimulate debate and make the attendees think about the nature of forensic anthropology education and practice.

This presentation will impact the forensic science community by discussing the reasons why more women enter university programs and become professional forensic anthropologists than men. It is undeniable that, both in the United Kingdom and abroad, undergraduate, and postgraduate programs are inundated with female applicants, and female students in courses outnumber male students in the order of at least 2:1, up to record numbers of even 25:1. This has tremendous implications for the future of forensic anthropology as a discipline, and for universities attempting to attract male, as well as, female students. Female-rich cohorts may positively or negatively influence selection criteria, numbers enrolled on part-time courses, completion rates, and the quality of learning. The high numbers of female professional forensic anthropologists may have positive or negative implications for career progression, deployment opportunities, membership of professional organizations, and acceptance by male-dominated institutions such as police and law enforcement agencies. Although the phenomenon has undoubtedly been noticed in the classrooms and laboratories of the United Kingdom and the United States, a systematic analysis of the reasons behind it has not been carried out to date, and it is vital in order to understand and prepare for the future of modern forensic anthropology.

This research aims to discover the cause of the undeniable, worldwide phenomenon that women dominate the global discipline of forensic anthropology today. There are more women than men training to be forensic anthropologists; in academic roles teaching forensic anthropology; and in professional forensic anthropology practice, in the United Kingdom, United States, and abroad, which begs the question "*why*"? This study is focused on establishing the various motivations for both men and women contemplating degrees and careers in forensic anthropology, and discusses their implications for the discipline.

Research questionnaires were circulated among male and female student and professional forensic anthropologists in the United Kingdom, United States, and Europe, in order to collate educational backgrounds and attitudes towards the subject and careers in the discipline. Admission and attendance statistics from United Kingdom, United States, and European universities and professional organizations were also gathered to amass data to chart the progression of the trend, the steady influx of women, and the decline of male students in undergraduate and postgraduate forensic anthropology courses since they began. Preliminary data has shown a steadily increasing majority of female applicants since the subject was offered as a university degree in the United Kingdom in 2002. Data from professional organizations in the United Kingdom and abroad was interrogated to determine whether applications from women outnumber those from men, and whether continued attendance and contribution has shown a gender bias over the last ten to fifteen years. In the handful of professional organizations for Biological and Forensic Anthropologists in the United Kingdom, for example, women outnumber men as many as 3:1.

The questionnaires pinpoint the factors that influence and encourage women to pursue a career in forensic anthropology, and to stay in it even if their life circumstances change. Preliminary results have offered some conflicting evidence, some of which suggests a career in forensic anthropology is flexible enough to accommodate raising a family, and some of which implies it may preclude it. It considers the attractions of the discipline to women, and whether these are different to those for men. The presentation explores this undeniable and extensive phenomenon, and investigates how long it has been occurring. It also discusses the motivation and impetus behind it. It will investigate the extent of the trend, and whether it exists only in forensic archaeology and anthropology, or whether it is true for forensic science as a whole, or indeed all the sciences in general.

Preliminary questionnaire responses raise some important questions: is the popularity of television crime shows to blame/credit? What is it about forensic anthropology that appeals to women? Is forensic anthropology perceived as glamorous? Does forensic anthropology represent a flexible career for women with families? Is it a recent phenomenon that reflects changing demographic distribution in most academic subjects? Is the trend to do with changing attitudes towards science, academic careers, women, or men, or all of the above? How long will it last? Already, there is a vast pool of opinion regarding these issues, which shows that although the trend is obvious, the reasons behind it are not, and/or they may be difficult to accept. Controversially, it will discuss whether women make better forensic anthropologists, or whether they are better suited to the subject for any reason, and whether men cannot compete in the workplace. Are men being put off the subject? Is there a stigma attached to the discipline for men? Do they feel at a disadvantage for any reason? Are they feeling 'crowded out' or unwelcome in any way? Are men put off by the sheer numbers of women in the discipline? And of course, does the dominance of women in the discipline matter at all?

The answers to these questions have considerable implications for the future of forensic anthropology in the United Kingdom and abroad, in terms of education marketing, compliance with Equal Opportunities legislation and the composition of professional organizations and the practitioner workforce. This research aims to answer these questions and more, and to determine the true nature of the apparent complete dominance of the discipline that has emerged in the last ten to fifteen years, and above all, it aims to stimulate debate amongst male and female, student and professional, forensic anthropologists, and "get to the bottom" of this important and remarkable phenomenon.

Forensic Anthropology, Women, Education

### H20 Development of the Colombian Skeletal Collection

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After attending this presentation, attendees will gain knowledge of the development of the Colombian Skeletal Collection, including the administrative and technical logistics behind its assembly, the antemortem information available, and the goals of research projects.

This presentation will impact the forensic science community by detailing the creation of a new modern skeletal collection in Colombia available for research with the goal of aiding in the identification process of victims of the Colombian conflict.

During the past two decades Colombia has been faced with sociopolitical problems which have led to innumerous violent situations, resulting in the deaths and disappearances of thousands of individuals. Many of the recovered individuals are skeletons from clandestine graves located all over the country. Once excavated and sent to the forensic laboratories, analysis is done by forensic anthropologists, dentists, and pathologists who must determine who each individual was and whether or not they died in a violent context.

As the majority of recovered individuals remain unidentified, it is necessary to develop a way to augment the ability to answer several questions, including positive identification and cause and manner of death. Due to the above, a research collection of modern skeletons is currently being organized in Colombia. The Colombian Skeletal Collection is being assembled for several reasons: (1) to develop standards and validation studies (for age-at-death, sex, stature, etc.) from the Colombian population because best practice states that standards developed from one population should only be applied to that particular population; (2) at this point, there are very few forensic anthropologyrelated population studies that have been done in Colombia, and therefore, the standards used to analyze forensic cases here are those based on American and European populations; and, (3) to allow for the generation of scientific knowledge with regards to physical/forensic anthropology for Colombia and to enable research to move forward here in the areas of physical/forensic anthropology, dental anthropology, radiology, osteopathology and taphonomy, among others.

In order to assemble the collection, an inter-institutional agreement was entered into between the National Institute of Legal Medicine and Forensic Sciences and the Public Services Administrative Unit, the entity which administers the cemeteries within Bogotá. The agreement states that instead of being placed into collective ossuaries, bodies not claimed by relatives once a four-year single burial period has lapsed will be donated to the Institute of Legal Medicine for the purpose of scientific investigations.

The first phase of the project has a five-year duration (2009-2013) and will be renewed in 2013 if both parties agree. The goal for Phase 1 is to assemble a collection of 600 individuals. Since 2009, 100 skeletons have been prepared for the collection (native-born Colombians, males and females aged 18-65, birth years 1940-1987, death year 2005). Skeletons are in good to excellent condition.

A large amount of antemortem information is available for each individual in the collection and is being placed in a searchable database. This information includes date of birth, place of birth, sex, stature, date of death, and cause and manner of death. In the instances where the individual died while under a doctor's care, the medical records that show the evolution of the patient's treatment are available. Likewise, when an autopsy was performed, this report is also available with postmortem photographs of the individual's face. Furthermore, information from each cemetery regarding where each person was buried (e.g., in a coffin in the ground or in a mausoleum); climate of the area where the cemetery is located; type of coffin or burial container; and the photograph of the individual at the time of exhumation is available as well.

The collection will be available for research once 150-200 individual skeletons have been prepared, which is anticipated within a year. The requirement for researchers will be that a project proposal be presented to and approved by the Division of Scientific Investigation of the National Institute of Legal Medicine and Forensic Sciences in Bogotá.

In addition to being the first of its kind in South America, the Colombian Skeletal Collection will rival other similar modern skeletal collections in terms of available antemortem information alone. This will greatly contribute to both the utility and variety of the research questions that will be investigated.

Colombia, Skeletal Collection, Modern Population

#### H21 A Bayesian Approach to Multifactorial Age-at-Death Estimation

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The goal of this presentation is to inform attendees about a new Bayesian approach to multifactorial age-at-death estimation.

This presentation will impact the forensic science community by presenting a new method for combing several indicators of skeletal ageat-death to arrive at a single age estimate.

Most forensic anthropologists rely on multiple skeletal indicators of age-at-death but lack a statistically sound method for combining individual indicators. Attempts at multifactorial aging (e.g., Brooks, 1955; Lovejoy et al., 1985) have had generally disappointing results because they typically rely on either non-statistical or linear statistical methods, creating problems with validity and applicability.

Recently, paleodemographers have been at the forefront of multifactorial age-at-death estimation. Boldsen and colleagues (2002) developed a computer program (ADBOU) that collects data on multiple skeletal indicators scored as discrete ordinal phases and uses Bayesian

at-death. Unfortunately, tests of the ADBOU program found it only moderately effective (Bethard, 2005; Uhl, 2008), in part because the trait scoring departs from the methods (e.g., Suchey-Brooks) that so many osteologists are accustomed to. Without extensive practice, intra- and inter-observer error can be problematic. Further, the ADBOU program comes with a small choice of prior age-at-death distributions "hardwired" into the program. Bayesian analyses rely on these prior probabilities, together with the osteological data, to estimate ages at death for individual cases. The current research makes use of a more diverse, and possibly more appropriate, reference sample and familiar skeletal scoring techniques to estimate age-at-death from multiple indicators when combined with an appropriate prior age-at-death distribution. The present data set consists of age indicator scores for pubic

inference to calculate the posterior probability density and estimate age-

The present data set consists of age indicator scores for public symphysis (6 phases; Brooks and Suchey, 1990), auricular surface (8 phases; Lovejoy et al., 1985), and sternal rib end (8 phases; İşcan et al., 1984, 1985) for 623 individuals from four collections: the Hamann-Todd Collection, the William M. Bass Collection, the R.J. Terry Collection, and the Pretoria Bone Collection.

**Results:** One initial issue to address is whether the original scoring follows a particular transition model. First, a Lagrange multiplier test indicated that the original six-phase pubic symphysis scoring and the eight-phase rib end scoring fit well in a cumulative log probit model. The auricular surface scoring did not fit well, so the first four phases in the Lovejoy et al. system were collapsed into a single phase. After making this collapse, the scoring did fit well in a cumulative log probit model.

Following initial testing, 100 individuals were randomly sampled structured on age-at-death using a Gompertz model of mortality estimated from the ages at death for Suchey's LA County male forensic data. This Gompertz model was also used as the informative prior in estimating ages for the 100 individuals. After forming this "hold out" sample, transition models were fit using the remaining 523 individuals, and the 95% highest posterior density region was found for each of the 240 morphological patterns (6 pubic symphyseal phases times 5 auricular surface phases times 8 rib phases) combined with the informative prior. The left and right boundaries were stored in a "lookup table" and then compared to the actual ages for the hold out sample. Ninety-five of the 100 individuals had ages that fell within the 95% highest posterior density regions, indicating proper coverage. The widths of the 95% highest posterior density regions were sometimes quite considerable, reaching a maximum of 50 years for anyone in the final phase for all three indicators. The right side for this region is entirely determined by the prior age-at-death distribution.

**Conclusions:** All analyses were done in "R," which is an open source package that can be downloaded for free. As such, the lookup tables, while they are easy to use can also be adjusted to meet individual researcher's needs. For example, the density regions can be changed (to, for example 50% highest posterior density regions) and the Gompertz model parameters for the prior age-at-death distribution can also be changed.

Age-at-Death Estimation, Bayesian Inference, Multifactorial Age Estimation

## H22 The Use of Vertebral Osteoarthritis and Osteophytosis in Age Estimation

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The goal of this presentation is to assess whether or not vertebral degenerative changes can be used for estimating age.

This presentation will impact the forensic science community by demonstrating that a significant but weak correlation exists between age and vertebral degenerative changes.

For more than 50 years, research has been conducted on various regions of the human skeleton to establish techniques for determining age at death; however, the accuracy of those age prediction techniques generally decreases as chronological age increases. While previous research on the vertebrae indicates that a correlation exists between age and osteophyte development (osteophytosis) (Snodgrass 2004, Stewart 1958),<sup>1,2</sup> degenerative changes (osteoarthritis) in the zygapophyses have not been assessed for patterns associated with age. Additionally, many of the past studies that assessed vertebral bodies in forensic and bioarchaeological settings were conducted on skeletal collections from more than 75 years ago.

The present study examined degenerative changes both in the bodies and zygapophyses in all 24 vertebrae using a modern forensic population from the Donated Collection at the University of Tennessee, Knoxville. Researchers independently examined and scored the superior and inferior borders of the vertebral bodies and the superior and inferior facets of each vertebra for 104 individuals aged between 30 and 90 years. Scoring techniques for osteophytosis and osteoarthritis were based on Ubelaker (1999).<sup>3</sup> Statistical analyses were used to assess relationships between age and degenerative change for the bodies and facets, both separately and in combination, for all vertebrae collectively, as well as for subcategories of vertebral types. Separate analyses also were conducted which included only the vertebrae in regions that are most commonly flexed (for osteophytosis, these regions included C5-6, T8-9, and L4-5; for osteoarthritis, C6-7, T1-5, L2-4).

Results using all 24 vertebrae indicate the following. Severity of osteophytosis is significantly correlated to age for all vertebrae collectively, as well as for each vertebral subcategory (p < .001); however, the association is not strong (R<sup>2</sup> values range from 0.244 for cervical vertebrae to 0.393 for lumbar vertebrae). With regard to osteoarthritis, severity is significantly correlated to age for all vertebrae collectively, as well as for the cervical and lumbar subcategories (p < .01); however, once again, the association is not strong (R<sup>2</sup> values range from 0.168 for all facets combined to 0.305 for cervical facets). Results do not improve when bodies and facets are considered together: severity is significantly but not strongly correlated with age in all categories (p < .05; R<sup>2</sup> ranges from 0.205 for thoracic vertebrae to 0.370 for cervical vertebrae).

Results of the analyses for areas of common flexion are only slightly better. Osteophytosis and osteoarthritis are significantly correlated to age for all categories of data when considered both separately and together (osteophytosis: p < .001 with  $R^2$  values ranging from 0.243 in the cervical vertebrae to 0.408 for combined subtypes; osteoarthritis: p < .01 with  $R^2$  values ranging from 0.116 in the thoracic facets to 0.244 in the lumbar facets; combined: p < .001 with  $R^2$  values ranging from 0.217 in the thoracic vertebrae to 0.319 in the lumbar vertebrae).

The current study assessed the presence and strength of the relationship between age and vertebral degenerative changes with the hope of generating predictive models for estimating age in older individuals. To differentiate from previous research, data from multiple indicators were considered both individually and collectively and a contemporary population, composed of individuals whose deaths post-dated 1980, was used. In general, results from this study add to, but ultimately mirror, previous research. That is, both osteophytosis and osteoarthritis are significantly but not strongly correlated with age (either singularly or in combination). Therefore, though both types of degenerative change are believed to be associated with repetitive movements and stress (and, thus, exacerbated by the aging process), the relationship is not strong enough to yield predictive power for establishing age estimates.

**References:** 

- <sup>1.</sup> Snodgrass JJ. Sex differences and aging of the vertebral column. J Forensic Sci 2004;49(3):458-463.
- <sup>2.</sup> Stewart TD. The rate of development of vertebral osteoarthritis in American whites and its significance in skeletal age identification. Leech 1958;28(3-5):144-151.
- <sup>3.</sup> Ubelaker DH. Human skeletal remains: excavation, analysis, interpretation. 3<sup>rd</sup> edition. Washington, D.C.: Taraxacum, 1999.

Age Estimation, Vertebrae, Osteoarthritis

#### H23 Error and Uncertainty in Pelvic Age Estimation Part II: Younger vs. Older Adult Females

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After attending this presentation, attendees will understand how the error rates of three commonly used pelvic age estimation methods differ among females of different age groups, and how to quantify uncertainty in forensic anthropological analysis. Additionally, error rates will be compared for males and females.

This presentation will impact the forensic science community by responding to Recommendation 3, of the National Academy of Sciences Report, *"Strengthening Forensic Science in the United States: A Path Forward,"* which calls for research determining causes of bias and work toward quantification of method error in forensic investigations.

This presentation is the second half of an aging study designed to test the assumption that adult skeletal age estimation methods have lower error rates when applied to younger versus older adults. It will focus on pelvic age estimation methods for adult females; the first part of the study concerning adult males was presented at the 2010 AAFS meeting.

Skeletal age estimation methods are widely understood to overage the young and underage the old. This paper supports these assertions by offering quantified measurements of error for three frequently used pelvic age estimation methods, as applied to a large sample of female individuals between the ages of 18 and 101 years. The methods include the following auricular surface and pubic symphyseal techniques: Lovejoy *et al.* (1985); Suchey-Brooks (1990); and Osborne *et al.* (2004).

The study sample was compiled from several sources: female individuals sampled from modern known-age Iberian skeletal collections housed at the Universidad de Valladolid and the Universidad Autònoma de Barcelona; and identified female individuals from the Forensic Data Bank (FDB) courtesy of Dr. Richard Jantz at the University of Tennessee, Knoxville. The combined sample was divided into two broad age categories: "younger" individuals ( $\leq$  39 years) and "older" individuals ( $\geq$  40 years). Error with respect to the methods' assigned means was analyzed in terms of bias (directionality of error:  $\Sigma$ [estimated age – actual age]/n) and inaccuracy (absolute mean error in years:  $\Sigma$ |estimated age – actual age range included the individual's actual age) was also calculated.

All three methods have low mean positive biases and mean inaccuracies close to five years for the group of females  $\leq$  39 years of age. Conversely, all three methods have substantial mean negative biases and mean inaccuracies greater than 17 years for females  $\geq$  40 years of age. In all three methods, levels of mean bias and inaccuracy were statistically significantly different for the two age groups (p  $\leq$  0.001; Student's *t*-test). Error rates were always greater for older than for younger individuals.

Use of the Suchey-Brooks method resulted in correct classification of 95% of individuals  $\leq$  39 years of age and 76% of individuals  $\geq$  40 years of age. For the Lovejoy *et al.* method, the percent of correctly classified individuals was 49% for individuals  $\leq$  39 years of age and 53% for individuals  $\geq$  40 years of age. The Osborne *et al.* phase modifications resulted in a higher amount of correct classifications than the Lovejoy *et al.* method for both age groups (90% and 71%, respectively). Full ranges of error (in years) for each method for individuals  $\leq$  39 are as follows: Suchey-Brooks (-11 to 31); Lovejoy *et al.* (-9 to 19); Osborne *et al.* (-14.9 to 24.8). For individuals  $\geq$  40, full ranges of error (in years) are as follows: Suchey-Brooks (-43.8 to 20); Lovejoy *et al.* (-53 to 25); Osborne *et al.* (-48 to 18.9).

As compared to adult males, adult females exhibit higher error rates for all three pelvic age estimation methods. In most instances, females are also more likely to be incorrectly classified than males when using these selected methods. An exception is the Lovejoy *et al.* method applied to individuals over the age of 40, which results in 53% correct classification of females and 30% correct classification of males. The full ranges of error for males and females are similar, though female ranges are always slightly larger.

This study indicates that three widely used pelvic aging techniques estimate age in younger adult females ( $\leq$  39) with lower error than older adult females ( $\geq$  40), but with higher error for females than males. Auricular surface methods are problematic regardless of age group or sex. Given that error increases with age, modifications of upper phases of the Suchey-Brooks method are warranted (e.g., Berg [2008]). It is important to recognize that there will always be error associated with age estimation and other forensic anthropology methods. Therefore, the focus should now move to understanding and quantifying error so as not to overstate method performance.

Adult Female Age Estimation, Pelvis, Error

#### H24 Assumptions and Bias in Recalibrating Age Standards Across Populations

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The goals of this presentation are to explore the application of Bayesian analyses in age estimation for human identification and to demonstrate possible evidentiary biases that result from incorrect assumptions about the data. This is particularly critical for research into population variation where investigators attempt to recalibrate age parameters based on ethnic variation.

This presentation will impact the forensic science community by reanalyzing published data on age estimation for dental methods. The correct application of Bayesian statistics and assumptions about population data are critically important when these methods are applied to estimate age among living subjects, for human identification among decedents, and for the courtroom admissibility of anthropological methods.

In recent years, there have been a number of published articles that argue for population specific standards, in other words, researchers suggest that aging methods should be recalibrated when applied across populations. While Bayesian analyses in forensic anthropology can be very useful in some contexts, it is generally agreed that if informative priors are used they need to be clearly delineated. Without making priors explicit, forensic anthropologists run the risk of introducing biases into evidentiary processes based on assumptions that may not fit well with what is known about a particular case. The use of dental age estimation methods often fail to account for implicit priors. The following study reexamines data from published studies to demonstrate how interpretations vary based on prior assumptions about the data and how results change based on explicit prior information.

For example, summary data from Kasper et al. (2009 Journal of Forensic Sciences 54(3):651-57) is re-analyzed. Kasper et al. present data on third molar development for 950 individuals ranging in age (at the last birthday) from 12 to 22 years including the mean and standard deviation for age within seven stages of third molar formation ("B" through "H" from Demirjian et al.'s 1973 scoring system). As Konigsberg et al. (2008:542) noted "a final problem with any method that conditions on stage to estimate age is that all of these methods contain an implicit prior distribution for age." This is seen in the present study, particularly for teeth where the root apex is complete (stage "H"). Kasper et al. assume that age within stage is normally distributed, but because their sample's age distribution is truncated at 12 and 22 years, the mean age within stage "H" must be less than 22 years.

With age data, it is difficult to justify the assumption that the age distributions within stages are normal, as these distributions depend on: (1) the age distributions for when individuals move to the next higher stage; and (2) the overall age distribution of the sample. By Bayes' Theorem:

$$f(a|i) = \frac{p(i|a)f(a)}{\int\limits_{a=0}^{a} p(i|a)f(a)}, (1)$$

where p(i|a) is the probability that someone at exact age "a" is in stage "i," f(a) is the probability density function for age, and f(a|i) is the probability density function that someone is exact age "a" given that they are in stage "i," and  $\omega$  is the upper limit of integration (i.e., the maximum possible age). If a researcher does not wish to include an informative prior then a uniform prior can be substituted, giving:

$$f(a|i) = \frac{p(i|a)}{\int_{a=0}^{a} p(i|a)} \cdot (2)$$

A critical issue for the presentation of aging methods in court when identifying the age of living suspects is the probability of being a certain age. Examples of court cases in which age methods have been disputed are discussed in this presentation. Additionally, other methods of determining the probability of a given age include the use of a parametric model for p(i|a). More specifically a cumulative probit model on the log scale ages can be used to model p(i|a). This is precisely the model that was used by Moorrees, Fanning, and Hunt in their classic studies of dental development.

This paper demonstrates that in using estimated "transition parameters" in log cumulative probit models, the probability that someone is over the age of 18 years is substantially different than the reported accuracy in published studies and demonstrates the need for discussion about the biases implicit in demographic data as well as the possible evidentiary biases that result from such assumptions about the data.

Age Estimation, Population Variation, Bayes' Theorem

#### H25 Sacral Epiphyseal Fusion at S1-S2: Classification, Comparability, and Error

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After attending this presentation, attendees will understand how sacral fusion can be used properly in age estimation, problems associated with current techniques, and proposals for best practices when using sacral fusion as an age estimation technique.

This presentation will impact the forensic science community by examining sacral fusion age estimation in response to critiques raised by the National Academy of Sciences Report (NAS) concerning the need to evaluate the reliability and accuracy of methods used in forensic science.

Epiphyseal fusion as an age estimation method is useful because fusion generally occurs at the same time for all individuals. The fusion of the anterior margins of the sacral vertebrae has further potential for age estimation in young adults because of the delayed union of the first two sacral segments (S1 and S2). McKern and Stewart's 1957 publication of *Skeletal Age Changes in Young American Males*, one of the first and most comprehensive analyses of age estimation, examined sacral fusion employing a five-stage system (e.g., Stage 0=nonunior; Stage 4=complete union). Several more recently published methods also examine sacral fusion, albeit with the use of different scoring systems (e.g., Coqueugniot and Weaver (2007) used a three-stage letter system [a=open, b=partial, c=complete], and Belcastro *et al.* (2008) used a fourstage numbering system [e.g., Degree 0=absence of fusion, Degree 1=less than 50% fusion]). These differences complicate comparisons of results from essentially the same technique.

The current study was designed to examine the performance of the sacral fusion age estimation method using the scoring system and age intervals given by McKern and Stewart (1957:148). The known age-at-death was compared to the predicted age-at-death based on the recorded stage of fusion for all individuals identified at the JPAC-CIL between 1972 and 15 June 2010 whose case documentation specifically referenced the McKern and Stewart (1957) method (n=40). Correct classification, or the percent of individuals whose known age-at-death fell within the assigned age interval, was used to test this method. Additionally, the sample was compared to the overall JPAC-CIL identified sample and the Korean War identified sample from McKern and Stewart (1957).

The JPAC-CIL sample for the McKern and Stewart (1957) sacral fusion method (n=40) has a mean age-at-death of 24.2 years, an age range of 12 years (youngest individual=18, oldest individual=30), and is entirely male. There is a statistically significant difference (p=0.002, Student's *t*-test) in mean age-at-death between this sample and the total known age-at-death sample of JPAC cases (n=979, =27.2); the sample aged using S1-S2 fusion is younger than the entire identified sample.

Of the 40 individuals whose case files referenced this method, 45% (n=18) were placed in Stage 0. The second largest group was comprised of individuals scored as Stage 2 (n=12). Stages 1, 3, and 4 each had three individuals, and one individual was scored as "Stage 1 or 2." Compared to the McKern and Stewart (1957) sample, there were considerably more individuals observed with nonunion of the S1-S2 joint in the JPAC-CIL sample.

The age distribution of the samples also differs. For example, in the McKern and Stewart (1957) sample, Stage 0 (nonunion) was observed only in individuals between the ages of 17 and 18, whereas nonunion was seen in the JPAC-CIL sample in individuals up to 30 years of age. Because of this, the sacral age estimation method based on McKern and Stewart's (1957:148) reported ages had a correct classification rate of 32.5% and an incorrect classification rate of 67.5% for the JPAC-CIL sample. However, when applying a simple "fused versus unfused" model, the percentage of correct classification increases to 95% for the

entire sample (n=40). This model classifies Stages 0, 1, and 2 as incomplete fusion and Stages 3 and 4 as complete fusion and categorizes individuals with incomplete fusion as less than 30 years of age and individuals with complete fusion as 17 years of age or older.

Analyses of the JPAC-CIL case files indicate that employing the age intervals provided by McKern and Stewart (1957:148) results in largescale misclassification of age when presented with an S1-S2 joint in any stage of incomplete (i.e., partial or open) fusion. It is therefore recommended that incomplete sacral fusion be regarded simply as an accessory to other more precise methods of age estimation. Incomplete sacral fusion can be used to establish an upper bound for the age estimate; in this sample, age 30 was found to be a useful sectioning point. However, further research in a more varied sample could modify this sectioning point. Additionally, there is a great need for anthropologists to agree on methods of age estimation, to include the use of identical scoring systems. This will alleviate unnecessary complications in data comparison and the continual redevelopment of these scoring systems. Sacral Fusion, Age Estimation, Error

H26 An Evaluation of the Chen et al. Pubic Aging Method on a North American Sample

Julie M. Fleischman, BA\*, Michigan State University, 560 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will understand the Chen et al. (2008) pubic bone aging method and its application for estimating age-at-death for a North American population.

This presentation will impact the forensic science community by exploring the utility of the Chen et al. (2008) aging method for males of European ancestry.

Accurately assessing the age-at-death of adult human skeletons is fundamental in creating biological profiles for unidentified remains. There are many methods available to forensic anthropologists to estimate age-at-death; the most widely used and generally accepted involve analysis of the pubic bones. Numerous aging methods using the pubic bones are available, including Chen et al. (2008) which is the focus of this study.

Chen and colleagues assessed age-at-death for Chinese Han males based on multiple pubic bone features. The features were scored for 262 pubic bones and were subjected to four types of statistical equations to estimate age: multiple regression analysis (MRA) and gradual regression analysis (GRA), with quantification theory model-I (QMI) and GRA to compare with MRA. One goal of the Chen et al. (2008) study was to improve upon the Suchey-Brooks method, which is currently the most accepted technique for estimating age from the pubic bone. For the Han sample Chen and colleagues claim that with the use of their statistical formulae, a large sample, evaluating males only, and subdividing each feature, age-at-death can be quantitatively estimated with a high degree of accuracy.

The objective of this research is to evaluate the Chen et al. (2008) method to determine if it can accurately evaluate age-at-death for individuals outside the original study population. This research addressed two primary questions: (1) Will the Chen et al. (2008) method accurately assess age-at-death for non-Chinese males?; and, (2) Will the revised Chen et al. (2008) method accurately assess age-at-death for males of European ancestry?

This research is based on a known sample of modern pubic bones curated at the Maricopa County Forensic Science Center (FSC) in Phoenix, Arizona. A sample of 296 left male pubic bones of European ancestry, between the ages of 18 and 70, was selected from the larger collection. These bones were scored based on nine morphological indicators (e.g. ridges and furrows on the symphyseal surface, ossific nodules, and bone density). Each pubic bone was scored blind by four observers with osteological experience ranging from 20+ years to 2 years.

This research generated statistical data concerning the accuracy, rates of error, and significance of the Chen et al. (2008) model's utility for aging male populations of European ancestry. The original Chen et al. (2008) equations were tested and then four revised equations were generated from the FSC scores. Accuracy for the revised equations was evaluated via the percentage correct within brackets of one, five, ten, and fifteen years from the actual ages. A higher percentage per bracket translates to higher accuracy.

Results indicate that the Chen et al. (2008) method is fully replicable for males of European ancestry. The most accurate equation varies by bracket—one year from actual age: original Chen et al. MRA+GRA (10.8%); five years: revised QMI+GRA (38.6%); ten and fifteen years: revised MRA+GRA (65.7% and 87.3%). The revised model demonstrates only incremental gains over the original model (revised model MRA+GRA R<sup>2</sup> = .491 and original model MRA+GRA R<sup>2</sup> = .440), and on average the revised model tends to slightly over-age the specimens. The revised model has an average error of 8 years from actual ages. Both the original and revised models have lower predictive values for the FSC sample than Chen and colleagues report for their sample (Chen et al. MRA+GRA R<sup>2</sup> = .978). All Pearson's correlations for inter- and intra-observer error were statistically significant indicating low error rates between observers.

The Chen et al. (2008) method is challenging and requires proficient knowledge of the nine pubic bone features and their development before implementation; however, the model does explain almost 50% of the variability in the FSC sample. An average error of eight years from actual age is acceptable for a forensic biological profile, and the model accurately estimates age within 15 years for over 87% of individuals. Therefore, this is a viable method for estimating age-at-death for males of European ancestry. Future research is required to determine if this method is more or less accurate than others, such as Suchey-Brooks.

Pubic Bone, Age Estimation, Male

#### H27 The Accuracy of the Lamendin Method of Dental Aging in Teeth With Fillings

Kristin E. Horner, MA\*, Secchia Center, 15 Michigan Street Northeast, Grand Rapids, MI 49503

After attending this presentation, attendees will understand the results and implications of a study to determine if the presence of a filling in a tooth effects the accuracy of the Lamendin method (Lamendin et al., 1992) of dental aging.

This study will impact the forensic science community by providing validation for the application of a commonly used aging technique to a unique subset of teeth. This validation is important in the post-*Daubert* era, where established error rates are important. Although error rates are known for the Lamendin method, and some dental aging studies have included teeth with fillings, no investigation has previously been made into the effects that these fillings might have on the accuracy of age estimation.

The purpose of this presentation is to discuss the effects of dental restorations on the accuracy of the Lamendin dental aging method. The Lamendin method uses two measurements, tooth root translucency and periodontosis. Tooth root translucency begins at the tip of the root and proceeds toward the crown with advancing age, and is believed to be caused by calcification within dentinal tubules (Bang & Ramm, 1970). This changes the refractive index within the dentinal tubule so that it is similar to that of the material surrounding the dentinal tubules, making the area appear transparent. It has been established that root canal

treatment can have a significant effect on the development of tooth root translucency (Thomas et al., 1994), but there has been no published work documenting the effects of fillings.

The utility of the Lamendin method is clear; it is fast, easy to use, does not require any special equipment, and utilizes a simple formula. The method provides a relatively accurate estimation of age that is useful in both forensic and archaeological contexts. However, it is important to determine if any external factors affect the rate of development of tooth root translucency. If any factors are discovered that do affect the rate of translucency, these factors would influence the accuracy of dental aging methods, such as the Lamendin method, that rely on tooth root translucency.

Premolar teeth (N = 100) from the William M. Bass Donated Skeletal Collection were used for this research. The sample consists of 50 teeth with no dental restorations and 50 teeth with fillings. All teeth were selected from individuals age 30 or older because the Lamendin method cannot be used in individuals younger than 25, and tends to be unreliable at younger ages. Measurement of periodontosis and root height were taken in millimeters with sliding calipers. Measurement of root translucency was observed using a light box and taken in millimeters with sliding calipers. Age-at-death was recorded from the collection database.

Age at the time of death was estimated using the Lamendin method. The difference between the estimated age and known age was calculated for each tooth. Error was compared between teeth with no restorations and teeth with fillings using a student's T-test. No significant difference (p < 0.05) was found between the errors of the teeth with fillings and the teeth without fillings.

It is concluded that the presence of dental fillings does not significantly impact the accuracy of the Lamendin method, and that teeth with fillings may be used to estimate age using the Lamendin method. **Lamendin, Dental, Aging** 

#### H28 Three-Dimensional Geometric Morphometric Analysis and Multislice Computed Tomography: Application for Adult Sexual Dimorphism in Human Coxal Bone

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The goal of this presentation is to present an assessment of the sexual dimorphism based on the study of the human adult coxal bone, by three-dimensional (3D) geometric morphometric analysis of clinical multislice computed tomography (MSCT) explorations. This presentation illustrates the potentialities of the MSCT with a particular anthropological tool, the 3D geometric morphometric analysis, and a particular anthropological application, sexual dimorphism.

This presentation will impact the forensic science community by providing an example of anthropological use of the 3D geometric morphometric analysis, based on clinical MSCT.

**Background and Introduction:** Multislice computed tomography is uncommonly used in anthropology and forensic anthropology. With this in mind, this study demonstrates that the 3D adult coxal shape differences related to sexual dimorphism can be identified and visualized objectively with geometric morphometric analysis based on clinical MSCT explorations.

**Materials and Methods:** Materials consist of a retrospective study of coxal bones from adult patients undergoing clinical MSCT in the authors' institution. Patients with a known history of bone disease were excluded. A total of 65 MSCT explorations were included, consisting of 30 males and 35 females, with 16 x 1.5 mm collimation. Scans were saved as DICOM files and a 3D post-processing was performed.

The methods included standard anthropometric techniques, 15 osteometric landmarks were chosen on the left innominate. The 3D coordinates of landmarks were identified on the MSCT 3D reconstructions. The three separate bones of the innominate (e.g., the anatomical pubis, the anatomical ilium, and the ischium) were first studied individually. Additionally, a modified ilium shape (consisting in the ilium and the ischial spine), and a modified pubis shape (including the ischiopubic ramus) were studied. Finally, complexes from bone parts were analyzed, including: the ischiopubic complex (consisting of the modified pubis and the ischium), the iliopubic complex (consisting of the ilium and the pubis), the ilio-ischial complex (consisting of the ilium and the ischium), and the complete coxal bone. Males and females were analyzed separately. Percentage errors were calculated for the 15 landmarks to examine the effects of intra- and inter-observer errors. For each analysis the recorded landmarks were scaled, rotated and translated using Generalized Procrustes Analysis. A consensus configuration, or mean shape configuration, was produced for males and females, so that sex differences could be compared. The landmark coordinates were analysed using Principal Components Analysis (PCA) and Canonical Variates Analysis (CVA). Finally, Goodall's F-test and Mahalanobis D<sup>2</sup> matrices were calculated.

**Results and Discussion:** Clinical MSCT explorations have not been previously used with geometric morphometric analysis to study sexual dimorphism of the adult human coxal bone, using 3D reconstructions. The advantage of geometric morphometric techniques is their ease of use, and their reproducibility. In the present case, intraand inter-observer variabilities were less than 3%. Goodall's F-test for all structures studied was significant, suggesting that the sexual dimorphism of the specific morphological structures of the skeletal elements, are similar to results achieved in previous studies.

Based on the results of the PCA, CVA, and Malahanobis  $D^2$  distances, the most sexually dimorphic anatomical structures were nonisolated bones: the complete coxal bone, the iliopubic complex, the ilioischial complex, and finally the ischiopubic complex. Our results agree with classical sex determination data. The 3D consensus shapes (masculine or feminine) are intrinsically composed of all the differences of lengths or length ratio, which explained the high sexual dimorphism of the innominate. Concerning the ischiopubic complex, our results completely agreed with previous results, demonstrating it is an important marker of sexual dimorphism. However, results were surprising in regards to the iliopubic and ilio-ischial complexes. Data were not found concerning the sexual dimorphism of those complexes, but those complexes were highly dimorphic, and particularly more dimorphic than the previous described ischiopubic complex.

The most discriminating isolated bones of the innominate with anatomical and embryological definition were the ilium and the pubis. The modified pubis, including the ischiopubic ramus, had Malahanobis  $D^2$  distances similar to those of the anatomical ilium. This feature had never been described before in the literature. Inclusion of the ischiopubic ramus within the pubis increased its sexual dimorphism. The modified ilium, including the ischial spine, provided supplementary information

concerning the greater sciatic notch, which agreed with the classical anthropological data. Based on the results of the PCA, CVA, and Malahanobis  $D^2$  distances, the isolated ischium presented a weak but significant sexual dimorphism.

**Conclusion:** The reliability of this method and determined innominate's areas with the greatest shape sexual dimorphism are demonstrated. All the results are on accordance with previous past studies' results but bring also new data for sexual dimorphism. Further studies will be done on supplementary individuals, immature populations. Furthermore, dimorphism analysis of the innominate shape with landmarks type III (semi landmarks) will be an additional way of research.

Forensic Anthropology, Geometric Morphometric, Multislice Computed Tomography

#### H29 Estimation of Stature From Foot and its Segments in a Sub-Adult Population of North India

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After attending this presentation, attendees will understand the usefulness of stature estimation in forensic examinations especially from foot and its segments as the literature on this aspect has been scanty.

This presentation will impact the forensic science community by presenting standards for stature estimation from foot and its segments when feet or their parts are brought for forensic examination.

Establishing personal identity is one of the main concerns in forensic investigation process. Estimation of stature forms a basic domain of investigation process in unknown and commingled human remains in forensic anthropology case work. The objective of the present study was to set up standards for estimation of stature from foot and its segments. Sample for the study constitutes of 154 male and 149 female adolescents from Northern part of India. The subjects were aged between 13 to 18 years old (Mean age in male and female was  $15.8 \pm 1.7$ and  $15.5 \pm 1.6$  years respectively). Besides stature, seven anthropometric measurements that included length of the foot from each toe (T1, T2, T3, T4, and T5 respectively), foot breadth at ball and foot breadth at heel were taken on both feet of each subject. All the measurements were taken with standard procedures and landmarks according to international texts and research papers. The results indicate that mean stature in adolescent males (163.1 + 10.1 cm) is significantly larger than mean stature in females  $(154.3 \pm 5.9 \text{ cm})$ . All measurements in the male foot are significantly larger than in females (p<0.05). Statistically significant sex differences exist between various anthropometric measurements of the foot. Significant side differences occur in foot breadth at heel amongst males and foot breadth at ball, and at heel in females. Foot length measurements (T1 to T5 lengths) do not show any statistically significant bilateral asymmetry. Karl Pearson's correlation coefficients (r) between stature and various foot measurements on the right and left sides in males and females were found to be statistically significant (p<0.001). Thus, the stature is positively and strongly related to various foot measurements. In males, various foot measurements show relatively higher values of correlation coefficients than in females. Linear regression models and multiple regression models (step wise regression models) were derived for estimation of stature from the measurements of the foot. The present study indicates that anthropometric measurements of the foot and its segments are valuable in estimation of stature. Based on Standard error

of estimate (SEE), it is observed that stature from foot measurements can be estimated more accurately in females than males. Among the foot measurements, T5 in males and T1 in females give the most accurate estimation of stature by linear regression analysis. Multiple regression models are derived for estimation of stature from foot length (T1 to T5) in males and females. Foot breadth measurements (BHEL and BBAL) are used to derive multiple regression models on the right and left sides in males and females. Multiple regression models tend to estimate stature more accurately than the linear regression models. It is observed that the multiple regression models derived from the measurements of the foot length (T1 to T5) estimate stature more accurately than models derived from the measurements of the foot breadth (BHEL and BBAL). The method may be applied successfully for estimation of stature whenever foot remains are brought for forensic examination that can help the investigating agencies primarily in narrowing down the pool of possible victim matches by establishing the partial identity of the deceased.

Forensic Anthropology, Foot Anthropometry, Stature Estimation

#### H30 New Linear Measurements for the Estimation of Sex From the Human Sacrum

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The goal of this presentation is to inform attendees about six new measurements of the sacrum that were proven to be the most useful for adult sex estimation through a discriminant function analysis.

The presentation will impact the forensic science community by demonstrating an accurate method of adult sex estimation from the sacrum through discriminant function analysis using new linear measurements derived from three-dimensional inter-landmark distances.

The accurate estimation of sex is crucial to the development of a biological profile for a set of unidentified skeletal remains. Previous metric and non-metric methods of sex estimation utilizing the sacrum have demonstrated the potential of this skeletal element for such an assessment; but classification rates derived from an appropriate sample are unknown. This research provides new linear measurements of the sacrum from a large sample that were shown to be the most useful in the estimation of sex through a cross-validated discriminant function analysis.

A geometric morphometric analysis of the sacrum was previously conducted (Passalacqua et al. 2010) in order to capture the sexual dimorphism visually apparent in this skeletal element. This method was able to estimate sex with an 85.75% cross-validated accuracy (89.40% for males, 82.10% for females). Unfortunately, the use of geometric morphometric analysis in forensic anthropology casework is often impractical due to differential preservation, skeletal trauma, or lack of equipment. Due to these factors, the development of new twodimensional linear measurements of the sacrum will allow for a wider application of this method. Thus, the current study utilizes previous geometric morphometric sacrum data to determine the most useful linear measurements for sex estimation (Passalacqua et al. 2010).

A sample of 163 adult sacra (85 males, 78 females) was collected from the Hamann-Todd Collection (Cleveland Museum of Natural History). Twenty-three three-dimensional (3D) landmarks were developed and collected on each individual using a digitizer. Interlandmark distances were then extracted from the 3D data providing 253 measurements for each sacrum. This method of extracting linear measurements allows for a vast amount of data to be collected in a short amount of time and in addition creates new measurements which may not have been otherwise analyzed. These measurements were analyzed through a forward step-wise (F = 0.05 to enter, F = 0.10 to remove) discriminant function analysis. This discriminant function analysis selected six measurements for estimating biological sex. These measurements focus on the alae especially in relation to the promontory. This suggests the majority of the sexual dimorphism exhibited in the sacrum involves this area and effective sex estimation is possible with fragmentary sacra as the overall size, shape, and curvature were not necessary. Results indicated an 89.0% cross-validated accuracy of the correct classification of sex (males were correctly classified at 89.4% and females were correctly classified at 88.5%). As noted above, this is slightly higher than the classification rate with 3D geometric morphometrics and these inter-landmark distances can be measured using standard sliding calipers allowing for this method to be easily utilized in the field or laboratory without access to a digitizer.

Sex Estimation, Sacrum, Discriminant Function Analysis

#### H31 Sex Discrimination Using Patellar Measurements: Method and Validation Study

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After attending this presentation, attendees will understand how to determine the sex of Americans using discriminant functions derived from common patellar measurements.

This presentation will impact the forensic science community by introducing a series of easily applicable discriminant functions for use in determining the sex of Americans using a single patella. Subsequent validation of the method, using an independent American sample, indicates the method is robust. The visual inspection of associated ROC curves provides a means of selecting among the available discriminant functions. Based on the results of this analysis, the patella is offered as an alternative method to determine sex when others are not applicable.

Although a number of previous projects have presented discriminant functions for sexing the patella, these methods are derived from African and European populations. To date, no specific patella based sex classification method in the scientific literature is available, which is both easily applicable and calibrated for use with Americans. This project addresses this issue using a sample of 182 individuals combining white and black males (100) and females (82) from the Hamann-Todd collection. Each individual possessed data on the left and right patella height, width, and thickness. The left and right values were later averaged for each measurement to make the applicable to a single bone of either side. Males possessed an average patellar height of 44.12 mm  $\pm$  2.93mm, an average patellar width of 44.57 mm  $\pm$  3.17 mm, and an average patellar thickness of 21.03 mm  $\pm$  1.57 mm. Females possessed an average patellar height of  $38.83 \text{ mm} \pm 2.94 \text{ mm}$ , an average patellar width of 39.10 mm  $\pm$  2.95 mm, and an average patellar thickness of 19.01 mm  $\pm$  1.57 mm.

The three measurements used in seven different combinations were examined using discriminant function analysis. The resulting discriminant functions generated average classification rates between 73.5% and 83.5% when cross-validated with average classification rates ranging between 73% and 83% for males and 74.4% and 85.4% for females. These results are similar to previous studies and generally indicate the method is robust, but a more powerful and convincing test of the method is by applying it to an independent sample. Here an independent American sample of patella measurement data from a series of 300 white and black males (147) and females (153) from the Terry collection, obtained by O'Connor (1996) was used to test the classificatory power of the Hamann-Todd patella discriminant functions. Upon testing, the efficiency drops an average of 3% to achieve values between 70% and 79%. The correction classification rate among males ranges between 68% and 80% and for females between 75% and 80%.

Reduced efficiency is a common result of validation but the overall classification rates remain relatively high. Among the seven discriminant functions, the most effective can be identified using the classification rate, but a visual method comparing ROC curves is used. The associated statistics indicate that all seven discriminant functions provide results that are significantly different from random guessing. The most consistent equations being those developed with patella width and height.

Although the present method does not yield correct classification rates of 90%, the best validated discriminant functions does a provide classification rate of 79%, which suggests the method has potential for sex discrimination. Since the patella is a small bone, with a dense structure, and is often recovered intact, the discriminant functions developed here are offered to the scientific community as an alternative method, applicable when other more powerful methods cannot be used due to recovery or preservation issues and as check on the results obtained using other methods.

Patella, Sex, Validation

### H32 Sex Estimation Using the Petrous Portion of the Temporal Bone By Linear Regression Anaylsis

Dolly K. Stolze, MA\*, 1900 Huntington Lane, Apartment 4, Redondo Beach, CA 90278

After attending this presentation, attendees will understand how the petrous portion of the temporal bone can be used to identify sex in fragmented skeletal remains.

This presentation will impact the forensic science community by giving the forensic anthropologist another method to estimate sex in fragmented skeletal remains and provide a jumping-off point for further evaluation of the use of the petrous portion of the temporal bone in sex estimation.

When bodies are heavily decomposed, chances increase that not all of the remains will be recovered. Lengthy postmortem intervals seen in heavily decomposed or skeletonized remains can impact identification efforts because essential bones for a biological profile may not be recovered due to human, animal, and environmental factors. A number of taphonomic processes that affect skeletal recovery include human and environmental processes, such as disfigurement of dead bodies, dismemberment to prevent positive identification, animal scavenging, and environmental disbursement. Since recent forensic anthropological studies have shown a metric relationship between temporal bone morphology and sex, this study investigates the quantitative relationship of seven measurements of the temporal and occipital bones and sex. 304 crania from the Bass Collection were measured for this study, including 92 females and 212 males. This study used the following seven measurements: (a) mastoidale to porion; (b)porion to asterion; (c) asterion to mastoidale; (d) asterion to the intersection of the parietal, temporal and sphenoid (PST); (e) PST to mastoidale; (f) the length of the petrous portion from the foramen lacerum (fl) to the mastoidale; and (g) from the mastoidale to basion. The base of the petrous portion (from its most anterior point in the foramen lacerum to the mastoidale) is an insertion point for the levator veli palatini. This muscle elevates, retracts, and laterally deviates the soft palate, and opens the auditory tube during swallowing. So the length of the base of the petrous portion may be larger in males because they have more robust muscle attachments than females. Five regression formulae were developed using these seven measurements of the temporal and occipital bones. The fifth regression equation [0.539 (fl-ms) + 0.265 (ms-po) + 0.157(ast-ms) -4.137] is statistically significant to determine sex in a fragmented skull. This formula correctly identified sex in 88% of the cases used for this study. Three measurements taken on petrous portion of the temporal

bone can be used to identify sex in skeletonized and fragmented remains: (1) the length of the petrous portion from the foramen lacerum to the mastoidale; (2) from the mastoidale to the porion; and (3) from the asterion to the mastoidale. This demonstrates the forensic value of the length of the petrous portion in sex identification in fragmented skeleton remains.

Sex, Petrous Portion, Linear Regression

### H33 Age Estimation Utilizing Postnatal Dental Mineralization: An Exploratory Analysis of Molar Development for a Contemporary Florida Population.

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After attending this presentation, attendees will understand that a more accurate construction of dental age estimation standards can be achieved by considering population age structure and by utilizing Bayesian analysis. The objectives of this study are to explore the patterns and timing of dental development for a contemporary Florida population, to test the accuracy of current dental age estimation standards for juveniles and young adults devised by Moorrees et al. (1963), and to evaluate the accuracy of age estimation utilizing third molar development.

This presentation will impact the forensic science community by presenting data related to estimating appropriate dental ages for unknown juveniles and young adults utilizing advanced stages of molar development. The accurate estimation of age utilizing molar development can have important legal implications for living individuals for which chronological age is unknown since the observation of advanced mineralization stages in third molars can provide insight into whether or not an individual is likely to have reached 18 years of age. This information can assist courts within the United States in determining whether or not an individual is legally considered a minor or an adult.

Due to the strong genetic component of dental development, research has shown that mineralization patterns of the human dentition are relatively buffered against environmental influences that normally affect bone growth and development (Cardoso 2007). It is because of this resistance to environmental factors and the continuous growth of the permanent dentition throughout childhood and adolescence that the evaluation of dental development patterns has become the preferred method of age estimation in living and deceased children.

While it has been suggested that the timing of dental development varies by ancestral descent and geographic populations, further exploration of the role of statistical modeling in the comparisons of dental development tempo and patterning among populations is necessary. For this study, 81 panoramic radiographs of individuals (33 males and 48 females) from a contemporary Florida population ranging in age from 7.7-20.4 years were reviewed. The mean age for males included in this study was 15.7 years, while the mean age for females was 16.1 years. Maxillary and mandibular third molars were observed and assigned a mineralization score ranging from 1-14 in accordance with dental development standards devised by Moorrees et al. (1963). Previous research (Demirjian 1978) suggests that dental development occurs symmetrically between tooth types in each dental arcade. Therefore, one score was obtained for each tooth type. Most scores were obtained from teeth in the left side of the mouth; however, in instances where the development stage of the left tooth was not clearly visible, the

development stage of the corresponding tooth on the right side of the mouth was scored. Similarly, most scores were obtained by observation of the mineralization stage of the distal root; however, in instances for which the mineralization stage of the distal root was not observable, the mesial root was scored. Of 246 molars observed, 53 were maxillary third molars, 77 were mandibular first molars, 77 were mandibular second molars, and 39 were mandibular third molars. Maxillary first and second molars were not scored due to the difficulty in observing advanced mineralization stages of maxillary teeth on panoramic radiographs.

Previous research has suggested that females achieve advanced dental development stages earlier than males (Tompkins 1996). Therefore, each sex was treated independently, and mean ages for attained development stages were calculated for each tooth. The mean age of complete root apex closure of the third maxillary molar (stage 14) for males was 19.5 years, while the mean age of complete root apex closure of the third manilular molar (stage 14) for males was 17.6 years. Similarly, the mean age of complete root apex closure of the third maxillary molar (stage 14) for females was 18.5 years, while the mean age of complete root apex closure of the third maxillary molar (stage 14) for females was 18.5 years, while the mean age of complete root apex closure of the third maxillary molar (stage 14) for females was 18.5 years, while the mean age of complete root apex closure of the third mandibular molar (stage 14) for females was 18.8 years.

The accurate observation and comparison of stages of molar development can serve as a noninvasive method for evaluating the probability of whether or not an unknown individual is likely to have reached 18 years of age. The refinement of existing dental age estimation standards can be achieved by incorporating a Bayesian statistical analysis, transitional analysis, and a cumulative probit model on the log scale ages.

Dental Mineralization, Age Estimation, Bayesian Analysis

#### H34 A New Method for Height Estimation Using Photogrammetry: Reliability and Validity

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After attending this presentation, attendees will gain knowledge of a new method, and its reliability, for height estimation using photogrammetry.

This presentation will impact the forensic science community by demonstrating how height estimation of the subject videotaped while in the act of robbery is a parameter that can be accurately estimated using the proposed method, respecting the experimental conditions described, and that it can consequently be utilized in probatory inquiries.

The identification of subjects by means of image comparison has already been used in the past; however, the advent of new software for the elaboration of images has provided a new impact and new resources useful for the application of techniques for the identification of the culprits. The sensitivity of the results of the investigations which, in association with other evidence, can point the judge towards a verdict of guilt or innocence, making the use of reliable scientific methods necessary, without neglecting to highlight the possible objective limits of the techniques used.

These scientific studies have had a particular impact in Italy, where the identification of the culprit by means of the comparison between the images of the arrested suspect and those of the subject videotaped in the act of robbery is allowed.

The application of such techniques; however, requires the permission of the suspect to be filmed by the bank surveillance system; in addition, the images filmed during the robbery need to be of excellent quality. When this permission is denied, it might be useful to collect the information regarding the robber's stature from the images taken during the robbery itself.

During this study, the possibility of determining the stature of a subject by means of photogrammetry was investigated; such technique is defined as the procedures that make use of photographs in order to obtain the position, the shape and the dimension of a subject.

Preliminarily, actual heights (in cm) were obtained by measuring a selection of 288 people including subjects of a height ranging from 150 cm to 200 cm with a metallic pole; they were all photographed while standing in a doorway, so as to simulate the images of subjects taken in the doorway of a bank.

The selected subjects were measured by a standardized method. They were photographed (wearing shoes) positioned both standing still and in movement; another operator measured the actual height by using a metric pole, standing still, wearing the same shoes. The photographs obtained were examined (by another operator who was unaware of the actual heights) using a professional image editing software to determine the height of the people selected using the grid technique.

In the assessment of the height of a person in motion, it was attempted to standardize the measurement by filming the subjects placed in such a position that their center of gravity corresponded with the threshold of the door.

From what has been seen so far, the use of photograph for forensic purposes can be considered useful only when the subject is filmed in a static position (i.e., inside the bank doorway). The mean differential values between the actual height and the height measured in people standing, ranging from -0.90 cm to +1.24 cm, confirm the reliability of the technique. However, the validity of the technique for the measurement in motion is unreliable, owing to the high variability between the actual heights and the measurements obtained by a professional image editing software (ranging from -3 cm to +6 cm).

Height Estimation, Photogrammetry, Reliability

#### H35 Contribution of the Maxillary Sinus Analysis for Human Identification

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After attending this presentation, attendees will understand and appreciate how maxillary sinus analysis can contribute to human identification in forensic cases.

This presentation will impact the forensic science community by showing one more human identification method, the maxillary sinus analysis.

The goal of this study is to evaluate the possibility of individual human identification and sex identification by means of maxillary sinus and propose three identification methods using the referred structures.

The sample was composed of 656 panoramic radiographs, from 328 adult individuals of both sex, more than 20 years, and divided in: Group I/ control: formed by radiographs of patients submitted to orthodontic treatment, but that did not need dental extraction in posterior teeth; and, Group II/experimental: formed by radiographs of patients submitted to orthodontic treatment that needed dental extraction in any posterior teeth. The radiographs had been randomly selected for the sample composition.

Two radiographs were used from each individual, one from the beginning of treatment and the other after a two-year-orthodontic treatment. After that, three methods were employed in each radiograph, in both groups.

In the manual technique I, the configuration of the right and left maxillary sinus was performed, using an acetate sheet on the panoramic radiography and after that.

Using the trace of maximum height and width in the transparency on the panoramic radiography from the previous reported technique, in manual technique II, the aerial cavity of the maxillary sinus was divided into four quadrants (Q1, Q2, Q3 and Q4). Based on that division, the quadrant morphology was compared using overlapping of the acetate sheet related to the radiographs of the same individual.

In the computerized technique, after panoramic digitalization, the configurations of the maxillary sinus were computer-generated and the respective areas and perimeters were calculated, using an image acquisition and analysis software. Besides the previously mentioned measures, the form factor measurement was also used. The form factor value is calculated through the relation between area and perimeter, and expresses how much the morphology of maxillary sinus was preserved if compared to the radiographs of the same individual.

In the analysis of the results, descriptive statistics techniques were used (average and standard deviation), Student's t-test with similar and non-similar variants and paired Student's t-test to quantitative variables. The level of significance used in the statistics tests was 5.0%. Statistical analysis software was used to obtain statistics calculations. The quadrant analysis was performed by visual comparison.

In the manual technique I, the measurement results in the initial radiography and in the radiography after a two-year- treatment were evaluated separately. In both radiographs, regarding all the variables, the averages were higher in male than in female sex.

Between sexes, the only significant difference was observed in the "left width" variable in the experimental group. Differences between the groups were observed in "right width" in both sex and "left width" in female. Regarding those variables, it was observed that the averages were positive in the experimental group and negative in the group control, except for "left width" in female sex.

In the comparison of the quadrant morphology of maxillary sinus in both kinds of radiographs, absence of alteration in the registered individuals as group control was observed. In the experimental group, the presence of alteration in the sinus morphology was observed in eight individuals, all of them in inferior quadrants, related to the loss of dental units.

The computerized technique results shown that in the initial radiograph and in the one after a two-year-treatment, in most of the variables, the averages were higher in male than in female sex.

In the experimental group, except from the "form factor" variables, in both sexes – that presented negative values, all the other averages were positive. Differences between the groups were checked for "right perimeter" in both sex; and "right area" in female. Regarding those variables, it was observed that the averages were positive in the experimental group and negative in the control group.

The present research techniques can be used in human identification cases where only skull fragment is available for anthropological analysis. The incorporation of the analysis added to other evidences may contribute in a decisive way to cases of forensic human identification.

Forensic Odontology, Human Identification, Maxillary Sinus

### H36 Evaluating the Performance of Population Estimation Methods in Commingled Skeletal Assemblages

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The goal of this presentation is to compare population estimation results yielded from commingled skeletal assemblages of different sizes. This presentation will impact the forensic science community by evaluating different methods of estimating the original case population size represented by skeletal assemblages produced by widely varying circumstances. Predictive models generated from intact mass graves and secondary burial mass graves excavated by the Joint POW-MIA Accounting Command - Central Identification Laboratory (CIL) anthropologists are compared to portions of large and heavily commingled skeletal assemblages unilaterally turned over to the CIL by the Democratic People's Republic of Korea (DPRK).

The CIL received a series of shipments of skeletal remains from the DPRK during the early 1990's. A total of 208 boxes of remains were turned over dubbed the "K208." The North Koreans claimed that these remains represented 208 U.S. servicemen. The original anthropological analysis of the remains revealed that most of the accessions represented multiple individuals. When the remains were sampled for mitochondrial DNA (mtDNA), it confirmed the suspicion that more people were present in the containers than previously estimated to a substantial degree. In the analysis and sorting of the K208 assemblage, it is important to create an accurate estimation of the original population.

Different population estimation methods were used in this study, with special consideration for the Most Likely Number of Individuals (MLNI).<sup>1</sup> The MLNI has been shown to estimate the original population size, while the Minimum Number of Individuals (MNI) estimates the recovered population size. In cases of taphonomic loss, the MLNI should provide a more accurate estimation than the MNI. In addition to a traditional MNI, the Grand Minimum Total (GMT) was also calculated.

The results of these different population estimation methods were generated from a series of CIL-lead excavations with the purpose of constructing predictive models. First, two excavations consisted of intact primary mass graves from the DPRK where commingling was slight; in both of these cases some remains were retained by DPRK officials. Second, another excavation consisted of poorly preserved and fragmentary remains from a World War II bomber crash. Finally, an excavation of a secondary burial mass grave from the DPRK where remains had been intentionally planted in the recent past was analyzed. The population estimation results derived from these models were either compared to the number of individuals archaeologically determined or to the minimum number of mtDNA sequences present (MNS).

In both primary mass graves and the World War II bomber case, all population estimators were accurate and in close agreement. The MNI only slightly underestimated the original population, while the MLNI estimated the true original population. This is to be expected given the high recovery rate. An exception to this trend was recognized in one mass grave where poor preservation and fragmentation restricted accurate pair matching, which inflated the population estimation. In the secondary mass grave, all population estimators drastically underestimated the original population size, including the MLNI.

For the K208 skeletal assemblage, population estimators usually underestimated the original population in each purported origin. The highest MLNI in Chongsung-ni was 23 individuals and the MNS is 22; however, the overall MLNI is 18. The highest MLNI in Okchang-ni is 5 individuals and the MNS is 12. In Kaljon-ri the highest estimation is 34 individuals and the MNS is 44. The highest MLNI from the combined villages is 58 individuals and the MNS is 67.

The underestimation is due to a number of factors. The use of mtDNA to aid in the pair matching of heavily fragmented remains has helped prevent the method from overestimating the population. In both the secondary mass grave and the K208, purported individuals were being constructed from a stockpile of remains of unknown number. This has produced an effect of selective data loss. While the MLNI can help more accurately estimate the original population in cases of normal taphonomic data loss, in situations such as the K208 and the planted mass grave, the MLNI alone is unable to do so. An avenue for future research is to increase the discrimination powers of the population estimator used by combining osteometric sorting and mtDNA analysis with the MLNI.

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**Commingling, Population Estimation, MLNI** 

### H37 Revising Revisions: Modification of the Measurement of the Sacral Body Height for Use in Fully's (1956) Anatomical Method of Stature Estimation

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After attending this presentation, attendees will understand how the pelvis should be properly reconstructed in anatomical position, and how measurement of the height of the first sacral body is unnecessary for use with a revision of Fully's Anatomical Method of stature estimation.

This presentation will impact the forensic science community by clarifying some of the uncertainty of Fully's measurement instructions. Increasing the precision of the Anatomical Method can provide numerous opportunities to conduct comparative group (including sex differences) studies using skeletal collections that lack records of living stature.

When applicable, the Anatomical Method can provide more accurate results than that of "mathematical methods" (i.e., single element regression-based methods). This is because measurements are taken for all bones contributing to stature, and varying allometric patterns within/among groups and between the sexes, therefore should not be affected by these factors. The method also compensates for individuals with extra vertebrae. Recent studies called for a revision of the protocols described by Fully (1956), as the method tended to underestimate living or cadaveric statures in known-stature skeletal collections (Bidmos 2005; Raxter et al. 2006). This revision is necessary in part, because utilizing the sacral height, rather than reconstructing the pelvis, does not compensate for a gap between the first transverse line of sacrum to superior margin of acetabulum. The Raxter et al. (2006) revision of the Fully method attempted to account for this gap; however, it is unclear which procedure for reconstruction of the pelvis was utilized, furthermore, it did not systematically employ a new measurement, but rather introduced a correction factor based on a subsample of their dataset to compensate for the gap.

The current study explores a revision of the Raxter *et al.* (2006) in order to measure the gap between the first transverse line of the sacrum and the superior margin of the acetabulum. First, the anatomical position of the pelvis was reconstructed following Hiramoto (1972) which substitutes the 2 mm thickness of cartilage with clay placed between auricular surfaces and sacroiliac joints, and approximately 7 mm between the pubic symphyses. The pelvis was placed in a sand box for support, while the anterior superior iliac spine of the ilium and pubic tubercle were held on same plane/perpendicular in lateral view (Bannister *et al.* 1995: 673). The pelvis was next turned toward the researcher in the anterior view, then a perpendicular scale and another scale to make a right angle for the measurement of the height of the first transverse line of sacrum and parallel line of the left and right superior margins of the acetabulum.

Measurements were taken using the standard Fully (1956) method with this revised criterion on a skeletal sample of 102 Japanese individuals (males: n=76 and females: n=36) from the University of Chiba School of Medicine and the University of Jikei School of Medicine. Paired-sample t-tests show that there are significant differences (p<0.01) in the first sacral body height in both males and

females between samples of unreconstructed sacra and those using the reconstructed pelvis. The former was 2.98 cm in males and 2.86 cm in females. However, after reconstructing the pelvis, the height of the first sacrum in anatomical position was 1.26 cm in males and 1.24 cm in females. Therefore, the average difference of the height of first sacral body between Fully's instructions and this study was 1.72 cm in males and 1.62 cm in females. The gap is 3.83 cm in males and 4.22 cm in females between the first transverse line of the sacrum to the superior margin of the acetabulum, which can be significant for assessments of living stature.

This study clarifies of the ambiguity of Fully's (1956) instructions of the measurement on the sacrum and increase the precision of the anatomical method of the stature estimation.

Fully's Anatomical Method, Stature Estimation, Sacrum

#### H38 Investigating Between Group Differences in Zygomaxillary Suture Form Using Fourier Analysis

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After attending this presentation, attendees will better understand how the zygomaxillary suture can be used for differentiating human groups and how 3D-model based analysis can enhance the capabilities of forensic anthropologists for human identification.

This presentation will impact the forensic science community by reevaluating the validity of widely perceived criterion of race determination and presenting a way to minimize its interpretive element. Moreover, this quantitative approach to zygomaxillary suture form signifies a larger trend in forensic anthropology towards computer-based methods, which can offer considerable advantages in terms of precision, repeatability, and objectivity.

The zygomaxillary suture is commonly viewed as one of the racial attributes of the human skeleton. Formed by the intersection of the zygomatic and maxillary bones of the skull, the suture can occur in "angled" or "curved" forms which have been associated with Caucasoid and American Indian crania respectively. It has even been suggested that Caucasoid and American Indian crania crania can be differentiated by zygomaxillary suture form alone. However, the dichotomous nature of this typology does not adequately describe the continuous scale of variation exhibited within and among crania of different groups, nor does it account for the ambiguity that may result from bilateral asymmetry in individuals. Furthermore, these distinctions in suture form are based on qualitative characteristics that require a subjective assessment of each skull. Such subjectivity can be problematic in a legal context, where the credibility of expert testimony requires highly reliable methods of analysis.

In this study, a quantitative approach was used to investigate between-group differences in zygomaxillary suture morphology. A sample of 120 human crania from northern European (n=60) and California Indian (n=60) populations were recorded with a threedimensional (3D) laser scanner, and the complete digital models were analyzed with 3D data analysis software. Each model was oriented in standard alignment with the Frankfurt horizontal and midline planes, using published protocols. The zygomaxillary sutures of the models were then traced with digital tools for defining a 3D contour. As a result, each suture was represented by a contour with a density of three equallyspaced Cartesian coordinates per millimeter, with endpoints at the craniometric landmarks of *zygoorbitale* and *zygomaxillare*. Both the right and left sutures of each cranium were traced, which yielded a total of 240 contours. Using an in-lab computer program, the projections of the contours along the XY and XZ planes were scaled to uniform length and subjected to Fourier analysis. Fourier coefficients were used to create discriminant functions that most effectively separate the European and American Indian crania in the sample by side and by sex, and the validity of the functions were tested with the leave-one-out technique.

The purpose of this study was to test the hypothesis that a quantitative analysis of zygomaxillary suture form is equally effective in discriminating European and Native American crania as a qualitative analysis. Thus, the results of the discriminant analysis were compared with the results of a traditional visual assessment, in which both evaluations identified "angled" or "curved" suture forms in the sample and calculated the within-group frequencies of each type. Based on these comparisons, it was possible to evaluate the relative merits of these methods of purposes of human identification. In addition, new information was obtained on the diagnostic capabilities of the zygomaxillary suture in males versus females and in the right side of the skull versus the left side, which has not previously been investigated. **Zygomaxillary Suture, Fourier Analysis, 3D Models** 

## H39 An Investigation and Critique of the DiGangi et al. (2009) First Rib Aging Method

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After attending this presentation, attendees will be familiar with the DiGangi et al. (2009) first rib aging method and will have been presented with a study that investigates and critiques this method.

This presentation will impact the forensic science community by making attendees aware of the pros and cons of this newly developed method and the direction of future aging research in the field of forensic anthropology.

Most commonly used methods of age estimation have several shortfalls. They tend to over-estimate the age of young individuals, under-estimate the age of older individuals, utilize terminal age categories, such as 50+, provide age ranges which are too precise or too wide to be of practical use in a forensic setting, and fail to provide prediction intervals based on an explicit probability. To address these issues, the DiGangi et al. (2009)<sup>1</sup> first rib aging method utilizes transition analysis on features of the first rib previously investigated by Kunos et al. (1999)<sup>2</sup> in the Hamann-Todd collection. The newly developed method was first applied to positively and presumptively identified males of Balkan ancestry collected in the former Yugoslavia (n=470). The application of the method, as described in the original publication, requires only that observers familiarize themselves with descriptions of the traits to be scored and the example photos found in the appendix, score the features of the ribs as described, and refer to the table of posterior densities provided in the article to find the appropriate age prediction range and the point estimate of age. The purpose of this study is to evaluate the performance of this method.

To assess inter- and intra-rater agreement, four graduate students with advanced osteological training scored 113 ribs of white males from the Hamann-Todd collection ranging from 21 to 88 years. Sub-samples of individuals were re-coded from the total sample by each observer to allow for the calculation of intra-observer agreement. The 'irr' package in R.2.10.10 (2009)<sup>3</sup> was used to assess levels of agreement for the costal face, tubercle facet, and combined scores. The data was analyzed using tests for both nominal and ordinal data. Despite the fact that the published 95% probability intervals for each combination of scores range from 35 to 50 years, individuals were only placed into an age range that contained their true age on average 87% of the time. With the exception of four younger adults between 20 and 35 years of age who were problematic for all observers, all individuals incorrectly aged were above 55 years of age.

Due to the large overlap in the age ranges provided for each unique combination of costal face and tubercle facet scores, it is possible for observers to correctly age an individual while having only minimal agreement in their scores for each rib feature. The highest inter-observer values for any agreement statistic (Cohen's Kappa) were 0.74 for the costal face and 0.56 for the tubercle facet. Despite the apparent simplicity of the coding system provided, the use of stages with multiple features and ambiguous descriptions results in high inter-observer error and a method that is generally unreliable. Also, the use of arbitrary stages containing multiple features that may or may not be present as opposed to specific ordinal variants directly violates the fundamental assumptions of transition analysis and is inappropriate.

The discrepancies between the performance of the method as described in the original article and the results of this study may be due in part to genetic differences between the males of Balkan ancestry in the original publication and the American white males of the Hamann-Todd collection used in this study. The definitions provided should also be reviewed and revised as necessary to lower inter-observer error rates to acceptable levels. Also, concentrating on ordinal features that change over time is preferred to using an agglomerated "stage" approach. Despite the disappointing performance of this method for age-at-death estimation, transition analysis and other statistically based methods of age-estimation represent the most promising new frontier for the development of new standards.

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Age-at-Death Estimation, Transition Analysis, Forensic Anthropology

### H40 Cervical Vertebral Centra Epiphyseal Union as an Age Estimation Method in Teenage and Young Adult Skeletons

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After attending this presentation, attendees will gain an understanding of the pattern, sequence, and timing of maturation of cervical vertebral centra and how it may be used to estimate skeletal age at death.

This presentation will impact the forensic science community by introducing a supplemental method of estimating skeletal age-at-death of teenagers and young adults, which when used with other skeletal age indicators may improve the accuracy of age estimation in human identification.

This study examined epiphyseal union of the inferior centrum of the second cervical vertebra (C2 or the axis), and the superior and inferior centra of the third though seventh cervical vertebrae, C3-C7. The sample comprised 55 individuals of known sex, ethnicity, and age at death. There were 23 females (2 American European, 21 African American) and 32 males (5 European American, 27 African American), aged 14 to 27 years from the Robert J. Terry Skeletal Collection housed at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

A four stage method was used to code the progress of epiphyseal union of the vertebral centra or "ring epiphyses". Stage 0 represented the absence of any epiphyseal union activity. Vertebral centra in Stage 0 were completely bare with no epiphyseal attachment. Stage 1 signified beginning union or union in progress. Beginning union was characterized by the slightest adherence of any portion of the epiphyses, and union in progress included partial to full epiphyseal rings present with gaps-adhesion in some areas and open spaces in other areas along the surfaces of the vertebral centra. Stage 2 denoted epiphyses that were almost completely united or recently united. Beginning union and union in progress were consolidated into one stage, Stage 1, and almost complete union and recent union were also consolidated into one stage, Stage 2, since the timing of fusion seemed to occur over the course of only a few months. Stage 3 corresponded to epiphyses that were fully fused for some time. The distinction between recently united epiphyses (Stage 2) and fusion that had been complete for some time (Stage 3) was important in that noting recent union allowed for more age information to be extracted from the sample and may yield greater accuracy in estimating age at death. That an individual may skeletally show signs of youth in adulthood (recently completed union, Stage 2) is more informative than simply recognizing an individual as adult (complete union, Stage 3) since that adult skeletal status could have occurred many years ago.

Results indicated that: (1) females matured at an earlier age than males; (2) there was no identifiable sequence of union-various ring epiphyses of C2-C7 fused in seemingly random order; and, (3) cervical vertebral ring epiphyseal union correlated with known age-at-death moderately well (r=0.63). Thoracic and first two lumbar vertebral ring epiphyseal union data for the same sample, however, yielded a higher correlation with known age-at-death (r=0.70) probably due to there being more data for thoracic and lumbar centra-28 epiphyses-versus 11 epiphyses for the cervical vertebrae. Cervical vertebral ring union data correlated rather poorly with thoracic and first two lumbar vertebral ring union data for the same sample (r=0.41). While these results may not fare as well as other skeletal age estimation methods as a stand alone method, cervical vertebral ring epiphyseal union is still a viable option inasmuch as it may be used to corroborate findings from other skeletal age indicators and or it can provide a general idea of an age range if cervical vertebrae are the only bones available for analysis.

Age Estimation, Epiphyeal Union, Cervical Vertebrae

#### H41 A Pilot Study in the Forensic Potential of the Health Index

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The goal of this presentation is to inform attendees about the potential utility of applying the Health Index to skeletal remains recovered from forensic contexts.

This presentation will impact the forensic science community by describing the results of a pilot study applying the health index to a small forensic sample of individuals with known backgrounds and health statuses. Further, a general comparison will be made of the overall health index score of an aggregate modern forensic population to health scores of past, bioarchaeological populations from North America.

In bioarchaeology the health index developed by Steckel et al. (2002), ranks aggregations of individuals clustered into sites, time periods, etc., in order to understand relative rankings in biological "health." However, Steckel and Rose (2002:62) speculate "if estimated for [single] individuals, it could be used to assess not only average health

but inequality of health within groups." Here, health is measured by a number of dental and skeletal variables including: age-at-death and stature, as well as presence and severity of dental and bony pathologies, degenerative joint disease and skeletal trauma (See Steckel et al. 2002 for further details).

Previous studies have had limited success when investigating applications of life history and activity pattern models to forensic remains. However, trends in skeletal pathology such as healed fractures, marked vertebral osteophytic activity, and/or poor dental care often appear in decedent's remains from similar cultural contexts such as homelessness and individuals with a history of drug addiction problems.

The goal of this project is to apply the health index to a number of known forensic cases which include some background of the decedent's health prior to death. Doing so will not only demonstrate potential differences in antemortem health status among individuals, but this will also serve as a test of the forensic efficacy of the Health Index. Nearly 50 forensic cases with antemortem health statuses ranging from what would be considered "good" to "poor" have been assessed and the sample size will increase by time of presentation.

These contemporary individuals will also be grouped into a forensic population and compared to other ranked health index scores of past bioarchaeological populations (bioarchaeological health index scores obtained from Steckel and Rose (2002)). This will demonstrate the relative health of a modern North American forensic population in comparison to historical populations from North America.

Preliminary individual health index results suggest that while less healthy individuals generally score below those considered healthier, there does not appear to be a strong enough trend to recommend the health index as a tool for interpreting individual forensic antemortem health statuses. When considering a single forensic population, the group "% of max" falls above the bioarchaeological mean value (using n=65 archaeological sites), but within a one standard deviation interval. This suggests the health ranking is not significantly greater than North American bioarchaeological scores. This is also true when only considering bioarchaeological heath index scores of samples within the last 200 years (n=20). It may be important to note that a forensic sample is likely biased and thus not a true representation of the health status of the entire contemporary United States population from which it is derived. Further research based on the potential of these results may be to examine a larger U.S. population from a contemporary donated nonforensic sample and thus compare these health index scores of those of the forensic sample.

Health Index, Pathology, Demography

### H42 Demographic Differences of Homicide Victims Examined by Forensic Anthropologists in Comparison to National Homicide Victim Trends

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After this attending this presentation, attendees will appreciate differences in demographic parameters, regional differences, and temporal changes between homicide victims examined by forensic anthropologists and national homicide statistics.

This presentation will impact the forensic science community by informing practitioners of the unique demographic profile of homicide victims examined by forensic anthropologists in comparison with national homicide trends.

This study examines the demographic parameters of individuals that were victims of homicide, examined by forensic anthropologists, and reported to the Forensic Data Bank (FDB). The FDB is a centralized database to which forensic anthropologists from around the country report information from recent cases. The data used in this study contains individuals that were examined by forensic anthropologists between 1961 and 1991. By virtue of the fact that individuals in the databank were examined by forensic anthropologists, the postmortem interval of the average homicide victim in the databank is longer in comparison to other homicide victims. This is because forensic anthropologists are typically involved in cases where individuals are partially to completely decomposed, and when identification through other methods is not feasible. The sex ratio, age, and ancestry of individuals in the FDB is compared with national homicide statistics in order to determine if the demographic profile of individuals examined by forensic anthropologists is unique in comparison to national homicide trends.

Preliminary results of the demographic characteristics of homicide victims show that 53% of homicide victims in the FDB are female, in comparison to national statistics where females make-up only 24% of homicide victims. In addition, the mean age of individuals in the FDB is 28.3 years, while nationally the mean age of homicide victims is 33.8 years. Finally, in the FDB, 65.8% of homicide victims were reported as White, 22.6% as Black, and 11.6% as other. Nationally, the ancestry profile of homicide victims is 52.9% White, 45.3% Black, and 1.7% other. In addition to these preliminary results, the FDB will be compared to national trends to examine changes over time in the demographic parameters of the two samples. Also explored is the regional variation in order to determine if there are differences in FDB demographic parameters in difference areas of the country.

These preliminary results suggest that in general, homicide victims that are examined by forensic anthropologists are more likely to be female than other homicide victims. Moreover, they are somewhat younger and more likely to be White in comparison to other homicide victims. Given that the main difference between individuals in the FDB compared to other homicide victims is that FDB individuals have a longer postmortem interval, a number of possible causes for these differences are explored. The clear female-bias in the FDB suggests that female victims of homicide are more likely to be concealed following death and the period between death and discovery is longer for females. Furthermore, the results suggest that female victims of homicide are more often killed in private settings, perhaps related to sexual violence associated with the homicide, and therefore there is a longer period until the body is discovered. Social theory research on intimate partner homicide and violence against women will be used to contextualize the results of this comparison.

Homicide, Demographics, Comparison

## H43 Ancestry Estimation Using Random Forest Modeling

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After attending this presentation, attendees will be introduced to the use of Random Forest Modeling (RFM) and the performance of RFM in ancestry estimation.

This presentation will impact the forensic science community by providing an additional method for the estimation of ancestry.

Compared to other exploratory and classification methods used in anthropological research, for example, principal component analysis (PCA) and linear discriminant function analysis (IDFA), Random Forests may be more appropriately applied to datasets frequently encountered in forensic anthropology. The suitability of Random Forest models to normal distribution, and homogeneity of variance), which do not hold for many of the datasets encountered during forensic anthropological research and method development. Fortunately, these assumptions are not required for nonparametric methods like Random Forest modeling. The most promising potential of Random Forest models is their ability to handle all variable types (categorical, continuous, count, etc.) seamlessly and to relate the various observations in highly non-linear ways to a response variable. Ancestry estimation as practiced by forensic anthropologists regularly incorporates both metric (continuous) and morphoscopic (categorical) data. In reality, most analysts prefer-or trust!-one method over the other. Only after one method (e.g., morphoscopic analysis) has provided results does the analyst turn to the next (e.g., metric analysis) for confirmation or refutation. Combining metric and morphoscopic predictor variables into a single classification analysis is generally not possible because of the differences in the distribution of the data. RFM avoids these issues using a nonparametric classification algorithm (a classifier consisting of a collection of treestructured classifiers) relying on majority voting and bootstrapping to assign cases to a response class after the initial model is produced from a randomly selected training set. Further randomness is introduced during initial variable selection and tree construction by randomly selecting predictor variables, resulting in a 'forest' of trees contrasted of randomly selected individuals. A classification matrix (and various classification statistics) is then constructed to assess how well the model classifies all individuals in the dataset. Two supplementary measures produced during Random Forest analysis provide additional information: a measure of the importance of each predictor variable and a proximity measure (measure of the internal structure of the data). These statistics provide the analyst a great deal of information on the structure of the data (proximity measure) while identifying the most important variablescontinuous and categorical, combined-to consider when estimating ancestry.

forensic anthropological data is due in large part to the rather rigid

assumptions of parametric methods (i.e., observation independence,

To examine the usefulness of Random Forest modeling in ancestry estimation, we applied the RFM classification algorithm to 34 standard cranial measurements and 16 standard morphoscopic traits collected from 149 crania. The sample represents modern American Whites (n = 72) and Blacks (n = 38) from the William M. Bass Donated Skeletal Collection in Knoxville, Tennessee and identified and unidentified border crossers representing Southwestern Hispanics (n = 39) from the Pima County Medical Examiner's Office in Tucson, Arizona. Using Random Forest, 89.5% of the cross-validated groups (by group: American Whites (AW) = 84.0%; American Blacks (AB) = 92.8%; Hispanic (H) = 92.6%) were correctly classified, substantially improving classifications compared to using traditional methods independently (craniometric = 76.1% [by group: AW = 81.0%, AB = 75.0%, and H = 69.2%]; morphoscopic = 72.7% [by group: AW = 70.0%, AB = 61.5%, and H = 85.7%]). Heuristically setting a threshold value at 0.50, thirtyfour variables (seven morphoscopic, 27 craniometric) derived from the RFM variable importance measure were examined for underlying patterns to better understand their significance. The significant morphoscopic traits are all mid-facial (NAS, INA, IOB, NBC, NAW, ORB, and NSF), quantifying Brues (1990) assertion that the mid-facial skeleton is the most important area to consider when estimating ancestry, at least anthroposcopically. The significant craniometric variables are facial breadth (ZYB), orbital breadth (OBB), alveolar length (MAL), vault width (WFB, STB, ASB) and vault length (NOL, GOL), and alveolar prognathism (BPL). The metric variables do not follow the same pattern of the morphoscopic variables as they are not isolated to one specific area, but rather the craniometric variables seem to describe overall cranial morphology.

The results of the analysis using Random Forest modeling to estimate ancestry indicate that the combination of morphoscopic and craniometric datasets—which have for so long been diametrically opposed—greatly enhances the estimation of ancestry, allowing researchers to quantify the process of variable selection. In other words, the advantage of Random Forest modeling as a practicable classification alternative to traditional methods, such as morphoscopic trait lists and discriminant function analysis, is that analysts are freed from the obligation of defending method selection while maintaining the principle of ancestry estimation.

Forensic Anthropology, Ancestry Estimation, Quantitative Method

#### H44 Ancestry Determination From Foramen Magnum

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After attending this presentation, attendees will become aware of possible cranial base changes and of the usefulness of the foramen magnum shape as a non-metric characteristic of ancestry to aid in the identification of unknown human remains.

This presentation will impact the forensic science community by presenting results that suggest possible localized change in cranial base dimensions and explore the potential for an eliminating non-metric characteristic for ancestry determination.

Ancestry estimation is a crucial part of creation of a biological profile in forensic anthropology. Improper classification of ancestry can affect other aspects of the biological profile, such as stature. Several metric and non-metric techniques are used by forensic anthropologists to determine ancestry of unidentified human remains. Some anthropologists believe the cranium to be an excellent indicator of ancestry (Rhine 1990).<sup>1</sup> Previous studies have explored the effectiveness of using the cranial base's occipital condyles for ancestry assignment of an individual. Holland (1986)<sup>2</sup> studied the Terry Collection, housed at the Smithsonian, to develop five multiple-regression equations for determining ancestry from osteological landmarks on the cranial base. The current presentation focuses on the utility of the foramen magnum region on the cranial base as a positive indicator of ancestry.

This research utilizes the same measurements as the study conducted by Holland (1986) to analyze four modern skeletal collections consisting principally of whites, blacks, and Hispanics. A total of 12 measurements were taken from 465 cranial bases from collections of modern forensic remains housed at the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville, the Louisiana State University Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory, Pima County Office of the Medical Examiner, in Tucson, Arizona, and the Maxwell Museum of Anthropology's Osteology Laboratory at the University of New Mexico.

All measurements were taken with a sliding caliper unless the osteological landmarks were missing or incomplete. These measurements included: length of the left and right occipital condyles, maximum width of the left and right occipital condyles, minimum width of the left and right occipital condyles, maximum distance between occipital condyles, minimum distance between occipital condyles, maximum interior distance between occipital condyles, foramen magnum width, foramen magnum length, and length of the basilar process. A Student's t-test indicates that variation of the foramen magnum width among blacks, whites, and Hispanics is significant (p < p0.05). Also, when comparing results of the measurements of blacks and whites from the modern forensic collections with those from Holland  $(1986)^2$  variation was significant (p < 0.05) in two of the 12 measurements for both sex and ancestry. These results suggest that localized changes on the cranial base may have occurred. The maximum distance between occipital condyles increased in length and the maximum interior distance between occipital condyles has decreased in length.

Finally, to assess non-metric variation of the shapes of the foramen magnum, five different shape categories were defined to classify each foramen magnum: Arrowhead, Circle, Diamond, Egg, and Oval. A Pearson's chi-square test showed a significant relationship between black, white, and Hispanic ancestral groups and foramen magnum shape (p < 0.05) based on shape analysis as defined by the researchers. To test the practicality of applying such a non-metric assessment of ancestry based upon the shape of the foramen magnum, a survey was conducted at the 62<sup>nd</sup> American Academy of Forensic Sciences Annual Meeting. That survey asked participants, ranging in experience from undergraduate students to experts, to classify a group of foramen magnums into one of the five categories. The results from the survey showed that the five foramen magnum shape categories are highly subjective and that the Diamond and Arrowhead categories should be combined. Interestingly, since none of the 37 presumed Hispanic skulls (either self-identified or defined by the Pima County Office of the Medical Examiner) possessed an Egg-shaped foramen magnum, an Egg shape has the potential to be used as an eliminating non-metric characteristic.

#### **References:**

- <sup>1.</sup> Rhine, Stanley. "Non-Metric Skull Racing." *Skeletal Attribution of Race: Methods for Forensic Anthropology*. Albuquerque: Maxwell Museum of Anthropology Press, 1990.
- <sup>2</sup> Holland, T.D. "Race Determination of Fragmentary Crania by Analysis of the Cranial Base." *Journal of Forensic Sciences*. Vol 31, No 2. (1986): 719-725.

Ancestry, Foramen Magnum, Biological Profile

#### H45 Group Classification Using Traditional Craniometrics, Angle Measurements, Geometric Morphometric Techniques, and the Potential Applications of These Methods to Fragmentary Crania

Jolen Anya Minetz, MA\*, and Jiro Manabe, MA, JPAC-CIL, 310 Worchester Avenue Building 45, Hickam AFB, Honolulu, HI 96853

After attending this presentation, attendees will have a greater understanding of the utility of various craniometric methods as they pertain to differentiating populations as well as associating fragmentary crania with specific groups. The goal of this presentation is to examine the morphological variation evident in the crania of three groups and the utility of several craniometric techniques: (1) traditional craniometric measurements; (2) angles acquired for cranial landmarks; and, (3) geometric morphometric techniques to differentiate between groups and assist with the assessment of race in a biological profile.

This presentation will impact the forensic science community by contributing to the continuous evaluation of the utilization of craniometric analyses and emphasize the importance of developing diverse craniometric methods for the analysis of fragmentary crania.

The purpose of this research is to test the discriminatory ability of these analyses in the classification of three groups, and evaluate the ability for these methods to classify fragmentary crania. The reference sample consists of 198 dry male skulls representing three groups: Japanese (n=105), American White (n=42) and American Black (n=51). Cranial landmarks were collected in Cartesian coordinates using a Microscribe G2X digitizer. The three dimensional coordinates were deposited into a formatted spreadsheet that computed inter landmark distances for 24 standard cranial measurements and angles between

landmarks for as 8 angle variables. A generalized procrustes analysis was also conducted on the data in Morphologika2 to obtain principle components for using in discriminant function analyses.

A discriminant function analysis was performed using SPSS statistical software. The classification rate for the three groups using the standard measurements alone ranged from 78.6% for American White to 90.2% for American Black. The classification rate for the angles was between 80.0% for Japanese and 86.3% for American Black, and when the analysis was performed in a combined model (standard measurements and angles), each of these groups were correctly classified above 90%. The 3D data classified the three groups at a higher rate than the standard craniometric analysis but not as well as the combined method; the predicted group member ship ranged from 82.4% for the American Black group to 94.3% for the Japanese group.

The utility of the different methods was tested in the analysis of several fragmentary crania. Different models were used depending on the portion of the cranium preserved. If portions of the crania, such as the craniofacial area or cranial vault are preserved, then measurements and landmarks are generally abundant enough to be analyzed using all of the models. However, in more heavily fragmentary crania where the midsagittal plane was compromised or lateral fragmentation obscured the contralateral point of a paired craniometric point, then metric analysis was only capable with geometric morphometric analysis. Heavily fragmentary crania that exhibited these patterns tended to retain very few non metric traits that could assist with race determination. The cranial fragments were analyzed by inputting a database into FORDISC 3.0 comprised of the principle components produced by the geometric morphometric analysis of the aforementioned groups and cranial fragment and running a discriminant function analysis. The results were then compared to the mtDNA haplogroup of the cranial fragment, and in some cases to the antemortem records. Overall, the classification results were useful, but the discriminating powers of the landmarks ranged based on the location and number of obtainable landmarks. For future research it would be valuable to assess the utility of all combinations of landmarks and how the combinations relate to the underlying morphology in order to better predict the classification potential for any fragmentary crania. It would also be valuable to compute and analyze a variety of inter landmark angles in order to understand the relationship of small areas of the cranium in relation to the overall morphology and provide more minute measurements to assist with the classification of fragmentary crania.

The development and validation of these methods in the future will greatly assist with the biological profile of fragmentary remains. Since the cranium is the most important aspect of the skeleton for determining race, advancing these techniques for the purpose of evaluating cranial fragments that retain little information otherwise, could be a great help in a variety of forensic contexts where remains have been compromised and may not yield an mtDNA sequence.

Geometric Morphometrics, Cranial Angles, Craniometrics

# H46 Sex and Ancestry Estimation Using the Olecranon Fossa

#### Michael W. Kenyhercz, MS\*, 6327 Catawba Drive, Canfield, OH 44406

After attending this presentation, attendees will learn methods for quantifying shape measures in the olecranon fossa through GIS and elliptical Fourier analysis and their application to sex estimation.

This presentation will impact the forensic science community by testing the Rogers method on the distal humerus and also offering a method to quantify complex shapes.

Forensic anthropologists generally work with either partially or fully skeletonized human remains. Their task is to generate a biological profile consisting of age, sex, stature, and ancestry for law enforcement to aid in a positive identification. Due to the *Daubert* standards for the admissibility of expert opinion, the measures of the biological profile should ideally have a quantifiable basis and associated levels of confidence (*Daubert v. Merrell Dow Pharmaceuticals, Inc* 1993). Therefore, forensic anthropologists are constantly looking for new techniques and also ways to improve previous methods (i.e., ways to quantify non-metric methods) to aid in their investigation.

The distal end of the humerus is often found through recovery efforts due to its durability in withstanding environmental factors. Through this durability, the distal humerus is frequently used in sex estimation. The morphology of the humerus has been previously studied by Rogers (1999, 2006, 2009) as a means to estimate sex in adults and adolescents. Using four criteria (trochlear constriction, trochlear symmetry, angle of medial epicondyle and olecranon fossa shape and depth), Rogers has reported sex estimation accuracy rates as high as 92%. The most significant of the aforementioned traits was determined to be the shape and depth of the olecranon fossa; however, her study is based on visual observations with no quantifiable measurements. For example, the male olecranon fossa form is described as a "shallow triangle," while the form of the female olecranon fossa is described as a "deep oval" and Rogers concluded that, "shape is more important than depth" (Rogers 1999). Beyond these simple descriptions, there are no guidelines on how this trait is to be examined and evaluated. Her method results in subjective characteristics by offering only qualitative data, especially regarding shape.

This study was conducted to evaluate the Rogers method in regard to olecranon fossa depth and shape for sex estimation. A sample of 140 (35 black males, 35 black females, 35 white males, 35 white females) left humeri were digitized from the Hamann-Todd Osteological Collection curated at the Cleveland Museum of Natural History, Cleveland, OH. The coordinate data for each humerus was uploaded into ArcGIS (ESRI 2008) and ArcMap was used to define the olecranon fossa outline, or rim, and to calculate the output variables maximum and mean slope, maximum curvature, and surface volume. Outlines were also analyzed using Elliptical Fourier Analysis (EFA) through the program Shape 1.3 (Iwata and Ukai 2002) where principle components of shape were calculated and visualized. The programs TPSdig (Rohlf 2010) and GMTP (Taravati 2009) were used to calculate centroid size from the outlines. In all, 13 variables representing size, shape, and depth of the olecranon fossa were obtained and used for sex and ancestry estimation.

FORDISC 3.0 (Jantz and Ousley 2005) was then used to perform discriminant function analysis from the 13 variables. Forward Wilks stepwise selection was used to select the appropriate variables for each analysis and all percent correct classifications were cross validated. A four-way sex and ancestry estimation for all groups classified 48.3% correctly and a two-way sex estimation of the individuals classified 82.5% correctly. Two-way sex and ancestry specific ranged from 58.3% to 82.5% correct classification.

The Elliptical Fourier results show that shape of the olecranon fossa rim had no correlation with either sex. Both sexes showed similar ranges of shape variation, only being separated by size. Between ancestral groups there were significant differences in shape through slope, curvature and the principle components generated through the EFA. This study presents an objective means to record the olecranon fossa form and demonstrates that sex and ancestry can be determined through the olecranon fossa alone while also meeting the *Daubert* standards for court admissibility.

Sex Estimation, Humerus, GIS

#### H47 Applicability of Femur Subtrochanteric Shape to Ancestry Assessment

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After attending this presentation, attendees will understand the advantages and limitations of utilizing femur subtrochanteric shape in distinguishing between ancestral groups during the analysis of fragmentary and/or incomplete skeletonized remains.

This presentation will impact the forensic science community by testing the applicability of the platymeric index, a relatively common postcranial ancestry determination method, on samples of modern Southeast Asian and white American individuals.

The determination of ancestry from postcranial skeletal remains presents a significant challenge to forensic anthropologists in the analysis of fragmentary and/or incomplete remains. Morphological and metric observations from the femur can be used to differentiate between broad ancestral groups. In particular, metric dimensions of the subtrochanteric region are believed to assist in distinguishing between individuals of Asian and non-Asian descent (Bass 2005; Brothwell 1981; Gilbert and Gill 1998; Wescott 2005). To determine the shape of the subtrochanteric region, the platymeric index is calculated by dividing the subtrochanteric antero-posterior diameter by the subtrochanteric mediolateral diameter and multiplying by 100 (Wescott 2005). It is believed that individuals of Asian descent typically exhibit a medio-laterally broad (platymeric) subtrochanteric region with platymeric indices below 84.9, while non-Asian individuals typically exhibit a more rounded (eurymeric) subtrochanteric region with platymeric indices between 84.9 and 99.9. Less frequently, individuals may exhibit an antero-posteriorly broad (stenomeric) subtrochanteric region with platymeric indices over 100; however, the data to support the association of platymeria with Asian ancestry were collected from small samples composed largely of pre-contact Native American individuals. This can be partially attributed to the makeup of the skeletal collections used for skeletal biology research in the United States, which lack significant numbers of Northeast and Southeast Asian individuals. Ancestry assessment methods derived from North American samples, such as the platymeric index, have not been rigorously tested on other Asian samples. Thus, it is unclear whether such methods can be utilized to identify individuals of Northeast and Southeast Asian descent in a forensic context.

This dearth in research is of particular concern to the forensic anthropologists at the Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL), where casework routinely requires ancestry assessment of highly fragmented or incomplete remains that were recovered from, or unilaterally turned over by, Asian countries. The primary goal of the JPAC-CIL is to recover and identify U.S. service members killed during past conflicts, including the World Wars, Korean War, and Vietnam conflict. The ability to distinguish between the remains of Southeast Asian and Black and White American males is integral to accomplishing this goal.

As many JPAC-CIL cases exhibit extensive peri-mortemtrauma (i.e., from aircraft crashes and projectile trauma) and originate from extreme postdepositional environments (i.e., highly acidic soils and humid jungle environments), fragmentation of remains is common. However, due to its robusticity, the femur is often represented in casework assemblages, making it an important skeletal element for sex, age, race, and stature estimation.

This study tests the applicability of the platymeric index on a sample of 128 modern Southeast Asian males (age 23-96 years) housed at Khon Kaen University (KKU), Khon Kaen, Thailand, and 77 White American males (age 18-41 years) identified by the JPAC-CIL, Hickam AFB, Hawaii. The KKU skeletal collection consists of more than 600 known individuals from northern Thailand. The JPAC-CIL sample

consists of U.S. servicemembers who died during World War II, the Korean War, and the Vietnam conflict. Measurements were obtained with standard anthropometric sliding calipers and rounded to the nearest millimeter. The platymeric index of the left femur was calculated; however, the right femur was substituted if the left was damaged or missing.

While results indicate that the KKU sample contains a larger number of platymeric femora, both samples exhibit variability in subtrochanteric form. In the KKU individuals, platymeric indices range from 64.1 to 109.6 and are normally distributed (mean = 83.9; S.D. = 7.36), with 58% exhibiting platymeric, 39% exhibiting eurymeric, and 3% exhibiting stenomeric femora. In the JPAC-CIL sample, platymeric indices range from 76.5 to 118.4 and are normally distributed (mean = 91.6; S.D. = 10.2), with 44% of individuals exhibiting eurymeric, 36% exhibiting platymeric, and 19% exhibiting stenomeric femora. Differences in the mean platymeric indices for the two samples are statistically significant ( $p \le 0.001$ ), with the KKU platymeric index range generally lower, and the JPAC-CIL range generally higher; however, the considerable overlap in the ranges urges caution when using platymeric indices in ancestry assessment.

Ancestry Determination, Femur, Southeast Asia

### H48 Improving Sex Estimation From the Cranium Using 3-Dimensional Modeling From CT Scans

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After attending this presentation, attendees will learn about the utility of alternative approaches in exploring and quantifying sexual dimorphism in the human skeleton, particularly the cranium. This presentation will offer the forensic community simple and effective measurement techniques for improving sex estimation from the cranium providing measurements with the highest discriminatory power for sex estimation, as well as precise descriptions of how to take the measurements accurately using radiographs and/or calipers.

This presentation will impact the forensic science community by facilitating more accurate sex estimation techniques for the cranium than those used most frequently in forensic practice today. In addition, this study uses a large sample of modern Americans, thereby increasing statistical power of the discriminant functions. Finally, reducing the number of measurements needed to accurately discriminate sex from the cranium will lend these functions useful for fragmentary crania, as well.

Among the skeletal elements used for sex estimation, postcranial elements are generally superior to cranial elements. Therefore, when cranial and postcranial elements are present forensic anthropologists typically give more weight to postcrania, especially os coxae; however, skeletal forensic cases often consist of a skull only, or a skull and fragmentary/incomplete postcranial remains. A query of cases submitted to the Forensic Data Bank showed that 45% of cases consist of a skull with no sexable postcranial elements.

Metric sexing of crania using statistical procedures came to forensic anthropology via Giles and Elliott's classic paper on the American population.<sup>1</sup> Their success rate was in the high 80%, a rate typical of subsequent cranial sexing analyses. In general, accuracy rates exceeding 90% are rare in sexing crania, whether using morphological traits or measurements. Several studies have shown lateral radiographic cephalometry to be more accurate than traditional techniques for cranial sex estimation.<sup>2-4</sup> Hsiao *et al.* reported 100% accuracy in sexing Taiwanese adults with 18 variables, as well as rates ranging from 94% to 98% using between one and three variables only. A subsequent validation study on a European population achieved 96% accuracy.<sup>4</sup> A unique aspect of lateral cephalometry is that it allows the practitioner to take endocranial measurements and calculate angles that are informative of skull shape. In view of these promising reports, the present study conducts a similar analysis on the William M. Bass Donated skeletal population using computed tomography (CT) scans and an innovative 3D bone-modeling algorithm (the bone atlas). CT scanning technology enables the crania to be examined more thoroughly than has heretofore been possible and to examine structures that are not easily accessible in traditional evaluation.

The study sample consists of CT scans of adult skeletons from the William M. Bass Donated Collection. Each scanned cranium was rendered as a 3D model and then added to the cranial atlas. A statistical bone atlas is an average mold that captures the primary shape variation in the bone and facilitates rapid and accurate generation of automated measurements. An atlas consists of a sample of bones that all contain the same number of points and share the same spatial relationship. At the writing of this abstract, the cranial atlas contains 40 individuals; the projected atlas size upon completion will be 600-700 individuals (January 2011). A combination of linear and angular measurements was calculated from the atlas and t-tests and discriminant function analysis with cross-validation and stepwise variable selection were performed on these measurements. T-test results showed basion-nasion, metopionglabella to glabella-basion angle, and the linear distance between the tips of the mastoid processes to be the most significant measurements (p<.05). Despite the small preliminary sample size, 90% accuracy was reached with just 7 measurements. Angular measurements capturing the inclination of the forehead and the cranial base angle, as well as measurements pertaining to mastoid size were selected as the best discriminators.

The analytical approach used in this study has been shown to improve sexing accuracy over traditional methods in postcranial bones, such as the patella, femur, and clavicle. Consequently, significant increases in discriminatory power is expected as the atlas sample size increases. Because CT scanning technology is not available to all forensic practitioners, measurements easily taken from lateral radiographs were used. Hence, the deeper understanding of sexual dimorphism can be introduced into forensic practice without the need for expensive equipment or programs. Results from an ongoing validation study on lateral radiographs will be included as part of the presentation, including observer error rates for the radiographic measurements.

This project was funded by the National Institute of Justice.

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Sex Estimation, Cranium, Discriminant Analysis

### H49 Dismemberment: Cause of Death in the Colombian Armed Conflict

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The goal of this presentation is to illustrate major findings in the recovery and analysis of victims, where dismemberment is a cause of death within the context of the armed conflict in Colombia.

This presentation will impact the forensic science community by providing useful analytical information and contributes to the correct interpretation of forensic analyses in cases of dismemberment and/or in the examination of remains within the context of the Colombian conflict.

Dismemberment has been described in the literature as a rare method used by a perpetrator to attempt to conceal a body and/or prevent identification of the decedent. To the contrary, in the country of Colombia, dismemberment is a common technique used by illegal armed groups to dispose of their victims.

The Colombian Justice and Peace Law of 2005 was enacted to bring an end to the ongoing armed conflict between the paramilitary groups and the government through providing incentives for members to lay down their arms and surrender. This law requires that demilitarized members confess to all their illegal activities publicly in a court of law. Known as "free versions," they reveal dismemberment as a widespread and recurrent form of murder. Some offenders have confessed to their actual participation in dismemberment while victims are still alive, while others claim to have witnessed these actions. In addition, artistic therapies as part of social programs meant to rehabilitate former paramilitary members have resulted in artwork illustrating dismemberment scenes. Family members of victims additionally report firsthand knowledge that their loved one was dismembered, as bodies are often left for family members to inter as a warning. Therefore, its use can also be symbolic, being used as an attempt to send a message to certain individuals and/or communities.

To date, over 2,000 clandestine graves have been exhumed in Colombia. These graves are usually rounded, small, and shallow, and bodies are found disarticulated, commingled, and incomplete. Laboratory analyses of some of these remains indicate that they exhibit evidence of multiple linear, sharp-edged cut marks, which primarily affect the distal and proximal ends of the upper and lower limb bones and cervical vertebrae. Other findings include blindfolds and gags covering both eyes and mouth, as well as ligatures on ankles and wrists.

The determination of cause of death of these individuals is a challenge because often no other injuries due to gunshots or other mechanisms are found that could explain the cause of death. Frequently the only injuries found are cut marks indicating dismemberment. Given this evidence and the lack of evidence of other trauma coupled with witness accounts, dismemberment and the vast exsanguination associated with it is then indicated as the cause of death.

It is important to note that the determination of dismemberment as the cause of death must be carefully supported with detailed descriptions of field and laboratory findings, analyzed within the context of the information available from investigators and witnesses.

Accurately determining the cause of death of victims is crucial because it provides answers for grieving family members and helps to repair the rift that the Colombian armed conflict has caused over the past 60 years. This information and reports will be used in current and future prosecutions and ultimately will help a country uncover the truth behind the illegal acts that have occurred.

Dismemberment, Armed Conflict in Colombia, Cause of Death

## H50 CPR Fractures in Infants: When Do They Occur?

Miriam E. Soto, MA\*, The University of Tennessee, 250 South Stadium Hall, Knoxville, TN 37996

After attending this presentation, attendees will be more aware of characteristics that may indicate which infants are more susceptible to CPR related rib fractures.

This presentation will impact the forensic science community by contributing information which may be helpful for differentiating between abuse and CPR related rib fractures.

The literature indicates that CPR related rib fractures in infants and children are rare, occurring in only 0-2% of studied samples. To test these findings, this study examined all autopsy records of infants and children, up to the age of two years, that came into the Harris County Institute of Forensic Sciences (HCIFS) office during a one year period (n=186). The purpose of this evaluation was to identify characteristics which may be contributory to CPR fractures. In addition, this study compared the bone quality of infants/children that had CPR fractures and those that received CPR but did not exhibit fractures. Since CPR fractures often occur in ribs 4-6, an effort was made to examine ribs from these positions; however, in a single case rib two had to be examined due to availability. There was no preference for side. The samples for this study were taken from the osteological material stored in the Anthropology Laboratory of the HCIFS. It is hypothesized that infants that were in poor health for extended periods of time will be more susceptible to rib fractures due to a lower quality of bone. Gross observations with and without a stereoscope of the ribs of infants with CPR fractures was used to assess bone quality.

Of the 186 infant/child cases that entered the HCIFS office in 2009, 162 received CPR. Only seven of these 162 cases had CPR related rib fractures. These results indicate that cases in which infants/children received CPR fractures are indeed rare, occurring in 4% of infant/child cases that received CPR.

Regarding the direct comparison of ribs for the evaluation of bone quality, the rib specimens of six infants aged two to five months that received CPR fractures were compared to the rib specimens of four infants aged two to four months that received CPR without receiving CPR fractures. All infants without CPR fractures were born at  $\geq 36$ weeks gestation while four out of the six infants with CPR fractures were born at <36 weeks gestation. Gross observation revealed that infants with CPR fractures had moderate to low bone quality and increased porosity in comparison to the infants that did not experience fractures during CPR. Five out of the six infants that had CPR fractures were also in a poor state of health for an extended period of time following birth. Prematurity was a contributing factor to the poor health of four of the five cases. In the fifth case, it was likely that the poor bone quality was a result of metabolic bone disease. Of the infants that did not get CPR fractures, three of four were reportedly healthy at birth and were in a good state of health prior to the circumstances causing death. Birth records were not available for one of the infants without CPR fractures, which was a premature (36 weeks gestation) twin birth.

In conclusion, this study found that CPR fractures may be rare, but that there are identifiable characteristics that may contribute to the susceptibility of infants to CPR fractures. Infants that are premature and/or have extended hospital stays due to serious illness are more susceptible to CPR fractures. Premature infants are likely to experience osteopenia of prematurity, causing brittle bones that are more susceptible to fractures. In addition, there is an increased likelihood that premature infants will experience an extended period of illness due to complications of prematurity.

Forensic Anthropology, CPR, Rib Fractures

### H51 The Relationship Between Directionality of Force and the Formation of Butterfly Fractures

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After attending this presentation, attendees will better understand the formation of butterfly fractures and the underlining factors affecting the pattern of their formation.

This presentation will impact the forensic science community by providing an understanding of the mechanisms of injury behind butterfly fractures found in relation to human skeletal remains.

Fractures are caused in a number of ways. Pathological conditions could weaken a bone to the point of breaking, or continual stress to a bone could lead to a stress fracture, but the cause of fractures most people consider first is an abrupt impact of some force that directly results in a break in the continuity of a bone. This third case is where there is a direct cause and effect to the trauma, and because of this the order of events leading up to a fracture can be traced. The location, angle, and severity of a fracture indicate the type of mechanism of injury involved. It is the variation between fractures that make it possible to determine if a person slipped and fell or if they were defending themselves.

Identifying the type of fracture being observed will aid in identifying the cause of the trauma. A butterfly fracture is a comminuted fracture that results from an abrupt impact to appendicular long bones. The butterfly fracture creates a butterfly fragment, which is a triangular piece of the bone that detaches when two main fracture lines meet forming what looks like a Y-shaped fracture. This study was conducted to focus on the formation of butterfly fractures and any influences differing blunt shapes and forces had on their formation.

Ninety-four sheep femora were broken at two force levels, one group in the 900s Newtons range and the other group in the 800s Newtons range, with either a rounded, flat, or edged blunt anvil to analyze the resulting fractures. The two force levels were measured by a force plate. The blunt anvils, connected to a metal bar and guided vertically perpendicular to the floor by a custom constructed apparatus, were dropped at consistent heights to control the force levels.

The cortical thickness of the bones and the general degree of the angle of the butterfly fragment were noted to see if an underlying pattern in the fracture's formation occurred. A high-speed camera was utilized to see the timing of the individual fractures that make up the Y-shaped characteristic of a butterfly fracture and their directionality with respect to the point of impact.

Preliminary examinations of the video revealed that the timing of when individual parts of the Y-shaped fracture began varied between bones, resulting in similar appearing fractures that had formed in different sequences. Inspection of the bones also showed that not all of the fractures exhibited the upwards Y-shaped patterning where the force was exerted from the open top of the Y; some created an inverted Y breaking initially at the point of impact in a straight line and then continuing on into two distinct fractures.

The results of this study show that many of the preconceived notions about directionality of force exerted and the formation of the Yshaped fragment in butterfly fractures are inconsistent and may therefore be unreliable. The variations seen during this study indicate that those preconceived notions could be skewed, resulting in a distorted interpretation of the mechanism of injury. Further experimentation and data collection are needed to show conclusively if there exists a consistent pattern to the formation of these fractures and how information that can be attained by their examination can accurately be used in the determination of mechanisms of injury.

Butterfly Fractures, Blunt Force Trauma, Mechanism of Injury

#### H52 Microscopic Analysis of Sharp Force Trauma From Knives: A Validation Study

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The goal of this presentation is to present error rates in determining blade class characteristics from tool mark impressions made by knives.

The presentation will impact the forensic science community by providing a baseline study with which known error rates for determining knife class characteristics from tool marks can be referenced.

Forensic anthropologists are often asked to examine defects on bone and cartilage created as a result of sharp force trauma. Previous research has established the precedent for analyzing cut mark morphology on bone and cartilage and has promoted the use of class characteristics (Andahl 1978; Symes 1992). While this research has been invaluable in advancing toolmark analysis in bone, it has largely focused on tool marks left by saws as they are more variable than knives and thus have the capability to leave more class characteristics. Although a general anthropological approach to tool mark analyses has been established and accepted, there is a lack of method validation and known error rates for correctly identifying these characteristics, particularly in reference to knives. This is necessary in light of the recommendations of the recent National Academy of Sciences Report (2009) and considering that analytical results are subject to Daubert standards of courtroom-acceptable scientific evidence (1993). Researchers have noted this deficiency, but previous efforts have suggested no correlation between serrations on a blade and the regularity of striation patterns in experimentally cut pig cartilage (Love et al. 2010).

This research attempts to establish a baseline study to assess the accuracy of associating a tool mark with a particular blade class under optimal conditions. Experimental defects will be evaluated for class characteristics that relate to only two blade characteristics: blade serration (serrated, partially serrated, and non-serrated) and direction of blade bevel (left, right, or both). A medium-to-soft casting wax is presented as an optimal material when it is necessary to transect material with an experimental cut. Wax blocks were impacted in two ways: (1) in a single impact transecting the wax block (to mimic a stab wound); and, (2) in a repetitive, reciprocating motion (to mimic dismemberment). Impacts were made for each of the fourteen knives in the study sample and coded to be unknown to the researchers (four partially serrated blades, five non-serrated blades, and five serrated blades with a variety of different bevels and serration patterns). The test cuts were then assessed by three researchers with varying degrees of experience analyzing sharp force trauma. Additionally, two microscopes were used to test the necessary level of technology for these analyses (a digital microscope offering an increased depth of field and the ability to reconstruct defects in three dimensions and a standard light microscope). Cuts were analyzed at the observer's preference between 10x and 50x magnification with the assistance of fiber optic lights to produce oblique lighting. In total, 168 cut marks were observed. Error rate was assessed as the number of misclassifications divided by the total number of observations.

Serrated blades were generally distinguishable from non-serrated blades due to their distinct, patterned striations whereas non-serrated blades leave fine, unpatterned striae. Distinct striations can generally be considered as equidistant, but the angle of the impact of the blade is the most influential aspect of the distance between striae left by blade teeth, so pattern recognition was favored over measurements. Two observers misclassified two of the four partially serrated blades as serrated blades while the third misclassified all four as serrated blades. Blade bevel was assessed by determining the corresponding direction change in the vshaped kerf. The average raw error rates (misidentification of class) for all three observers was 19% for the assessment of blade serration using both the digital and light microscopes. Partially serrated blades were particularly problematic in this study as the impact may not have left both a serrated and a non-serrated signature. When the adjusted error rates are considered from only serrated and non-serrated blades, the average error rate for determining blade serration was 2% on both the digital and light microscopes. Average error rates were 18% for determining blade bevel on the digital microscope and 10% on the light microscope, but this corresponded to the experience level of the observer. These preliminary results show that, under optimal conditions and an appropriate level of experience, assessments of blade serration and blade bevel can be made with a high level of accuracy. This research will be supplemented by experimental cuts in bone and cartilage.

Tool Marks, Sharp Force Trauma, Validation Study

## H53 Strontium Particles: Confirmation of Primer Derived Gunshot Residue on Bone in an Experimental Setting

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After attending this presentation, attendees will appreciate the potential of using Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDXA) to confirm both visually and by elemental composition, the presence of primer derived gunshot residue (GSR) on bone.

This presentation will impact the forensic science community by discussing how the use of an SEM and EDXA on bone fractures has the potential of providing a means of determining whether a bullet was involved and a mechanism of trauma.

Motivation for this project was derived from recent research that resulted in two papers presented at the 60th annual meeting of the American Academy of Forensic Sciences held in Washington, DC. This research was first undertaken to demonstrate the presence of primer derived GSR deep within the wound tract (Berryman *et al.*, 2010); a finding that is counter to the generally-held belief that it is found only on clothing, skin, or at the subcutaneous level. The GSR examined in this study is solely primer-derived and not other general soot-related particles that can arise from multiple sources including propellant, lubricants, and metals found in the bullet, bullet jacket, cartridge casing, and gun barrel. It is vital to differentiate the sources of GSR, as it is only primer derived GSR that are considered unique to the shooting environment. It is these unique particles measuring 0.1  $\mu$ m to 55  $\mu$ m in diameter that are examined in this study.

The current research was directed at confirming the original findings of primer derived GSR (Berryman *et al.*, 2010). The experimental design is essentially the same as the original one. Pork ribs with intact muscle tissue were used in an experimental attempt to identify bullet wipe on bone at distances from one to six feet. Instead of the barium/antimony/lead-based primers used in the initial study, bullets with strontium-based primers were used since this element is not readily present in the shooting environment. In addition, the authors devised a rigorous protocol both in the shooting and processing environments to eliminate the potential risk of contamination. The presence of strontium therefore, would confirm that the GSR particles observed on bone are derived solely from primer components, and not from elements present in the bullet, bullet casing, or gun barrel.
After processing, which involved the forceful removal of periosteum and drying of the ribs, each fragment was placed in the Hitachi S-3400 SEM for visual analysis. With backscattered electrons, the intensity of the signal is directly related to the atomic number of the material being illuminated by the electron beam. By adjusting the contrast, brightness, gain, and scan rate, particles containing heavy elements, having a higher atomic number, in this case Strontium, will glow brightly as compared to the rest of the field, specifically the bone. Particles that glowed brightly using this process were then examined for their elemental composition using the Oxford INCA Energy 200 Dispersive X-Ray Analyzer.

Strontium particles were found on ribs shot at gun-to-target distances of one to six feet confirming the original findings of Berryman et al. (2010), that primer derived GSR occurs well below the level of subcutaneous tissue and is present on bone, even after the forceful removal of the periosteum in gun-to-target distances of up to 6 feet. This research is ongoing with expanded sample size and an increase in gunto-target distances to determine the maximum range primer-derived GSR can be detected on bone. Further research could provide a method for determining gun to victim distance although this could be extremely complicated due to the wide variety of ammunition available, including variations in primer composition, caliber and bullet type; however, this technique could prove useful in situations where ammunition type is known permitting test firings to establish case-specific distances. Additionally, if GSR particles are present after decomposition then these observations can be used to verify a gunshot wound to bone in the absence of a typical gunshot wound fracture pattern.

This research was supported by the Forensic Science Foundation Lucas Grant.

**Gunshot Residue, Terminal Ballistics, Gunshot Trauma** 

## H54 Determining the Epidemiology of Hyoid Fractures in Cases of Hanging and Strangulation

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After attending this presentation, attendees will become familiar with literature in forensic science concerning injuries to the hyoid bone following hanging or strangulation; will learn about local hanging or strangulation cases from recent years exhibiting fracture of the hyoid bone; and, will learn about possible epidemiological causes for the trauma seen in the hyoid following hanging or strangulation.

This presentation will impact the forensic science community by providing age ranges for unilateral and bilateral fusion of the greater cornua to the hyoid body, discussing the effect of demographic variables on the fusion patterns, and improving the interpretation of traumatic injuries to the neck.

A review of the forensic literature on neck trauma in hanging and strangulation cases showed two distinct patterns. Overall, there appears to be little debate that hyoid fractures are more common when the cause of death is strangulation. Traumatic injuries to the hyoid bone following strangulation have been described as being frequent and previous studies have shown that up to one third of strangulations cases lead to a fracture; however, in cases of hangings, opinions are much more varied. Some studies argue that fractures in hanging cases are much fewer than in strangulations cases, while other authors mention that trauma to the hyoid bone is common following hanging. Population variation may be responsible for the divergent literature and this study attempts to identify the variables that may be responsible for the variation.

To study local cases of hanging and strangulation, data collection was performed at the Hillsborough County Medical Examiner's Office in Tampa, Florida. The Hillsborough County Medical Examiner's Office services a population of more than one million people and investigated an average of 1,915 cases between 2004 and 2009. A total of 148 cases between fall 2004 and spring 2010 listed hanging, ligature strangulation, manual strangulation, asphyxia, or compression of neck as the cause of death. Autopsy reports were analyzed to obtain a series of variables from each case. In addition to sex, age, and ancestry of the victim, cause and manner of death, past or present history of substance abuse, description and location of the hyoid bone trauma if present, and if noted by the medical examiner, fusion of the hyoid bone were collected.

The vast majority of cases, 134 out of 148 (91.0%), were classified as hangings. An additional eight were indicated as strangulations, two as ligature strangulation, and four were classified as a combination of suffocation, asphyxia, and compression of neck and chest. Similarly, 134 cases were listed as suicides. Nine cases were homicides, four were classified as accidents, and one case remained undetermined. From the 148 cases reviewed, only eight contained a fractured hyoid bone while another two autopsy reports made no mention of the hyoid bone. Six of the eight fractured hyoid bones were from hanging cases while the remaining were classified as manual strangulations. Overall, 2.05% of strangulations cases contained a traumatic injury to the hyoid bone, while damage was present in only 4.0% of suicides cases analyzed. In half of the hanging cases exhibiting trauma to the hyoid, force exerted on the ligature implement appeared to be a significant cause for the damage. In one case, the victim hung them self from a bridge while in two additional cases, the men weighed well over 200 lbs. Age could possibly be a factor as an ossified hyoid bone is more prone to traumatic injuries than an unfused one. Unfortunately, the autopsy report discussed the fusion of the hyoid in only five cases. Seven out of eight fractured hyoid bones were males, but this is representative of the sample used. Through a better understanding of the variables that affect hyoid fractures in hanging and strangulation cases, forensic anthropologists may be able to better interpret a fracture found on a skeletonized hyoid.

Hyoid Bone, Hanging, Strangulation

#### H55 Fusion Patterns in Modern Hyoid Bones

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The goal of this presentation is to examine how unilateral and bilateral fusion patterns in the hyoid bone vary with age within a population, and how the ossification process can help forensic anthropologists understand fracture patterns of the hyoid in traumatic cases.

This presentation will impact the forensic science community by providing age ranges for unilateral and bilateral fusion of the greater cornua to the hyoid body, discussing the effect of demographic variables on the fusion patterns, and improving the interpretation of traumatic injuries to the neck.

The fusion of primary and secondary ossification centers is one of the commonly used methods by forensic anthropologists to age adolescents and young adults due to the specific age ranges at which elements of long bones and vertebrae fuse together; however, few studies have looked at the fusion process in the hyoid bone. Ossification of the hyoid bone occurs slowly over time and as the greater cornua fuse with the hyoid body, chances of traumatic injuries increase. This project was designed to study the fusion pattern of the greater cornua to the body of the hyoid bone using a modern North American sample, determine how variation arises between individuals of various sexes and ancestries, and determine probabilities of trauma to the hyoid bone from patterns of unilateral and bilateral fusion of the greater cornua.

Data collection was performed in collaboration with the Hillsborough County Medical Examiner's Office in Tampa, Florida. During a five-month period, all hyoid bones were collected during autopsy for the study regardless of demographics or cause of death. A sixth month was later added to collect additional hyoid bones to increase the percentage of juveniles and African-Americans in this sample. For each hyoid bone, demographic information, cause and manner of death, and past or present abuse of alcohol and drugs were noted. A total of 264 hyoid bones were processed and used for analysis. The hyoid bones were processed by removing the majority of the excess soft tissue and then boiling each hyoid to facilitate the removal of the remaining tissue. A mobility test was performed during processing to assess the fusion of each greater cornua: a positive test occurred when the cornua was still slightly movable while a negative result was associated with a greater cornua that was completely immobile. Once the hyoid bones were dry, photographs and radiographs were taken of each hyoid using superior and posterior views to observe the joints between the hyoid body and each greater cornua. The radiographs were used to assess the fusion of each greater cornua to the hyoid body. Each cornua was scored independently by two anthropologists: a score of "0" indicated a completely unfused cornua, while presence of fusion, whether complete or incomplete, was scored as "1." In addition, a linear regression was used to determine how much variation in age can be explained through unilateral and bilateral fusion.

Results indicate that a wide variation exists in the unilateral and bilateral fusion patterns of the hyoid bone. Unilateral fusion was observed as early as at eight years of age while bilateral fusion was first visible in a 23-year-old. As a previous study demonstrated, the majority of hyoid bones are fully fused in the elderly but in some cases the hyoid may remain only partially fused. Two males from our sample, one in his 70s and one is his 80s, still exhibited a unilaterally fused hyoid at the time of death. Overall, the number of individuals displaying unilateral fusion increased steadily until the 40-49-age bracket and decreased afterwards. Conversely, the percentage of individuals with bilateral fusion constantly increased from 65.0% in the 20-29-age bracket to over 90.0% in the 70-79 and 80+ age ranges. In both ancestral groups the mean age for bilateral fusion occurred approximately five years earlier in men, and in both sexes, African-American individuals exhibited bilateral fusion two years earlier. The regression formula demonstrated that 30% of the variation in age is explained by greater cornua fusion patterns. Through the understanding of the pattern in which the greater cornua fuse to the hyoid bone, anthropologists can better understand estimate the risk of fractures to neck structures according to the ossification of the hvoid bone.

Hyoid Bone, Fusion Pattern, Age Estimation

# H56 The Prosecution of a 28-Year-Old Case of Shaken Baby Syndrome

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After attending this presentation, attendees will have addressed the events leading to the successful prosecution of an older male who was responsible for the death of a nine-month-old infant 28-years earlier.

The presentation will impact the forensic science community as well as those family members who have lost an infant in a suspicious death by suggesting that after a lengthy period of grief, noteworthy skeletal evidence of a death due to shaken-baby can be revealed following an earlier diagnosis of death as being due to a case of SIDS.

On November 28, 1979, a nine-month-old infant, David Drew Dickson, died while temporarily in the care of his day-care provider's husband. It appeared the day-care provider's husband demanded his wife leave him in charge of three infants while she left to purchase beer. When the wife returned, one of the children was found dead in an adjacent room. The husband did not provide an immediate explanation other than to suggest the child must have fallen from the sofa where he was sleeping. Furthermore, the husband stated that he had attempted CPR when he noticed the child had stopped breathing. The infant was taken to the local hospital and pronounced dead. The death was deemed suspicious and the infant's body was autopsied. In spite of evidence of trauma (i.e., broken ribs and cranial hemorrhage), the pathologist concluded that the death was due to SIDS.

Although the child's parents were suspicious of the incident, no further investigation was conducted and David Drew Dickson was buried the following week. Years later a forensic pathologist from a neighboring county requested the exhumation of the infant's body. The forensic pathologist stated that new testimony from the day-care provider suggested a different interpretation of the evidence. On May 2, 2006, the requesting pathologist, various staff of the California State University, Chico Human Identification Laboratory (CSUC-HIL), along with local sheriff and district attorney investigators conducted the exhumation.

The infant's remains had very little flesh and only a minor amount of adipocere adhering to the largely skeletonized remains; therefore, the remains were transported to the CSUC-HIL for skeletal analysis. The analysis revealed a complete set of skeletal remains of a nine-month-old infant still in their correct anatomical position. Of particular note, greenstick and complete rib fractures were noted among right ribs Nos. 2, 3, 5, 6, and 7, as well as left ribs Nos. 2 and 3. Furthermore, a deformed fracture was discovered on the right side of the occipital bone near the temporal-occipital junction of the lambdoidal suture. All the fractures were determined to be peri-mortem due to the combination of their location, deformation, and/or lack of healing. The result of the skeletal analysis suggested that the child likely died from a combination of being shaken and/or squeezed around the chest/abdomen along with blunt force trauma to the head.

Armed with these two new lines of evidence (testimony from the day-care provider, as well as the skeletal analysis) the day-care provider's ex-husband was charged with the murder of David Drew Dickson.

The suspect pled guilty to California Penal Code 192a. In addition to murder, the Code addresses voluntary manslaughter, or the killing of a human being without malice, and in such an instance permits a maximum term of eleven years in state prison. In this specific case, the defendant's sentence was suspended; he was placed on five-year probation, and ordered to pay a fine of \$5,200.00.

Exhumation, Shaken Baby, Cold Case

# H57 Infanticide and Unclear Law: The Death of Four Infants

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The goal of this presentation is to provide details on the investigation, recovery, and forensic examination of the remains of four infants, three of which were discovered in a skeletal state. Examination

procedures involving locating hidden or buried remains will be discussed in addition to the sorting and aging of the skeletal remains of three full term babies. Also, to be discussed is the importance of social services, law enforcement, and community in observing clues to infanticide behavior

Impact of this presentation will provide present and future forensic investigations insight as to the forensic analysis of decomposed and skeletonized remains of newborn and term infants. The forensic community will benefit from the knowledge that specialized recovery is required for remains of fetus and infants, and that proper handling and detailed analyses is required when dealing with such cases

Recovery of decomposed remains of late term fetus and newborns can be quite challenging for forensic scientists and investigators. The small size of such deceased and their skeletal elements, and the limited ossification of skeletal elements, provides for easy disposal and rapid degradation of the remains compared to that of adults.

In July of 2007 hospital personnel in Ocean City, Maryland alerted authorities of a possible infanticide of a newborn child as a result of treating a 37-year-old woman who had arrived at the emergency room with vaginal bleeding. Examination of the local resident by physicians revealed that the woman had recently given birth, which she denied. The police searched the woman's residence and discovered a deceased newborn hidden beneath a bathroom vanity. Further searching of the residence by police led to the discovery of two additional infants wrapped in plastic and placed in a clothing trunk; a fourth body was found in a motor home parked at the residence. The finding of the multiple sets of remains prompted law enforcement officials, including the FBI, to conduct an intensive search of the suspect's house and entire property. A search of the house included removal of various walls and ceilings. Fiber-optic cameras and cadaver dogs were utilized to search inside of the residence. A preliminary search of the ground property was conducted utilizing a combination of ground penetrating radar, cadaver dogs, and soil probing. Suspicious areas of the ground property were examined by establishing a marked grid followed by hand excavation. Fetal skeletal elements can be difficult to recover; thus, this multipronged approach to recovery allows complex surfaces and subsurface areas to be examined thoroughly and efficiently.

The female suspect was arrested and initially charged with murder for the death of one of the infants a male fetus (aged at 26 weeks of gestation), recovered from beneath the bathroom vanity. The woman, who worked as a cab driver and was the mother of four other children, was never suspected of being pregnant by her live-in boyfriend during the four pregnancies. According to neighbors, the suspect always wore extra large and loose fitting clothing and made up various excuses during her pregnancies to hide her condition.

Anthropological examination of the three sets of skeletal remains revealed them to represent full term fetuses, between 37 to 40 weeks of age based on osteological development. No apparent skeletal abnormalities were noted and no clear evidence of skeletal trauma was present. At the Grand Jury hearing the criminal charges against the woman were dismissed as a result of insufficient evidence after a medical examiner's report. The defendant did admit the children were hers; however, she insisted that she did not kill them. An additional complication leading to dismissal of the charges is that "Maryland law expressly protects woman who abort their own unborn children from criminal prosecution." Also, it unclear whether it was a crime to retain the remains of miscarried children. As a result of this case, the Maryland law was modified to address the two issues.

Anthropology, Fetal Remains, Pediatric

# H58 Proficiency and Competency Testing — What They Are, What They Are Not

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After attending this presentation, attendees should be able to understand the basic differences between competency and proficiency test programs. Attendees will learn the basic concepts and procedures related to competency and proficiency testing, especially as they relate to the human identification discipline, and how to meet the criteria and expectations of laboratory accreditation agencies. Additionally, drawing from experiences and lessons learned from the JPAC Central Identification Laboratory, participants will learn best practices and practices to avoid. Attendees should be able to utilize the material presented to formulate and manage competency and proficiency test programs for their staff.

This presentation will impact the forensic science community by advancing quality assurance in forensic laboratories and forensic programs. It will allow human identification laboratories to expedite the planning and implementation of competency and proficiency test programs in their organizations. These programs, when properly established and managed, will ultimately strengthen and elevate the forensic profession as a whole.

Quality assurance programs in forensic laboratories and activities have been a growing trend over the past decade. The publication of the National Academy of Sciences Report, "Strengthening Forensic Science in the United States: A Path Forward" and its recommendations have made quality assurance programs and accreditation relevant and thus an increasing priority for forensic human identification laboratories. Since 1999, the Joint POW/MIA Accounting Command, Central Identification Laboratory (JPAC-CIL) has implemented a stringent quality assurance program to ensure the scientific integrity of its casework. The CIL's quality assurance program ultimately led to its accreditation by the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB) Legacy Program in 2003-the first forensic skeletal identification laboratory to be so credentialed. In 2008 the CIL was re-accredited under the ASCLD-LAB International Program using ISO 17025 (General Requirements for the Competence of Testing and Calibration Laboratories) and criteria from the ASCLD-LAB supplement, Supplemental Requirements for the Accreditation of Forensic Science Testing and Calibration Laboratories.

Informal surveys and queries within the human identification discipline includiing discussions during sessions of the Scientific Working Group for Forensic Anthropology (SWGANTH), reveal that there are many misconceptions and misunderstandings about competency and proficiency test programs. During its accreditation efforts, the CIL gained vast experience with competency and proficiency testing programs. While these programs are a key component to any successful quality assurance program and its accreditation, at the same time they have the potential to negatively consume resources if not properly understood and effectively managed and administered. To that end, the CIL recognizes that it is imperative that laboratories first understand the basic differences between competency and proficiency testing programs-what they are, and what they are not. As such, this presentation will demonstrate the differences between competency and proficiency test programs from a standpoint of their intents and purposes, discuss minimal program requirements that human identification laboratories need to meet for ASCLD-LAB accreditation, outline criteria and considerations for training, testing, and corrective action, as well as discuss similarities between the two programs.

Administration and management considerations of competency and proficiency test programs are also addressed. For example, competency and proficiency test programs need to strike a reasonable balance between their intended outcomes, the resource expended, laboratory productivity, and satisfying accreditation requirements.

Competency Test, Proficiency Test, Quality Assurance

#### H59 Errors, Error Rates, and Their Meanings in Forensic Science

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After attending this presentation, attendees will gain a clearer understanding of the different classes of errors pertinent to forensic methods and practice, and will be provided a better taxonomy for method development, validation, and quality issues in their daily work.

This presentation will impact the forensic science community by providing a clearer understanding of the different types of errors. This understanding will make error easier for practitioners to identify, control for and discuss, and will provide the courts with a better understanding of how to interpret the classes of error introduced in scientific testimony. Overall, this presentation will result in a higher quality of forensic practice.

The discussion of errors and error rates has gained momentum in forensic science following the rulings from the Daubert trilogy (Daubert v. Merrell Dow Pharmaceuticals, Inc., General Electric Co. v. Joiner, and Kumho Tire Co., Ltd. v. Carmichael) and has accelerated with the National Academy of Sciences National Research Council's Report "Strengthening Forensic Science in the United States: A Path Forward." While the concepts of testing, standards, peer review, and general acceptance are fairly easy to understand, identify, and evaluate, the issue of error has proven to be more problematic. It has become clear that a discussion of what "error" means and how it is applied in forensic sciences is warranted. Furthermore, the convergence of science and law has made the identification and interpretation of error in the courtroom an even greater challenge. This paper presents an overview of the concept of method error as it pertains to forensic science techniques and attempts to clarify the difference between method error and other types of error that may be encountered in a forensic examination. As part of this clarification, the notion of the so-called "zero error rate" is addressed, and why this is an impossible and inherently non-scientific claim.

Too often, forensic practitioners themselves misunderstand the meaning of technique or method error (method validity), often confusing it with practitioner (human) error. Statistical error (unexplained variation) inherent in a statistical model is yet another type of error that the practitioner needs to consider. Misunderstanding or conflating different classes of error may lead practitioners to be reluctant to address the issue of error as it relates to their discipline or their individual case results. This confusion can also been seen in the courts, where attempts have been made to derive a measure of method error from things like practitioner proficiency testing results. Certainly the courts are concerned with both method error and practitioner error, but practitioner error is not error in the scientific sense and, for the most part, does not relate to method validity.

Misunderstanding (and misuse) of the concept of method error by forensic practitioners is particularly evident in claims of a "zero error rate" for particular forensic techniques. What some practitioners fail to realize is that despite the strength of the basis for certain forensic association techniques (e.g., the uniqueness of fingerprints as a basis for their use in identification), experts can still make false matches. The issue of method error does not relate to the uniqueness of a particular feature, but to how reliable and valid the methods of comparison are in determining a positive match, exclusion, or concluding that there is no scientific basis for either determination. Most forensic examination results require tempered conclusions, and practitioners need to demonstrate caution and distinguish errors from uncertainty and probability.

Error, Daubert, Validation

## H60 A Simulation for Exploring the Effects of the "Trait List" Method's Subjectivity on Consistency and Accuracy of Ancestry Estimations

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After attending this presentation, attendees will have a clear understanding of the specific aspects of applying the trait list method that potentially incur bias. The sources of bias are tested by simulating the application of the trait list method, and the results will provide attendees with empirical information on the overall consistency of the trait list method.

This presentation will impact the forensic science community by providing a mathematical analysis of the bias embedded in a commonly used forensic anthropological method for assessing ancestry. The results of the analysis allow the authors to make specific conclusions regarding the consistency of the method, as well as recommendations for how to avoid bias when utilizing the trait list method.

The nonmetric "trait list" methodology is widely used for assessing ancestry of skeletal remains. Although, recent valid critiques have been made,<sup>1-3</sup> the method has endured because of its ease of application and the familiarity of traits to the anthropologist. For a given unidentified skeleton, a checklist of traits is completed, noting each trait's state of expression. The distribution of the trait states among three geographic ancestries (Asian, African, and European) are typically used in conjunction with additional lines of evidence (such as metric analysis) to arrive at an ancestry estimation for the unidentified skeleton. While the application seems straightforward, there are both theoretical and logistical issues with this approach. Trait states are not exclusive to a single ancestry; instead, the trait list method is grounded in the belief that individuals of a specific ancestry more often express a particular trait state than other ancestries. Because nonmetric traits are considered heritable, albeit to various degrees, genetic drift and gene flow must be considered when accounting for shifts in distributions of trait state expressions. The polygenic nature of nonmetric traits maintains a complex path for variation in expression. The evolutionary premise of trait state distribution within a population and the influential genetic nuances are often lost in the application of the trait list method, such as with the use of "mixed" or "admixed" ancestries.<sup>4</sup> This designation implicitly relies on the concept that trait states are unique to a given ancestry and that "Asian," "African," or "European" parental ancestries actually existed at some point in the past.5

Choosing to incorporate the admixture approach into their research, because whether this is a theoretically sound approach or not, it is an approach that has been generally practiced over the decades. Thus, this research is based on the typical application of the trait list method, not the theory-bound approach that has recently found support.<sup>6,7</sup> The effects of the method's embedded subjectivity on subsequent accuracy and

consistency are largely unknown. Trait list ancestry assessment involves a series of decisions (how many and which traits to use) and interpretations (how to describe the ancestry based on the trait states), but there is no protocol.<sup>4</sup> For example, if 10 out of 10 observed traits express the Asian state, the associated skeleton would typically be classified as being of Asian ancestry, but what if only 9, 8, or 7 out of 10 are associated with Asian ancestry? What is the threshold for considering the conventional admixture estimations when using the "trait list" methodology?

Using a mathematical simulation that realistically represents the possible analytical variations of trait list ancestry estimation. The simulation explores: (1) trait selection; (2) number of traits employed; and, (3) ancestry choice thresholds affect the ancestry estimation of a skeleton. The relative accuracy of the trait list method in actual casework has not been comprehensively examined. The current study is a simulation of this accuracy, and tests how ancestry estimations for a given skeleton can differ from practitioner to practitioner when methodological choices vary. Using two temporally and geographically diverse samples comprising over 100 individuals, the simulation demonstrated that trait selection, quantity of traits, threshold choices, and the exclusion of high-frequency traits within a given sample had minimal effect on consistency in ancestry determination. For all datasets and Runs, accuracy<sub>AS</sub> was maintained above 90%.

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Nonmetric Cranial Traits, Ancestry Estimation, Bias

#### H61 The More the Better?: Evaluating the Impact of Fixed Semi-Landmark Number in Cranial Shape Variation Analyses

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The goal of this presentation is to refine the applicability of fixed semi-landmark-based techniques in biological profiling.

This presentation will impact the forensic science community by highlighting several important factors which contribute to the effectiveness of fixed semi-landmarks in characterizing morphological variation in cranial curvature.

Geometric morphometrics (GM) often utilizes anatomical landmark coordinates, in either two or three dimensions, to capture biological shape. Anatomical landmark coordinates exhibit biological homology across specimens and are categorized into three groups: Type I - discrete juxtapositions of tissue; Type II - points of maximal curvature; and Type III – extremal points (Bookstein, 1991). However, some anatomical regions, such as boundaries and surface curvature, lack well-defined landmarks. GM addresses this obstacle via the application of semilandmarks to such regions. The simplest form of semi-landmark data are represented by the placement of equally-spaced points on curves or surfaces. An algorithm resamples raw curve data into a manageable number of evenly-distributed fixed points. The number of fixed semilandmarks is user-defined and reflects just enough points to maintain the original curve's "shape." However, exactly what constitutes *enough* points is arbitrary and thus entirely at the researcher's discretion.

The present study examines the relationship between the number of fixed semi-landmarks utilized and their effectiveness in detecting sex and population differences in cranial curvature. Three-dimensional fixed semi-landmarks were captured on the orbital rims, zygomatic arches, nasal aperture, and maxillary alveolar ridge of 193 crania embodying a mix of socially-determined race (Black, White) and biologicallydetermined sex from the Terry, Hamann-Todd, Maxwell Museum, and W.M. Bass skeletal collections of known individuals. Curvature data was gathered as continuous stream data using a portable digitizer. Fixed semi-landmarks were then extracted utilizing a beta program (Slice 2005), which applies an algorithm that re-samples each curve into a userdefined number of evenly-distributed points. Two separate resampling sessions were performed on the original dataset. During the first session of resampling (S1) the following numbers of fixed semi-landmarks were extracted for each region: orbits = 40; zygomatic arches = 64; nasal aperture = 16; maxillary alveolar ridge = 16. These amounts were halved during the second resampling session (S2) (orbits = 20; zygomatic arches = 32; nasal aperture = 8; maxillary alveolar ridge = 8).

The resultant regional semilandmark data from each session were fit into a common coordinate system via a generalized Procrustes analysis (GPA), which filters out the effects of location, scale, and rotation. A principal component analysis (PCA) was performed on the covariance matrix of the GPA-aligned coordinates in order to reduce dimensionality. The resulting principal component (PC) scores, which accounted for 85% of the total variance in each region, were employed in subsequent multivariate statistical analyses. A multivariate analysis of variance (MANOVA) of the PC scores from both sessions detected significant race and sex effects in all of the regions (Pr>F=<0.05). A discriminant function analysis, which calculates the effectiveness of a set of variables in predicting group membership, was then conducted on the PC scores from both sessions using crossvalidation or n-1 method. The error count estimates (proportion of group misclassifications) for race exhibited minimal to no difference between data from Session 1 and 2 in all of the regions (S1 vs. S2 - orbits: Black= 0.3435 vs. 0.3435, White= 0.3387 vs. 0.3226; zygomatic arches: Black= 0.0611 vs. 0.0611, White= 0.2097 vs. 0.27426; nasal aperture: Black= 0.0992 vs. 0.084, White= 0.1613 vs. 0.1935; and, maxillary alveolar ridge: Black= 0.2901 vs. 0.2901, White= 0.2419 vs. 0.3065). The semi-landmark data from Session 1 and 2 produced a similar pattern of error count estimates in terms of sex (S1 vs. S2 - orbits: Female= 0.3284 vs. 0.3433, Male= 0.3095 vs. 0.3095; zygomatic arches: Female = 0.5224 vs. 0.4776, Male= 0.2302 vs. 0.1984; nasal aperture: Female = 0.3582 vs. 0.084, Male= 0.3889 vs. 0.3413; and, maxillary alveolar ridge: Female = 0.4328 vs. 0.5075, Male= 0.3968 vs. 0.3968). Thus, the statistical impact of reducing the number of fixed semi-landmarks in each region was negligible.

These preliminary results indicate that there is little statistical advantage to employing a large number of fixed semi-landmarks to capture shape variation. Moreover, when employing semi-landmark data to discriminate between populations and the sexes, the area from which they are collected, and not their number, is of primary importance. Incorporating such information into standard forensic practice may allow for a more informative assessment of race and sex in unidentified human crania.

Geometric Morphometrics, Semi-Landmarks, Crania

#### H62 A Performance Check of Ear Prediction Guidelines Used in Facial Approximation Based on CT Scans of Living People

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The goal of this presentation is to report quantified data on established ear prediction methods used in facial approximation.

This presentation will impact the forensic science community by communicating to peers the strength and weaknesses of ear prediction methods.

Facial approximation is the method used to predict the appearance of the face from a skull. This face can then be advertised in the hope that somebody recognizes it, possibly generating leads that may assist in the identification of the skeletal remains. For facial approximation to work effectively, it is important for prediction methods to be valid and accurate. This applies to all components of the face, including the ears. Currently several prediction rules have been published and widely employed with respect to the *pinna* or outer part of the ear, but few empirical validation studies have been conducted.

In this study, previously published ear prediction methods using seventy-eight living individuals of known age and sex who had been subjected to CT-scans were examined. The sample is composed of 43 males and 35 females with a mean age of 41.4 years (18-84 years, SD = 18.8 years). Osseous and cutaneous surfaces were reconstructed using the half-maximum height algorithm of the TIVMI software (Treatment and Increased Vision in Medical Imaging, developed by Bruno Dutailly, Université de Bordeaux). Landmarks and associated angles and measurements were collected to quantify the orientation and size of the mastoid process, nasal bones, nose and outer ear regions. Lobe attachment and supramastoid crest development were also visually assessed. These data enabled us to examine the following well-known ear prediction rules:

- 1. The main axis of the ear is oriented parallel to the major axis of the posterior mandibular ramus (Welcker 1883).
- 2. The height of the ear approximates the height of the nose (and a variation using an additional two millimeters (Ullrich and Stephan, in press) and the width of the ear equals half its height (Gerasimov 1949, 1955).
- 3. A large and broad ear is related to a massive and prominent mastoid process and the inverse regarding small ears; upper ear protrusion is also related to a strong development of the supramastoid crest (Gerasimov 1955).
- 4. Anterior projection of the mastoid process is associated with free lobes; and inferior projection of the mastoid process with attached lobes (Fedosyutkin and Nainys 1993).
- 5. The ear is oriented parallel to the profile angle of the nose (Wilkinson 2004).

Student *t-tests*, correlation matrices and cross table analysis were performed to evaluate the above mentioned prediction rules and to assess asymmetry, sexual dimorphism, and age trends within the sample. None of the empirical rules concerning the reconstruction of the ears reported in the literature proved reliable in our sample. The gross approximation of the height of the ears from the height of the nose was observed (mean error = 5 mm), however, no correlation was found between these two measurements. Although the width of the ear is not half of its height, the two were correlated (r = 0.56), and the width averaged 0.6 of the height (mean error of the estimate = 2 mm). In addition, no bony dimensions collected on the mastoid region were found to correlate with ear dimensions (r < 0.3). The only additional relationships between the soft and hard tissue that we observed were that a strong supramastoid crest appears to be linked with a free ear lobe ( $\chi^2 = 5.65$ ; df = 1; *p-value* =

0.02). However, the inverse is not true, subtle mastoid crests are associated with both free and attached ear lobes and height and width of the ear appears to be influenced by age (r < 0.38) and sex (*p*-value < 0.01).

These findings indicate that classic ear prediction rules hold little value for accurate prediction in facial approximation. Future efforts should be made to examine other relationships between the ear and the skull.

Facial Approximation, Facial Reconstruction, Pinna

#### H63 The Importance of Testing and Understanding Statistical Methods in the Age of *Daubert*: Can FORDISC Really Classify Individuals Correctly Only One Percent of the Time?

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After attending this presentation, attendees will avoid future misunderstandings in the use of FORDISC and will be better able to use the program correctly and effectively.

This presentation will impact the forensic science community by exploring common misunderstandings in statistical analysis, particularly FORDISC.

Fordisc 3.1 (Jantz and Ousley 2005) uses discriminant function analysis, as has previous versions, and FORDISC has provided more and more additional information in addition to the classification results during its evolution. Failure to understand this additional information has led to a claim that challenges the accuracy of FORDISC. The recent publication of a series of FORDISC tests by Elliott and Collard (2009) is a result of their failure to appropriately interpret statistical results.

Elliott and Collard classified individuals from five groups in the Howells database (Berg, Northern Japan, Santa Cruz, Tasmania, and Zulu) into all Howells groups. They used all 56 craniometric variables in FORDISC as well as three groups of 10 variables from different cranial regions (basicranium, neurocranium, and face). Due to a misunderstanding of posterior probabilities, they reported very low percentage correct classification in general and concluded that FORDISC classifies less than 1% of individuals correctly. Their criterion was that classifications showing a typicality probability of less than 0.01 or a posterior probability of less than 0.8, no matter which group was most similar, were considered incorrect. Unlike typicality probabilities, the posterior probability does not have a threshold requirement. Higher posterior probabilities generally reflect higher probability of correct classification, but there are no specific recommendations or discrete cut-off values. In the statistical literature, having a posterior probability of at least 0.8 is merely considered a "strong" classification. Their test conditions seem rigged for failure: when using 56 variables, they were using more variables than many of Howells sample sizes, resulting in lower typicality probabilities, and when using only 10 variables form certain areas of the cranium, they were extremely unlikely to get high posterior probabilities. Additionally, they classified Howells individuals from the five groups using every other Howells group to ascertain if groups showed geographic similarity. However, they designated only one specific group from each region that should theoretically be the most similar one, and any other classifications were deemed incorrect. For instance, in Europe, only a classification of Howells' Berg individuals into Norse was considered correct.

Elliott and Collard's methods were followed as closely as possible using both FORDISC 3.1 (2005) and SAS 9.1 (2003), using the Howells

database. Individuals from the same five ancestral groups were used, and run against all individuals in the Howells database. The analyses were conducted with all 56 variables and the same three groups of 10 variables representing the basicranium, neurocranium, and face. Because Elliott and Collard did not state which typicality probability was used, the chi-square typicality was used in this study. Correct analysis of the results resulted in correct cross-validated classification percentages of 18 to 32%, which is significantly greater than random, and greater than 1%. Classifications with higher posterior probabilities showed higher correct percentages, and regionally patterned variation was strongly indicated. The disparity between Elliott and Collard's conclusions and those of the current study is clearly a result of their misuse of posterior and typicality probability thresholds. Further, the geographic affinities of the test groups were confirmed when a more flexible criterion of regional similarity was accepted. Unlike Elliott and Collard's results, the current study showed that when the reference group is excluded, the percentage correct regional classifications is comparable to or slightly higher than the percentage correct classifications when the group is included in the analysis.

With the advent of *Daubert* standards, it is critical for forensic anthropologists to validate methods. The current analysis has shown that it is imperative to thoroughly understand the statistical underpinnings of any method, and that faulty criteria and test procedures can lead to false conclusions of low validity for a method. The number of measurements and stipulations for classification correctness used by Elliott and Collard resulted from a statistical misunderstanding that virtually guaranteed a low classification accuracy rate.

FORDISC, Discriminant Function Analysis, Statistics

## H64 Forensic Interviews: Corroborating Evidence and Collecting Data for Anthropological Field Work

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After attending this presentation, attendees will learn which forensic interviewing techniques can be applied by anthropologists who are interviewing international populations in anticipation of field excavation. The same techniques can also be considered by investigators in the United States who are reviewing cold case homicides and missing person investigations involving immigrant and migrant populations.

The presentation will impact the forensic science community by discussing the validity of memory recall from survivors or witnesses of genocide, massacre, torture, murder, and other acts of violent crime after an extended period of time has elapsed. This presentation will examine techniques for developing a forensic interview strategy when approaching witnesses and survivors of violent crimes and international atrocities.

From November of 2008 through July of 2010, investigators from the University of South Florida conducted interviews with Nigerians who survived or witnessed a massacre that occurred in Asaba, Nigeria on October 7, 1967. The process of creating an oral record began with a review of the known literature and the development of an interview strategy. The interviewers established a general interview protocol that was used to establish the witness' *role* during the event, ascertain specific information that could assist with a field excavation, and open a forum that offered the interviewees the opportunity to recount their experience. The research team conducted more than 40 interviews for this project and these interviews were used for the analysis presented in this research paper. This paper will address the benefits of conducting a directed, goal oriented interview that also allows interviewees the opportunity of tell their stories (in the oral tradition). This technique allows for the broadest opportunity to gather information and identify evidence. Investigators who attempt to gather *legal truth* from events which have occurred in the past are faced with the challenge of corroboration and will be limited by the existence of evidence. These types of forensic interviews are a starting point for the anthropologists who are searching for physical evidence, or it can be used by law enforcement to corroborate physical evidence.

Interviewing survivors or witnesses to genocide, massacre, torture or other acts of violent crime presents specific problems of memory recollection, legal reliability, and/or credibility. The role of anthropologists in these types of investigations is more varied than in typical American casework. Therefore, the ability of anthropologists, pathologists, and investigators to successfully interview family members and survivors is critical to successfully completing the mission.

Each interview for the Asaba project was developed in a unique manner and the interviewers were required to direct the process so the established interview goals were met, while not interfering with the natural process of the interview. The interviewers were also required to connect the common points of each interview (in real time) and to address any points of discrepancy between the interviews.

The investigators who are developing a forensic interview strategy must consider the following factors before they consider the validity of episodic memory: the elapsed amount of time between the event and the interview, the psychological effects of trauma, the potential of outside influence upon the memory, cultural perspectives of the interviewees, the age of the interviewee at the time of the event and the time of the interview, and any other hidden agendas brought to the interview process. The investigators and anthropologists have to understand that information (or evidence) provided these witnesses has the greatest potential for being unreliable and this presents very specific challenges to the interviewer. This challenge is the purpose for creating a defined forensic interview strategy.

The paper will also address the methodology for documentation of the data, including the creation of missing person(s) questionnaire and victim databases, and the best practice standards for obtaining, sharing, and analyzing such data.

The initial results from this interview project suggest that the forensic interview strategy did establish the reliability of the witness, corroborated known facts, and directed the anthropologists with the field excavation plan.

Interview, Massacre, Cold Case

# H65 Archaeological Methodology Used at the World Trade Center Site During the 2006/2007 Recovery Excavation

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After attending this presentation, attendees will be presented an overview of the archaeological methods used during the 2006/2007 Human Remains Recovery Operation at the World Trade Center (WTC) site. Additionally, some of the findings, including the relationships between the buried deposits of WTC material and human remains recovered during the excavation and specific situations and activities conducted during the 2001/2002 recovery operation that lead to their omission will be presented.

This presentation will impact the forensic science community by providing a body of information and protocols, developed and tested in the field that could be adapted to future mass disaster situations, built upon, and potentially used to improve recovery efforts, especially where a high degree of fragmentation is involved.

This presentation will provide an overview of the archaeological methods used during the ongoing Human Remains Recovery Operation at the World Trade Center site conducted by the New York City Office of Chief Medical Examiner (OCME) since 2006 and will discuss findings from the excavation. The topics addressed will include the archaeological methods used to identify, delineate and document the site, the identification of WTC debris patterns and their relationship to the recovered human remains concentrations, and what these patterns reveal about the original recovery effort that took place immediately following the terrorist attacks.

The primary objective of the WTC operation was to recover victims' remains and personnel effects for identification. The excavation also presented an opportunity to test which archaeological methods would be most effective in a large-scale mass disaster recovery operation. In addition, the archaeological investigation provided insight into aspects of the initial response and recovery conducted in 2001/2002. Although the OCME operation was not intended to analyze or critique the original recovery operation, but when understood within the larger site context it provided general explanations for why much of the remnant WTC material and remains were not originally removed from site.

A total of 952 potential human remains have been recovered from the excavation during sifting operations conducted in 2006 and 2007. Potential human remains were recovered from 49 (83%) of the 59 excavated units. In addition, 29 potential remains were recovered from subterranean structures located on and adjacent to the site. The three main contexts where WTC debris and human remains were found were: (1) sections of intact paved pre-9/11/2001 surfaces; (2) pre-9/11/2001 unpaved areas; and, (3) voids caused by debris impact, machine excavation and pre-9/11/2001 subterranean structures. Many of these contexts were found to be partly the results of recovery activities carried out during the 2001/2002. Photographs of the original recovery effort clearly support the excavations findings and illustrate these relationships.

Many of the archaeological contexts exposed during the excavation were not necessarily unique to the WTC disaster and could be encountered in other mass disaster situations. These insights regarding the original recovery effort including the assessments of the strategies used in the OCME operation provide a body of information that could be adapted to future situations, which could be used to improve mass disaster recovery efforts, especially where a high degree of fragmentation is involved. The OCME WTC operation demonstrates the strength and practicality of using archaeological methods as a framework for systematic mass disaster recovery operations. In addition, the operation demonstrates that archaeologists properly trained in forensic protocols are uniquely effective at carrying out the variety of tasks it takes to ensure that the scene has been accurately defined, cleared and documented. Attendees will gain an appreciation for the practical benefits of archaeological methods in such tasks as defining horizontal and vertical site boundaries using stratigraphic analysis and artifact identification, as well as some conceptual ideas regarding how pre- and post-disaster land use factor into an urban mass disaster recovery operation.

It is not suggested that a mass disaster response and recovery operation similar to the WTC disaster should, or could, be conducted solely by archaeologists, but that those leading forensic investigations and recovery operations might consider the benefit of adopting archaeological methodology, as well as including uniquely trained professional archaeologists in future mass disaster response teams.

Archaeology, World Trade Center, Mass Disaster

#### H66 World Trade Center Revisited: A Bayesian Approach to Disaster Victim Identification

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The goal of this presentation is to demonstrate to attendees the potential of a multivariable identification process using Bayesian statistics in a mass fatality context. The specific variables presented include anthropological estimates of age and sex, dental information, and recovery location and uses the World Trade Center disaster as an example.

This presentation will impact the forensic science community by providing an alternative approach to victim identification for disasters featuring highly fragmented and/or degraded remains and where traditional identification methods have reached their limits. The approach presented utilizes fragments of data deemed insufficient for identification based on a single modality.

Disaster victim identification (DVI) for mass fatality incidents involving highly fragmented and/or degraded remains is a difficult process because the individuating information that is required by the various identification disciplines is often limited. For example, the roughly 9,000 unidentified remains from the World Trade Center (WTC) disaster possess varying amounts of anthropological, odontological, and/or DNA data that go unused because none is sufficient for identification on their own. Further, information such as recovery location is often available but never directly utilized in the identification process. Steadman et al. (2006)1 recently demonstrated how age, sex, stature, pathology, and dental data may be combined in a Bayesian framework to generate a statistical statement regarding the relative strength of a single circumstantial identification. The goal of this research is to apply the Steadman et al. approach to combine age, sex, dental, and recovery location data in a mass fatality context, where a defined victim population presents practical and statistical advantages. This approach incorporates the concept of a statistical threshold for identification as currently used for direct DNA-based identifications of WTC remains (Posterior Odds  $> 4x10^9$ ).

Ante- and postmortem data from the WTC disaster were utilized in this research. Likelihood ratios were calculated for each theoretical combination for the following variables:

Age: Likelihood ratios were calculated for age based on estimates using the Suchey-Brooks  $(1990)^2$  method for the pubic symphysis for each theoretical combination of Age and Phase. The WTC victim age distribution was used as the prior odds and data collected by Hartnett  $(2010)^3$  was used as a reference sample.

**Sex:** Likelihood ratios were calculated for sex based on estimates using the Phenice  $(1969)^4$  characteristics with the WTC victim sex distribution as the prior odds. Data collected by Konigsberg et al  $(2002)^5$  was used as a reference sample.

**Dental:** Likelihood ratios were calculated for the available dental patterns of the unidentified WTC remains based on the expected pattern frequencies in the "population-at-large" according to the Odontosearch application (Adams 2003).<sup>6</sup>

**Recovery Location:** The WTC victim population was divided into subgroups based on known location at the time of the incident (Tower 1, Tower 2, AA 11, UA 175, etc). Likelihood ratios were calculated for membership within a particular WTC subgroup based on the recovery location of the remains (grid system established by the New York City Fire Department). Likelihood ratios were determined for each combination of group and location using the location of identified remains as a known sample and the total number of remains recovered at each location as the prior odds.

The average likelihood ratios calculated for age, sex, and recovery location are comparable to the contribution of individual CODIS STR

alleles. The available dental patterns were more informative, with likelihood ratios ranging from just above 1 up to 37,956. Combining these variables using the Product Rule under a theoretical "best-case" scenario produces a likelihood ratio of  $8.3 \times 10^6$ , which does not meet the established threshold for identification ( $4 \times 10^9$ ). However, it does result in a smaller required contribution from any potential DNA evidence. These results suggest that partial DNA profiles may be sufficient for identification if other available information is considered within a Bayesian framework.

The consideration of additional variables beyond DNA in a quantitative manner allows for a truly multidisciplinary DVI process and has the potential to allow for identification of highly fragmented and/or degraded remains that might not otherwise be identified. The quantification of these variables also has the potential benefit of providing a mechanism for ranking of database search results similar to dental identification applications.

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- <sup>4.</sup> Phenice, T. W. 1969 A newly developed visual method of sexing the os pubis. *Am J Phys Anthropol* 30(2):297-301.
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Disaster Victim Identification (DVI), World Trade Center (WTC), Bayesian Statistics

#### H67 New Forensic Archaeological Recovery Protocols for Fatal Fire Scenes

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After attending this presentation, attendees will be familiar with new forensic archaeological techniques applicable to fatal fire scenes that result in efficient and effective evidence recovery.

This presentation will impact the forensic science community by pointing out the benefits of employing forensic archaeology in the documentation and recovery of all types of outdoor crime scenes, including fatal fire scenes.

Victim remains at fatal fire scenes are typically difficult to detect, recover, and handle. All of the burned material at the scene, including biological tissue, is often modified to a similar appearance and bones, in particular, become discolored, brittle, and highly fragmented. As a consequence, these remains are often missed, disturbed, altered, or even destroyed during scene processing with standard crime scene protocols. The added postmortem fracturing, fragmentation, and bone loss resulting from these recovery techniques hinder the already difficult task of autopsy and laboratory analysis of burned human remains. This is especially problematic for bone trauma analysis, as its most immediate goal is distinguishing peri-mortem(forensically significant) trauma, from Lack of on-scene recordation of relevant information related to body positioning and contextual relationships of the remains as well as of other physical evidence at the scene, further complicates trauma analysis, biological profile estimation, and event reconstruction.

New scene recovery protocols drawn from forensic archaeological methods are described in this presentation. Six tests of specific scene recovery methodologies were conducted in the last two years in which evidence, including spent bullet cartridges, knives, and euthanized pigs, were placed in house structures that were then burned to the ground. Following a search for evidence by trained fire investigators, forensic archaeologists then excavated the burned matrix and carefully mapped the evidence found in situ. The method that yielded the most efficient and effective recovery involved the hands and knees "search/excavation" in which burned matrix was excavated using a "cake-cutting" (i.e., cutting a vertical face) technique working from the outer edges of the excavation corridor or room, inward. This process allowed for the rapid excavation of debris in areas where no significant evidence was located. The excavated debris was removed by buckets and placed on tarps, sorted by provenience unit, where it was quickly sorted by hand and discarded if no evidence was detected or sieved. The matrix from these areas bypassed the tarp hand sorting and was directly sent for careful screening on 1/4 in mesh screens. The debris over the victim was removed via a "top-down" excavation method, thus exposing fully the remains. The remains were photographically documented in situ, and mapped both by hand and with electronic instrumentation such as a total station or survey-grade GPS units. Head, distal limbs and any other fragile body regions were protected with heavy-duty plastic wrap to maintain the integrity of the bone. The remains were then placed on a sheet of plywood in a body bag in efforts to reduce further disruption of the remains during transport.

These new protocols were demonstrated to: (1) improve evidence detection and recovery; (2) limit disturbance and further fragmentation of the remains during the recovery; and, (3) provide precise and detailed information regarding the position and orientation of the body and related evidence, as well as on their contextual relationship at the scene. Further, these improvements are realized within an efficient timeline.

This project was funded by the National Institute of Justice, U.S. Department of Justice.

Forensic Archaeology, Fatal Fire, Human Remains

#### H68 Using Spatial Analysis to Recognize Normal and Abnormal Patterns in Burned Bodies

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After attending this presentation, attendees will be introduced to the utility of spatial data in recognizing normal and abnormal patterns in burned bodies.

This presentation will impact the forensic science community by quantifying the progression of heat alteration in burned bodies, independent of the circumstances of death or area of heat exposure, thus suggesting criteria for identifying normal and abnormal burn patterns. A mapping approach will demonstrate the utility of geographic information system (GIS) technology for the analysis and quantification of heat alteration to human remains. Recognizing the typical pattern of heat alteration in burned bodies under normal circumstances is important in a forensic context. Deviations from this pattern may imply special burning conditions such as protective shielding, the presence of accelerants or pre-existing trauma. Symes and colleagues (2008) provided a preliminary model for the normal sequence of bone exposure and heat alteration resulting from tissue thickness and limb position (i.e., body posture). While an invaluable resource, that model is merely derived from observation (based on extensive case experience) and has not been empirically quantified or tested. This study employs GIS to achieve these goals.

The sample data were collected at the Office of Chief Medical Examiner (OCME) in New York City. Burned body information with detailed documentation and known circumstances of death were compiled from cases spanning from January 2005 to July 2009. After excluding superficially burned victims and deaths due to smoke inhalation with little or no heat alteration to the body, the final sample included 74 forensic cases. Cases consisted of accidents (including vehicular accidents), homicides, suicides, and undetermined manners of death at both indoor and outdoor crime scenes. The burn patterns were charted in homunculi diagrams, with an anterior and posterior chart for each body. The degree of heat alteration was coded into five categories: 1 = no burning/minimal burning; 2 = charred tissue; 3 = charred tissuewith burned bone visible; 4 = charred tissue with calcined bone visible; and, 5 = missing or fragmentary bone. Polygon shapefiles of the body outlines and burn patterns were created in a GIS application for each case. The vector data were converted to raster data and added together, and the surface areas for each heat alteration category for each case were calculated.

A composite image of the 74 cases illustrates the areas of the body that are more severely altered by heat, as well as the extent of this modification. As predicted by Symes and colleagues (2008), the degree and anatomical pattern of heat alteration can be most accurately predicted from tissue thickness, principally in relation to the sequence in which the areas exposed to heat will attain a particular degree of alteration. In this way, deviations from this sequence can be marked as suspicious, regardless of the overall degree of heat exposure. In order to test this, individuals were ranked based on degree of burning and that rank was compared with the total area burned. Results indicate a strong correlation ( $R^2 = 0.98$ , *p*-value < 0.001) between the degree and extension (area) of heat alteration, in such cases where the whole body was exposed to fire, but not at a temperature or period long enough to result in the alteration of the entire body surface. These bodies, therefore, provide a baseline for the normal sequence and intensity of heat alteration. After approximately 80% of the body shows heat alteration, any degree of burning to the body is not uncommon. Abnormal burn patterns are recognized when less than 80% of the body is burned yet a category of 3 or higher of heat alteration is observed. The examination of cases meeting this proposed criteria for the detection of abnormal patterns revealed that they include a homicide with the victim's legs bound by a ligature, a vehicular accident in which the victim sustained extensive blunt force injuries, and accidents with evidence of substantial clothing on the body.

This research was partially funded by a grant from the National Institute of Justice.

Burned Body, Pattern Recognition, Spatial Analysis

#### H69 Recovery and Identification of a WWI American Doughboy in Rembercourt-sur-Mad, France

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The goal of this presentation is to provide the attendee with a case example involving the remarkable recovery and identification of an individual from World War I. After this presentation, attendees will gain a better understanding of the complexity of cold cases, a greater awareness of the importance of the multiple lines of evidence that are required for identification, and a heightened appreciation for community responsibility.

This presentation will impact the forensic science community by providing an example of a successful forensic recovery in the international setting, by demonstrating how a positive identification was attained despite having multiple name associations, by broadening our understanding of factors that may influence preservation of remains, and by further demonstrating the significance of proper archaeological techniques and methodical data collection. With this case, the forensic community may gain additional approaches that can be applied to a variety of cold cases or cases in the international setting.

The Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL) has the mission to search, recover, and identify service-personnel still missing as a result of past U.S. conflicts. Anthropologists at the CIL regularly conduct recovery missions around the world related to World War II and the Korean and Vietnam conflicts. However, recoveries and identifications from earlier conflicts are uncommon. Even more uncommon are those that result in a well preserved burial of a World War I American Doughboy Marine whose identification was obfuscated by dog tags recovered with his remains that were inscribed with the name of another marine.

Shortly after a discovery by French relic hunters, JPAC was notified in September 2009 of an alleged burial of a World War I American Marine in the village of Rembercourt-sur-Mad in northeastern France. Initial verification of the plausibility of the burial involved investigation of archival records. This confirmed that the first U.S.-led offensive of the war by the American Expeditionary Forces, under the command of General John J. Pershing, occurred on September 12, 1918, at St. Mihiel, approximately 17 miles northeast of Rembercourt-sur-Mad. Among the approximately 7,000 Allied casualties were 2,000 American KIA. Fortysix U.S. Marines are memorialized at the Saint Mihiel cemetery (with 11 listed as unaccounted for).

Anthropologists from the JPAC-CIL traveled to Rembercourt-sur-Mad, France, where they recovered a superbly preserved human burial. Thirty-five kinds of artifacts were recovered from the burial; their *in situ* locations on the skeleton mimicked where they would have been worn on the body during life, including a wallet, dog tag, and badge in the front left breast pocket, a first-aid kit, shaving kit, canteen, and side arm ammunition on the hips, and six complete clips of rifle ammunition still slung across the chest. In addition, tree roots had grown, over time, through the burial site. Some had penetrated the thorax, but rather than damaging the remains, they gently moved and shifted skeletal elements.

This preservation was welcome, as roots can be a very disruptive taphonomic force. All archaeological signs pointed to a considerate burial by friendly forces.

After international transport, the skeletal remains and artifacts were analyzed at the CIL. A name engraved on the badge and initials inside the wallet were consistent with one of the 11 unaccounted-for U.S. Marines, but a different person's name engraved on the dog tag warranted caution of any presumptive identification. After further investigation, and using numerous lines of evidence, the individual was identified in March 2010. Personnel records included a letter written to the individual's brother by a witness to the death incident. In that letter, the witness recounted their hasty but respectful burial of the individual, as well as a map of its location. Shortly after the war, a search for his burial using this map was unsuccessful. Ninety-two years later, his remains were found, recovered, and identified. He was buried in June 2010 in Arlingtion National Cemetery with full military honors.

Cold Case, WWI American Doughboy, Presumptive Identification

#### H70 The Fromelles Project – The Recovery and Identification of British and Australian WWI Soldiers From Mass Graves in Northern France

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After attending this presentation, attendees will have a greater understanding of how forensic anthropological and archaeological methods can be used to not only excavate eight mass graves, document, and recover 250 sets of remains and thousands of artifacts, but also manage to positively identify almost 100 of the soldiers through interdisciplinary evidence collection.

This presentation will impact the forensic science community by demonstrating up-to-date and progressive excavation and anthropological analysis methods and by showing the possibilities of positively identifying individuals after being buried almost 100 years.

On July 19 and 20, 1916, British and Australian Forces fought a hopeless battle against German forces trying to draw attention away from the Somme. The outcome of this battle was the catastrophic loss of over 7,000 soldiers in less than 48 hours.

In the past two decades professional and amateur historians managed to locate eight possible mass grave pits adjacent to a small village of Fromelles in Northern France. The presence of multiple remains was confirmed in 2007-08 and in February 2009, Oxford Archaeology (OA) was awarded the contract to carry out the recovery at Pheasant Woods. A team of OA staff and external consultants was assembled, including forensic archaeologists and anthropologists, osteoarchaeologists, finds experts, crime scene investigators, anatomical pathology technologists, radiographers, IT experts, and many more.

A second contract was awarded for analyzing ante- and postmortem DNA samples. The goal was to extract sufficient amounts of uncorrupted DNA from the soldiers as well as trying to find second or third generation direct relatives. Both aspects of the program were extremely challenging but turned out very successful.

After the site was made secure in April, two teams of around five to six archaeologists and one supervisor each began excavations of the first two graves. All stages of the excavation were carefully documented by professional surveyors and photographers. The excavation was conducted under strict forensic archaeological rules. All data was immediately entered onto a secure database system and therefore instantly available to all relevant staff in the anthropological laboratory.

DNA sampling was carried out using a specifically developed protocol that ensured that samples were taken within a few minutes of being uncovered and exposed to oxygen and to eliminate contamination as much as possible. All personnel involved on site had to wear full personal protective equipment at all times when within less than 10 meters of the grave.

To ensure that all human remains and artifacts were recovered, metal detectors were used extensively throughout the excavation and all soil that was removed from around remains or artifacts was scrutinized in great detail. Soil was collected from around and underneath remains and x-rayed to make certain that even the smallest finds would not be lost. The excavation and recovery phase resulted in 250 sets of human remains and over 6,000 artifacts.

The laboratory, store rooms, and office space was set up in March and April. The layout guaranteed a secure and efficient workflow as well the dignified and respectful treatment of the human remains. Sets of remains and associated artifacts were transferred from the excavation to the anthropological laboratory using a documented handover procedure witnessed by a crime scene investigator to guarantee the continuity and integrity of all evidence. Remains and artifacts were first x-rayed using a direct-digital x-ray unit, operated by an experienced radiographer. All images were stored digitally and moved onto the secure database to give access to the anthropologists.

Human remains were then carefully cleaned to prepare them for anthropological analysis. All anthropologists had their own workstation, consisting of a fixed table, a digital SLR camera permanently fixed to the ceiling above the table, a PC workstation connected both to the camera and the database server.

The newly build cemetery is located in close proximity to the mass grave site. Each soldier was buried individually with full military honors. DNA analysis took place throughout the project and the results, together with the anthropological and artifact analysis results were presented to an Identification Commission in March 2010. To date, 97 soldiers have been positively identified. The cemetery was officially dedicated and opened in a ceremony in July 2010.

Forensic Anthropology, Forensic Archaeology, DNA Sampling

#### H71 Validation of X-Ray Fluorescence (XRF) to Determine Osseous or Dental Origin of Unknown Material

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After attending this presentation, attendees will understand the results of a study conducted to validate the use of x-ray fluorescence (XRF) in determining whether unknown material is osseous (bone) or dental (tooth) in origin or some other type of material (such as mineral, plastic, wood, etc.).

This presentation will impact the forensic science community by supplying an additional analytical tool for forensic anthropologists or other experts to quickly and effectively assess the potential skeletal origin of unknown material.

Forensic anthropological examinations typically involve the analysis of human skeletal remains, but it is sometimes necessary to first determine whether the material in question is even osseous or dental in origin (i.e., whether it is, in fact, part of a skeleton). This is especially relevant in cases where the material may be submitted for DNA analysis.

Tissue identification can usually be achieved through visual macroscopic and/or microscopic (and in some cases radiographic) examination by a trained anthropologist when specimens are sufficiently large and in good condition. Occasionally, however, specimens are very small and/or taphonomically compromised, making this determination difficult. X-ray fluorescence spectrometry (XRF) is a technique that reveals the elemental composition of materials and is hypothesized to have utility in these analyses. Validation of the XRF technique for identifying osseous or dental tissue would impact the forensic community by supplying an additional analytical tool for forensic

anthropologists or other experts to quickly and effectively assess the potential skeletal origin of unknown material.

In this study, XRF analysis was conducted on a variety of tissues of known osseous and dental origin in good condition including human bones, human teeth, non-human bones, non-human teeth, and ivory. In addition, other biological hard tissues such were analyzed as horn, beak, coral, and shell, as well as other materials that may appear similar to osseous or dental tissue when in small fragments or altered states such as wood, minerals, plastic, metal, and glass. XRF was also conducted on these same tissues and materials in thermally, chemically, and taphonomically altered states. These states included various degrees of burning (e.g., charred, calcined), weathering (e.g., bleached, exfoliated), antiquity (up to 9,000 years old), and exposure to several destructive chemicals.

Analysis of the human and non-human osseous and dental tissues in good, burned and weathered conditions revealed characteristic levels of calcium and phosphorous. Osseous and dental tissue samples also commonly (though not always) contained trace levels of strontium. Significantly compromised osseous and dental tissue, such as ancient samples, showed very low or virtually absent phosphorous levels, as did the coral and shell samples. Horn, plastic, wood, metal, and other materials in either good or compromised conditions did not contain these characteristic levels of calcium, phosphorous or strontium. Because there was no sample preparation involved in the analysis, many specimens contained low levels of various other elements due to surface contamination. These levels did not substantially affect the results.

Materials were accurately identified as osseous or dental in origin based on the calcium and phosphorous levels identified by XRF using the analytical parameters of this study, with no other material showing profiles that might be mistaken for osseous or dental tissue. In other words, preliminary results suggest that osseous and dental tissue in altered states may be misclassified as some other material (due to its similarity to materials like shell and coral), but non-bone or non-tooth materials are unlikely to be misclassified as osseous or dental tissue. It is concluded that XRF analysis is a valid and effective means of determining osseous or dental origin of unknown material.

Forensic Anthropology, X-Ray Fluorescence, Elemental Composition

## H72 The Condyle Connection: Forensic Implications for the Association Between the Condyles of the Femur and Tibia

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After attending this presentation, attendees will have observed the results of comparisons between the condyles of the distal femur with those of the proximal tibia for a given individual as well as the developed predictive formula which have practical applications for the medical community, archaeological research, and forensic casework.

This presentation will impact the forensic science community by providing an analysis of the relationship and correlation between the individual skeletal components of the functional unit of the knee.

The knee is one of the most functionally important and largest joints in the body. Previous research has investigated the distal femur and proximal tibia with regards to sex assessment, ancestry and morphological differences (Waxenbaum et al., 2007), but the relationship among the condyles specifically has not been addressed. Given the robusticity with which these components of the lower limb survive in both archaeological and mass disaster scenarios, this investigation into the degree of their association is particularly important. The populations examined include segments of the Terry White (n=94) and Terry Black (n=100) anatomical collections, a component of the South Dakota Arikara (n = 120) and Native Alaskan groups (n = 201) (all remains included in this analysis are housed at the National Museum of Natural History, Smithsonian Institution). Individuals were sampled from both sexes and were separated into "older" and "younger" categories for age analysis given the archaeological nature of the Alaska and South Dakota remains. Measurements of the left medial and lateral condyles of the distal femur and proximal tibia were taken on all individuals and compared through correlations analysis, Tukey's procedure and reduced major axis regression.

The present research found that the medial and lateral condyles of the proximal tibia and the distal femur show a statistically significant relationship across sex (p<0.0001) and ancestry (p<0.0001) for all components compared, and for age (p<0.0299) in three out of four comparisons. Insight from Tukey's analysis highlighted significant, specific variation between the four ancestries. Native Alaskan populations were distinct in femoral condylar surfaces from all other populations but indistinguishable from Terry White individuals for tibia condylar measurements. Terry White and Black groups could not be statistically separated in all analyses given the present sample. Additionally, archaeological remains (Arikara and Native Alaskan remains) could be significantly separated from modern, anatomical specimens (Terry Whites and Blacks) in three out of the four condylar surfaces compared.

Through reduced major axis regression, a series of 15 equations were developed that were able to predict the size of the opposing bone's condyle. The equations are general and specific to age, sex, and ancestry. The value of this observed variation is its ability to differentiate individuals of diverse populations or identify sex in mass disaster scenarios where a large number of decedents may be highly fragmented and/or commingled. In addition, the equations could be employed in a clinical setting to improve the fit of knee prosthesis during total knee arthroplasties. This would help reduce lateral over- and underhang, correcting improper fitting prosthesis, reducing discomfort and increasing flexibility for the patient. The results of this research provide an invaluable addition to forensic mass fatality recovery and identification as well as insight into skeletal variation for both clinical and anthropological research.

Knee, Condyles, Mass Fatality

## H73 Craniometric Variation in the Caribbean and Latin America as Influenced by the Trans-Atlantic Slave Trade

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After attending this presentation, attendees will have a better understanding of the craniometric diversity within Caribbean and Latin America as influenced by the Trans-Atlantic slave trade.

This presentation will impact the forensic science community by highlighting the importance of investigating biological diversity in regional samples. Such investigations are paramount in refining identification methods, which would allow forensic anthropologists to determine ancestry more accurately and aid in narrowing the pool of missing persons during an investigation.

Timely and accurate identification of unidentified remains is integral to the progression of medico-legal and human rights investigations. Determination and/or estimation of sex, age, stature, and ancestry narrows the list of missing persons, potentially leads to the positive identification of unidentified remains, aids in the success of criminal investigations, and provides family and friends with closure. As the application of forensic anthropology increases worldwide, the need for population specific methods and population specific research has become more paramount, particularly those concerned with ancestry. Until recently, ancestral categories have been loosely based on linguistics, regional, and/or continental affinity. For example, the terms Hispanic and African provide broad categories which assign a missing person as coming from a Spanish speaking population or the entire continent of Africa. Increasingly, investigations have shown that humans are far more diverse than these broad categories account for and have shown that modern statistical methods can more narrowly identify intra-regional variation as well as answer broader questions concerning human migration and expansion (Ousley 2010, Spradley et al. 2008, Kenyhercz et al. 2010, Ross et al. 2003, Ross et al. 2008).

During the 16<sup>th</sup> and 19<sup>th</sup> centuries, nearly 10 million African slaves were transported to the Americas drastically changing the biological composition of the region. This event brought together Europeans, indigenous Americans, and various African groups to create a blend of cultural and biological diversity. One approach to investigating this biological diversity is through the comparison of cranial inter-landmark distances.

In order to investigate the biological diversity found within the Caribbean and Latin America and elucidate the specific African origins, several samples of African origin, contemporary Mexicans (n=21), nineteenth-century Cubans (n=23), contemporary Panamanians (n=12), contemporary Afro-Antillean Panamanians (n=6), and contemporary Ecuadorians (n=54) were compared using traditional craniometrics. The African data include the Teita from Southeast Kenya (n=83), the Dogon tribe from Mali West Africa (n=99), the Zulu from South Africa (n=101), the Bushman from South Africa (n=90), individuals from Angola (n=68), individuals from São Tomé (n=5). All African data (excluding Angola and São Tomé) were collected by W.W. Howells and can be easily accessed online at http://konig.la.utk.edu/howells.htm. Inter-landmark distances (ILDs) from the Howells data were collected using the traditional 2D caliper-derived methods. On nearly all of the remaining crania, 3D data was collected using a Microscribe digitizer in which the traditional ILDs were simultaneously recorded. To evaluate group similarities and differences, Mahalanobis  $D^2$  were calculated using SAS 9.13 (2001). Mahalanobis  $D^2$  is a function of the group means as well as pooled variances and covariances that measures the degree of differentiation observed between the considered populations. Results show that all African groups are significantly different from one another at the <0.05 level (nearly all with *p*-values <0.0001). Interestingly, Afro-Antillean Panamanians are not significantly different from Angolans (p $value=0.1793, D^2=3.27$ ) or the S. Tome sample (*p*-value=0.4904,  $D^{2}=4.69$ ). However, this may be the result of a small sample size and evokes further investigation as S. Tome and Angola were controlled for long periods of time during the slave trade by the Portuguese. While Mexico was significantly different from all African samples, Mexico was not significantly different from the Afro-Antillean Panamanians (pvalue=0.1950, D<sup>2</sup>=2.15) and contemporary Panamanians (pvalue=0.0818, D<sup>2</sup>=1.68), possibly suggesting a similar indigenous and African origin. While exploratory, these results indicate that not only are the various African populations significantly different from one another, this diversity has also contributed to the diversity evident in the Caribbean and Latin America.

Ancestry, Craniometrics, Mahalanobis Distance

#### H74 Regional Variation of the Proximal Femur in the United States: Analysis of Data From NHANES III

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After attending this presentation, attendees will understand the impact that regional variation has on the morphology of the proximal femur and the expression of sex and group differences in the United States.

This presentation will impact the forensic science community by providing an alternative means of studying the proximal femur through the use of data from living populations to identify factors relating to regional, group, and sex variation.

The femur is one of the most studied bones of the appendicular skeleton. A large body of published works has accumulated over the decades with information concerning major aspects of the biological profile. Anthropologists have focused on the determination of age, sex, ancestry, stature, and secular change by analyzing traditional measures from the femur. The present study contributes to this literature by testing the hypothesis that regional variation in the United States has a strong influence over proximal femur morphology, which may affect identification and clinical practice. To test this hypothesis, the present study utilizes hip geometry data (Beck, 2002) from the National Health and Nutrition Examination Survey (NHANES) III database made available to researchers by the Center for Disease Control and Prevention. All the data used in the present study were collected by using a hip structural analysis program and a bone mineral density and structural geometry methodology. The selected sample for this study consists of 13,006 individuals (6,415 males and 6,591 females). All data were organized into four major regions: Northeast, Midwest, South, and West and represent non-Hispanic whites, non-Hispanic blacks, and Mexican Americans.

Nine measurements, in centimeters, collected by a hip structural analysis program provided the basis for conducting the present study. The measurements are: hologic femur neck width; femur neck shaft angle; femur neck length; narrow neck width; narrow neck endocortical diameter; intertrochanteric width; intertrochanteric endocortical diameter; femoral shaft width; and femoral shaft endocortical diameter. To test the proposed hypothesis, a MANOVA first tested the main effects of interaction for region, group affiliation, and sex. A canonical discriminant function analysis was then performed on the entire sample for sex, on males for group and regional variation, and on females for group and regional variation. Significance was observed at the .05 level in all of the analyses.

According to the MANOVA procedure, regional, group, and sex differences are statistically significant. Moreover, the MANOVA procedure shows statistical significance in the interaction between region and group, but no statistical significance in the interaction between region and sex. The discriminant function analyses support the results of the MANOVA procedure. The discriminant function analysis for sex suggests that sex can be identified with 88% accuracy when all groups and all regions are pooled together. The most meaningful variables for sex identification are intertrochanteric width, narrow neck width, and intertrochanteric endocortical diameter. Group affiliation affects the pattern of sexual dimorphism, but region has no effect.

The male discriminant function analysis suggests that group affiliation can be identified with 56% accuracy when region is a factor. In CAN1, narrow neck endocortical diameter and intertrochanteric endocortical diameter account for group differences in the sample. In CAN2, femur neck length and femoral shaft width account for regional differences. Similarly to the male analysis, the female discriminant function analysis suggests that group affiliation can be identified with 56% accuracy when region is a factor. In CAN1, intertrochanteric

endocortical diameter and narrow neck endocortical diameter account for group differences in the sample. In CAN2, narrow neck width and femoral shaft width account for regional differences.

The results of the present study are consistent with previous works by demonstrating that regional variation has a strong effect in the morphology of the proximal femur. While the overall pattern in sexual dimorphism is not affected by region, the pattern of group affiliation is, which in turn, influences sex variation. In both males and females, the sample breaks down according to group affiliation. However, the pattern of group affiliation is determined by regional membership. This study demonstrates the importance of using data from living populations to create biological profiles of skeletal remains. The creation of biological profiles is not possible without an understanding of variation from living populations.

Proximal Femur, Sexual Dimorphism, Group Affiliation

#### H75 Morphometric Evaluation of Nasal Characteristics in 20th Century White and Black South Africans

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After attending this presentation, attendees will gain a variation of knowledge in mid-facial characteristics of black and white South Africans, and will understand the statistical framework used to describe similarities and/or differences within these groups.

This presentation will impact the forensic science community in contributing to knowledge of human variation within a modern South African population, in providing a more scientific evaluation of this variation, and in presenting a mathematical approach to the classification of population groups.

With more than 49 million people of various social identities, languages, and belief systems, South Africa is an ideal country in which to evaluate human variation and the statistical relationship between social identity and biological characteristics. With the world's highest rate of homicide and a large number of unidentified persons, a need exists for accurate and reliable methods to assess ancestry from skeletal remains of sub-Saharan Africans. Since patterns of variation within and between populations are shaped by culture, language, geography and secular change, it is necessary to define the effect these parameters on the reliability and accuracy of our methods for estimating ancestry as well as sex, stature, and age at death.

With a large database of population groups, FORDISC 3 has addressed problems regarding osteometric differences among populations. However, the accuracy of non-metric features, such as inter-orbital breadth and nasal aperture width, in describing variation among black and white groups outside of North America has not been adequately described. In North American populations, mid-face and nasal morphology has been shown to be the most accurate region of the cranium from which to sort population groups.

The purpose of this study was to assess variation in mid-facial shape, namely nasal bone structure, interorbital breadth and nasal shape, among black and white South Africans using Elliptical Fourier Analysis, Discriminant Function Analysis (DFA) and Geometric Morphometrics (GM).

The mid-facial region of 151 crania of black and white South Africans (75 males; 76 females) from the Pretoria Bone and Raymond A. Dart research collections were photographed in the Frankfort plane, at a distance of 46 cm, using an Olympus 305 digital camera. Standard landmarks, which include subspinale, inferior point of nasal borders,

alare, nasale inferius, dacryon, nasal superius, nasion and glabella, along with three nasal arcs were digitized using a MicroScribe G2. Inter- and intra-observer error was evaluated.

Geometric Morphometric (GM) analyses including Procrustes fit and Elliptical Fourier analysis (EFA) were used to obtain shape variables. These variables as well as linear measures were imported into FORDISC 3.1 for linear discriminant function analysis (DFA). Statistical significance was assessed within and between ancestral groups. Each group was tested for normality and each was proven to be normally distributed. Outliers were identified through box plots. Student's *t*-test between whites and blacks were performed for each measurement and each proved to be statistically significant. A two-way analysis demonstrated 95% correct cross-validated classification. The differences observed between these groups may be used as a tool for estimating ancestry among South Africans.

To approach the evaluation of ancestry from unknown skeletal remains, the relationship between social and biological race has to be examined, understood, and continually evaluated on modern groups. Large databases are needed, and an understanding of the cultural history of the population is crucial for the interpretation of these differences.

Morphometrics, Nasal Aperture, South Africans

# H76 Can Femoral Shape be Used to Estimate Weight?

#### Gina M. Agostini, MA\*, 83 Newton Street, Greenfield, MA 01301

The goals of this presentation are to investigate the relationship between body mass index and femoral shape, and to determine the utility of using cross-sectional measurements in body mass index estimations.

This presentation will impact the forensic science community by providing evidence that being overweight or obese significantly impacts external femoral shape in a specific pattern. Despite poor classification results, these significant shape differences necessitate further investigation into the use of long bone shape to estimate weight, a visible trait that could be integrated into the biological profile and used to aid in forensic identification efforts.

It has been known for several decades that long bone shape is affected by body mass; however, there has been limited investigation into the impact that obesity has on load-bearing bones despite its high prevalence in modern populations. Given that obesity is a condition that clearly affects how an individual appeared to others in life, this research can benefit the forensic community by investigating whether bone geometry is sufficient to estimate weight, potentially adding another trait for use in biological profile determinations.

Previous research in this concentration demonstrated a significant positive relationship between body weight and mediolateral (ML) dimensions of the proximal femur. Using a larger sample with increased representation of obese individuals, this project sought: (1) to further investigate the relationship between body mass index (BMI) and femoral shape; and, (2) to determine the utility of using cross-sectional measurements to estimate BMI classification. This project was designed under the null hypothesis that individuals from all BMI classes would have the same mean anteroposterior (AP) and ML dimensions. Using standards largely devised by Ruff (1983), external AP and ML measurements were taken at 20%, 35%, 50%, 65% and 80% bone shaft length, with 20% and 80% indicating the distal-most and proximal-most measurement, respectively.

Four categories were formed based on BMI: underweight (BMI < 17.5), normal weight (BMI = 19 - 24.5), overweight (BMI = 26 - 30) and obese (BMI > 31.5). Age was controlled for in all statistical tests. Control for ancestry, sex and secular trends was effectuated through sampling, as only males of European ancestry with a date of death within the last century were included for this research. The final sample

consisted of 268 total individuals, 37 obese, 88 overweight, 86 normal weight and 57 underweight.

After controlling for age, multivariate statistics show a significant (*p-value* < 0.01) relationship between midshaft and proximal ML dimensions and BMI. MANOVA results also report a significant Wilk's  $\lambda$  (*p-value* < 0.05) for BMI. T-tests with an LSD correction for uneven sample sizes confirm ML dimensions are significantly larger in the overweight and obese BMI classes (*p-value* < 0.05). Additionally, size and shape variables were computed according to Mosimann and colleagues (Mosimann 1979; Darroch and Mosimann 1985). ANOVA results show that BMI has a significant effect on overall ML size (*p-value* < 0.01). MANOVA results report a significant effect of BMI on shape-standardized variables at all five ML locations (*p-value* < 0.05) with a significant Wilk's  $\lambda$  (*p-value* < 0.05).

There was a significant effect of BMI on AP dimensions at all five diaphyseal locations (*p-value* < 0.05) using the raw data. However, a significant interaction between age and BMI was observed at all five AP locations (*p-value* < 0.01) when using the transformed size-standardized data, invalidating any further analysis of BMI effect alone. These results suggest that the femora of overweight individuals undergo abnormally high rates of ML stress irrespective of age, but that both age and BMI operate in conjunction to impact AP dimensions. It is also possible that pelvic movements in overweight/obese individuals create abnormally high ML torques of the femur, rendering any age effect irrelevant.

Finally, a discriminant function analysis with cross-validation was conducted to assess the classificatory power of using ML measurements to discern BMI status. Poor classification results were obtained, with 58% correct classification for underweight, 57% for normal weight, 50% for overweight and 36% for obese. Collapsing underweight and normal weight individuals into one category and overweight and obese individuals into another resulted in little difference, with 58% correct classification into the underweight/normal weight BMI category, and 45% into the overweight/obese BMI category.

Three important conclusions are drawn from this research: (1) there is a significant relationship between femoral shape and BMI, but this relationship differs for AP and ML dimensions; (2) this relationship has poor use in classifying individuals into their respective BMI categories; and, (3) these femoral shape changes correlate well with documented biomechanical modifications made by overweight/obese individuals during locomotion. Given that previous research has demonstrated the importance of internal cross-sectional geometry in bone strength properties, it is possible that use of external measurements alone is not sufficient to estimate BMI classification.

**Obesity, Bone Morphology, Weight Classification** 

# H77 Osteometric Analysis of the Vertebral Column

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After attending this presentation, attendees will have a greater understanding of the relationship of measurements within a vertebral column, as well as, the relationships and correlations of those measurements with other postcranial elements.

This presentation will impact the forensic science community by introducing the utilization of novel measurements for osteometric analysis.

The Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL) is analyzing a heavily commingled skeletal assemblage that was unilaterally turned-over to U.S. officials from North Korea. Prior to their turnover, the remains were manipulated in such a way as to appear as single individuals, but gross observations detected major discrepancies. Multiple rounds of mtDNA sampling were conducted in order to sort major elements. Now, in addition to the mtDNA results, analysts use a variety of tools including osteometric sorting, pair matching, articulations, taphonomic and radiographic comparisons to sort the remains. These methods are very useful for sorting the major elements, but are not used as often with the association of vertebrae. In this context, a vertebral column can be associated with a set of remains if a single vertebra in a column segment is cut for mtDNA and yields a valid sequence, or if the column is continuous and has a terminal end that articulates with a major element. However, isolated vertebra and fragmentary columns are much more difficult to associate to individuals or combine.

The purpose of this research is to determine how well a metric analysis classifies a vertebra within a column and how well vertebrae correlate with one another and other postcranial elements within an individual in order to determine the associative value of these measurements and analyses. The sample consists of 19 intact vertebral columns of unknown race, sex and age, sixteen of which have associated postcranial remains. Twelve to eighteen discrete measurements were taken per vertebra throughout the entire vertebral column. Canonical discriminant function analyses were conducted to evaluate the classification and discriminating powers of the combined measurements of vertebrae within a column. In the primary analysis, all of the compatible measurements (14 distinct variables) for C3 through L5 were utilized. Wilks' lambda was significant in the testing of the first seven iterations of discriminant functions ( $\lambda = 0.000$ , df = 308, p < 0.001, based on functions 1 through 14 and  $\lambda = 0.518$ , df = 128, p < 0.001, based on functions 7 through 14). The first fourteen canonical discriminant functions were used in the analysis and the first four of those functions account for 95% of the variation, with the first accounting for 64%. The variables that are the most correlated with this discriminant function and therefore the most discriminating are related to the vertebral centrum. The classification results for predicted group membership revealed 76.1% of the original grouped cases were classified correctly, and 58.0% of the cross-validated grouped cases were correctly classified. The discrepancy is due in large part to the small sample size. The overall results of the canonical discriminant function analyses reveal that it is best to run the analyses using the optimum number of variables that are the most significant and highly correlated. The level of inter observer error was evaluated for the vertebral measurements by comparing the differences in the measurements of a single column between analysts. Overall the error was minimal (<1 mm), but the error in the pedicles was less than 2 mm.

The vertebral measurements correlate with other postcranial measurements to varying degrees. Several significant correlations (as determined in a bivariate correlation matrix using Pearson's correlations with a p value < 0.001) between appendicular elements and vertebrae were assessed using linear regressions. The purpose of this analysis was to test the null hypothesis that isolated vertebrae are similar enough in size to other isolated elements to have derived from the same individual. This method was tested on a fragmentary vertebral column, and was found to have good potential for sorting vertebrae within a commingled assemblage. The development of this method for correlating vertebrae could serve two purposes when sorting comingled remains: it provides supplemental data for associating unarticulated vertebrae and has potential to provide a size range for each measurement that can be searched within a database developed for the assemblage.

**Osteometric Sorting, Postcranial Measurements, Vertebrae** 

#### H78 Variation in Browridge and Chin Morphologies: Sexual Dimorphism and Covariation With Body Size

#### Heather M. Garvin, MS\*, Johns Hopkins University, 1830 East Monument Street, Room 302, Baltimore, MD 21205

After attending this presentation, attendees will be presented with a new method to quantify sexual dimorphism in browridge and chin morphology using 3D surface scans and geometric morphometrics. Upon attending the presentation, attendees will have a better understanding of brow and chin shape differences exhibited in males and females of American black and white ancestries. Knowledge will also be gained regarding the relationship between these features and postcranial body size.

This presentation will impact the forensic science community by discussing how in traditional sex determination methods, the browridge and chin are scored using ordinal categories presented in a universal set of line drawings, as well as, objectively quantifying these shape changes, thus providing an opportunity to formulate population-specific standards and overall gain a better knowledge of existing morphological variation.

Sexual dimorphism exists among modern humans in body size and cranial features, but varies in degree of expression across populations. Robusticity of craniofacial traits has been shown to be related to ancestry and geographic origins, but variations between groups in degree of sexual dimorphism of these traits have not been well documented. Besides degree of expression, studies have also shown that populations differ in the pattern of craniofacial traits. For example, one population may display robust brows and gracile chins, while another displays the reverse pattern. Therefore, documenting the variation of within and between population differences could be important for sex determination methods.

While linear cranial measurements have been proven to be correlated with postcranial size, evidence for a relationship between body size and discrete craniofacial traits is equivocal. This suggests that craniofacial traits may be influenced by different factors or display a different degree of plasticity than postcranial size. Understanding the relationship between body size and craniofacial traits, dimorphism in these variables, and the factors affecting each, will provide knowledge regarding population differences and observed secular and evolutionary trends.

This project examines variation in human skeletal sexual dimorphism using metric and morphometric approaches. The sample consisted of 19<sup>th</sup>-20<sup>th</sup> century blacks and whites from the Terry collection. A method was developed to isolate and quantify sexually dimorphic craniofacial traits (browridge and chin morphology) using 3D laser surface scans and geometric morphometrics. Once quantified, co-expression between the traits was evaluated and the relationship between the craniofacial traits and postcranial size was analyzed for allometric effects. The use of semi-landmarks across the brow and chin regions and principal component analyses, allowed visualization of shape changes between sexes and ancestries.

Preliminary results suggest that the browridge can be sexually differentiated by size, volume, and degree of projection relative to size. Morphometric analyses also suggest shape changes across the brow differ between the sexes. The chin, however, displayed a much higher degree of morphological variation and asymmetry. The majority of the variation between the sexes was confined to the lateral tubercles, which are responsible for the traditional "squared" or "pointed" chin shapes. When individual principal components were compared to body size, it was found that some of the most sexually diagnostic components displayed no significant relationship with postcranial or cranial size. However, Spearman rank correlations between cranial trait and body size discriminant function scores suggest there is a relationship between overall "maleness" and "femaleness" between the variables. Terry whites displayed a significantly lower degree of postcranial dimorphism than the blacks. Overall dimorphism in cranial robusticity was not significantly different between the groups, although trends suggest more masculine brows in the Terry white males thereby slightly increasing dimorphism. This could be evidence for separate factors affecting postcranial and craniofacial trait dimorphism, or different responses in the skeletal regions to the influences.

Browridge, Chin, Sex

# H79 Taphonomic Changes Observed on Skeletal Remains in Southeast Texas

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After attending this presentation, attendees will better understand taphonomic changes, in particular how sun-bleaching of skeletal remains can be attributed to the clothes worn by the decedent during decomposition.

This presentation will impact the forensic science community by potentially providing a link between the last known attire worn by a missing person to sun-bleached patterns on skeletal remains.

Few studies focus directly on the taphonomic process of subleaching and the resulting patterns produced on human bone (Calce and Rogers 2007, Janjua 2008, Quatrehomme and Iscan 1997). The goal of this study is to expand upon the current taphonomic literature as well as enhance the interpretation of sun-bleaching patterns observed on skeletal remains. During the course of a year in Southeast Texas, it is expected that an individual exposed to the environment would undergo taphonomic alterations during decomposition.

Taphonomy is the study of processes affecting an organism from the time of death to the time of discovery (Calce and Rogers 2007, Haglund and Sorg 1997). Forensic anthropologists, as well as other death investigators, rely on taphonomic studies in order to properly interpret alterations sustained by skeletal remains. The extent of alteration to skeletal remains is dependent upon weathering processes, such as the amount of moisture, sun, and soil type to which the remains are exposed (Janjua 2008, Calce and Rogers 2007). Taphonomy also includes modifications by carnivore activity, fluvial transport, and trampling (Haglund and Sorg 1997, Janjua 2008), though these processes were not the focus of the present study.

This study revolved around an individual involved in a fatal vehicular accident. The remains of the individual were donated to the Southeast Texas Applied Forensic Science (STAFS) Facility where they were clothed in shorts, a short-sleeved button-up shirt, and flip-flops. The remains were placed within the maximum security outdoor research facility on July 14, 2009 and were removed on June 2, 2010. Throughout the eleven-month duration of the study, scavenger activity was inhibited by the placement of a hardware cloth cage over the remains. At the time of removal, the remains were skeletonized with mummified tissue remaining on the skull and both hands and legs. A visual examination of the remains revealed sun-bleaching on areas of the bone that were not protected by clothing, thereby creating a color pattern consistent with the decedent's postmortem attire. The sun-bleached bones include facial bone fragments not covered by desiccated tissue, clavicles, cervical and lumbar vertebral bodies, sternal rib ends, distal portions of both arms, portions of the pelvic girdle, and portions of the distal legs not covered by desiccated tissue. Algal growth was also observed on two left ribs, several vertebrae, the superior sacrum, distal portions of the left ulna, two carpals and eleven tarsals and metatarsals. It is important to note that algal growth occurred only on bones that were bleached by the sun.

Facial bones sustained blunt force trauma with comminuted fractures as a result of the vehicular accident in which the individual was involved. Several of these facial bone fragments, as well as the proximal and distal ends of the left ulna and the proximal end of the left radius, exhibited uniform sun-bleaching. It was determined in a recent study that perimortem-inflicted blunt force trauma to pig skulls (*Sus scrofa*) also revealed uniform sun-bleaching of fracture sites (Calce and Rogers 2007). Postmortem trauma; however, can result in inconsistent sunbleaching or coloration of the fractured bone (Quatrehomme and Iscan 1997). Acknowledging this phenomenon could be potentially beneficial to the forensic investigator by providing a means to differentiate between peri-mortem and postmortem trauma of skeletal remains.

The sun-bleaching patterns observed on the skeletal remains can possibly reflect clothing worn by the decedent which, in turn, can provide seasonal clues as to when the person may have died. This information can be related to clothing details provided in a missing persons report and can thereby link skeletonized remains to an individual's identity.

Taphonomy, Sun-Bleaching, Blunt Force Trauma

#### H80 Using the Freeze-Thaw Cycle to Determine the Postmortem Interval: An Assessment of Pig Decomposition in West Central Montana

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The goal of this presentation is to educate attendees about the variables of human decomposition in west central Montana during the freeze-thaw cycle of winter and spring. Previous decomposition studies have found that temperature is the most influential factor on the rate of decomposition; however systematic research in cold, dry climates has yet to be conducted. West Central Montana represents a climatic region characterized by cold winters and hot, arid summers. This presentation provides information from the second half of a two-part systematic study conducted on two *Sus scrofa* in west central Montana.

This presentation will impact the forensic science community extending the knowledge and comprehension of decomposition patterns in an array of geographic climates. Establishing the postmortem interval, or PMI, is one of the most important factors used when attempting to identify human remains for the medicolegal field as it can establish the time period and context surrounding a death. The study of human decomposition is crucial to understanding the postmortem interval, and outdoor human decomposition has been most notably assessed at the Anthropology Research Facility at the University of Tennessee in Knoxville, an area characterized by warm temperatures and high humidity.

This study investigated the decomposition pattern of pig carcasses during the freeze-thaw cycle of winter, spring, and summer in west central Montana. Data collection focused on the particular physical changes associated with colder temperatures and the decomposition stasis that occurs during the late fall and winter months. The preliminary findings suggest the stasis that occurs during periods of subfreezing temperatures can result in an inaccurate assessment of decomposition and an imprecise estimation of the PMI. Analysis of the pattern of the decomposition cycle after the spring thaw was carried out to assess differential patterns of previously frozen remains.

This baseline project suggests that the rate at which a body becomes frozen during the freeze-thaw cycle of the Montana winter and spring alters the expected decomposition pattern. These observations can be used to differentiate between remains that experience the freeze cycle quickly as opposed to remains that experience a delayed freeze. This indicates whether or not remains had become exposed to taphonomic forces before or after the onset of subfreezing temperatures in this region. To establish an accurate postmortem interval in west central Montana the impact of the freeze-thaw cycle and the unique decomposition patterns associated with delayed and rapid freezing of remains must be understood.

Decomposition, Freeze-Thaw Cycle, Postmortem Interval

## H81 Animal Scavenging and Taphonomic Interpretation: An Evaluation of the Role of Scavenger Behavior and Environmental Context in Outdoor Forensic Scenes

#### Lisa N. Bright, BS\*, 1259 Hobart, Chico, CA 95926

The goals of this presentation are to provide an assessment of taphonomic predictions in relation to animal scavenging behavior, an evaluation of the role of animal behavior and environmental factors on human remains; and a discussion of actualistic experimental data that demonstrate patterns of damage and dispersal of skeletal elements from outdoor contexts.

This presentation will impact the forensic science community by providing a critical evaluation of taphonomic theory and discussing the benefits of a multidisciplinary approach to taphonomic analysis.

The goals of this research include: (1) an assessment of taphonomic predictions in relation to animal scavenging behavior; (2) an evaluation of the role of animal behavior and environmental factors on human remains; and, (3) a discussion of actualistic experimental data that demonstrate patterns of damage and dispersal of skeletal elements from outdoor contexts.

Taphonomy has become an integral area of research within forensic anthropology since the late 1980s. Previous studies have generally focused on field or laboratory-based experiments, retrospective case reviews, or case reports. However, little attention has been devoted to evaluating aspects of taphonomic theory, such as scavenging behavior, using rigorous experimental methodology. Sorg and Haglund (2002)<sup>1</sup> advocate the use of a bioenvironmental and idioecological approach to forensic taphonomic analysis. By focusing on the context specific features of an environment and the scavengers inhabiting that area, a more accurate interpretation of outdoor forensic scenes is possible. The incorporation of models from other disciplines, such as ecology and paleontology, provides a more detailed perspective.

This research was conducted at the Big Chico Creek Ecological Reserve (BCCER). The BCCER, owned and maintained by California State University, Chico, currently encompasses 3,950 acres of diverse habitats, which support more than 140 animal species. Common carnivores include the black bear, western spotted skunk, gray fox, coyote, raccoon, as well as the domestic dog and cat. Although the black bear and raccoon are omnivorous, they are known to actively scavenge animal carcasses, and are treated as carnivores for the purpose of this study. Less common scavengers include the bobcat, mountain lion, marten, fisher, and badger. Mountain lions excepted, these carnivores are thought to minimally contribute to the scavenging of human remains in northern California.

A pilot study was conducted in October of 2009 to document animal scavenging behavior at the BCCER. The carcass of a single adult mule deer (*Odocoileus heminous*) was placed at a site where animal scavengers are known to frequent. A motion sensitive digital infra-red game camera was positioned within the site to identify carnivore species and to monitor scavenger behavior. The site included a 15 meter clearing protected by vegetation, which provided a suitable environment for scavengers to feed without being disturbed. This location is also a known popular habitation spot for bears inhabiting the BCCER, with easily visible game trails and access to water. To prevent the immediate

removal of the carcass from the camera position, it was tied down to rebar stakes with lengths of barbed wire wrapped around the forelimbs and hind limbs. The site was monitored for three days, and data such as time, temperature, and precipitation were recorded as well as notes on decomposition, surface scatter, and scavenging damage. The carcass was collected at the end of the three days, and was completely devoid of soft tissue. Many of the elements were scattered within the site, and a few elements, including the right and left scapula, left forelimb, eight ribs, thoracic vertebrae No. 12, and a small portion of the skull could not be located. The remains were transported to the CSU-Chico human identification laboratory for analysis of scavenging patterns. .

The game camera recorded two different species: black bear (*Ursus americanus*) and the gray fox (*Urocyon cinereoargenteus*). The black bear was the only species documented actively scavenging the carcass. The carcass was not investigated until it had been exposed for seven hours, but once scavenging began it continued unabated for more than an hour. A majority of the activity occurred at night, and during the 24-48 hour exposure period. Bears ranging from a first year cub to the reserve's alpha male were documented feeding. The camera recorded significant damage, including tooth impact marks and breakage resulting from manipulation of the carcass.

The photographic, climatic, and osteological data in conjunction with biological, ecological, and anthropological theory, is being used to generate a model for scavenging behavior of human remains in Northern California. This model will be further tested using the remains of ten feral pigs (*Suc scrofa*) as models for human remains.

**Reference:** 

<sup>1.</sup> Marcella Sorg and William Haglund. Advancing Forensic Taphonomy: Purpose, Theory, and Process. In: Haglund W and Sorg M, editors. Advances in Forensic Taphonomy Method, Theory, and Archaeological Perspectives. Boca Raton: CRC Press, 2002;3-30.

Taphonomy, Scavenging, Animal Behavior

#### H82 A Longitudinal Study on the Outdoor Human Decomposition Sequence in Central Texas

Joanna K. Suckling, BS\*, Texas State University, 601 University Drive, San Marcos, TX 78666

After attending this presentation, attendees will gain a better understanding of human decomposition in the climate of Central Texas as learned through a longitudinal experimental study.

This presentation will impact the forensic science community by presenting the observed stages of decomposition for a specific climate while providing insight regarding how geographic regions may influence human decomposition. Results from this study will allow forensic anthropology practitioners to better understand environmental factors present in Central Texas and similar geographic regions that complicate the estimation of the postmortem interval, or PMI, also known as the time since death.

Forensic anthropologists may be consulted in the estimation of the PMI. In this estimation forensic anthropologists employ decomposition stages. PMI is dependent upon local climate conditions, such as temperature, humidity, and scavengers (Megyesi et al. 2005; Reeves 2009). Although decomposition sequences have been proposed for specific geographic regions including Tennessee (Mann et al. 1990; Vass et al. 1992; Love and Marks 2003), New Mexico (Rhine and Dawson 1998), and Arizona (Galloway 1997), these sequences may not be applicable in other regions. The Forensic Anthropology Research Facility (FARF) at Texas State University-San Marcos is located in an area subject to various weather conditions characteristic of a sub-tropical

climate, in which the humid climate may be punctuated by periods of drought leading to semi-arid conditions (Dixson 2000).

A systematic longitudinal study on the decay of the human body was conducted with a sample of ten donated human cadavers (N = 10). The objective of the research was to monitor each donated cadaver upon arrival at the FARF by using the decomposition scoring method developed by Megyesi et al. (2005) based on Galloway's (1997) arid environment decomposition stages. For each day, decomposition was scored using the same scoring categories developed by Megyesi et al. (2005) to represent the overall condition of remains and determine if there is a sequential order to human decomposition. Decomposition stage was assessed for the torso, limbs, and head separately to account for different areas of the body decomposing at different rates (Megyesi et al. 2005). The decomposition stages used were divided into Fresh, Early, Advanced, Skeletonization, and Extreme Decomposition (Galloway 1997; Megyesi 2005). Donations were observed until skeletonization, defined as less than one half of the skeleton covered by desiccated or mummified tissue (Galloway 1997).

Previous decomposition studies (Galloway et al. 1989; Rhine and Dawson 1998; Megyesi et al. 2005) could not control for scavengers in a natural environment, but longitudinal data collection permitted a comparison between scavenged and non-scavenged human remains. Both caged donations and those exposed to scavenging were included in the sample. Time delayed photography on a wildlife camera was used to photo document specific scavengers and their effect on the rate of decomposition. Accounting for the behavior and effects of scavengers will provide anthropologists and future researchers' data on how to properly evaluate the postmortem interval when scavengers have access to a body (Reeves 2009).

The present study supports Galloway's (1997) assertion that beyond broad categories of early decomposition, advanced decomposition, skeletonization, and decomposition of skeletal material, secondary characteristics, such as coloration and mummified tissues, do not necessarily follow a sequential order of appearance. Decomposition in Central Texas also appears to coincide with incidences of high humidity as observed by Galloway et al. (1989), with a rapid onset of advanced decomposition, high rates of maggot activity when avian scavengers do not have access, accelerated autolysis, and rapid skeletonization or adipocere formation. The results of the study also demonstrate that decomposition in Central Texas can progress rapidly. In one case of an autopsied donation, without the variable of scavenging, the first sign of bone exposure was noted within four days after placement outdoors. With scavenging, bone exposure to the point of skeletonization can occur within 24 hours. The results of the study also assert that the variable of insect activity is not the only major factor that may disrupt or accelerate decomposition estimates. Temperature, humidity, and access to scavenging animals can all significantly distort time since death estimations in outdoor environments.

Forensic Anthropology, Decomposition, Postmortem Interval

# H83 Taphonomy Reader Beta-Version: A Software to Help in Taphonomic Syndromes Diagnosis

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After attending this presentation, attendees will value the benefits of using a free software that assists reading the alterations on human bones to reconstruct the taphonomic process.

This presentation will impact the forensic science community and students in this discipline by offering a Beta version of a free software

that presents the possibility of recognizing taphonomic imprints on bones, a combination of which can indicate one or more taphonomic syndromes and is useful to redevelop the postmortem life of skeletal remains.

As demonstrated by numerous articles and the AAFS Workshop in 2010, taphonomy has an important role in the study of skeletal human remains from historical and forensic settings. The natural and artificial alterations readable on osseous remains can make understandable the changes and the events that a body suffered in its death onwards; to individuate taphonomic imprints on bones and their classification in taphonomic syndromes, it is necessary in forensic anthropological investigation to estimate the postmortem interval and forensic pertinence, to contextualize the remains, and to recognize the natural and human agents that acted upon the remains.

Taphonomy is a very complex discipline and in its application on archaeological and forensic study, it is possible to recognize two different lines: depositional and contextual taphonomy. The depositional taphonomy analyzes the position of the remains and the preservation of articulations that helps to recognize the type of the burial (i.e. primary, secondary, a dressed or nude body, in an empty space). The contextual taphonomy analyzes the specific imprints left on the bones by the interaction between the remains and the environment such as soil, water, atmosphere, fire, and human activity as well. The software presented is called *Taphonomy Reader* and has been developed to help the anthropologist (students or professionals), the archaeologist, and the forensic investigatory in the contextual taphonomy analysis by creating the base for a common language in this discipline.

*Taphonomy Reader* has been written in HyperText Markup Language (HTML), the predominant markup language for web pages; this language allows the software to be used on every kind of computer and platform (Windows, Linux, Macintosh) using a simple browser. The HTML has embedded scripts using JavaScript, a different language that affects the behavior of HTML pages, which creates an elaborate "results page." JavaScript has been used to avoid developing a database that requires use online; in this way, users can download the software on their computer and use it either on- or off-line.

When run, Taphonomy Reader shows the "main window" with the most common taphonomic imprints: marrow or soft tissue presence; complete degreasing; fossilization and sub-fossilization; soil adherence; soil staining; mineral incrustation; warping; melted elements adherence; roots invasion; roots erosion; soil infiltration on fractures; mineral staining; unilateral soil adherence; unilateral soil staining; bleaching; algal growth; punctures; parallel striations (rodent gnawing marks); longitudinal splitting; weathering; blackening with smoke; ash accumulation, calcination; carbonization; crisscross cracking; shrinkage fractures or bull-eye fractures; sand adhesion; water staining; circumferential staining; surface erosion; rounded margins; marine taxa incrustation. If the user is uncertain about the meaning of each pattern, it's possible to click on the pattern's name to open a new page with a synthetic description and a picture of the typical appearance of each taphonomic modification. The pictures are from several Italian historical or forensic cases and from the sample collection of the MercyHurst College.

Examining a case, the user can tick off the patterns that are singledout on the analyzed remains and then push the verification button. The script recognizes the choices of the user and creates a new virtual "result page." The taphonomic changes present in the software can correspond to eight different taphonomic syndromes: forensic interest; possible forensic interest; historical remains; burial; surface exposure; animal activity; cremation or fire alteration; and, aquatic taphonomy.

The "result page" presents the selected taphonomic changes tabulated in a schedule where it can be visualized and each taphonomic syndrome imprint is presented. The syndromes are clickable to open a page with a short explanation and references. As some imprints are common to more than one syndrome, the user can choose the most appropriate syndrome or if two or more possible syndromes are present, they can be listed sequentially.

A preliminary version of the software will be presented that is able to analyze 32 different taphonomic imprints tracing them back to eight different taphonomic syndromes. The software is free and downloadable at the web site www.restiumani.it. As it is an open source, the software can and will be improved by the scientific community over time.

Taphonomy, Software, Forensic Anthropology

#### H84 Comparison of Fresh Tissue Autopsy and Skeletal Analysis Reports in Colombia

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After attending this presentation, attendees will be presented with a set of recent cases from Colombia that provide the rare opportunity to compare postmortem examinations of skeletonized remains with freshtissue autopsy reports of the same individuals at the time of death. The attendees will be able to compare and identify disagreements, additions, and omissions between types of analysis. This presentation will help the forensic scientists who are focused on human identification and determination of circumstances of death to anticipate differences in reports and adjust expectations and methods accordingly. It will also highlight the value of multidisciplinary teams in postmortem exams.

This presentation will impact the forensic science community by demonstrating the need for flexibility and cooperation between experts in the analysis of human remains. Problems concerning both human identification and circumstances of death are highlighted.

In the summer of 2008, eighteen cases of unidentified combat dead from 1995-2003 were disinterred from a cemetery in the Department of Antioquia, Colombia. All were analyzed by standard anthropological methods. The skeletal analyses were then compared with the records of forensic autopsies (necropsies), which had been conducted locally near the time of death. The autopsies provided a superficial description of the remains, including skin and hair color, tattoos, and scars. The autopsy reports then focused on superficial peri-mortem trauma (recent wounds), but did not include radiographs. The anthropological analyses included a basic description of sex, age, race, and stature. They then focused on a more detailed analyses of bone trauma, both antemortem and perimortem. (Postmortem trauma did not present a problem because the bodies had been maintained in crypts and carefully removed from the original coffins.)

Of the eighteen research cases, the autopsy reports of fifteen individuals contained enough material for serious comparison. Of the fifteen, there was complete agreement in sexual identity, but 31 percent disagreement regarding age at death. Part of this can be attributed to the differences in method of reporting. The autopsy reports tended to state a unique age and the anthropology reports stated an age range. Another problem is the lack of local population data. The stature estimates were surprisingly inconsistent and cannot be easily explained. The error might have been introduced by overly rapid estimations during autopsy, or the errors may be the result of the lack of local population data. It was almost impossible to compare the conclusions regarding race. The vocabulary used by the pathologists was based on skin color and local terminology. The vocabulary used by the anthropologists was based on cranial observations and measurements described in terms of global populations. The anthropological reports revealed 54 elements of additional information potentially critical to success in personal identification.

The most significant report differences were in the analysis of perimortem trauma. The anthropology reports revealed that many of the discrepancies and errors were in the original (autopsy) trauma analysis. Forty-nine injuries were not recorded in the autopsies; fourteen gunshot trajectories were reported to have occurred in the wrong direction; and two injuries were recorded in autopsy on the wrong side of the body (left/right error). There were also five differences of opinion about the specific weapon of injury, and several inconsistencies in the general interpretation of injuries.

Methods, materials, and often motives differ between fresh tissue autopsy and skeletal analysis. The result is a separate set of findings, sometimes congruent, but sometimes very different. As expected, autopsies provide more information about soft tissues, whereas osteoanalyses provide more information about both antemortem and peri-mortem trauma to osseous tissues. The combination of reports provides a more complete description of the circumstances of death, significantly improves accuracy, and increases the probability of personal identification. Cooperation between professionals as utilized for example, in DMORT post-disaster operations, is recommended. Osteoanalysis, Autopsy, Colombia

## H85 Conditions for Breaking Down Mummified Tissue and the Subsequent Implications for Time Since Death

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The goal of this presentation is to inform attendees of the methods and conditions favorable to soften mummified tissue for easy removal from bone.

This presentation will impact the forensic science community by demonstrating how the application of this technique will quicken and simplify the bone cleaning process for skeletal collections. Moreover, the findings from this project suggest that traditional time since death estimates may need to be adjusted based on the state of mummified remains.

As more decomposition facilities are launched across the nation, the need for establishing effective techniques for removing debris, tissue, and organic matter from skeletal remains increases. In order to run a successful facility, the director must maintain a delicate balance between incoming donations and those ready to be cleaned for curation. Mummified tissue has a tough texture, is challenging to remove, and slows the process of cleaning skeletons. Efforts to soften the tissue typically occur after the skeleton is removed from the outdoor environment and transferred to an indoor lab for cleaning. Attempts to remove tissue can include heating it in water and picking it off the bone. Unfortunately, these strategies still result in an increase in the time it takes to clean donations. Thus, the need for finding a more suitable means to remove this type of tissue is necessary to prevent a processing backlog. Based on observations of processing techniques, the most effective variables for softening mummified tissue are heat and moisture. This project involved testing a variety of variables, including heat and moisture, prior to the removal of the donation from an outdoor environment. As a result of the effectiveness of heat and moisture application in indoor lab procedures, the author expected these variables to play a similar role in the outdoor environment.

Over the course of one year, donations were observed at the Anthropological Research Facility (ARF) at University of Tennessee, Knoxville. Each donation was prescreened to ensure that at least one anatomical region (e.g., a hand, etc.) was mummified. After the donations were collected, they were placed into two bags: the inner bag was clear plastic (allowing for observations of changes in the tissue); and, the outer bag was a tan biohazard bag (to designate the nature of the contents). Observations of moisture, light, insects, and amount of soil were made at the time of placement into the bag and approximately every week until the remains were deemed suitable for processing or were skeletal. Donations were placed in several locations at the ARF to provide differing amounts of exposure to the variables. Categorical statistics were applied to the data to isolate the most effective variables for breaking down the toughness of the tissue in the shortest amount of time.

The results of this project were consistent with the findings during processing; both light and moisture (in conjunction with one another) were significant variables in speeding up the decomposition process. Surprisingly, partial sun was the most effective amount of light, while both small and medium amounts of moisture were successful. This discovery is consistent with the hypothesis that both heat (as generated by the amount of light) and moisture are crucial elements for the decay of mummified tissue. Donations that are placed in bags in a location with partial sun and light to moderate moisture will break down the tough tissue in intervals between two weeks and two months, based on seasonality.

The purpose of this methodology is to ease the burden of the processing crew. Instead of spending weeks working on removing tissue from one mummified skeleton, the processing crew can focus on mostly skeletal remains that only require a few hours to clean. The mummified donations can be placed into bags with the optimal conditions listed above and left while the natural moisture and light break down the tissue. The implications for these findings extend beyond mere processing techniques; they are also significant for time since death estimations. Assessing individuals with tissue that appears mummified (discoloration, patches of toughness, etc.), except for a soft texture, should take into account the possibility of a longer postmortem interval than those remains that are mummified with a tough texture. This project demonstrates that softening of mummified tissue can occur after a body has mummified and been exposed to key environmental elements.

Mummification, Time Since Death, Decomposition

# H86 Comparing Human and Porcine Infant Parietal Histomorphology to Facilitate Research on Pediatric Cranial Trauma

Lindsey L. Jenny, MA, Paige V. Wojcik, BS\*, and Todd W. Fenton, PhD, Michigan State University, Department of Anthropology, 354 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will understand similarities and differences in histomorphology between human infant and porcine infant parietal bones as well as histomorphology's relationship to trauma research.

This presentation will impact the forensic science community by indicating that infant porcine parietal bones are an appropriate analog to human infant parietal bones up to 24 months of age when conducting pediatric cranial trauma research. Because it is difficult to obtain human pediatric skeletal material for trauma and biomechanics research, it is important to understand the histological relationships between the human infant skull and the porcine infant skull, which is often used as an analog in these types of studies.

This study established the baseline histomorphological properties of the human infant parietal bones and the porcine infant parietal bone, which did not previously exist. Histomorphology, the study of microscopic features of bone, is useful in determining the growth and developmental patterns of the porcine infant and human infant skulls. This information is essential to understand the manifestations of cranial injury on a histomorphological as well as biomechanical level.

Previous research at Michigan State University suggests a correlation in cranial bone development between 1-day-old porcine skulls and 1-month-old human skulls based on biomechanical properties. This research tests the hypothesized correlation by comparing porcine bone histology to human bone histology. By comparing the appearance

and thickness of diploe, endocranial thickness, ectocranial thickness, and secondary osteon size, number, and arrangement, it was determined if the hypothesized correlation in cranial bone development is valid in a histomorphological context.

Human infant and porcine infant parietal bones were thin sectioned using a saw and examined using light microscopy at 100x. Images were captured using image analysis software. Image analysis software was also used to measure endocranial and ectocranial thickness and osteon size and density.

The main goal of this research was to determine whether the porcine infant skull is a reasonable analog for the human infant skull in conducting research on pediatric cranial trauma. It is necessary to use the porcine skull as an animal model of the human skull due to a lack of pediatric cadaveric material available for research and experimentation.

Paired t-tests found no significant differences between the human and porcine skulls in the ratios between the ectocranial, endocranial, and diploe thickness to total parietal thickness. Results indicate that there was a correlation in the appearance of diploe between nine-day-old pigs and twelve month old humans. However, parietal development permanently diverged at two years in humans and twenty-four days in pigs. There was no correlation in osteon density as infant pigs had more osteons than human infants. Differences in osteon size and density have been reported between species in long bones (Hillier and Bell, 2007) but the skull requires further exploration.

In conclusion, this study indicates that infant porcine parietal bones are an appropriate analog to human infant parietal bones up to 24 months of age when conducting pediatric cranial trauma research. Because it is difficult to obtain human pediatric skeletal material for trauma and biomechanics research, it is important to understand the histological relationships between the human infant skull and the porcine infant skull, which is often used as an analog in these types of studies.

Pediatric, Trauma, Histology

## H87 Identification vs. Cause of Death in Mass Graves Where Individuals are Commingled in Colombia

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The goal of this presentation is to demonstrate the importance of determining cause of death of commingled individuals from mass graves from the Colombian armed conflict.

This presentation will impact the forensic science community by discussing the approach used to individuate remains that will impact the forensic community in those countries with mass graves issues and low resources.

In Colombia, graves were commingled and/or incomplete individuals were buried to increase the complexity of forensic analyses. Of the reasons that can explain the existence of these type of graves is the practice used by paramilitary groups where victims are dismembered alive or as a method to dispose and hide the remains. Consequently, several individuals are sometimes buried in one or more graves in a disorganized and random manner. Other reasons are the cultural traditions of the community and circumstances of the victim's family, who bury their loved ones in one grave on their own property or in local cemeteries, even when they have died in different events or circumstances, including natural death. Sometimes the community is forced to carry out mass burials in a short period of time when threatened by armed groups that may strike again. On other occasions, forcibly displaced families exhume their dead relatives in order to rebury them in the area where they relocate. Lastly, particularly in rural cemeteries, bodies are often exhumed and disposed of with other remains, in order to

\* Presenting Author

open space for the recently deceased. No record is kept on the conditions in which these individuals are buried or found.

These graves are characterized by the presence of commingled and incomplete bodies at different stages of preservation and with various types of injuries. Frequently, the climate of the tropical forest where the conflict takes place deteriorates the bony structures and hinders the determination of the biological profile and prevents articulation of body segments.

During lab analyses, remains are individualized based on the morphological and metric characteristics in order to determine the minimum number of individuals. The most likely number of individuals is not used because graves usually have three to five bodies. In cases where individualization/sorting is impossible, the remains of each individual are classified as INDIVIDUAL X; groups of anatomically associated structures where it is impossible to determine to which individual they belong are classified as GROUP X; and disarticulated structures that may correspond to any individual are classified as MISCELLANEOUS. Both INDIVIDUAL and GROUP skeletal samples are kept for purposes of genetic analysis. However, due to the high cost and volume of cases, DNA labs give higher priority to INDIVIDUAL samples than to GROUP samples. Consequently, INDIVIDUALS samples are identified, but in most cases the cause of death may not be established, particularly if the injuries that caused death remain unassociated.

The medical-anthropological teams are frequently faced with these two problems: sorting and determination of the individual's cause of death. Returning complete skeletons to family members is very often not possible and cannot rely on genetics because of sample processing limitations. Additionally, the cause of death of these individuals is frequently undetermined. The proposal is to take bone samples from the skull and injured structures for identification purposes. This would require the enhancement of DNA extraction techniques from small structures, and a coordinated effort with DNA experts. Processing samples in this way may contribute to victim identification and explain the circumstances of death. Additionally, it would meet the requirements of the administration of justice and each family's need to know the truth about what happened to their loved ones.

Commingled, Cause of Death, Armed Conflict in Colombia

#### H88 Positive Identification Through Comparative Panoramic Radiography of the Maxillary Sinuses: A Validation Study

Angela Soler, MA\*, Michigan State University, Department of Anthropology, 354 Baker Hall, East Lansing, MI 48824

After attending this presentation, attendees will learn the results of a validation study among forensic anthropologists and forensic odontologists conducting positive identifications by comparing antemortem and postmortem panoramic radiographs of the maxillary sinuses.

This presentation will impact the forensic science community by providing accuracy rates for the use of panoramic radiography and the maxillary sinuses to establish positive identification of unknown decedents. In addition, this presentation will discuss the effects of educational background and experience in comparative radiography when making a positive identification using panoramic radiographs and the maxillary sinuses.

According to *Daubert v. Merrell Dow Pharmaceuticals* (1993) all court admissible forensic techniques must comply with four standards: they have been or have the potential to be empirically tested; have known error rates; have been subjected to peer review; and have been generally accepted in the scientific literature. Furthermore, with the 2009 recommendations of the National Academy of Sciences and the 2010

Draft Outline of Forensic Reform Legislation there is an increasing push for the standardization and validation of forensic methods. As a result many studies have been conducted to validate positive identification in forensic anthropology and odontology. Validation studies on the use of comparative radiography have included a range of anatomical regions including: the lumbar spine (Wankmiller 2010); chest (Keuhn et. al. 2002); hand (Koot et. al. 2005); hyoid (Cornelison 2002); frontal sinuses (Christensen 2005); and the dentition (MacLean et. al. 1994, Soomer et. al. 2003). In recent years panoramic radiography has become a standard of care in many dental offices throughout the United States and now a larger percentage of positive identifications are being made through the comparison of panoramic antemortem radiographs. In addition, new technology is increasing the efficiency and use of postmortem panoramic radiography in the medical examiner setting (Du Chesne et. al. 2000, Mincer et. al. 2008). Although the dentition in panoramic radiographs has been validated as a viable positive identification method (Lee et. al. 2004), the use of the maxillary sinuses and other osteological features has never been investigated.

This study evaluates panoramic radiography and the maxillary sinuses in positive identification by comparing antemortem and postmortem radiographs. Twenty fully skeletonized skulls from Michigan State University were selected for this project. Simulated "antemortem" panoramic radiographs were obtained using a Panorex radiography machine. The skulls were placed on a table and propped up with foam blocks. All crania were positioned in the Frankfurt horizontal plane with the central incisors resting on a notched bite stick. The frontal was placed onto a forehead block and the skull was steadied using the device's parietal head supports. Horizontal linear light beams on the lower border of the nasal aperture and vertical linear light beams on the left canine were used to maintain consistent positioning of the skull. Five crania were then randomly selected to simulate the "postmortem" matching films. Each of the five skulls were repositioned in the machine using the above method. All radiographs were digitized and then cropped to exclude viewing of the teeth. A web-based study was designed to invite forensic anthropologists and odontologists to match five "postmortem" radiographs from one of the twenty "antemortem" films. Data regarding the most helpful features, like the borders of the maxillary sinuses, nasal aperture, and inferior orbits was collected. Additional data regarding the participant's field of expertise, level of education, years practicing in forensics, and experience with positive identification was also analyzed.

Although the study is ongoing, so far a total of thirty-five forensic anthropologists, odontologists, and graduate students have participated in this study. The overall accuracy rate for correctly matching all five of the postmortem films was 65.7%; however, when the most challenging radiograph was withdrawn, the accuracy rate reached 91.4%. Only 57.1% of participants accurately identified postmortem film C, and 14.3% chose not to answer based on the poor quality of the antemortem film. Postmortem radiograph C was the most difficult to match due to a slight difference in the radiographic angulation and distance from the xray; however it highlights an important point. The ability to accurately identify individuals based on panoramic radiography of skeletal features other than the teeth relies heavily on the clarity of the films and exact duplication of the antemortem position of the skull within the panoramic radiography machine. Contingency tables and chi-square test results suggest that observer education, years in the field of forensics, and experience in positive identification did not significantly affect the ability to accurately identify the correct match for any of the postmortem radiographs, including postmortem film C. In addition, there was no significant difference in the way that experienced forensic anthropologists or odontologists performed in the study. However, it does appear that the key to correctly matching antemortem and postmortem films in this research was the ability to determine whether the antemortem films were of sufficient quality to perform an identification, which often comes with experience in comparative radiography.

Positive Identification, Panoramic Radiography, Validation

#### H89 New Method of Identification Based on Computer-Assisted Radiograph Comparison

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After attending this presentation, attendees will have received a detailed description of the basis for the computer-assisted radiograph comparison method, explanation of the proposed practical application of the method in medical examiner/coroner offices, and a discussion of the pilot study results.

The presentation will impact the forensic science community by introducing a new and practical identification method that is responsive to the recent National Academy of Science Report recommendations and post-*Daubert* evidence admissibility standards.

A crucial need exists for a statistically validated, time-sensitive, and relatively inexpensive scientific identification method for routine use in the medical examiner/coroner setting. Medical examiner/coroner reliance on scientific identification extends far beyond the completely unknown medicolegal decedent. Tentatively identified decedents are often unrecognizable due to disfiguring facial trauma, charring, or advanced decomposition. In cases of multiple fatalities, scientific identification methods are used because there is increased potential for incorrect assignment of identity. In the aftermath of a mass fatality event, scientific identification of the casualties becomes paramount. This presentation describes a new quantitative approach to identification achieved through modification of existing clinically-tested computer technology. Attendees will receive a detailed description of the basis for the computer-assisted radiograph comparison method, the proposed practical application of the method in medical examiner/coroner offices, and a discussion of the pilot study results.

The foundation of the proposed identification method is "Quantitative Motion Analysis" (QMA<sup>®</sup>) software (Medical Metrics, Incorporated). The performance of the software has been validated in multiple clinically based peer-reviewed studies of spinal biomechanics and spinal treatments. QMA<sup>®</sup> allows for computer-assisted matching of specific skeletal elements, such as vertebral bodies, by tracking them through multiple radiographic images. The first step in transforming QMA<sup>®</sup> into a forensic identification tool has been to program the software to generate quantitative match scores for a statistically sufficient number of tracked skeletal elements per comparison.

The ongoing pilot study began with the development of a processing algorithm that provides QMA® with the ability to successfully calculate the required match scores. The fourth cervical vertebra (C4) was selected as the first test element. The goal of this initial work was simply to develop and test the algorithm, a timeconsuming process that required testing of a large number of rejected algorithms. Once a satisfactory algorithm was developed, it was tested on five unique sets of lateral cervical radiographs or fluoroscopic images of 10 individual subjects. The anonymized radiographs were assembled from archived spine research image sets. There were no implants or surgical alterations in any of the views. The sets of images were of subjects between the ages of 22 and 89 years, loosely grouped into "younger" and "older" subjects for comparison purposes. Each set of images contained two different images of the same index subject and an array of nine images from nine different subjects. During the QMA® tracking process, variations in magnification between images were adjusted so that the C4 was approximately the same size in all of the images. Following the tracking process, a region of interest was defined

around the C4, the images were rotated so that the endplates were approximately horizontal, and histogram equalization was applied to all of the images, using the combined histogram for the entire set of images.

This process standardized the orientation of the vertebrae and equalized brightness and contrast of the images in each set. A contrast enhancement filter was then applied that maximized contrast for horizontally aligned image features. This filter weights the contribution of the vertebral endplates more heavily than other features. An image match score was then calculated between the first image and each of the other images in the set.

For each set of 10 images, the maximum match score was returned for the two index images, representing a correct comparison match for the index subject in each set. The maximum score was scaled to 100 and all other match scores were normalized to that score. After normalization, all matches returned a score of 99-100 and non-match scores ranged from 77-98. The results of the pilot study show that QMA<sup>®</sup> can successfully be adapted to establish positive identification in the medical examiner/coroner system.

Radiograph Identification, Forensic Anthropology, QMA®

#### H90 Test of Osteon Circularity as a Method of Human/Non-Human Identification

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After attending this presentation, attendees can expect to learn how new osteon area and circularity tests can help with the identification of fragmentary remains and how to utilize the proposed method on fragmentary skeletal casework.

This presentation will impact the forensic science community in terms of knowledge, competence, and performance by describing to the attendees how osteon measurements can be used to distinguish between human and non-human fragmentary remains, educating the attendees on the proper method of application of the proposed method, and providing a standard operating procedure for the implementation of this method in forensic practice.

Due to increased need for the identification of highly fragmentary skeletal remains, more laboratories are turning to histological techniques for fast, accurate species identification tests. This research adds to the body of knowledge by focusing on the overall shape and areas of the osteons. Human bone specimens from known individuals were utilized to develop reference data. Each of the limb bones from five individuals was histologically sampled, yielding slides from 30 specimens. Digital images of a random location on each slide were captured at 100x magnification using a color digital camera.

Osteon area was measured with Image J program (NIH). The program requires the perimeter of each osteon be traced. The perimeter outline provides the basis for calculation of area and other measurements. The assessment of shape, called the circularity index (CI), was obtained using Image J's circularity measurement option. Area and circularity data were obtained for 1204 osteons from the 30 samples from five individuals to form the reference data set. These individuals were selected from the Bass Collection (UTK). The same procedures were used to obtain area and circularity from 1,442 osteons in 37 samples from 35 individuals from JPAC-CIL (calculating the mean values for area and circularity from a random sample of 30 osteons drawn from each sample image). This mean is compared to the mean and "standard error of the mean" calculated from the reference data. A bootstrap step was taken to generalize the reference data and make the statistical tests meaningful (i.e., to avoid the trap with large sample sizes whereby any difference is statistically significant). The 1,204 osteon

measurements for each variable had independent samples of size 30, randomly drawn 1,000 times. Each time a sample was selected, the mean was calculated. Specimen mean values were compared to the grand mean (e.g. mean of means) utilizing the standard deviation (SD) of the 1,000 means (bootstrap standard error of the mean) as the basis for a formal test of the null hypothesis that the case specimen is human (e.g. has osteons that are within the bounds of natural variation for area or circularity).

The grand mean value obtained for human osteons was 37,365 microns and the standard deviation of the bootstrap means was 2,728 microns. The distribution closely approximates normality. Consequently, it was decided to conduct a one-sided test of the null hypothesis (e.g., specimen is human) in the direction of smaller mean areas. The resulting cutoff values for osteon area were set as follows: mean < 31,909 for p<0.025; mean<29181 for p<0.005. The circularity values for humans deviate downward from perfectly circular (e.g., value of 1). The grand mean value was 0.94 and the SD of the bootstrap means was 0.0053. The distribution closely approximates normality. A two-sided test was used with the following referents: mean >0.96 or mean <0.92 for p<0.01; mean >0.95 or mean <0.93 for p<0.05.

Application of the two tests to the independent test sample (N=37) yielded no rejections of the null hypothesis for osteon area. For circularity, there were 17 (46%) rejections of the null at the p < 0.05 (4 below and 13 above the mean) level and 4 (11%) rejections at the p < 0.01level (3 below and 1 above the mean). Of the 13 specimens above the mean in the p<0.05 level test, all but one were approximately the same value as the referent (e.g. 0.95). While the osteon area test meets or exceeds expectations, the circularity test did not perform as well. Two potential explanations, neither mutually exclusive, are plausible. First, the protocol for avoiding measurement of osteons that have been sliced at an oblique angle was not consistently followed between derivation of the reference data and the test data. This scenario is unlikely to be a sufficient explanation, since the same individuals measured both specimen sets and were trained by the senior author. Second, the reference data incorporated measurements from 5 individuals while the test sample included measurements from 35 different individuals. Thus, the test sample demonstrates variation among individuals that must be captured in the reference data for the test to be effective. The optimal solution is to incorporate the test data into the reference data prior to publishing the final recommended test.

Physical Anthropology, Bone Histology, Osteon Area and Circularity

#### H91 The Evaluation of Bone Area as a Histomorphometric Variable for Estimating Age at Death

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The goal of this presentation is to determine if measuring bone area rather than the traditionally used cortical area has the potential to improve histological age estimation methods for the sixth rib.

This presentation will impact the forensic science community by exploring improvements in sixth rib histomorphometry. Measurement techniques used in histological age estimation have previously excluded trabecular bone area. This preliminary study is designed to investigate the relationship of bone area (cortical area + trabecular area) to individuals of known age.

Age-related bone loss has been extensively documented in the medical literature. The fragility of the human skeleton increases with age owing to changes in bone quality, bone remodeling properties, and microarchitecture. While age-related changes in cortical and trabecular bone microarchitecture have been reported in the anthropological literature, the evaluation of trabecular bone area has not been included in histological age-estimation methods.

With advancing age cortical bone is architecturally modified through the process of trabecularization. This conversion increases the endosteal area and displays a network of interconnecting trabecular struts that are not included in the measurement of cortical bone area. The traditional measurement of cortical area requires the observer to subjectively determine where cortical bone ends and cancellous (trabecular) bone begins, producing measurement variability between observers. By including trabecular bone in area measurements, this subjectivity is removed. Furthermore, it is believed that incorporating both cortical and trabecular bone in area measurements to produce a bone area variable will provide more information for histological analysis of age-related bone loss.

It has been demonstrated that histological age estimation methods based on osteon counts (OPD) are limited when applied to older individuals. This is attributed to the nature of bone remodeling and the osteon population density (OPD) variable used for histological age estimation. Osteon counts from the rib eventually reach an asymptotic value in individuals of advanced age. Two factors exist that determine the age at which osteon counts will reach an asymptote value: (1) cortical area; and, (2) osteon area (size). Including trabecular bone area in histological measurements may improve future histological age estimation methods for the ribs, especially in older individuals where the OPD value becomes less reliable. The objectives of this study are: (1) to remove the subjectivity caused by measuring cortical area; and, (2) to determine if bone area has a stronger correlation with age than cortical area alone.

The sample consisted of midshaft sixth rib cross-sections from 31 known age individuals, ranging from 16-87 years of age (mean age 46.4 +/- 3.2 SD years). These included 26 men and 5 women. All data was collected from a pre-existing collection of sixth rib bone samples at the Office of Chief Medical Examiner in New York City. The following histomorphometric variables were collected: (1) total subperiosteal area (TA); (2) cortical area (CA); (3) bone area (BA); (4) relative bone area (rBA); (5) relative cortical area (rCA); and, (6) endosteal area (EA).

The rib cross-sections were prepared following standard protocols for histological sample preparation. The cross-sectional area of two rib samples per individual was evaluated. Only thin sections with suffice microstructural preservation and an intact cortex were utilized. Thin sections were photographed using a transmitted light microscope and a mounted digital camera attachment (x40 magnification). A series of sectional photographs were merged using digital imaging software. Cortical and trabecular bone areas were measured using a modular imaging software and a digitizing tablet.

Statistical analyses were completed using statistical software. A one-way analysis of variance (ANOVA) with age as the predictor variable was fit for rBA, rCA, CA, BA, EA, and TA to determine the strongest correlation with known age. When significant effects were detected, paired t-tests were utilized to identify significant differences between age categories. Relationships between known age and the respective variables were evaluated by linear regression analysis.

Results from this preliminary research indicate that cross-sectional area measurements were found to decrease with advancing age. Although the sample size is small, individuals greater than 40 years of age were found to have significantly smaller mean BA than those who were less than 40 years of age. Overall, a negative correlation was observed between known age and sixth rib BA (R=-0.34), and CA (R=-0.35). rBA and rCA displayed highly significant negative correlations with increasing age. The correlation strength was slightly higher between known age and rBA (R=-0.65) compared to known age and rCA (R=-0.59). Known age and TA displayed a positive correlation (R=.0.25). With one-way ANOVA, both rBA and rCA show significant difference among age groups at P=.039 and P=.025, respectively.

Previous studies have demonstrated that sixth rib cortical bone area significantly decreases with age and therefore, the variable has been applied to more recent age estimation equations. This overall age-related decrease in bone mass can be attributed to trabecularization of the cortical bone which results in endosteal expansion. The results of this study suggest that evaluating bone area may improve the accuracy of histological methods. Including rBA in future studies will provide a more relevant measure of bone area as it is independent of bone size. Finally, subjectivity and interobserver error may be reduced if the BA and rBA variables are included in future methods.

Histology, Histomorphometry, Bone Area

#### H92 Improving Forensic Facial Reproductions Using Empirical Modeling

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After attending this presentation, attendees will understand the benefits of using empirical modeling to produce more accurate soft facial tissue thicknesses that are used in facial reproductions.

This presentation will impact the forensic science community by presenting how empirical modeling improves the accuracy of forensic facial reproductions and can have a positive impact on the identification rate of unknown skeletal remains.

Forensic facial reconstruction has been used for many years to identify skeletal remains. The face of the unknown person can be reproduced based on the soft facial tissue thickness, which overlays the bony structure of the skull. Currently, forensic artists place average facial tissue markers at 21 specific anatomical locations on the skull and use clay to model the face based on the length of the markers. The purpose of this study was to develop a new method for estimating the facial soft tissue thickness at the 21 traditional craniometrical landmarks used in forensic facial reconstruction. This newly developed method uses a non-parametric modeling technique to predict the facial tissue depths based on a unique skull input.

Computed Tomography (CT) images of 100 American White male subjects' skulls were used to build a database of facial tissue thicknesses and input predictors for the non-parametric model. The inputs to the model are various cranial bone thicknesses and measurements along specific anatomical lines, which are then used to predict the facial tissue thicknesses at the traditional landmarks using a Non-Parametric Kernel Regression model. The tissue and bone measurements were performed using a software package being developed at the Center for Musculoskeletal Research (CMR) at The University of Tennessee. Hetero-Associative Kernel Regression (HAKR) and Inferential Kernel Regression models were built using the measurements from the 100 male subjects.

Two results were computed for each model; one including age, height, weight and BMI as predictors in the model and the other removing them from the model. This was done because, in many cases, the demographics of an unknown skull are not known. The Root Mean Squared Error (RMSE) when not using the demographics as an input to the model was 2.21mm for the HAKR architecture and 2.19 mm for the inferential model. When including the demographics, the RMSE for the HAKR architecture was 2.04mm and 1.89mm for the inferential architecture. The HAKR and Inferential model's RMSE were both less than the currently used tabled tissue thickness RMSE from the actual measured tissue thicknesses of 3.07 mm. The developed inferential

model provided forensic facial tissue thickness approximations with an average of 38% less error when using demographics or 29% less error when not using demographics. The error reduction is based on the tabled tissue thicknesses that are used in facial reconstructions today. The average prediction uncertainty from the LOOCV was computed to be 19.7% for the HAKR model and 20.5% for the inferential model.

Three male skulls from the William Bass Donated Collection at The University of Tennessee were used to visualize the model's performance. A certified forensic artist performed the facial reconstructions. The facial reconstructions using tabled tissue thicknesses were compared to the reconstructions of the same subject using the inferential model's predicted tissue thicknesses. The reconstructions were then compared to an actual photograph of each respective subject. The results show an overall more accurate representation of the actual subject face when using the empirical model tissue thicknesses. The findings from this pilot study have shown a proof of principal for using non-parametric empirical modeling to predict facial soft tissue thickness. This technology has the promise, with further research, to produce more accurate forensic facial reproductions. More accurate facial reproductions will hopefully have a positive effect on the identification rate of unknown skeletal remains.

**Facial, Reconstruction, Modeling** 

#### H93 Prediction of Mouth Shape Using Geometric Morphometrics for Facial Approximation

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After attending this presentation, attendees will be introduced to the advantages of geometric morphometrics when applied to facial approximation issues and the possibilities to enhance the accuracy of a prediction with the mouth shape as a practical example.

This presentation will impact the forensic science community by emphasizing the importance of taking the bone morphology into account in a multivariate way prior to the reconstruction of a face based on the skull.

Facial approximation specialists base their research on the principle that the facial form is directly related to the morphology of the underlying bony features. The applicability of such techniques is hindered by a lack of accuracy and reproducibility. The new advances and software availability in the geometric morphometrics field contiguously, with the development of 3D medical imaging, offer many possibilities to enhance the precision of forensic techniques. The purpose of this research is to introduce a semi-automatic face approximation method, using objective analysis strictly based on skull morphology.

Several hundred computed tomography (CT) scans were collected in French hospitals. A homogeneous sub-sample of 140 known age and sex individuals was extracted. The mean age of the sample is 54 years (min = 18; max = 96; SD = 21) and the sex ratio is 1:1. Patients showing pathological or traumatic conditions were excluded. Forty 3D landmarks were collected on the mouth region of each individual directly on the reconstructed osseous and cutaneous surfaces. TIVMI software was used to obtain reliable surfaces, based on the half-maximum height algorithm in 3D (Dutailly *et al.* 2009). The extracted coordinates were imported into MorphoJ software (Klingenberg 2008) for analysis. The 7 cutaneous and 33 osseous landmarks were checked for intra- and interobserver error and the configurations were normalized with a Procrustes superimposition. The resulting residuals and centroid sizes were used to study sexual dimorphism (with discriminant function analysis), age trends (with canonical variate analysis), asymmetry (with a Procrustes ANOVA), allometry (with multivariate regression) and covariation between the different sets of landmarks (with Partial Least Squares or PLS analysis). Results indicate the lower facial skeleton is influenced by age (mainly reflected by bone remodeling due to loss of teeth), subtle localized allometry (size-related shape changes) and sexual dimorphism (more pronounced on the mandible and consistent with allometric shape changes). Lips show some specific changes with age (wider mouth and thinner lips in older individuals) and a subtle allometry (mouth corners more posterior and thinner lips when centroid size is high) but no significant differences between males and females. PLS results suggest a good correlation between shape changes of the lips and shape changes of the facial skeleton; significant covariation in the regions studied was detected by this method.

Based on these results, a prediction technique was employed (by multivariate regressions) using principal components of the osseous landmarks configuration and the Procrustes coordinates of the cutaneous landmarks. The mean correlation (r) of the regressions attains 0.69 ( $r^2 =$ 0.48). Based on its specific morphology, it is possible to extract the shape variables from a skull to make an approximation of the coordinates of the lips. This methodology was applied to 5 CT scans (out of the 140 sub-sample) in order to compare the estimated shape of the lips to the true coordinates. The differences can be evaluated through a Principal Component Analysis in order to visually assess the distance between the true shape and the predicted shape. In terms of metric proportions, distances between landmarks can be calculated and multiplied by the centroid size of the true subject to get appreciable data. For example, the width of the mouth which is the largest measurement that can be extracted from the lips, displays a mean absolute error of 2.3 mm (4.3 % of the mean mouth width in the tested individuals).

This methodology offers an accurate and objective tool to approximate the shape of facial features from the skull. Further assessment of the shape of facial features using outlines or semilandmarks might enhance the precision of the results. In order to implement this approach, the next step is to develop software for computer-assisted (semi-)automatic technique for facial approximation. **Facial Reconstruction, Stoma, Procrustes Superimposition** 

#### H94 The Effects of Avian and Terrestrial Scavenger Activity on Human Remains in the Piney Woods of Southeast Texas

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After attending this presentation, attendees will understand the impact scavenger activity has on human decomposition rates in the Southeast region of Texas. By recognizing soft tissue and skeletal trauma caused by scavengers, as opposed to those caused by humans, investigators will be able to interpret taphonomic events more accurately.

This presentation will impact the forensic science community by presenting research which illustrates the impact several species of scavenging animals, native to the Southeast area of Texas, has on human decomposition. Investigators, such as pathologists, law-enforcement officers, and forensic anthropologists must understand all of the factors which influence decomposition, including wildlife, in order to correctly interpret taphonomic events for the establishment of accurate postmortem interval (PMI) estimates.

There have been many studies conducted which examine the effects scavenger activity has on human and nonhuman decomposition. However, to date, no such research has ever been conducted which examines these effects in the subtropical humid climate of Southeast Texas. This research project will, for the first time, examine the effects terrestrial and avian scavenger activity has on human decomposition. Data collected during this study will not only help investigators understand the agents involved in scavenger modified scenes, but also help to establish the time standards of decomposition as they relate specifically to the Piney Woods of Southeast Texas. This information is crucial to investigators such as medical examiners, law-enforcement personnel, and forensic anthropologists who need to determine the postmortem interval (PMI) for recovered human remains.

During the time period of August 2009 to January 2010, ten cadavers were placed outdoors at the Southeast Texas Applied Forensic Science facility for observation. The research subjects were photographed with a camera for decomposition stage assessment while scavenger activity was monitored via motion-sensing stealth cameras. These stealth cameras recorded a sequence of still photos each time an object moved into the camera's field of vision throughout the day and night. Scattered skeletal elements were observed and distance from primary placement was recorded utilizing photographs, linear measurements, and mapping software taken in the field. By recording activity with a stealth camera, the authors were able to unobtrusively observe several different scavenging animals in the field including the American black vulture (Coragyps atratus), turkey vulture (Cathartes aura), bobcat (Lynx rufus), and opossum (Didelphis virginiana). The results showed that patterns in scavenger activity leading to complete disarticulation seem to have been influenced by environmental conditions such as temperature, lunar phases, vegetation, and levels of sun exposure. Those placed outdoors during the fall season achieved disarticulation much faster than those placed during the winter. For example, one cadaver placed during the fall reached skeletonization and complete disarticulation in approximately two months whereas those placed during the winter required over six months. Among those cadavers included in winter placement, scavenging activity and decomposition noticeably slowed during the colder months until warmer temperatures returned in the spring. By this time, vegetation in two of the placement units had grown tall enough to obscure the location of the cadaver. This growth in vegetation seems to have camouflaged the area and negatively impacted scavenger activity.

Findings regarding scavenger behavior showed that turkey vultures were among the first to arrive but would observe the area of cadaver placement from a tree nearby before their descent. Once their interaction with the cadaver began, they would visit throughout the day along with the American black vultures. These two species of vultures would often scavenge together however it was usually the American black vulture who took the initiative to disarticulate the skeletal elements. Their forceful behavior often led to turkey vultures leaving the scene while American black vultures flocked in and dominated the area. One fallplaced cadaver was photographed with as many eighteen vultures during one such scavenging scene. Over the course of one weekend, this same cadaver was almost completely disarticulated through vulture activity alone. At the completion of this research subject's participation in the field, the skeletal elements were recovered and processed in the lab. By observing the photographic evidence along with the skeletal analysis, the trauma was able to be associated with the animal that caused it and at the specific stage of decomposition it occurred.

This study shows the effects environmental conditions and scavenger activity has on human decomposition. As more data is compiled with the completion of future studies, a time-line of activity along with a decomposition stage assessment specific to this region of Texas will be established. By understanding and correctly interpreting the trauma on modified remains and the scene of activity, investigators will be able to establish a more accurate PMI based on the taphonomic evidence documented from research such as this.

Scavengers, Taphonomy, Postmortem Interval

#### H95 Scavenging Impacts on the Progression of Decomposition in Northern New England

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After attending this presentation, attendees will better understand the potential effects of scavenging on differential decomposition, and how these modifications may influence estimations of postmortem interval (PMI).

This presentation will impact the forensic science community by illustrating how regional ecological variation can affect accurate estimation of PMI.

Many researchers acknowledge the critical role scavengers can play in the scattering of remains and postmortem modification. However, few studies document the impact of scavenging on PMI estimates. Recent research and experimental studies in the Midwest have demonstrated the utility of estimating accumulated degree days (ADD), by assessing of decomposition. Megyesi et al. (2005) and Sheil (2008) have proposed an ordinal scale ("total body score," referred to as TBS) to assess the extent of decomposition in three body zones (head and neck, torso, and limbs) and use the resulting score in a regression formula to calculate an ADD range.

With more than 90 percent of its land covered in forest, Maine's outdoor forensic cases are usually (70 – 80% percent) modified by scavengers, making it an extremely important taphonomic issue. Often, whole regions of the body are consumed, scattered, or missing. Megygesi's methodology was piloted with a small sample of nine forensic cases from Maine with a known PMI and an ADD calculated from nearest weather station data. A recently developed Regional Taphonomy Geographic Information System (GIS) was used to assess the macroenvironmental context and calculate the weather station-based ADD for the PMI dates in each case. Five cases lacked representative elements for one or more of the anatomic regions. When all regions are represented, these preliminary data show that formula-calculated ADD ranges tend to be much lower than the actual ADD range.

Maine's "scavenger guild" includes both mammalian and avian animals, predominantly coyote (Canis latrans), black bear (Ursus americanus), fox (Vulpes vulpes), bobcat (Lynx rufus), raven (Corvus corax) crow (Corvus brachyrhynchos), turkey vulture (Cathartes aura) and bald eagle (Haliaeetus leucocephalus), as well as smaller mammals. In far southern and coastal areas, as well as during winter months statewide, the scavenger guild is coyote-dominant. In spring, summer and fall, it is potentially bear-dominant in most of the state. Particularly in early spring, bears are more apt to seek protein-rich meals when they first emerge from hibernation. In warmer weather, bears are more likely to be attracted to a carcass by a maggot mass, rather than decomposing flesh. Raptors and corvids participate in the scavenger guild throughout the state. Corvid presence can signal other scavengers, particularly eagles, which lack olfactory capacity, that there is a meat source in the proximity. With their sharp beaks, corvids easily "open up" a body, even when frozen, enhancing access to the viscera for other avian taxa. Coyotes are common throughout the state and are very frequent scavengers, along with other canids.

Similar to canid patterns modeled by Haglund (1997) for the Northwest, limbs are often removed beginning with the upper limbs, which are easier to disarticulate and lighter to carry (if removed from the site). Bones with a thinner cortex and narrower diameter, such as ribs, require little expenditure of energy on the part of the scavenger and are generally consumed on-site early on in the process. The cranium and limb bones are often scattered, and the limb bones and extremities are often completely consumed, missing, or cached. Although most scatter tends to be within a 50 meter radius, it can range up to a mile, creating extreme challenges for search and recovery.

Results indicate that scavenging can both accelerate and decelerate progression towards skeletonization, potentially interfering with estimates of PMI. In Maine cold temperatures as well as the involvement of large mammalian scavengers can preclude, or considerably diminish, insect infestation. Frequently, one or more anatomic regions are entirely missing, which can also bias decomposition assessment and PMI estimates.

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Scavenging, Decomposition, Taphonomy

#### H96 Decomposition Patterns of Human Remains Within Enclosed Environments: A Comparative Analysis of the Midwest and Southeast

Melissa A. Pope, MA\*, University of South Florida, Anthropology Department, 4202 East Fowler Avenue, Tampa, FL 33620

The goals of this presentation are to explore the patterns and timing of the effects of decomposition for enclosed settings in both the Midwest and Southeast, and to identify taphonomic factors that will be useful for predicting the accumulated degree days (ADD). Attendees will be presented with predictive models for estimating ADD and a blueprint for retrospective decomposition studies.

This presentation will impact the forensic science community by presenting trends in human decay within two distinct geographical regions for an environment that has been largely unexplored. The identified patterns accentuate the need for generating comparative samples and engaging in collaborative research to create context-specific standards for estimating the postmortem interval (PMI).

Retrospective case studies from autopsy records were utilized to assess human decay trends in different geographical regions. The 2003-2008 Nebraskan autopsy records revealed 69 cases within enclosed settings, and Florida's Hillsborough County Medical Examiner records for 2009 yielded 87 cases. Five outliers were removed (Nebraska: n=67; Florida: n=84). The reliability of Bass' (1997) model was tested for a correlation between time ranges (first day, 2–7 days, 8–31 days, and >31 days) and decay stages (fresh, bloated, advanced) to determine whether a context-specific standard can be applied to enclosed settings in varied U.S. regions. To test variation in decomposition, relationships between PMI (time in days) and Bass' stages were tested using Spearman's Correlations for each state, and differences in PMI among decay stages were tested with Kruskal-Wallis and Mann-Whitney U tests. The role of insect activity was described by location. Spearman's Correlations were further used to identify factors that may be powerful in predicting ADD in Nebraska and PMI in Florida. For Florida, preliminary trends were identified. For Nebraska, a multiple regression model was constructed for the prediction of ADD.

For Nebraska cases, the investigation of decompositional phases revealed that there were 49.3% (33/67) fresh, 37.3% (25/67) bloated, and 13.4% (9/67) advanced. PMI ranged from one to 66 days (*mean PMI=4.84 days*, n=64). For Florida cases, there were 6.0% (5/84) fresh, 77.4% (65/84) bloated, and 16.7% (14/84) advanced. PMI ranged from 2–26 days (*mean PMI=6.27 days*, n=81).

For both regions, Bass' decay stages were significantly correlated with time ranges (Nebraska: r=0.829, n=64,  $p\leq0.000$ ; Florida: r=0.366, n=81, p=0.001). For both regions, relationships between PMI and decomposition were identified (Nebraska: r=0.772,  $p\leq0.000$ , n=64; Florida: r=0.512,  $p\leq0.000$ , n=81). For Nebraska, there were significant differences in PMI days among all decomposition stages ( $X^2=37.818$ , df=2,  $p\leq0.000$ ). Florida possessed differences in PMI between bloated

and advanced cases (*MW-U=165.500*,  $p \le 0.000$ ). Fresh cases could not be considered, due to small sample size.

For Nebraska, only 12.3% (7/57) cases with fly colonization were documented: 11.1% (2/18) within bloat and 71.4% (5/7) within advanced. The Florida sample included flies, ants, gnats and beetles; 73.56% (64/87) of cases had insect activity. Insect activity was more prevalent among Florida depositions.

For Nebraska, several factors were identified as significantly correlated with the transformed  $\log_{10}$ ADD. Ultimately, brain liquefaction, marbling, decompositional odor, mummified tissue and the use of A.C. or heat were selected for a predictive model (*Adjusted R*<sup>2</sup> = 0.952; *F*=40.807, *df*=5, 5 and p≤0.000). For Florida, taphonomic variables identified as having a relationship with PMI include: necrophagy (*r*=0.268, *p*=0.015, *n*=81), livor mortis (*r*=0.234, *p*=0.035, *n*=81), bloating (*r*=-0.251, *p*=0.024, *n*=81), mummified tissue (*r*=0.527, *p*≤0.000, *n*=81), brain liquefaction (*r*=0.285, *p*=0.038, *n*=53), and organ decomposition variables may be powerful predictors of ADD for Florida.

Correlations between stages of decay and PMI, as well as the disparity in PMI days among stages indicate that retrospective data are well suited for identifying what taphonomic effects have a relationship with time and would serve as powerful predictors of the PMI. Although Bass' model for outdoor Tennessee decomposition accounted for a significant portion of the variation for both locations, the low Spearman's rho score for Florida indicated that Bass' model cannot adequately address decomposition rates for enclosed settings in Florida. The disparity in insect activity and the selection of different decomposition variables as predictors of PMI and ADD for both locations suggest that taphonomic factors vary in influence over decay rates by geographical region. Collectively, these results lend support for the need to create quantitative models for predicting ADD that are geographically and contextually specific. Predictive models can be constructed by identifying taphonomic influences and effects that best characterize decompositional change in a given environment.

Florida Decomposition, Enclosed Environments, Postmortem Interval

#### **H97** Using Algae to Estimate Postmortem Submersion Interval in a Louisiana Bayou

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After attending this presentation, attendees will recognize the utility of algae in the estimation of postmortem submersion interval (PMSI).

This presentation will impact the forensic science community by highlighting an often overlooked resource for the prediction of time since death, promoting collaboration between forensic anthropologists and algologists for the estimation of PMSI.

When a body is found in a terrestrial setting, the forensic anthropologist has a wide array of taphonomic information available to assist in the estimation of postmortem interval (PMI). Yet, decomposition in water is far less understood, with the estimation of PMSI more difficult to determine. While insects are a principal contributor to the data on terrestrial decomposition, few truly sarcophagous aquatic insects have been documented (Wallace et al. 2008).<sup>1</sup> Water is a highly variable habitat, being affected by numerous factors such as sunlight, temperature, wind, pollution, and geographic location. The position of a body in water is also highly variable, with some bodies floating or sinking naturally, while others are trapped or deliberately weighted to sink to the water floor. Depth, salinty, pH content, current, and micro and macro organisms all affect how quickly a body will decompose, and disarticulation coupled with fluvial transport make submerged cases particularly challenging. However, researchers have identified stages of underwater decomposition, such as the six-stage system suggested by Payne and King (1972).<sup>2</sup>

The current study used fetal pigs as models for humans to assist in evaluating the potential for using algae growth as an indicator of PMSI, examining three questions: (1) will a decomposing pig have different algae growth than a non-decomposing object; (2) does a clothed substrate have different algae growth than an unclothed substrate; and, (3) is algae growth different in spring and fall? Pigs were placed in a Louisiana bayou in spring and fall, some clothed in cotton and others unclothed. Each season, one clothed pig and one unclothed pig were sampled for algae accumulation daily for ten days, then once weekly until no flesh remained. In addition, submerged clothed and unclothed slate tiles were sampled for algae during the same time period, acting as comparative, non-decomposing objects. In laboratory, the algae samples were quantified through chlorophyll a concentration, while microscopic analysis qualified diatoms as the primary algae in both seasons. Additionally, each season a neighboring clothed pig and an unclothed pig were not sampled but rather visually assessed for stages of decomposition. These unsampled pigs provided a control for observation into the effects of algae sampling on the rate of decomposition.

Statistical analyses were conducted to examine the effects of clothing and season on chlorophyll a on the pigs and tiles. Positive, linear relationships existed between the amount of time submerged and the accumulation of chlorophyll a l substrates in both seasons, meaning that chlorophyll a potentially can indicate time since submersion. Results also demonstrate that the presence of clothing has a greater impact on algae growth rate than the presence of decomposing matter, with the clothed pigs and clothed tiles having more growth than the unclothed pigs and tiles, especially in spring. Season was also found to highly impact algae growth, with the light rainfall and warm temperatures of spring creating ideal growing conditions compared to the heavy rainfall and cool temperatures of fall. Finally, the act of experimental algae sampling did not influence the process of decomposition.

In summary, this study shows that algae growth, measured through chlorophyll *a*, has tremendous potential for the estimation of PMSI. **References:** 

- <sup>1.</sup> Wallace JR, Merritt RW, Kimbirauskas R, Benbow ME, McIntosh M. Caddisflies assist with homicide case: determining apostmortem submersion interval using aquatic insects. Journal of Forensic Sciences 2008;53(1):609-614.
- <sup>2</sup> Payne JA, King EW. Insect succession and decomposition of pig carcasses in water. Journal of the Georgia EntomologicalSociety 1972;7:153-162.

Postmortem Interval, Algae, Taphonomy

# H98Profiling of Marine Microbial Communities<br/>AssociatedWith<br/>Decomposing<br/>Remains<br/>Can<br/>IndicateDecomposing<br/>Postmortem<br/>Submersion Interval

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After attending this presentation, attendees, will have a better understanding of the role that extrinsic microbial communities play in the rate and pattern of decomposition of remains in New Zealand coastal marine environments and how such information may enable forensic investigators to more accurately determine the postmortem submersion interval (PMSI).

This presentation will impact the forensic science community by extending the currently limited knowledge of aquatic decomposition of bodies and/or body parts mediated by marine microorganisms while introducing a novel framework through which PMSI may be determined by analyzing the marine microbial community present on submerged remains at the time of body recovery.

Microbial communities play a central ecological role in the recycling of nutrients and organic matter in aquatic ecosystems. Despite the clear importance of bacteria in the decomposition process, a detailed understanding of the postmortem microbiology of decomposing remains in aquatic environments is currently lacking. A previous study in a coastal marine environment showed that marine microbes display successional colonization patterns which can be linked to particular submersion intervals, but highlighted the need to develop a more high-throughput methodology, if this concept is to be utilized in a forensic setting.

One such methodology is terminal restriction fragment length polymorphism (TRFLP) analysis. TRFLP is a molecular fingerprinting technique that examines the 16S rRNA genes of virtually all bacteria in a microbial community, producing a community profile or "fingerprint" which consists of different length fragments for different bacterial phyloypes. TRFLP has the advantage of being a fast, reliable and relatively quick and inexpensive technique that produces digital output and could potentially be used for forensic analysis.

This study aimed to identify, using TRFLP analysis, successional changes in microbial community composition on submerged pig remains as they decomposed in the sea, and to assess whether unique components of the community existed for particular stages of decomposition or periods of submersion.

Adult domestic pig (S. scrofa L.) carcasses, used as models for partial human remains, were placed in cages encompassed by mesh, so as to deny larger scavengers access and achieve the longest postmortem submersion interval possible for the collection of colonizing bacteria. Cages were submerged in the Otago Harbour in water 5-7 m deep in January (summer) 2009 and July (winter) 2010, and in Wellington Harbour in water 8-10 m deep in February (summer) 2009. The study was performed in two geographic coastal locations, and in one of these locations, during two seasons, in order to explore the general applicability this technique would have across time and space. Bacterial samples were taken by swabbing the skin of the carcasses before entry into the water, after 1, 2 and 3 days, then at 2-4 day intervals until skeletonization. DNA from the bacterial community present on the carcass at each time point was extracted from the swabs and subjected to On sampling days, observations of gross TRFLP analysis.

decomposition changes and the presence of any small marine scavengers in or on the cage were also noted. During the course of the experiments, environmental data such as seawater temperature and pH were also monitored.

Clustering analysis of TRFLP microbial community profiles from early, mid and late periods of submersion formed distinct clusters and could be distinguished based on the presence of certain bacterial phylotypes. There appeared to be very little difference in the pattern of community change over time between carcasses deployed in the two different geographic locations, suggesting this concept has broad spatial applicability. Many phylotypes present on the skin before submersion disappeared within the first few days, indicating significant and rapid disruption of the original skin microbiome following submersion of the remains in salt water. Extrinsic marine microbes colonized the remains immediately. Dynamic shifts in the structure of the microbial community present on the remains were seen during the early submersion period (Fresh to Early Putrefaction stages), with a number of short-lived, time-specific phylotypes observed. The mid-submersion period (Advanced Putrefaction) was characterized by much more gradual shifts in community composition, with colonization by unique phylotypes not observed before particular submersion intervals, but which persisted on the remains for longer periods of time. As well as the continued presence of mid-phase colonizers, the late submersion period (Advanced Decay and Skeletonized Remains) saw the arrival and subsequent disappearance of many short-lived, time-specific phylotypes; thus the microbial community present on the decomposing remains once again underwent noticeable and rapid compositional changes. Preliminary results from a comparison of decomposition events during summer and winter in Otago Harbour found substantial differences in bacterial phylotypes within TRFLP profiles, suggesting there is a strong seasonal aspect to colonization. However the overall pattern of compositional change over time was similar.

The use of TRFLP as a microbial community profiling tool has enabled the first characterization, at a community level, of the postmortem microbiology of submerged mammalian remains over the course of an aquatic decomposition event. This reproducible, highthroughput technique is cost-effective and could be easily implemented in the modern forensic laboratory. Microbial community presence, abundance and successional dynamics, coupled with temperature data, have the potential to provide detailed information regarding length of submersion time of immersed bodies and/or body parts recovered from coastal marine waters of New Zealand and beyond in cases where a specific PMSI is in doubt.

Decomposition, Postmortem Submersion Interval, Microbial Communities

#### H99 Scavenging and Its Relationship to Decomposition in the Northern Rockies

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After attending this presentation, attendees will better understand the need for assessing scavenging as an aid to discerning the postmortem interval in regions such as Montana, where mummification is a normal part of the early decomposition process and often becomes the static, long term condition of remains not exposed to scavenging.

This presentation will impact the forensic science community by demonstrating many cases of partially and completely skeletonized remains in Montana are likely the result of large carnivore scavenging as opposed to extended periods of exposure as previously thought. Accurate estimation of time since death can be an important component of a forensic investigation and the processes by which soft tissue is lost from the skeleton need to be well understood, particularly for areas with variable decomposition patterns and the presence of large carnivores.

Recent research by Parsons (2009) and Dudzik (2009) has demonstrated that decomposition rates and patterns in the Northern Rockies of Montana vary from those known elsewhere in the United States. The arid and cool climate not only slows the general rate of soft tissue decomposition, but also results in mummification of the external tissues very early in the decomposition process. Nevertheless, partially or fully skeletonized remains are recovered, even when the postmortem interval has been relatively brief (3-6 months). Typically these remains have been scavenged by one or more of the large carnivores native to Montana, including brown bears, grizzly bears, mountain lions, and wolves.

A retrospective review of twelve cases, with established postmortem intervals, analyzed by the medical examiners of Montana's Forensic Science Division and forensic anthropologists at the University of Montana over a six year period was undertaken. Based on reports, case notes and photographs, degree of mummification was scored by percentage of the body retaining mummified tissue and degree of scavenging was scored as percentage of the skeleton bearing evidence for carnivore scavenging activity. Eight (66%) of the total cases reviewed had either not been subject to scavenging or were only minimally scavenged (scored as 10% or less) with postmortem intervals ranging from three weeks to two years. Of these cases, 75% of the individuals retained mummified tissue covering more than 50% of the body. In the cases where scavenging had significantly affected the remains (scored as 25% or greater), the retention of mummified tissue was limited to 50% or less, with two of these cases having postmortem intervals of less than one year. In summary, remains that do not experience scavenging tend to retain the external layer of mummified tissue, even years after death, while remains that are subject to large carnivore scavenging can be completely or partially skeletonized within a few months.

Therefore, estimations of the postmortem interval for human remains in Montana and similar regions must consider not only the unusual decomposition pattern associated with the climatic conditions of the Northern Rockies, but also the degree of scavenging by large carnivores as this is a likely factor responsible for the absence of soft tissue as opposed to decomposition due to an extended period of exposure. This will assist efforts to identify human remains or to pinpoint the timeframe for a crime.

**Decomposition, Scavenging, Postmortem Interval** 

#### H100 Anaerobic and Aerobic Decomposition in 55-gallon Oil Drums: A Two-Year Study on the Deliberate Concealment of Remains

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After attending this presentation, attendees will learn about the unique stages of decomposition occurring inside an above ground, manmade container used for concealment purposes. Using pigs as human models, this research focuses on both anaerobic and aerobic decay inside an above ground container and reveals how such decomposition can mimic antemortem or peri-mortemtrauma.

This presentation will impact the forensic science community by providing new criteria for estimating the postmortem interval for bodies concealed in above ground, man-made containers such as an oil drum. Gaining insight into this under-researched decompositional context is important for forensic anthropologists involved in estimating the postmortem interval (PMI).

Numerous studies have focused on the process of decomposition, but few studies have focused on decomposition within above ground containers used for concealment purposes. Moreover, no forensic studies have focused on decomposition within 55-gallon oil drums.

For the past two years, three, one-year-old male pigs weighing 80.0, 80.8, and 84.2 pounds, respectively, have been decomposing inside 55-gallon black, metal, oil drums placed on a cattle farm in Baton Rouge, Louisiana; each drum contains one pig. Oil drums were chosen for this study because federal regulations require that oil drums have an airtight seal to prevent the leakage of chemicals and oil during transport (U.S. Department of Transportation, 49 CFR 178.540(6)).<sup>1</sup> Prior to the start of this study, 1-ft<sup>2</sup> lexan observation windows were installed in the lids of two of the three oil drums to allow observations of anaerobic decomposition. Drum A (containing pig A) was sealed at the initiation of the project and then was opened one month later. Drum C (containing pig C) was sealed at the top but had four holes drilled in the sides to allow for insect access.

During the first six months of this study (September 22, 2008 – March 13, 2009), daily visits were made to the research site and detailed observations of the anaerobic and aerobic decomposition stages occurring inside each oil drum were recorded. A full collection effort of forensically-important insects was made during each visit; over 1,000 arthropods were collected and identified. Seasonal visits to the site have been made since March 13, 2009. A brief summary of the findings for each of the three pigs provides insight into the great variation in decomposition seen in each of the three drums.

Drum A was sealed on September 22, 2008; pig A currently is still sealed inside, and oil drum A has never been opened. The lexan window on the oil drum's lid has allowed the process of anaerobic decay to be observed. During the first two weeks after placement, pig A's intestines were forced outside of the body as a result of decompositional gases, but neither bloating nor the expected color changes of the skin occurred during the first year of concealment. Pig A gradually began to liquefy during the second year of concealment. No insects have been observed inside the oil drum.

Drum B was also sealed on September 22, 2008; pig B underwent one month of anaerobic decay prior to intentional removal of the drum's lid. During the first two weeks of placement, the pig's abdomen exploded as a result of gas pressure build up. This "explosion" was vastly different from the intestinal expulsion viewed in pig A with pig B's body tissue adhering to the drum's walls. No insects were observed inside the oil drum during the month of anaerobic decay. However, once the drum was opened, insects were immediately attracted to the exposed carrion following lid removal. Nevertheless, unlike pigs A and C, pig B gradually mummified during the five months following lid removal.

Drum C contained pig C and was sealed September 22, 2008. Prior to the pig's placement in the drum, four evenly spaced ½-inch holes were drilled into the oil drum's upper perimeter. Within 24 hours of placing pig C inside the oil drum, the pig's intestines were found resting outside of the body, though no explosion had taken place. Complete skeletonization occurred in one week; however, the liquefied remains and bones have undergone a series of taphonomic changes during the past two years. Most noticeably, the bones changed from black in color to white without ever having been removed from the oil drum or exposed directly to the sun. Two years after beginning this study, the skeleton inside this oil drum is no longer visible because the liquefied soft tissues have solidified and expanded to the top of the drum.

Finally, results from this study provide new insight into the effects anaerobic and aerobic environments have on decomposition within above ground containers used for concealment purposes. This research reveals that attempts to conceal remains can result in liquefaction of tissues, mummification, and, under-certain conditions, postmortem explosion of soft tissue.

Reference:

<sup>1</sup> U.S. Department of Transportation, Research and Special Programs Administration. *Code of Federal Regulations*. Title 49, vol. 2, pt. 178, sec. 504, Standards for Steel Drums. Washington, D.C.: Government Printing Office, 1999, (49CFR178.504).http://frwebgate.access.gpo.gov/cgibin/getcfr.cgi?TITLE=49&Part=178&SECTION=504&YEAR=1999 &TYPE=TEXT

Forensic Anthropology, Postmortem Interval, Anaerobic Environment

#### H101 Potential Impact of Regional Ecologies on the Estimation of Postmortem Interval: Case Comparisons From Northern New England

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After attending this presentation, attendees will better understand the potential effects of microenvironment on differential decomposition, and how regional differences can influence taphonomic condition and estimation of postmortem interval (PMI).

This presentation will impact the forensic science community by illustrating how regional ecological variation can affect accurate estimation of PMI in a medicolegal context.

Estimation of postmortem interval is among the most important and among the most complex—tasks performed by forensic anthropologists. An accurate PMI can help the medical examiner to determine a timeline for events surrounding a death and can inform the direction of medicolegal investigation. However, PMI estimation is complicated by numerous factors, many of which vary geographically, including temperature, precipitation, elevation, terrain, and soil characteristics. These combined factors all contribute to the ecology of plant, insect and animal communities that impact the condition of a corpse. A multi-year research initiative to develop regional taphonomic standards for northern New England has selected comparative case examples where time of death and ecological context is known to illustrate representative patterns.

This presentation examines the effects of regional ecosystem variables using a Maine case involving the body of a young woman found partially submerged in a shallow stream in a area of light-growth, mixed deciduous-evergreen woods. Data from a weather station six miles away was used to calculate the accumulated degree days (ADD). Investigation of daily temperatures showed a cold October, with below-freezing temperatures, but a warmer-than-average November. With a late September death, a 65-day PMI, and an ADD total of 536, the body demonstrated four distinct taphonomic zones, ranging from fleshed with intact internal organs to skeletonization of the head and arms. Large scavengers, primarily coyote, had removed flesh from the arms, back, and buttocks. The head was skeletonized but showed no evidence of carnivore activity, suggesting that this area may have been defleshed by insects or putrefaction prior to canid involvement. However, evidence of insect involvement was absent.

Microenvironmental factors help to explain differences between this case and selected comparison cases from the same geographic area and/or the same timeframe. This case is compared with two other cases found in the woods in the same local area. One is from roughly the same autumn timeframe, with a shorter PMI (about 30 days) and no evidence of mammalian scavengers. Decomposition is at the decay stage, and larvae are present but not abundant. The other comparison case is from the same area, and has a similar amount of decomposition on average, but was exposed in the summer and for a shorter time frame (15 days). Mammalian scavengers were minimally involved, and fly larvae were abundant on the body.

The importance of scavenger involvement and the cold, often freezing temperatures are critical variables in interpreting cases in the northeast. Many actualistic taphonomic studies used to calibrate the PMI estimation have been conducted at the scavenger-protected University of Tennessee Anthropological Research Facility, yet natural experiments in the arid regions of the southwest United States (Galloway 1997; Rhine and Dawson 1998), the southeast (Manhein 1997) and the northeast (Sorg et al., 1998) amply demonstrate tremendous regional variations in the taphonomic factors that influence the rate and characteristics of decomposition. Temperature has been demonstrated to alter the speed of decomposition, and Micozzi (1997) has shown that freezing temperatures can cause cellular breakdown and reverse the order in which decomposition occurs within a body, so that more external and peripheral parts decompose earlier. Comparative rates of thawing of anatomical parts may also reverse scavenging order, and colder temperatures and resulting food scarcity can enhance the intensity of scavenger activity (Klepinger 2006; Bass 1997).

In sum, regional variation in ecological and climatological factors necessitates a more fine-grained examination of case context in order to estimate PMI. Results of actualistic studies, particularly those conducted in other regions, need local testing and adjustments before they can be applied.

This research was supported in part by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

Taphonomy, Postmortem Interval, Regional Ecology

#### H102 The Relationship Between Ambient Temperature and the Temperature of Maggot Masses on Decomposing Pig and Rabbit Carcasses

Amanda B. Troy, MSc\*, 13 Castlerock, Tulla Road, Ennis, IRELAND; and Colin Moffatt, PhD, and Tal Simmons, PhD, School of Forensic & Investigative Sciences, University of Central Lancashire, Preston, PR1 2HE, UNITED KINGDOM

After attending this presentation, attendees will gain an understanding of the effect of maggot mass temperature on the development of Diptera larvae and will be aware of the need for a more accurate measurement of accumulated degree hours (ADH) from Diptera larval development in maggot masses.

This presentation will impact on the forensic science community by outlining the influence ambient and maggot mass temperatures have on Diptera larval development, which will aid in establishing a realistic postmortem interval (PMI) from entomological specimens originating within maggot masses at crime scenes.

Forensic anthropologists often estimate PMI by determining the accumulated degree days (ADD) to which a cadaver has been subject during decomposition. Insect activity is one of the most significant factors affecting the decompositional process. The examination of larval maturation is particularly important, as investigators have previously used the correlation of insect development rates over ADD to obtain an estimation of PMI. Diptera are of a particular forensic importance, as they are among the first insects to colonize a corpse. Temperature has a major influence on the rate of development of Diptera larvae. To date, estimating PMI from Diptera larval development has been proven to be

\* Presenting Author

inaccurate. The heat generated from maggot mass formation accelerates maggot developmental rate, significantly reducing the maturation time, and therefore altering the estimated PMI.

This study was conducted in the North West of England using domestic pigs (*Sus scrofa domestica*) and European rabbits (*Oryctolagus cuniculus*). Two experimental groups, one pig group (N=6) and one rabbit group (N=6) were left to decompose on a grass surface. When maggot masses formed, a thermal probe connected to a data logger was inserted into a mass on each carcass to record the temperature generated by the maggot activity. A pig control group (N=3) and a rabbit control group (N=3) were placed outdoors in a cage constructed from a wooden frame and covered in a fine wire mesh, in order to exclude insects and therefore assess the temperature generated by non-insect mediated decomposition processes. The internal body temperatures of the control groups were recorded.

The results demonstrated that the maggot masses were comprised of three species of Diptera, *Calliphora vomitoria, Calliphora vicina* and *Protophormia terraenovae*. Oviposition occurred at natural creases at the limbs, in the head, and along the back of the neck in the pig carcasses.

ADD was used to standardize all calculations in order to compare groups. The ADD of the maggot masses in the experimental groups was calculated, as was the ADD of the corresponding internal temperatures in the control groups. The results were statistically analyzed against the ADD of the ambient temperature.

Analysis of the mean daily temperatures in the pig control group compared to mean daily ambient temperatures showed no significant difference (ANOVA, p= 0.07). This would indicate that any resulting rise in temperature in the experimental pig group over ambient temperature is from maggot mass activity. The increase in the experimental pig group ADD over ambient ADD was 38.5%. An estimation of ADD from larval development, based on the ambient temperature, would have lead to an overestimation of PMI by over 13 days in a 6-week period. The difference between ambient ADD and experimental pig ADD for this research can be defined as:

Difference in Ambient ADD Versus Exp. Pig ADD ( $^{\circ}$ C) = (0.0461x + 0.2106)<sup>2</sup> where R<sup>2</sup>=0.96301: The rabbit carcasses yielded similar results, with the ADD of the rabbit experimental group showing an increase of 29.4% over the ambient temperature ADD, with no statistical difference between the mean daily ambient temperatures and the control rabbit daily temperatures (ANOVA, p=0.8). The difference between ambient ADD and experimental rabbit ADD for this research can be defined as:

Difference in Ambient Versus Exp. Rabbit ADD ( $^{0}$ C) = 0.4335x - 2.6092 where R<sup>2</sup> = 0.99254: Rabbits were shown to be poor models for this type of research, as it was not feasible to visually examine the maggot masses due to the fur and the maggot masses could not be sustained due to the size of the carcasses.

Investigators must be aware of the ineffectiveness of using previous ADD calculations of Diptera larval development in order to establish a PMI. It is recommended that the investigator should determine the ADD of maggot masses as opposed to ambient ADD when establishing PMI. Temperature, Maggot Mass, Postmortem Interval



# **PSYCHIATRY & BEHAVIORAL SCIENCE**



#### I1 Making the Diagnosis of Paraphilia in Sex Offender Evaluations

#### Dean De Crisce, MD\*, Coast to Coast Forensic Associates, 41 Schermerhorn Street, #325, Brooklyn, NY 11201

After attending this presentation, attendees will be able to understand the categorization of common diagnoses found in sexual predators, appreciate the current changes proposed for paraphilias in the upcoming DSM V, and have some practical guidelines on differential diagnosis of sex offenders and the use of paraphilia diagnoses.

This presentation will impact the forensic science community by discussing how sexually violent predator legislation has increased throughout the United States and frequently utilize psychiatric and psychological expertise to guide legal decision-making. The process of evaluation routinely requires the broad determination of a "mental abnormality" in addition to risk determination for the purposes of sentencing, Megan's Law, and civil commitment.

Evaluators must combine a thorough review of discovery material, such as police investigations and prior treatment records, with a mental status examination and knowledge of the increasing literature on sexual crimes to render psychiatric diagnoses in these cases. The process of a thorough evaluation, in essence, is comprised of three phases: (1) Information gathering - such as document review and a mental status examination; (2) information integration - involving the organization of information in a useful manner to provide for a consistent approach to evaluations; and, (3)information interpretation - in which all factors in a particular case are considered.

There is a good deal of controversy surrounding the use of paraphilia diagnoses in sex offender evaluations. It is seen by some groups that these diagnoses are made haphazardly and are inappropriately used to civilly commit individuals. While the majority of sex offenders do not suffer from paraphilias, there are some that most certainly typify that class of diagnoses. Recently proposed changes in diagnostic criteria for paraphilias, as intended for the DSM V, indicate more stringent thresholds to prevent misuse of the diagnoses.

The psychiatric examination of sexual crimes can be intensive, lengthy, and bring about strong counter transferential reactions. Nevertheless, forensic experts should be meticulous in their investigation of evidence and discriminate between true paraphilia diagnoses and other potential causes for sexual misconduct such as substance abuse, mania, psychosis, antisocial personality disorder and other personality disorders. A growing body of psychological and psychiatric sex offender literature indicates "clues" and "red flags" which might be used as guidelines to differentiate the paraphilic offender from other types of sexual offenders. Expert witness conclusions and testimony should demonstrate thoughtful conclusions that consider alternative explanations for misconduct. This maintains the integrity of mental health expertise and is appropriate when significant liberties are generally at stake with these cases.

Paraphilias, Forensic Psychiatry, Sexually Violent Predators

#### I2 Fatal Attractions: Women Who Have Affairs With Their Inmate Patients

Vivian Shnaidman, MD\*, Jersey Forensic Consulting, LLC, 181 Cherry Valley Road, Princeton, NJ 08540

After attending this presentation, attendees will be able to: (1) identify risk factors in females working in secure facilities that lead them to sexual entanglements with inmates; (2) identify the types of inmates who are likely to attract female staff members; (3) understand the psychodynamics of these relationships; and, (4) become familiar with legislation in various jurisdictions applicable to these relationships.

This presentation will impact the forensic science community by identifying individuals and institutions at risk for these dangerous liaisons between therapist or corrections staff and inmates, and helping institutions and supervisors implement programs to prevent this type of behavior.

Psychiatrists, psychologists, and all other types of therapists know that romantic and sexual involvement with patients is inappropriate, unethical, and not covered by malpractice insurance. Nevertheless, these types of relationships do occur and occasionally are described in the media. Recent years have inundated us with stories of teachers having sex with their pupils and the media have sensationalized many of these cases, especially when the teacher is someone young and attractive. The teacher-pupil relationship is a good model for the therapist-patient relationship, but of course it is not exactly the same.

Some of the inappropriate romantic and sexual relationships that can and do occur between mismatched participants will be discussed. Some of these relationships are clearly morally and legally reprehensible, such as a sexual relationship between an adult and a child.

The history of some of these relationships will be examined – at one time, sex between professor and student was somewhat acceptable, especially between a male professor and a female student. Then that type of relationship was re-defined as inappropriate and abusive of the female. Once, a male doctor could have sex with a female patient, and if they ended up married, there was no inappropriate relationship. Today, this sort of situation would be scandalous. If the doctor is a psychiatrist, then this behavior is inappropriate and unethical, and in some states, illegal. The gender of the doctor and the gender of the patient are irrelevant. Some of the features of and statistics about these relationships will be reviewed which may be surprising to some. Anecdotal information will be presented, of course with all identifying information changed far beyond recognition.

What happens when a therapist works with a patient inside a correctional institution or a quasi-correctional facility such as a forensic hospital or sex-offender facility? These therapists have even more of a responsibility to their patients because frequently their input is utilized in assessing dangerousness and making recommendations for parole or community release. Do therapists (of any degree level) get involved with their inmate patients? Unfortunately, they do.

In this presentation, first, the laws and the ethical implications and applications of sex with inmate patients will be reviewed and discussed.

Then the research into this type of behavior will be reviewed – the general research into sex with patients, and then the research about specific sexual activity between therapist and patient in correctional institutions. More surprising statistics will be presented. Then cases in the popular media will be reviewed and discussed with the audience. There will be no attempt made to diagnose the individuals discussed, but rather, the presentation will be an educational experience in which the participants will attempt to identify some of the risk factors presented in

the literature review in the cases that came to the attention of the popular media, to identify the psychodynamics of these relationships, and perhaps identify some potential ways to prevent this type of behavior.

#### Female Psychopaths, Sex With Inmates, Borderline

#### I3 Necrophiliac as a Morgue Attendant: Paraphilia in the Dead of Night

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After attending this presentation, attendees will understand the nature of paraphilias and those who exhibit such behaviors. Specifically, this presentation will concentrate on a paraphilia not otherwise specified, necrophilia.

This presentation will impact the forensic science community by assisting in the understanding of the psychological makeup of a necrophiliac. In better understanding of who will gravitate toward this aberrant sexual practice, whether or not there are certain signs and symptoms that one should look for when hiring morgue attendees and others working in sensitive legal and highly personal positions will be explored.

This presentation will explore the case of a morgue attendant with necrophilia. The individual's case will be described in detail. This will include details of the criminal case as well as results of an in-depth assessment of the individual. The prevalence and typologies of necrophilia will be explored. This presentation will include original footage of interviews with the morgue attendant.

Using this case example as a departure point, a detailed review of the history of necrophilia in both documented case law (ancient to modern) and the occult will be presented. Also discussed will be proscribed traditions for dealing with corpses of the deceased from various cultures. Furthermore, in modern day U.S. law, cross jurisdictions consider necrophiliac behaviors with considerable difference in severity of offense. That is, some states code abuse of a corpse as a misdemeanor while others consider it a felonious action.

The ethics of treating necrophiliacs who are criminally prosecuted with such disparity of punishments will be considered as well. Treatment for necrophiliacs will be reviewed in light of more recent breakthroughs in the rehabilitation of sex offenders.

Necrophilia, Coroner, Paraphilia

#### **I4** Facebook: Friend or Foe? Cyberbullying, Stalking, and the Practical and Forensic **Applications of Social Networking Sites and Technology Facilitated Crime**

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The goal of this presentation is to review the criminal justice literature on social networking and other internet facilitated sites to gain a better understanding of the uses and dangers of these internet sites from a forensic psychiatry and criminal justice perspective. Cases on cyberbullying will be reviewed; sexual misuses of these sites especially among teenagers will be discussed, along with cases involving stalking. Finally, the potential uses of social networking in forensic cases to solve crimes will be discussed, along with the risks vs. benefits and ethical dilemmas of professionals in the forensic disciplines and medicine using these sites for their own personal and professional networking.

This presentation will impact the forensic science community by illustrating the growing importance of internet sites on multiple forensic disciplines and the relevance for all forensic scientists to have a basic understanding of social networking and other internet sites.

Facebook, Twitter, and other social networking sites (SNS) have become a central aspect of the social life of many teenagers as well as adults. An estimated 14 million children ages 12-17 used social networking sites in 2006. An additional 35% of American adults had a profile on a social networking site in 2008 (Mitchell, Finkelhor, Jones, Wolak, 2010). With the growing popularity of these sites, there has been significant media attention to crimes and other dangers that may be associated with them. The number of social networking site continues to grow, yet there is little research on exactly how or if these social networking sites may be facilitating criminal activity. There is a need for more data on the actual relationship between SNS and crime.

The literature and cases in the media pertaining to the relationship between social networking sites and sex crimes, child abduction cases, stalking, and other types of online predation will be discussed. Recent media attention to prominent cases of suicide as a result of cyberbullying will also be discussed. The potential dangers of online victimization will be reviewed from a psychiatric and criminal justice perspective. The importance for those in the forensic sciences to have a basic understanding of the internet when investigating criminal activity related to the internet or evaluating victims and predators involved with social networking sites will also be discussed.

The use of SNS in criminal investigation will be discussed. While advances in technology and the increasing amount of information which can be recovered through that technology may aid investigations in some circumstances, there is little empirical research on the role of the internet and technology in investigations. Another investigational challenge is the wide range of crimes that may be technology facilitated, and how to effectively link the offender to the victim. In addition, the importance of recognizing the special nature of the internet when performing forensic evaluations pertaining to internet related offenders and victims will be addressed.

Finally, the risks and benefits and ethics of the personal and professional use of SNS by those working in healthcare and the forensic sciences will be discussed. The role that social networking may play professionally in communications and collaborations in business and in higher education will be discussed. The problems and special considerations that these internet sites may create for forensic evaluations will also be discussed. Because of the fluid, ever changing nature of the internet and social networking sites, it is vital that law enforcement and forensic professionals come together to effectively explore the role we play both personally and professionally in cyberspace.

Additional and continued research in this area is needed. In particular, careful attention should be placed on online harassment prevention programs, parental education on how to keep children safe online, the changing nature of social dynamics online, better reporting mechanisms for victims to report technology facilitated crimes to police, and increasing the public accountability for behavior on the internet. Finally, further training on the internet and SNS should be made available to law enforcement personnel to assist them in their investigation and response to technology facilitated crimes.

Social Networking, Internet, Crime

# I5 Sexual Sadism: Its Association With Paraphilia and Psychopathy Traits

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After attending this presentation, attendees will have a better knowledge of sexual sadism and its association with other paraphilias and psychopathy. There will be additional clarification on how sadistic fantasies and behavior coalesce.

This presentation will impact the forensic science community by shedding further light on how to discriminate between sexual sadism and other disorders in order to facilitate its assessment.

**Introduction:** Sexual sadism can be described as the experience of sexual pleasure produced by acts of cruelty and bodily punishment (Krafft-Ebing, 1886). Characteristics such as torture, mutilation, humiliation, and sadistic sexual interest are often associated with sexual sadism. It is almost impossible to determine the true prevalence of sexual sadism due to a lack of reliability and validity. One reason behind this lack of reliability and validity is the overlap of sexual sadism with other disorders such as psychopathy and other paraphilias.

Hart and Hare (1997) assumed a relationship between psychopathy and sadism. Since then, little research has been conducted on this topic, which is surprising considering the commonalities between psychopathy and sexual sadism. According to Porter and Woodworth (2006), the relationship between these two disorders is sexual pleasure derived from violent acts. At the affective level, sexual sadists and psychopaths share a number of characteristics such as a lack of remorse, a lack of guilt and a lack of empathy. In other words, they are cold hearted and emotionally detached. According to Cooke (2001), most sadists are likely to show significant psychopathic traits, while not all psychopaths are sadists.

On the other hand, sexual sadism is commonly co-morbid with other paraphilias. Sexual sadism is classified by the DSM IV TR (Diagnostic and Statistical of Mental Disorders) as a paraphilia, on axis I. Early works from Abel and al. (1988) show that most paraphiliacs have significant experience with as many as ten different types of deviant sexual behaviors. However, there is a tendency of some research to disagree with this assumption (Marshall, 2007; Nietschke, Blendl, Otterman, Osterheider & Mokros, 2009).

**Material and Methods:** The research was conducted on a sample of adult sexual offenders (n = 528) who were assessed in forensic institutions located in Massachusetts and Minnesota with the Multidimensional Inventory of Development, Sex and Aggression (MIDSA). The MIDSA is a computerized, self-report inventory that provides a clinical report to support therapeutic interventions with sexual offenders. Three dimensions (12 sub-scales) from the MIDSA were used: the sexual sadism (sadistic fantasy & sadistic behavior), the psychopathy-related and hypermasculinity scales (lack of empathy, lack of perspective taking, cunning and superficial charm, impulsivity, negative masculinity/toughness & hostility towards women) and the paraphilia scales (voyeurism, exhibitionism, transvestism, scatologia & fetishism).

**Results:** *Sadism Scales:* Analysis on the sadism scales shows a strong correlation (r(526) = 0.793, p<0.001) between the presence of sadistic fantasies and behavior.

*Psychopathy-related/Sadism Scales:* The analyses shows small to moderate correlations between the presence of sadistic fantasies and lack of perspective taking (r(526) = 0.361, p<0.01), impulsivity (r(526) = 0.377, p<0.01) and the presence of callous-unemotional (r(526) = 0.334, p<0.01) and cunning traits (r(526) = 0.381, p<0.01). Similar correlations were observed between sadistic behaviour and lack of perspective taking (r(526) = 0.408, p<0.01), impulsivity (r(526) = 0.355, p<0.01) and the

presence of callous (r(526) = 0.336, p<0.01) and cunning traits (r(526) = 0.380, p<0.01).

*Paraphilia/Sadism Scales:* The analysis shows fairly high correlations (r(526) = 0.532, p<0.01) between sadistic fantasies and behavior and the presence of other paraphilia. A moderate correlation was obtained between sadistic fantasy and voyeurism. Moreover, similar correlations (r(526) = 0.469, p<0.01) were observed between sadistic behavior and voyeurism.

**Conclusion:** This study presents interesting points which clarify the concept of sexual sadism. Sadistic fantasy and behavior seem to work in similar fashion and correlate with the same scales. The diagnosis of sexual sadism is often associated with severe sentences, such as the designation of Dangerous Offender (Canada) or Civil Commitment (USA). Therefore, it is necessary to have a better understanding of sexual sadism in order to adequately assess the risks and needs of offenders. Accurate diagnosis is essential for effective treatment of any condition.

Sexual Sadism, Psychopathy, Paraphilia

#### I6 The Elderly: Two Cases of Rape

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After attending this presentation, attendees will gain information relating to unusual sex crimes committed by the elderly described in the literature.

This presentation will impact the forensic science community by presenting an isolated socio-cultural milieu that seems to have played a fundamental role in these cases.

Many investigations conducted in recent years (UK Home Office, 2003; Fazel & Jacoby, 2002; Uzoaba, 1998; Greenfield, 1997) have confirmed an increase in the number of sentences for sexual offenses committed by elderly subjects. It is also known that unlike other crimes, the impulse to commit rape tends to persist despite advancing age (Alston, 1986).

Moreover, epidemiological studies have long since pointed out a high prevalence of mental disturbances among elderly subjects committing sexual offenses (Essen-Muller, 1956; Kivela et al., 1988), exceeding 50% according to some authors (Fazel & Grann, 2002). In such cases the most common diagnosis is Dementia (Series & Dègano, 2005), often associated with behavior defined as "hypersexuality," characterized by a poor control of sexual impulses and a marked sexual disinhibition. This can culminate in criminal acts.

However, other authors (Alston, 1986; Eysenck & Gudjonsson, 1989) have pointed out that the elderly are more likely to commit "non violent" sexual crimes (indecent exposure or pedophilia) than sex with violence (rape and murder), that are more commonly perpetrated by younger subjects (Taylor & Parrott 1988). The elderly prefer to commit such acts against minors (Hucker, 1984; Poortinga et al. 2007) because young victims are less able to defend themselves. The crime is generally committed in the elderly rapist's home or that of the young victim (McNamara & Walton, 1998).

By contrast, the elderly are also often the victims of violent acts including rape (Faugno et al., 2010), largely due to their reduced autonomy and isolation within the home.

Two cases of rape committed by elderly subjects were reviewed, as expert consultants for the judge, leading to a more in-depth reflection on this issue.

**Methods:** The two cases described are unlike those in literature but are both similar, in terms of various factors including: the socio-cultural

context (small highland villages in Southern Italy), the characteristics of the rapist (retired widowers over the age of 70 with a family and adult children, with no mental disturbance or homosexual experience) and of the victim (adult males over 50 years of age, with mental retardation and speech difficulties, unfit for work, and well known in the village), the type of crime (rape episodes continuing for more than one year), the final involvement of the police (after the crime was reported by unrelated inhabitants of the village), the rapist-victim relationship (simple acquaintances), the police investigations (video-recording of meetings); the medico-legal examinations (of the victims) and psychiatric-forensic investigations (of the victims and rapists).

Various points that emerged from a close study of these cases will be described with their common features and peculiarities.

**Conclusions:** In the two cases report, that are both similar, but different from the usual types of sex crimes committed by the elderly described in the literature, the same isolated socio-cultural milieu seems to have played a fundamental role, not only in terms of the type of crime committed but also of how the authorities finally came to be notified. **Elderly, Rape, Mental Disorder** 

#### **I7** Coexistence and Independent Relation Between Mental Disturbance and Stalking

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After attending this presentation, attendees will understand the relation between psychopathology and criminal motivations that are at the basis of the female stalking.

This presentation will impact the forensic science community by presenting an unusual case of a female stalker diagnosed with bipolar disorder.

**Introduction:** There are many known features of stalking, ranging from the definition (Meoly, 1998), characteristics of reiteration (Hege, 2005), continuity and persistence over time (Hall, 1998), to the underlying motivations. In this scenario, some have reported a significant correlation with mental disturbances, including erotomania (Zona, 1998), psychosis (Silva, 2000), personality disorders (Meoly, 1998), although the stalking is considered to be less strongly correlated with mental disorders.

Most stalkers are male (Budd, 2000), and the most dangerous are "rejected" and "resentful" (Mc Ewan, 2009), as defined by Mullen (1999), namely men who cannot accept the end of a relationship or are rejected by their partner and develop the habit of tailing their victim – generally a woman – in a sadistic game (Hege, 2005), while they get ready to strike (Palarea, 1999). A meta-analysis (Spitzberg 2002) confirmed the data.

Few studies have been made of female stalkers.

**Objectives:** Objective of this work is to contribute to the knowledge of female stalking. The data on this phenomenon depend on the population considered: the prevalence in the general population is 12-13% (Tjaden, 1998), whereas in selected samples from the psychiatric-forensic community there is a higher percentage, ranging from 28% (Purcell, 2001) to 32-33% (Zona, 1993; Harmon, 1995). In the latter group the male-female stalker ratio is 4:1 (Purcell, 2001).

Unlike male stalkers, few female stalkers have a criminal record (Purcell, 2001); typically, they are young, white, heterosexual, single, childless and highly educated (Meloy, 2003). In an overwhelming majority of cases the woman knows her victim personally, often on a professional basis (Purcell, 2001). She is acting in revenge in 2/3 of cases (Meloy, 2003) and the closer the pre-existing relationship between the stalker and her victim, the greater the risk of violent acts of

persecution. In his meta-analysis, West (2008) underlines the "smart" tactics adopted by the female stalker to torment her victim. There is no reason to believe that female stalkers are less dangerous than their male counterparts. Characteristically, a high proportion of victims of the same sex has been reported (Purcell, 2001).

Moreover, studies have shown that unlike in male stalkers, in females there is a high incidence of severe mental disorders and insanity. According to Meloy (2003), at the start of their stalking acts, 50% of these cases have a diagnosis of "psychosis." These are Axis I Disorders (Delusional Disorder, Paranoid Schizophrenia, Mood Disorder). There are also a high percentage of personality disorders, especially cluster B (Kamphuis, 2000). However, there are a lower percentage of disorders due to substance addiction than in male stalkers (West, 2008). Thus, there seems to be a greater prevalence of psychopathology motivations among female stalkers.

**Methods:** The case is a 43-year-old woman, university professor, single and childless, with a history of a long affair with the victim that ended in 2007. In July 2009 she was reported by the ex-partner's mother for acts of persecution (continuous phone calls, slanderous remarks, stalking, murder threats) against herself and her son, who had moved to another city.

Diagnosed with a bipolar disorder that had developed in 2003, the woman was in treatment since 2005. The woman's mother, who had committed suicide by throwing herself out of the window in 2003, was also affected by a bipolar disorder. The acts of stalking had started in January 2009, and lasted for six months, during which period the woman was not taking her prescribed drugs. After being reported to the police, she resumed treatment, and derived benefit. In December 2009, despite her medicated condition and the fact that she was still under forensic-psychiatric observation, she resumed the same stalking behavior she had previously abandoned.

**Conclusions:** The peculiar characteristic of this case is the fact that despite being well medicated the woman continued the same criminal acts. This demonstrates that even when there seems to be an evident relation between psychopathology and criminal motivations, it is always necessary to determine if, or to what extent, the mental disorder, even if severe, is at the basis of the criminal behavior.

Female Stalker, Mental Illness, Insanity

#### I8 An Unusual Homicide by Ligature Strangulation – Incaprettamento (a.k.a. Trussed Goat) Committed by a Retired Foreign Legionnaire

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The goal of this presentation is to identify some of the correlations between anthropological and cultural factors associated with a particular kind of homicide, where a rope is used to tie the neck of the victim to his or her wrists and ankles behind the back. This case study, offers the opportunity to analyze the interaction between these factors and those derived from the experience of a rigid military environment such as that of the Foreign Legion.

This presentation will impact the forensic science community by bringing to light possible further necessary research in the cross-cultural perspective, as well as, analysis of the typical dynamics of a rigid military environment.

The case presented here concerns an unusual type of strangulation homicide whereby a rope is passed around the victim's wrists, ankles, and throat resulting in suffocation by auto-asphyxia. The literature usually refers to this method as "incaprettamento" (i.e., "trussed up like a goat"), a method employed by organized crime groups such as the Italian Mafia, but also used in the execution of war crimes, or in various other cultural-anthropological contexts. This method of killing is meant to impart a particularly degrading and humiliating revenge on the victim. It also serves as sign of intimidation and a warning to others.

To all appearances, the case presented here is different than those usually reported in the literature. The lifeless body of a 48-year-old Tunisian man, found hanging from an iron rod placed between two electrical pylons, was discovered in the countryside of a small southern Italian town.

The perpetrator of the crime was identified as a 32-year-old Tunisian man. He had earned his university degree in philosophy in 2002. In 1998, he began a military career as a non-commissioned army officer, subsequently enlisting into the Foreign Legion. Training in the Foreign Legion is particularly rigid and severe. Service in the Legion is based on the highest trust in both the institution and fellow soldiers. Betrayal is considered to be an act of the greatest dishonor.

Following psychiatric evaluation, the subject was diagnosed with a personality disorder. It was discovered that the victim, a man whom the perpetrator had previously trusted, had "betrayed" the perpetrator on several levels.

The unusual way in which the killing took place, along with its symbolic meaning, seems to have both military and anthropological origins that reflect the culture to which the perpetrator belonged.

Homicide, Ligature Strangulation ("incaprettamento"), Foreign Legion

# **I9** Study on P300 and P50 and Cognitive Disorder of Patients After Brain Trauma

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After attending this presentation, attendees will learn about the application of Event Related Potentials (ERP) in forensic science in mainland China.

This presentation will impact the forensic science community by discussing the new study results of the ERP application for cognitive dysfunction patients.

Background: The assessment of cognitive function in patients with brain trauma in forensic psychiatry was mainly depended on psychiatric interview, inquiry materials and medical history. Although some items of neuropsychological testing, the relatively objective indicators, were applied in the disability evaluation after brain trauma, the reliability and veracity of the tests were influenced by the cooperation of experimenters and the qualification of evaluators. In recent years, the Event Related Potentials (ERP) have received more attention as more objective indicators for cognition. Some studies had proved that the latent period of P300 (a component of ERP) was extended, the amplitude was declined, and the amplitude of P50 (another component of ERP) was significantly different form the normals. However, these existing studies were mostly grouped and contrasted by the degrees of primary injury, the relationship between cognitive dysfunction caused by brain trauma and ERP was explored very little. Therefore, it is necessary to determine the relationship and to provide more objective auxiliary indicators for the disability evaluation after brain trauma.

**Aim:** The goals of this research are to: (1) to explore the application of neuropsychological tests during the evaluation of cognitive disorder in brain trauma patients; and, (2) to analyze characteristics and differences of waveforms of P300 and P50 of brain trauma patients with different degrees of cognitive disorder respectively,

in order to provide more scientific and objectives auxiliary indicators for the identification of cognitive disorder of patients after brain trauma.

Method: (1) the subjects were the interviewed in the Institute of Forensic Science from July 1, 2009 to December, 31, 2009. The subjects were selected based on the following criteria: six months after brain trauma, dextromanuality, and could coordinate the test, excluding those with neuropsychiatric disease existing before the trauma and those with psychotic symptoms after the trauma; (2) the following neuropsychological tests were conducted on the subjects: the items of block design, picture completion and similarities in the Wechsler Intelligence Scale for Adult-China Revised (WAIS-RC), the items of long-term memory (LTM) and short-term memory (STM) in the Wechsler Memory Scale - China Revised (WMS-RC), and the visual retention, simple visual reaction time, length discrimination and digit cancel in the fourth set of Computer - administered Neurobehavioral Evaluation System (NES-4). The P300 and P50 were examined by the BrainMaster, and the sites of electrode was according to the international 10-20 electrode system; and, (3) the subjects were grouped into mildinjury, moderate-injury and severe-injury groups according to the degrees of their primary injury and grouped into mild-cognitive dysfunction, moderate-cognitive dysfunction and severe-cognitive dysfunction groups according to the experts' opinion. Finally, the data were analyzed using analytical software: one-way ANOVA for differences among groups and the Least-Significant Difference (LSD) method for advanced pairwise contrast.

**Results:** (1) No significant difference was discovered in the tests of block design, information, similarities, length discrimination and digit cancel among the mild-injury, moderate-injury and severe-injury groups. The difference in the tests of picture completion, LTM, STM, visual retention and simple visual reaction time among groups were proved to be significant. The scores of LTM, STM, visual retention and simple visual reaction time among youp were higher than that in the moderate and severe-injury groups (p<0.05). In the advanced pairwise contrast; however, no significant difference appeared between moderate and severe-injury groups. The scores of picture completion in the mild-injury groups were lower than the moderate and severe-injury groups (p<0.05). The above results suggest that part of the neuropsychological testing, especially the intelligence tests, could not reflect the correlation between the degree of primary injury and the cognitive dysfunction correctly.

(2) The scores of LTM, STM, visual retention, visual reaction, length discrimination, and digit cancel were decreased following the ingravescence of the cognitive dysfunction, and the differences in the LTM, STM, visual retention, and simple visual reaction time among the mild, moderate and severe-cognitive dysfunction were identified to be significant. The significant differences also proved to exist in the advanced pairwise contrast (p<0.05). The scores of block design, picture completion, information and similarities between the mild and severecognitive dysfunction group were similar and were lower than that in the moderate-cognitive dysfunction group. Significant differences existed in the picture completion, information and similarities tests. The indicators of neuropsychology, such as the memory, memory-visual, memoryneurobehavioral, psychomotor performance, and apparent reaction rate could reflect cognition effectively.

(3) No significant differences were discovered in the latent period and amplitude of N200 and P300 potential in the sites of Fz, Cz, Pz, T3 and T4 in the control group by one-way ANOVA analysis, so as the P50 potential examination.

(4) In the N200 potential, the latent period was extended and the amplitude was declined following the severity of cognitive dysfunction.

(5) The differences in the latent period and amplitude of P300 potential between the cognitive dysfunction group and the control group proved to be significant, and its latent period was previously prolonged in the moderate and severe-cognitive dysfunction group compared to the mild-cognitive dysfunction and control group (p<0.05). However, no significant differences proved to exist between the control and mild-
cognitive dysfunction group, or between the moderate and severecognitive dysfunction group. The differences in the amplitude of P300 potential between the cognitive dysfunction group and the control group were significant, and the amplitudes were lower in the moderate and severe-cognitive dysfunction group compared to the mild and control group (p<0.05). No significant differences were found between the mild and the control group or between the moderate and severe group.

(6) The correct rate of P300 in the control group and cognitivedysfunction group was significantly different (p<0.001), and the significant differences also existed in the advanced pairwise contrast analysis (p<0.05).

(7) No significant differences in the latent period and amplitude of S1-P50 were discovered between the cognitive dysfunction group and the control group. The difference in the amplitude of S2-P50 proved to be significant; however, this difference was not discovered in the latent period performance of S2-P50. The amplitude of S2-P50 was higher in the moderate and severe-cognitive dysfunction groups compared to the mild and control groups (p<0.05). However, this difference between the moderate and the severe-cognitive dysfunction groups and the severe-cognitive dysfunction groups, was not significant.

(8) The differences in the inhibition rate of P50 potential between the cognitive dysfunction group proved to be significant, and it was higher in the moderate and severe-cognitive dysfunction groups compared the mild and control groups (p<0.05). No significant differences were found between the mild and the control group, or between the moderate and the severe-cognitive dysfunction group.

(9) The difference in the correct rate of P50 potential between the cognitive dysfunction group and the control group proved to be significant (P<0.001), and it was lower in the mild-cognitive dysfunction group compared to the moderate-cognitive dysfunction group (p<0.05). No significant difference was found between the mild and the severe-cognitive dysfunction group ot between the moderate and the severe-cognitive dysfunction group.

**Conclusion:** (1) The degree of primary brain trauma was not absolutely parallel with the degree of cognitive dysfunction: The patients with mild-cognitive dysfunction were more likely to exaggerate or feign dysfunction in the neuropsychological tests; (2) the P300 potential could be used as an objective indicator to reflect the degree of cognitive dysfunction, while the latent period of P50 potential could not measure the degree of cognitive dysfunction effectively; and, (3) the P300 and P50 potentials all could act as objective indicators for the identification of feigning symptoms, especially in the mild-cognitive dysfunction patients.

Forensic Psychiatry, Event Related Potential, Brain Injuries

### I10 Implications of Antisocial Parents: The Effects and Impact of Antisocial Parents on the Developing Child

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After attending this presentation, attendees will become familiar with distinguishing characteristics of antisocial personality disorder, psychopathy, and sociopathy. Attendees will also gain a better understanding of the development of antisocial behavior from a genetic, biological, and environmental perspective. Finally, attendees will learn about the effects of parents with antisocial behavior on children and the impact that this behavior has on the development of children.

This presentation will impact the forensic science community by helping to further understand the development of antisocial behavior in children who have antisocial parents as well as the transmission of antisocial behavior from parents to children. Antisocial behavior is a socially maladaptive and harmful trait to possess in the general population. This can be especially injurious for a child who is raised by a parent with this personality structure. The pathology of antisocial behavior implies traits such as deceitfulness, irresponsibility, unreliability, and an incapability to feel guilt, remorse, or even love. This is damaging to a child's emotional, cognitive, and social development. Parents with this personality makeup can leave a child traumatized, empty, and incapable of forming meaningful personal relationships.

There is a significant stability of antisocial behavior across generations, and both genetic and environmental factors influence the development of antisocial behavior. Numerous twin studies as well as studies involving adopted children have confirmed the strong heritability of antisocial behavior.<sup>1,2</sup> Moreover, the child with a genetic predisposition to antisocial behavior who is raised with a parental style that triggers the genetic liability is at high risk for developing the same personality structure. Parental negativity and warmth moderate the influence of genetic factors on the development of antisocial behavior in adolescents.<sup>3</sup>

In addition, adolescents' perceptions of parents' activities and inept parenting practices<sup>4</sup> provide two nongenetic routes of transmission of antisocial behavior from parents to adolescents. The child's awareness of the parent's behavior establishes a cognitive mechanism through which behavior is transmitted. Antisocial individuals are impulsive, irritable, and often have no concerns over their purported responsibilities. As a parent, this can lead to erratic discipline, neglectful parenting, and can undermine effective care-giving. Therefore, these parents lack consistency and discipline skills and are more likely to use hostile and coercive methods.<sup>5</sup> Thus, they are likely to inadvertently reinforce aggressive and antisocial behavior.

Other factors such as abuse,<sup>6</sup> head injury,<sup>7,8</sup> and separation<sup>9,10</sup> also influence the development of antisocial behavior.

Simply having knowledge of this will not necessarily alter the behavior or attitude of antisocial parents. The parents may be likely to minimize the evidence of the effects of their antisocial behavior on their children, thus allowing the behavior to continue. Moreover, inherent in the personality makeup of these individuals, be it antisocial, psychopathic, or sociopathic, are a lack of remorse, impulsivity, and disregard for others. These traits further lessen the chance that a parent will modify his or her behavior because of the likelihood that his or her child is modeling after it.

Therefore, not only is there trauma and often abuse in these families, but also there is a transmission of antisocial behavior from parents to children. This presentation will focus on the implications of parents with antisocial behavior and the impact that this behavior has on attachment as well as on the development of antisocial traits in children. **References:** 

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Antisocial Parents, Transmission of Antisocial Behavior, Good Parenting vs. Poor Parenting

#### I11 An Update on Juvenile Psychopathy and Development

Christopher R. Thompson, MD\*, 10850 Wilshire Boulevard, Suite 850, Los Angeles, CA 90024; and Lauren Reba-Harrelson, PhD\*, University of Southern California Institute for Psychiatry and Law, Keck School of Medicine, PO Box 86125, Los Angeles, CA 90086-0125

The goals of this presentation are to briefly review the construct of psychopathy and youth psychopathy instruments, examine developmental concerns that arise from application of the concept to youths, and to explore the validity and utility of this concept in the juvenile population.

This presentation will impact the forensic science community by helping forensic evaluators learn the limitations of the concept of psychopathy in juveniles and learn the limitations of certain psychopathy instruments in predicting recidivism in juveniles, particularly females and ethnic minorities.

Psychopathy is a construct with which most forensic practitioners are quite familiar. The presence of this "condition" in adults is considered by many one of the best predictors of general and violent recidivism and, to some extent, amenability to treatment. Recently, the concept of juvenile psychopathy has been proposed and numerous instruments have been developed to qualify and quantify the component traits in children and adolescents.

Labeling a child or adolescent as a psychopath, however, may be problematic for a variety of reasons. Transient, normative developmental phenomenon, and behaviors may be mistaken for fixed, maladaptive, malignant personality patterns. Since "amenability to treatment" and future dangerousness are often important considerations in the juvenile justice system (and to some extent, the adult criminal justice system), this may lead to lengthy, and perhaps unnecessary periods of incarceration for juveniles erroneously identified as psychopaths.

Currently, researchers are attempting to determine whether psychopathy is a valid construct in juveniles and whether its component traits are stable over time (and if so, which ones). However, this research is relatively new and has not yet confirmed or disconfirmed the validity or predictive utility of juvenile psychopathy, particularly in adolescent females and ethnic minority members. Over time, instruments may be developed and employed that will more accurately predict which juveniles are likely to continue offending as adults (particularly violently offending). This should lead to more just legal outcomes for minors, help protect the general public, and preserve the dignity and integrity of the legal process.

Psychopathy, Antisocial Behavior, Juvenile

#### I12 Juvenile Crimes and Substance Abuse

Manuel Lopez-Leon, MD\*, New York University School of Medicine, 276 5th Avenue, Suite 307-A, New York, NY 10001

After attending this presentation, attendees will become aware of the trends in drug related crimes perpetrated by juveniles, as well as, the trends of the types of drugs involved in juvenile arrests, and will be familiarized with the role and effectiveness of juvenile drug courts.

This presentation will impact the forensic science community by raising awareness of the alarming trends in drug related arrests in juveniles, and the effectiveness of juvenile drug courts and diversion programs.

There has been an upward trend in the number of teenagers arrested for drug-related crimes. Recent statistics reflect that almost 12% of all arrests for drug-related crimes have been juveniles. More than twomillion juveniles have been arrested for drug related crimes within the last 15 years. However the majority of them were arrested for possession of drugs. The number of arrests has increased; however, the types of drugs involved have changed over the recent years. The arrests relating to drugs such as cocaine and heroin have declined, while arrests relating to marijuana have increased significantly. The offenders older ages are directly related to the number of arrests. Recent statistics also demonstrate that white teens accounted for almost three-quarters of all the arrests, followed by black teens at almost one-quarter, and teens of Native American, Asian, or Pacific Islander descent account for less than two percent.

Juvenile justice agencies have embraced the drug court concept as a relatively new method of addressing adolescent substance abuse. This popular innovative idea was imported from adult courts. Specialized drug courts provide judicial supervision and coordinate substance abuse treatment for drug-involved juvenile offenders. Traditional juvenile courts still handle the vast majority of cases, however, fortunately the juvenile drug programs have been rapidly increasing changing the way practitioners and politicians think about the challenge of adolescent drug use. Drug courts provide juveniles the opportunity to benefit from drug diversion programs, change their behaviors, and stop their illegal drug use. As in adult drug courts, those who complete the program may have their sentences reduced, and in some cases even completely dismissed. Even though up until recent years drug courts were considered experimental, there is evidence demonstrating substantial reduction in new adjudicated charges, as well as significant reductions in the proportion of youth categorized as chronic offenders. This has been achieved through the incorporation of many evidence-based principles demonstrated to be effective at decreasing delinquency and adolescent substance use through comprehensive treatment planning, judicial supervision, family engagement, community partnership, and cognitivebehavioral approaches.

Juveniles, Drugs, Crimes

## I13 Problematic Sexual Behavior Among Juveniles: A Research Update on Identification, Risk Assessment, and Treatment

Abigail M. Judge, PhD\*, Children & the Law Program, 388 Commonwealth Avenue, Lower Level, Boston, MA 02215; and Fabian M. Saleh, MD, Law and Psychiatry Service, Massachusetts General Hospital, Boston, MA 02114

After attending this presentation, attendees will be familiar with the most current empirical literature on Problematic Sexual Behavior (PSB) in juveniles across three domains: phenomenology, risk assessment, and

treatment. Attendees will understand the clinical implications of these findings and develop an appreciation for the strengths and limitations of the available scientific literature.

This presentation will impact the forensic science community by providing attendees with a synthesis of the most current scientific literature on this pressing public health concern.

This presentation will provide a research update on three key topics related to PSB in juveniles: identification, risk assessment, and treatment. First, this presentation will review data on the prevalence and phenomenology of juvenile PSB, including common comorbid conditions, and possible etiologies. The importance of a developmental perspective will be emphasized, including knowledge about normative sexual development during adolescence, and the complexity of defining non-normative sexual behavior in this cohort. Second, this presentation will articulate a model for the practice of developmentally sensitive risk assessment with youth who engage in PSB. Given that PSB is not in itself a psychiatric diagnosis and no definitive actuarial measure of risk exists, empirically supported principles of risk assessment will be presented. Third, this presentation will conclude with a critical review of published outcome research on the treatment of PSB, including the limitations of extant data and future directions.

Juvenile, Sexual Behavior, Risk

#### I14 Adolescents, Sex and the Forensic Psychiatrist

Eugene Lee, MD\*, and Stephen B. Billick, MD\*, 11 East 68th Street, Suite 1B, New York, NY 10065-4955

After attending this presentation, attendees will be able to appreciate complexities inherent in the evaluation and understanding of adolescent sexuality, list key differences between adolescents and adults which would be considered in the comprehensive forensic evaluation, and give examples of human behaviors scientifically accepted as normal which may not always be legal or socially acceptable.

This presentation will impact the forensic science community by increasing awareness that adolescent expressions of sexuality warrant special considerations for forensic scientists.

**Hypothesis:** The forensic evaluation of developmental sexuality in adolescents raises several special issues different from the evaluation of adults.

Interest in sexuality and sexual behaviors increases throughout one's normal human development from birth into adulthood. Sigmund Freud postulated on the stages of psychosexual development, but it is now scientifically known that "latency" does not exist in real children. Rather, children learn that adults are often uncomfortable with childhood sexual behaviors, and so the children learn to keep sexuality private. As the child's body and mind mature—and especially with the onset of puberty—there is an escalation in sexual interest and activities during adolescence. Helping adolescents navigate their sexual desires, behavior, and identity can be clinically challenging.

Complicating this clinical treatment are forensic aspects of adolescent sexuality. The age of consent for sexual activity is a legal, cultural, and societal construct; socially accepted commencement of clinically mature sexual behaviors has changed over time and varies by country. The legal imposition of laws and regulations are sometimes at odds with physiologic and psychological understandings of human behavior. Biologically, humans are designed to have sexual behaviors in adolescence; in the past adolescents regularly married and procreated. This is still the case today in many cultures. Even as teenage pregnancy may be biologically normal, it can be quite disadvantageous for a developing teenager in a technologically advanced society where education is often required well beyond adolescence. Despite this fact, there is controversy in America regarding sexual education, knowledge and access to contraception, and the right to abortion for adolescents. The disparity between autonomous sexuality versus legal and societal controls can create psychological stress for adolescents. The rights and responsibilities of parents will be discussed, as well as self-determination and autonomy in the developing adolescent. Adolescence is a period of time when there is generally decreasing parental controls with simultaneously increasing adolescent autonomy. The balance at any given moment in the process can be difficult for the adolescent, the parents and the clinician. This presentation will include several clinical vignettes as springboards for discussion and sharing of clinical strategies amongst the audience.

Controversial American policies and practices reflect the conflicting findings of science, ethics, morality, and religion. One example is in the minimization of harm, specifically with efforts for the prevention of infection as well as unplanned or unwanted pregnancies. As another example, 18-year-old males who are sexually active with 16year-old females may face statutory rape charges in some jurisdictions. Furthermore, although it is scientifically accepted that same-sex desires, behaviors, and identity are normal occurrences within the broad spectrum of normal human sexuality, homosexuality is illegal in some jurisdictions of the United States and in the world. Lastly, there is controversy regarding the presence of Gender Identity Disorder in DSM-IV-TR as a mental illness. Psychiatric diagnoses sometimes are necessary to obtain medical and surgical interventions. In the early days of receiving legal abortions, some jurisdictions required a psychiatric clearance prior to performing them. Psychiatric consultations are also obtained frequently in evaluating kidney donors. Issues of stigma and necessity for psychiatric diagnosis will be discussed especially as related to adolescent sexuality.

Forensic Psychiatry, Adolescent, Sexuality

#### I15 Evolution of the Psychological Autopsy Over 50 Years: Los Angeles County Coroner Medical Examiner's Experience

Timothy Botello, MD\*, University of Southern Cailfornia, Institute of Psychiatry & Law, PO Box 86125, Los Angeles, CA 90086-0125; Thomas T. Noguchi, MD, 1135 Arlington Avenue, Los Angeles, CA 90019-3515; Lakshmanan Sathyavagiswaran, MD\*, Los Angeles County, 1104 North Mission Road, Los Angeles, CA 90033; Linda E. Weinberger, PhD\*, University of Southern California, Institute of Psychiatry & Law, PO Box 86125, Los Angeles, CA 90086-0125; and Bruce H. Gross, JD, PhD\*, University of Southern California, Institute of Psychiatry & Law, PO Box 86125, Los Angeles, CA 90086-0125

After attending this presentation, attendees will understand the history of the psychological autopsy method used by the Los Angeles County Coroner Medical Examiner's office over the past 50 years, and understand how legal challenges to the confidentiality of the psychological autopsy were handled.

This presentation will impact the forensic science community by encouraging the adoption of the psychological autopsy method in other Coroner Medical Examiner's offices in contested and equivocal suicide cases.

The nation's first known psychological autopsy, as an investigative tool, was first used in 1960 at the Department of Chief Medical Examiner-Coroner, in the County of Los Angeles. A historical review of the past 50 years of the psychological autopsy will be presented, which began as a collaboration between the then Chief Medical Examiner-Coroner, Dr. Theodore J. Curphey, and mental health professionals from the L.A. Suicide Prevention Center, including Drs. Litman, Schneidman, et al. A National Institute of Mental Health (NIMH) grant on suicide prevention provided funding for the project in order to find clues to suicide. The earliest psychological autopsies were performed on equivocal cases where accident or suicide could not be clearly determined. Several equivocal suicide cases per year were selected and reviewed. Face-to-face as well as telephone interviews and record reviews were conducted. In 1960, the Office of the Chief Medical Examiner-Coroner did not have its own investigator program, and had to rely on police reports regarding the death scene and background information, which often were less than optimal and not reliable for the conclusion about the manner of death. Meetings were held twice a month to review cases and to better understand the causes and potential treatment for suicide. In 1962, following the high profile death of Marilyn Monroe, Dr. Curphey appointed a panel of psychological experts (dubbed by the newspapers as "The Suicide Panel"), in addition to a large number of staff, who performed face-to-face interviews of Monroe's relatives, friends, business associates, and treating psychiatrist. The people interviewed were promised confidentiality in order for them to speak openly on intimate matters-a key element in the investigation of death. From 1967-1982, when Dr. Noguchi became the Chief Medical Examiner-Coroner, he was able to pay a small fee per case to replace the NIMH funding for psychological autopsies, which had been streamlined using telephonic interviews. Dr. Noguchi fostered the use of the psychological autopsy by using mental health experts, as he utilized other consultants in difficult cases, to help resolve the manner of death. In the early 1970s, Dr. Noguchi named the meeting where equivocal cases were discussed as the Mode Conference to help the Chief Medical Examiner-Coroner resolve the manner of death. It has been the tradition of the LA County Chief Medical Examiner-Coroner's Office to continue to name it as the Mode Conference. Also, in the early 1970s, Dr. Noguchi approached Dr. Seymour Pollack, Director of the USC Institute of Psychiatry, Law and Behavioral Science, to be his consultant for a few years. In 1987, Dr. Kornblum, the Chief Medical Examiner-Coroner from 1982-1990, contracted with the USC Institute of Psychiatry, Law and Behavioral Science, under the directorship of Dr. Gross, to perform psychological autopsies in contested cases of suicide and equivocal (between suicide and accident) cases. Since 1987, in-depth interviews, both face-to-face and telephonic, of family, friends, business associates, and others, along with a review of documents (e.g., medical and mental health records) are conducted by a team of mental health experts (Drs. Botello, Weinberger and forensic psychiatry/psychology fellows). Since 1992, the current Chief Medical Examiner-Coroner, Dr. Sathyavagiswaran, has continued to further develop the psychological autopsy process by encouraging the mental health experts to visit the scene of death as warranted. He has formalized the Mode Conference maintaining the confidentiality of the process with the Coroner's investigator presenting the details of the death scene, the deputy medical examiner reporting on the autopsy/examination finding, the toxicologist interpreting the toxicology screen results, and the mental health expert presenting, using a power-point format, the findings of the psychological autopsy. A discussion follows and the Chief Medical Examiner-Coroner uses the consensus of the attendees to assist in his final determination of the manner of death. Certain legal challenges to the confidentiality of the psychological autopsy report have lead to a formalized written contract outlining the nature of the process, the psychological risks involved, and the confidentiality of the report. It must be signed by the next-of-kin if the psychological autopsy is to be conducted. Both a paid for and department of coroner sponsored contract are available.

Psychological Autopsy, Coroner Medical Examiner, Suicide

#### **I16** Competence to be Executed: An Analysis of Ethical Issues and a Comparison With the Complexities of the Forensic Role

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After attending this presentation, attendees will be aware of the special ethical considerations and care that should be taken in competence to be executed assessments and better aware of the ethical complexities of the forensic psychiatric role.

This presentation will impact the forensic science community by increasing ethical sensitivity and awareness of the special considerations in competence to be executed assessments.

Competence to be executed evaluations present difficult ethical dilemmas. They require forensic psychiatrists to function in a role in which the goals of medicine and law are most disparate and likely to conflict. The death penalty is an example of a conflict in forensic psychiatry, as in general American society, with the additional factor of the forensic psychiatrist being a physician. Evaluators can potentially stop an execution or alternatively effectively remove the last meaningful impediment to proceeding with it. Forensic psychiatrists have primary duties to the legal system and truth and honesty. However, like all other areas of medical practice and consultation they also need to consider and balance conflicting secondary traditional medical ethical duties. The death penalty is so contrary to traditional medical values that participation in a legally authorized execution is ethically prohibited according to both the AMA and APA.

There are several steps in the legal proceedings leading toward the death penalty sentence. These include investigation, determination of competence to stand trial, verdict determination, sentencing, evaluation for competence to be executed, treatment leading to competence to be executed, execution itself, and finally certification of execution death. The ethical dilemma lies in whether a forensic psychiatrist's involvement in any of these stages ultimately leads to his involvement in the death of the prisoner. Both organizations interpret the prohibition of the AMA and APA to include treatment if such treatment is intended to restore competence to be executed. However, even if the primary treatment intent is something otherwise appropriate like relieving suffering or fostering prison safety, if competence to be executed would almost predictably be achieved as a result of the treatment of the prisoner, it should still be unethical.

A claim that it is merely an unintended consequence if such treatment results in competence to be executed is not persuasive and does not abrogate ethical responsibility. If competence to be executed is predictable, at least the willingness to take the strong risk of treatment leading to the execution of the prisoner needs to be considered as intended. In contrast, however, competence to be executed assessments can be ethical and appropriate. Diamond's approach is an option for an ethical approach to such assessments. Although arguably the purest ethically, it is likely to be a challenge to persuade most judges and juries that such honest legitimate assessments are objective if performed only for the defense as Diamond would recommend. As a result, most practitioners likely will be willing to assess and testify as to competence to be executed for either side. That also is ethical, but the ethical hazards of this position necessitate sensitivity to potentially conflicting ethical duties and roles. While ethical guidelines can help clarify what is ethical, in these ambiguous instances, the best guide for ethical conduct must be the integrity of the professional persons themselves, who in forensic psychiatry, face the challenge of confronting and evaluating many conflicting values. This is one of the many complexities that forensic psychiatrists face as we are at the interface of two very different

disciplines with differing goals and ethics, and issues surrounding the death penalty are an area where the differences are most marked. **Execution Competence, Ethics, Role** 

#### I17 Forensic Psychiatry in Japan: Personal Observations

#### Richard Rosner, MD\*, 140 East 83rd Street, Suite 6A, New York, NY 10028

After attending this presentation, attendees will have learned about Japanese forensic psychiatry.

This presentation will impact the forensic science community by fostering cross-cultural understanding between forensic psychiatrists in Japan and the United States.

On November 18, 2009, upon arriving at Narita Airport in Tokyo, this author was met by the host for the visit to Japan, Dr. Saburo Matsubara, and by Ms. K. Ota, a counseling psychologist, who functioned as a personal guide and interpreter.

During the visit, this author toured and spoke at several centers. These included the National Center of Psychiatry and Neurology where the physical facilities were impressive: they were modern and were designed to match the clinical progress of the patients from initial admission. The staff was multidisciplinary (psychiatrists, counseling psychologists, social workers, nurses, and activities therapists) and functioned as a team for the on-going evaluation and treatment of each of the patients. The visit to the Centre for Forensic Mental Health at Chiba University will be discussed where the presentation on "Legal Regulation of Psychiatry and Forensic Psychiatry: Clarifying Categories for Clinicians" was made. The members of the audience asked relevant questions regarding the mental health laws in the USA as compared and contrasted with those in Japan. After the formal presentation, there was an elegant traditional Japanese dinner, where informal professional discussions were continued.

Next, this author had the privilege of speaking to an astute audience at the Workshop Program on Forensic/Criminal Psychiatric Examination at the National Center of Sciences.

At the Okayama Psychiatric Medical Center, among the medical professionals present were: (1) Toyoji Nakashima, MD, Chief Executive Officer and Director of the Okayama Psychiatric Medical Center, President of the Japanese Society of Forensic Mental Health, and Vice-President of the Japan Municipal Hospital Association; and (2) Yoshiki Kishi, MD. The forensic psychiatry facilities was toured as well as the facilities for the treatment of adolescents, substance abusers, and "difficult-to-treat" patients. The facilities were modern and were adapted to the clinical needs of the patients. For example, the adolescent facilities housed fewer patients than the adult facilities, so that the adolescents would have more room for their activities. The forensic psychiatry facilities demonstrated four different types of accommodations, each of which was designed to meet the needs of the patients as they progressed from initial admission through the subsequent stages of treatment. This author met with the staff in two separate conferences. Among the issues that were considered were dualdiagnosis cases (for example, persons who had both mental illness and substance abuse problems), the care and management of persons with Asperger's Syndrome, and problems in the treatment of persons with Personality Disorders (for example, serial sex offenders).

Overall, the visit provided a valuable opportunity to learn about forensic psychiatry in Japan. The goal is to engage in on-going communication with colleagues in Japan regarding the practice of forensic psychiatry in Japan and in the United States of America. It was a richly rewarding professional experience.

Japanese, Forensic Psychiatry, Japanese Mental Health Law

#### I18 Assessment, Not Judgment: A Cautionary Tale and Its Current Forensic Psychiatric Implications

John L. Young, MD\*, Yale University, 203 Maple Street, New Haven, CT 06511-4048

After attending this presentation, attendees will be able to understand and integrate the causal elements of alleged criminal acts with improved clarity as they utilize the information for the assessment of responsibility and related issues.

This presentation will impact the forensic science community by demonstrating to attendees how to perform more relevant, reliable, and valid assessments of alleged acts of their evaluees by dissecting the pertinent causal elements, weighing their relative contributions, and integrating the results along with recognition of the pertinent limitations.

Forensic psychiatric work can bring its practitioners into contact with individuals accused of crimes that evoke strong reactions in normal individuals. The stronger the reaction is, the more difficult it can be to avoid the implicit formation of moralizing judgments extraneous to the forensic task at hand and possibly interfering with it. In this regard, Dostoevsky tells a cautionary tale in *The Brothers Karamazov*. Dostoevsky suggests that a judge cannot judge properly unless he recognizes that he himself could be more responsible for the crime in question than is the defendant standing before him. This could be the reality if the judge long ago gave a scowling look and hurtful word to an innocent child in need of a small gesture of love. The resulting damage could have resulted years later in the defendant's crime.

In order to avoid passing judgment on others and strive successfully to provide valid, relevant, and reliable assessments of criminal behavior for legal purposes; it is useful to recognize that its causality is likely to be complex. To organize the complexity of human behavior it is useful, following Aristotle, to consider the breakdown of causes into four kinds: material, formal, efficient, and final. We readily recognize final cause as the motive and efficient cause as, ordinarily, the accused and any accomplices. It is the concepts of formal and material causes that most interestingly engage our expertise.

Formal causes are what give behavior its shape or quality. In forensic psychiatric work these are usually manifold. In Dostoevsky's tale, the judge appears as a formal cause of the criminal behavior he is judging because of the overwhelming hatred felt years earlier by his innocent victim the future defendant. In a similar way, it is incumbent on forensic behavioral experts to gather and evaluate thoroughly the potential shaping influence of early upbringing on their evaluees' behavior. For example it is becoming increasingly clear that violence in the various media have a shaping influence, a formal causality, on the future behavior of their young audiences.

Material causes of human behavior can be understood as its bodily or physiological substrate. This includes genetic endowment, some of the realm of neuroscience, and peripheral elements. There is great diversity here, and new information is accumulating rapidly. It is up to forensic behavioral science experts to attend to the findings being made as well as to understand at a reasonable level the technology involved. Some of this technology is already appearing in the courtroom, both in reality and in portrayals on television.

Generally, then, each human act arises from multiple causes operating simultaneously and unequally. The current discussion regarding the definition and measurement of depravity raises questions about what may be the passing of judgment rather than the careful assessment of both material and formal causes contributing to particularly heinous behavior.

It is being made increasingly clear, the many current challenges to professional objectivity require energetic and informed vigilance. Our response will be effective in proportion to the clarity with which each causal component of the behavior in question is identified.

Assessment, Criminal Responsibility, Causality of Behavior

#### I19 When Rights Collide: Right to a Speedy Arraignment vs. Right to Psychiatric Care

Susan M. Gray, MD\*, 400 East 34th Street, OBV-CD 230, New York, NY 10016; and Elizabeth Ford, MD\*, New York University School of Medicine, Division of Forensic Psychiatry, Bellevue Hospital, 462 First Avenue, New York, NY 10016

After attending this presentation, attendees will be able to describe the tension that exists between an arrestee's right to a speedy arraignment and their right to medical treatment, including psychiatric treatment. Specifically, attendees will be able to identify the opposing ethical principles involved in this tension and be familiar with at least two landmark legal cases that have delineated these rights in the constitution and case law. By learning about a current study at Bellevue Hospital identifying the legal repercussions of admission to an inpatient psychiatric unit prior to arraignment, including the delay in length of time to arraignment, attendees will learn about the diagnostic and risk assessment considerations that go into pre-arraignment evaluations. An in-depth look at several examples of psychiatric admission that led to a significant delay in arraignment will help the attendees appreciate the complexities of balancing patient care with criminal rights in psychiatry.

This presentation will impact the forensic science community by highlighting the complexities of providing psychiatric admission to police detainees prior to arraignment. The data will systematically show the time to arraignment for detainees psychiatrically hospitalized and identify any delay as compared to the general population. This data will be used to help contribute to the understanding of the treatment of the mentally ill in the criminal justice system, specifically whether legal proceedings are significantly delayed and if a change is needed in the system so that the right to a speedy arraignment and the right to medical treatment are not so opposed.

**Proposition:** Every person has a right to a speedy trial under the Sixth Amendment of the United States Constitution. People with psychiatric symptoms who are under arrest may require hospitalization, which results, at least in New York City, in a dual commitment situation - civil and criminal commitment. Hospitalization necessarily involves a delay in arraignment, thus delaying their first contact with an attorney and their opportunity for release (either by posting bail, accepting a plea, or having the charges dropped). For those who would be released at arraignment, their time in custody may be significantly extended by hospitalization. However, while it is true that admission to the hospital delays an individual's criminal proceedings, it fulfills another right, which is the right to medical treatment while in custody. The Bellevue Hospital CPEP (Comprehensive Psychiatric Emergency Program) sees approximately 8,600 visits per year, about 30% of whom are prearraignment NYPD detainees. About 8% of those detainees are then admitted to the Bellevue Hospital inpatient forensic psychiatric, which is an average of 20-30 per month. This study will describe the population of police detainees who require psychiatric admission prior to arraignment and will compare the average length of time to arraignment following psychiatric hospitalization with the average length of time to arraignment for the general population in New York City. In-depth case review will help illustrate what is considered a significant enough psychiatric emergency to necessitate a delay in arraignment.

**Method:** The sample population will include all pre-arraignment detainees brought to the Bellevue Hospital CPEP and then admitted from April 2010 to August 2010. Descriptive variables, including age, race, diagnosis, psychiatric history and preliminary charge, will be collected and analyzed. Data will be collected about the length of time from arrest and admission to arraignment. This will be compared across the five boroughs in New York City, as Bellevue Hospital serves as the only inpatient forensic service in New York City for men in police custody requiring acute psychiatric admission. Clinical experience has indicated that a key factor in determining time to arraignment is the borough in which the instant offense was committed, in part because of various

geographic and systems barriers. Analysis will also involve exploring the disposition outcomes for those arraigned during hospitalization comparing the subgroups of those released with those retained in custody.

**Conclusion:** This presentation and study are designed to highlight the complexities of providing psychiatric admission to police detainees prior to arraignment. The data will systematically show the time to arraignment for detainees psychiatrically hospitalized and identify any delay as compared to the general population. This data will be used to help contribute to the understanding of the treatment of the mentally ill in the criminal justice system, specifically whether legal proceedings are significantly delayed and if a change is needed in the system so that the right to a speedy arraignment and the right to medical treatment are not so opposed.

Psychiatry, Arrest, Arraignment

## I20 Early Childhood Caries and Child Neglect: A Preliminary Report

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The goal of this presentation is to describe the correlation between Early Childhood Caries (ECC) and the phenomenon of child neglect.

This presentation will impact the forensic science community by underlining the importance in order to sensitize oral health physicians to this problem and prevent child maltreatment.

**Introduction:** Dental caries is an infectious, contagious, and multifactorial disease that was recently identified as the single most common chronic childhood disease. Increasingly dental caries in infants or ECC, if persistently left untreated, is recognized as part of the phenomenon of child neglect, in particular dental neglect, since dental caries, periodontal diseases, and other oral conditions, if left untreated, can lead to pain, infection, and loss of function, thus reducing an infants' quality of life.

Moreover, the effects of EEC can be long term, increasing risk for dental problems later in life, and interfering with basic social functioning, as well as optimal growth and development.

This kind of child maltreatment, resulting in failure to seek or obtain appropriate dental care, often results from many risk factors that usually interact, such as: parental ignorance or depression, intimate partner violence, lack of finances, family isolation, child's disability, and lack of perceived value of oral health.

Some researches reported a relationship between abuse and oral care among samples of children who underwent maltreatment and other studies highlighted that income seems to influence infants' medical and dental care access and quality.

The goal of this work is to describe the correlation between ECC and the phenomenon of child neglect, analyzing causes, consequences and manifestations, in order to inform and sensitize oral health physicians to the problem of dental neglect and prevent this kind of child maltreatment.

**Materials and Methods:** A random sample of 63 parents/guardians afferent to three pediatric services in Bari was delivered a self-report questionnaire. Over half of the sample (37 subjects) were males and 26 were females. Their mean age was 4.9 years.

The questionnaire recorded socioeconomic, demographic, and dental service-use data about their family and children. Information about general health, diet, and oral hygiene habits were also collected. Each questionnaire was completed by a clinical dental examination carried out on each child of the family. For the description of the dental conditions we followed Wyne nomenclature for ECC.

**Results:** The analysis of data collected showed: of examined children, 63% had EEC; 48% were coded type I, 13% type II, and 2% type III. Among infants fed with baby bottles (36.5%), 78% were affected by type I EEC and 22% by type II ECC, while children not fed with baby-bottles had lower percentage of ECC (30% type I and 7.5% type II), as 60% of infants were ECC free.

Concerning plaque debris, this sample revealed that 51% had poor debris and 49% medium or large debris. With reference to plaque, it is important to assess possible coexistences of plaque debris and EEC.

Infants' oral hygiene habits were analyzed, which showed results that among subjects who never used toothbrush 2 showed type I and type II EEC. Of children who reported to brush their teeth once a day, 68% were affected by type I and type II ECC, becoming 46% among infants who reported to brush their teeth twice a day, while 70% of subjects that stated to brush their teeth three times a day were suffering from type I EEC.

The last factor analyzed was socioeconomic status, considering first father/mother employment/unemployment and secondly the category of job (mean income), and EEC type.

Among the analyzed sample, 50% of infants unaffected by EEC belonged to low income families, while the remaining 50% could be divided into: 37.5% of children belonging to medium income families and 12.5% belonging to high income families. 43% of infants with type I ECC belonged to low income families, 40% to medium income and 17% to high income. Type II ECC in 87.5% of cases was observed in children belonging to low income families, while in 12.5% type II was observed in medium income families' infants. The only case of type III ECC belonged to a low income family.

The greatest part of parents/guardians appeared aware of the importance of infants' oral hygiene, and informed about preventive measures to avoid dental problems, especially ECC.

In conclusion, even though it is a preliminary report, the study presented provides a contribution in the prospective view of sensitize dental specialists, since recognizing and reporting child neglect, or even child abuse, can be achieved only with an accurate knowledge and information of maltreatment phenomenon

Early Childhood Caries, Child Neglect, Socioeconomical Status

#### I21 Man or Women? – Forensic Medical Experts Help Decide

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After attending this presentation, attendees will understand some principles of transsexualism, a known gender identity disorder in which an individual identifies himself with a gender that is different from their biological sex. A true transsexual experiences discomfort as a result of a desire to live and be accepted as a member of the opposite sex. Being such an uncommon disorder, the case study presented offers an opportunity of literature review on the necessary elements for the recognition of these situations as well as the status of legislation concerning this issue in numerous countries.

This presentation will impact the forensic science community by serving as an acknowledgement of the vital contribution of forensic medicine in a systematized format via interdisciplinary communication and collaboration, in assisting courts in specific cases that fall out of the range of the existing legislation.

The present case is of an individual, born male, with no morphologically ambiguous genitalia, no chromosomal abnormality, and

no sex hormone anomaly. At the age of 32, he filed a request in a Portuguese court to legally change his name and gender, alleging he was in fact a women and would therefore, like to be recognized as one by society. He presented himself as being a male-to-female transsexual and expected the court to allow the identification amendment.

The information gathered was the following, although being raised as a boy, his gender identity was that of a female, sensing he belong to the opposite sex not only biologically but also psychologically and socially. He stated to have been aware of this gender incongruity from very early childhood. He acknowledged an intense and persistent desire to participate in the stereotypical games and activities of the female sex, not feeling comfortable with the gender role that society expected him to play based on the body he was born with.

He strongly believed himself to be a victim of a biologic accident which made him cruelly imprisoned in a body incompatible with his subjective gender identity, having developed negative feelings toward his own genitals, even trying to mutilate them and harming himself.

Early on he looked for the company of girls and secretly found ways to cross-dress in women's clothes, namely his mothers', as a way of exploring and enjoying his feminine gender feelings.

Given his feelings of shame and humiliation by his female tendencies, he hid these secret longings from everyone, until about two years prior. As a consequence his general day to day life turned out to be a constant internal conflict. Thus, when he became aware of his options for social gender-change and for medical help, he immediately started hormone therapy and searched for advice, guidance, and gender counseling. He later initiated a "real life experience" of one year of living and working in the new gender with permanent cross-dressing and social changes, undergoing a "transgender transition." Then, before undergoing sex reassignment surgery, there was need for a psychiatrists' certification declaring that the patient was a true and pure case of transsexualism. It also stated to his strong motivation not only to undergo surgery but also for the long journey to total rehabilitation. In addition he certified to the patients stability, ruling out any other important mental disorders. Furthermore there was a medical certification acknowledging an orchiectomy, penectomy, urethral opening reconstruction, labiaplasty, clitoroplasty and vaginal opening and neovaginal canal construction.

The main issue in place was the attempt to legally change his identification into his new name and gender. In many countries, as in Portugal, there has not yet been created specific legislation concerning this matter. This allied to the fact that it is a relatively unknown and controversial matter, not well accepted in certain religious and cultural backgrounds, led the court where the appeal was filed, to request for help from medico-legal experts.

After careful observation of this individual and cautious interpretation of the physical and psychiatric findings of the medical exam preformed, a report was sent back to the judge, stating the opinion of the experts. Was this in fact a new born woman or was he to remain a man?

Transsexualism, Gender Identity Disorder, Forensic Medicine

#### I22 Investigating Correlations Between Drug and Alcohol Intake and Fire Fatalities

Niamh NicDaeid, PhD\*, Centre for Forensic Science, Department of Pure and Applied Chemistry, Royal College, University of Strathclyde, Glasgow, G11XW, UNITED KINGDOM; and Sarah Bergin, MSc, Centre for Forensic Science, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow, UNITED KINGDOM

After attending this presentation, attendees will have an insight into the correlation between intoxication and specific circumstances of fire fatalities. This presentation will impact forensic science by presenting information which will provide policy makers with data to inform community fire safety strategies for vulnerable groups, furthermore, this information will be of great value for fire investigators when involved in fatal fire investigation.

Approximately two people per day are killed in fire incidents in the United Kingdom. While the circumstances of each event may be very different a common trend is the intoxication of the victims either by drugs or alcohol. This work investigates the correlations between such intoxication and the circumstances of the fire incident in terms of variables such as age, gender, location of the fire, type of dwelling, etc.

Data was gathered for 744 fatal fire cases in London, England. This data included information relating to drug use alcohol consumption of the victims involved. The data was coded and analyzed using analytical software and the correlations between the specific variables are presented. This has facilitated the elucidation of specific trends in the data which can inform community fire safety strategies for vulnerable and at risk groups.

Fire Death, Toxicology, Investigative Correlations

#### I23 Major Drugs of Abuse: Comparative Analysis of Indicators From National and Local Data Sources

Liqun Wong, MS\*, Department Of Justice, Drug Enforcement Administration Office of Diversion Control, 8701 Morrissette Drive, Springfield, VA 22152

After attending this presentation, attendees will: (1) gain a better understanding of various indicators used to track the drug abuse culture in the United States; (2) will understand different aspects based on comparative analysis of data and information from law enforcement activities control drug availability; and, (3) will better understand drug demand and attitude toward drug misuse/abuse, drug adverse effects, and drug addiction treatment.

This presentation will impact the forensic science community by serving as an essential aspect of various indicators that measure the drug abuse situation in the United States, and will provide key information to support the drug policy that effects public health and well being.

Multiple data and information sources have been used to determine new and changing drugs of abuse and drug-related health and social problems, help determine priorities for government action, and identify areas for collaboration and monitor progress in achieving reductions in those problems as various policies and practices are implemented.

The national data infrastructure including measurements of morbidity and mortality such as the Drug Abuse Warning Network (DAWN), Emergency Department (ED) and Medical Examiner (ME) components, and Nation Vital Statistics Data (NVSD); drug testing programs such as Arrestee Drug Abuse Monitoring system (ADAM) and Quest Drug Testing Index, self reporting surveys including National Survey on Drug Use and Health (NSDUH), Monitoring the Future (MTF), and Youth Risk Behavior Survey (YRBSS); drug treatment programs such as Treatment Episode Data Set (TEDS), and Nation Survey of Substance Abuse Treatment Service (N-SSATS); and drug interdiction information such as National Forensic Laboratory Information System (NFLIS), System To Retrieve Information on Drug Evidence (STRIDE), Federal-wide Drug Seizure System (FDSS), National Seizure System (NSS), and Uniform Crime Reports (UCR).

In order to understand the drug abuse situation, effectively combat the continuously escalating and changing drug problem that plague our communities, assess progress following implementation of an initiative or policy; various indicators and measurements should be intergraded and analyzed consistently and systematically to derivate conclusions. A study of various comparative data analyses utilizing practical tools and techniques for major drugs of abuse including cocaine, heroin, and methamphetamine will be presented.

Drug Abuse Indicators, Drug Databases, Drug Seizures

#### I24 Preliminary Study of the Mentally Disordered Civil Capacity Rating Scale in China

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After attending this presentation, attendees will learn about the status of civil capacity assessment in China.

This presentation will impact the forensic science community by presenting the standard assessment of mentally disordered civil capacity in China.

**Objective:** To create the mentally disordered' civil capacity rating scale, and explore it's feasibility during the forensic psychiatric expertise.

**Methods:** Civil capacity-related items were analyzed, then the last items were abstracted after discussion and consultation, the mentally disordered civil capacity rating scale was created when the last items were arranged according to the logistic sequence of the assessment, and the manual was accomplished. The rating scale was used during the civil assessment in four institutes.

Results: There were 14 on the mentally disordered' civil capacity rating scale. 202 subjects were recruited, organic mental disorder was the maximum diagnosis (123, 60.9%). Based on the experts' opinion to the subject's civil capacity, the objects were divided into three groups. The subjects in the full, partial, and no civil capacity group were 28(13.9), 47(23.3%), 127(62.9%). The mean scores of the full, partial, and no civil capacity groups were 2.32±2.45, 11.62±4.01, 25.02±3.90 respectively, and the difference of the mean scored among the groups and between each groups was significant. The Cronbach  $\alpha$  of the rating scale was 0.9724, and during the split-reliability test, the two-splited part of the rating scale were high correlated, (r=0.9729,P=0.000). The Spearman correlative coefficient between the items and the score of the rating scale was from 0.643 to 0.880. According to the cut-point score of the rating scale, the objects were graded into three groups, and the result was consistent with that of the expert's opinion (kappa=0.841, P=0.000). When the discriminate analysis were used, seven areas were included into the discrimination equation, and 92.6% objects were graded into the correct groups using the equation.

**Conclusion:** There is better reliability and validity of the mentally disordered' civil capacity rating scale and the rating scale can be used as an effective tool to grade the civil capacity of the mental disorder during the forensic expertise.

Forensic Psychiatry, Civil Capacity, Rating Scale

#### I25 Maintaining Viability and Relevance of a Forensic Psychiatric Court Evaluation Service in an Era of Fiscal Constraint

#### Steven Ciric, MD\*, 38 Gramercy Park North, Suite 1A, New York, NY 10010

After attending this presentation, attendees will be familiar with the scope of services that are available and possible within an urban forensic psychiatric court evaluation service, the systems challenges faced in a time of economic stringency, and the need to balance efficiency and cost effective operations with the delivery of accurate and quality forensic examination services.

This presentation will impact the forensic science community in considering the potential effects of economic forces upon organizations providing forensic examination services, and the necessary response of remaining conscientious about systems efficiency and fiscal responsibility while still adhering to the principles of careful and appropriate forensic services.

The tightening of budgets that has affected virtually every organization and government entity over the past several years has also impacted health care systems and the delivery of forensic services. In New York City, the Forensic Psychiatry Clinic has provided embedded forensic psychiatric evaluation services to the New York County Criminal and Supreme Courts since the 1930's. Its scope of service has included the examination of competency to stand trial, pre- and postsentencing mental health evaluations, and evaluation services for probation. Its chief stakeholders include the criminal justice system, those defendants who become the subjects of examination, and the city health systems that provide for the administration of the service. Given consolidation of health care services across hospitals and clinics, reductions in staffing and services, and careful appraisal of the utility and efficiency of services by oversight and administrative agencies, it is critical for systems providing forensic evaluation services to be proactive in examining their existing programs and procedures, to be flexible in considering alternatives that may promote greater efficiency and costs savings, and to be creative and open to development of additional services that may increase the contribution and value of the system to its stakeholders. The issue is a pleading refrain that the current structure and program of services is under strain due to growing demand, but level or reducing staff, is unfortunately unlikely to yield increased resources. Rather, becoming aware of and being responsive to the specific concerns and needs of stakeholders is essential in guiding the development of relevant programs of service. For example, as pertains to the Forensic Psychiatry Clinic, consideration may be given to expanding the array of forensic psychiatric evaluation services to include examinations of defendants being considered for diversion from their criminal court proceedings into the mental health system, or augmentation of probation evaluations to provide for specific treatment recommendations, referrals to appropriate mental health services under conditions of probation, and interval reassessment and monitoring conjointly with probation. In proposing alternative or new approaches, institutional, financial, procedural, and political forces may pose barriers and need to be considered. Finally, whether undertaking to streamline or expand services within a forensic evaluation system, it is proposed to avoid shortcuts, procedures, and strategies that may be more fiscally sound, but hold potential to compromise the integrity, accuracy, and quality of the forensic examination process. In competency to stand trial examinations, by way of example, the high importance of maintaining the integrity of this process in ensuring both due process of defendants and the accuracy of legal proceedings is underscored.

Forensic Psychiatry, Forensic Evaluation, Court Service

### I26 Testamentary Capacity and Undue Influence: Challenges and Opportunities for Experts and Attorneys

#### Sanford I. Finkel, MD\*, 3127 Greenleaf, Wilmette, IL 60091

The goal of this presentation is to present a summary of the current state of thinking on issues related to contested wills.

This presentation will impact the forensic science community by leaving attendees with a clearer knowledge on the topic of contested wills

As longevity increases, certain mental disorders also increase in frequency. Older testators may lack testamentary capacity or may be more susceptible to undue influence.

The first part of this presentation will focus on published papers of the Task Force, including contemporaneous and retrospective evaluations of cases, indicia of undue influence, and deathbed wills. Cultural and trans-national differences in approaching inheritance will be included. Other cultures and countries—even with the Anglo-Saxon system often allow less autonomy to the testator. Concepts of taskspecific criteria for testamentary capacity, as situation-specific capacities will be described.

The second part of this presentation will focus on the challenges which experts face in working with attorneys, as well as issues that rise in the course of drafting wills, as well as litigating will contests.

The presentation will include risk factors for undue influence, including the social environment and circumstances, including isolation, change in family relationships and dynamics, recent bereavement, and family conflict. Moreover, there are circumstances in which the testator may be more vulnerable-including physical factors, loneliness, sexual bargaining, deathbed wills, dementing illness, substance abuse, personality disorders, mental disorders-including depression, mania, and psychosis. Ironically, certain personality traits and illnesses may render the testator as less vulnerable to undue influence.

The presentation will also compare contemporaneous reviews and retrospective reviews. In the former, assessments generally include: medical and psychiatric history, family input, mental status assessment, current cognition, and possible presence of psychiatric illness. How does the expert (or the attorney) determine that a testator is competent to execute documents? What are "insane delusions," and what role do they play in executing or nullifying a will? When is videotaping the execution of the will desirable, and what are the potential pitfalls?

For the attorney—when does one work primarily with a family member? How does one ask about or interpret medical information? What do you need to know about delirium and deathbed wills? What is the relevance of prior wills? When and how does one approach a testator to obtain a professional consultation prior to the final execution of a will?

Regarding retrospective review, this author will describe methodological approaches—how does one assess medical charts and fact witness depositions? What methods can facilitate a testator's level of cognition and capacity when the testator is demented? What are the challenges and pitfalls for the expert working with attorneys in cases of contested wills? What issues do many lawyers miss in which the expert can provide important assistance?

Among the cases, which will be discussed:

- 1. Two separate cases in which the testator left the entire estate to the government-in one case the expert was retained by the plaintiff; in the other by the defense.
- 2. A case of "undue influence," in which the testator clearly had capacity.
- 3. Cases in which testators suffered from various severity of dementias.
- 4. Cases in which the testator disinherited the natural objects of one's bounty to leave their estate to their stock broker or the attorney's mother.
- 5. Cases in which a change of family dynamics resulted in a radical change in the final testament.
- 6. Cases of deathbed wills and delirium.

**Testamentary Capacity, Undue Influence, Contested Wills** 

<sup>\*</sup> Presenting Author

#### I27 Linguistic and Paralinguistic Markers of Lying: Science and the Market

Karen B. Rosenbaum, MD, 262 Central Park West, Suite 1A, New York, NY 10024; Robert E. Remez, PhD\*, Barnard College, Department of Psychology, 3009 Broadway, New York, NY 10027; Kelly R. Damphousse, PhD\*, University of Oklahoma, Associate Dean, College of Arts and Sciences, 633 Elm Avenue, 323 Ellison Hall, University of Oklahoma, Norman, OK 73019; and James D. Harnsberger, PhD\*, University of Florida, Department of Linguistics, 4131 Turlington Hall, PO Box 115454, Gainesville, FL 32611

The goals of this presentation are to present and discuss recent technology and research in speech and voice analysis as a means of detecting lies.

The presentation will impact the forensic science community by presenting the significant scientific challenges that must be met in order to develop a practical speech and voice analysis technology that can be used in forensic evaluations.

Longstanding folklore and scientific ambition encourages the belief that an intention to deceive is betrayed in physical expression during the act of deception. Although this belief spurred the development of the polygraph, the failures of such devices in the laboratory and the field are now well recognized and summarized in a report in 2003 by the National Research Council. Whether the motivation is scientific or practical, alternative instrumental methods have been sought for detecting implicit markers of deception. Apart from the durable scientific interest in human behavior, the potential is great for marketing a successful technology to detect deception, for use in settings that require safety and security, and those that depend on truthfulness whether the proceedings are formal and juridical, or informal and commercial. Among many measures of autonomic and voluntary movement, research and forensic practice has focused from time to time on speech acts. These projects aim to identify the physical effects of speech that are attributable to stable linguistic and personal characteristics and to distinguish these from instance-specific aspects of speech that hypothetically reflect direct consequences of an intention to deceive, or indirect consequences of mendacity in acoustic effects of arousal or affect secondary to intended deception. In this panel, two recent research projects to contrast scientific practices and standards of evidence with the practices and standards recommended for commercial products and their uses will be discussed. While prior research on the detection of stress, emotion, and deception from speech and language has shown only limited progress, this has not dampened enthusiasm for marketing of commercial devices that purport to detect these states to a variety of customers. For the major products currently on the market, independent studies to date have failed to verify their efficacy with a wide range of speech materials collected under various experimental conditions, ranging from laboratory studies with carefully controlled speech to mock crimes to speech produced under realistic levels of jeopardy. This literature will be reviewed and it will be discussed with regard to policy debates conducted among private manufacturers, elected officials and their staffers, academic researchers, and federal bureaucrats. It can hardly be surprising that scientific evaluations of nascent technologies can sometimes provide findings that run counter to the experience of early-adopters who are using the technology in the field. The discussion will also address this conflict by identifying causes of this dissonance, including the problem of approximating the field in the laboratory and then generalizing the laboratory to the field. The significant distinction that different standards of efficacy have on such debates will also be addressed. This issue will be discussed within the context of recent evaluations of voice stress analysis programs. Finally, the long-term potential of the speech modality in deception detection will be discussed, with a focus on the constraints imposed outside the laboratory and the problem of countermeasures. Lie, Detection, Speech

# I28 Functional Magnetic Resonance Imaging (fMRI): Role In Forensic Psychiatry

Muhammad Saleem, MD\*, Gabriella Obrocea, PhD, Tai P. Yoo, MD, and Conrado Sevilla, MD, University of California, Los Angeles Kern Psychiatry Residency Program, 1700 Mount Vernon Avenue, Room 3057, Bakersfield, CA 93305

After attending this presentation, attendees will be able to: appreciate the history of functional magnetic resonance imaging, basic concepts, and how it works; understand the advantages of functional magnetic resonance imaging and basic research findings; understand the role of functional MRI as court evidence; and learn about the current and potential future uses of functional MRI in forensic psychiatry.

This presentation will impact the forensic science community by discussing how Blood Oxygen Level Dependant functional Magnetic Resonance Imaging (BOLD fMRI), is a newly developed diagnostic modality, which indirectly measures the brain activity during different brain functions, by measuring the blood flow to a particular areas of brain and hence localizes the areas involved in a specific act or behavior including, sociopathy, lying, pedophilia, or other crimes.

This principle is based on the long known fact that when a certain part of brain functions during brain activity, it uses up oxygen at a higher rate than its surroundings. To compensate this deficit, more oxygen is delivered to that part of brain via increased blood flow. This difference in blood oxygen level is picked as a signal by functional magnetic resonance imaging. Functional magnetic resonance imaging has become a topic of interest for forensic experts. There is a great deal of ambiguity regarding the use of functional magnetic resonance imaging as court evidence.

A literature search in duration ranging from 1991 to 2010 was performed. Pub med, Psychiatry online, and Internet web was explored. The search was limited to English language. The search words used were functional MRI and Forensic psychiatry, functional MRI and Sociopathy, functional MRI and Lie detection, functional MRI and Pedophilia and functional MRI in court proceedings. Several countries have been identified that carried out and published this kind of research including the United States, United Kingdom, Germany, France, Switzerland, and Israel.

Number of total articles, publications and abstracts obtained with the search and considered relevant were 177. Many articles have discussed the use of functional MRI in finding neurological basis for sociopathy, lying, pedophilia and mock crimes and have consistently shown functional MRI as useful tool in mapping neurological basis of behaviors.

BOLD fMRI is an imaging modality, which can show brain functioning during thought processes and behaviors. FMRI is safe with no radiation exposure, BUT expensive. Despite all the caveats, questions and concerns, BOLD imaging is well correlated with results from other methods of measuring brain activity. BOLD fMRI currently being used in psychopharmacological research and may potentially be used to track treatment outcomes: for example repetitive trans cranial magnetic stimulation (rTMS) or psychotherapies etc. BOLD fMRI can be used to find the neural basis correlates of the neuro-psychiatric illnesses including, sociopathy, pedophilia, aggressive and lying behaviors. BOLD fMRI is being used for Lie detection but with skepticism. With increasing frequency, criminal defense attorneys are integrating neuro-imaging data into hearings related to determinations of guilt and sentencing mitigation. This is of concern, since not all brain lesions and abnormalities indicate a compromised mental state that is relevant to knowing whether the act was wrong at the time of commission, and juries may be swayed by neuro-scientific evidence that is not relevant to the determination of the legal question before them. It is important to emphasize that the use of imaging studies in forensic

matters requires a careful scientific foundation and a rigorous legal assessment.

fMRI, BOLD fMRI, BOLD fMRI in Forensic Psychiatry

### I29 Communicating Effectively Through the Use of an Interpreter During Face-to-Face Interactions

Robert M. Tovar, MA\*, Marketstreets, Inc., PO Box 4331, Chicago, IL 60680-4331

After attending this presentation, attendees will gain an effective practice for the use of interpreters during face-to-face interaction. This approach has been used with a variety of cultures such as Spanish, Polish, and Middle Eastern subjects during criminal investigations. The concept of this interviewing approach is to focus the cognitive and behavioral mechanisms used during communications to the same communicative path between for greater communicative efficiently.

This presentation will impact the forensic science community by allowing attendees to gain an immediate interpreter protocol allowing greater investigative control and communicative efficiency with subjects of different cultures during interviews and interrogations.

During interviews and interrogations sessions, interpreters may tend to take over control of these interactive sessions between subject and investigator. Due to this intended or unintended control, the communicative paths between investigator and subject may be altered, as well as investigative strategy aversively influenced and hindered

While assigned to the Chicago Police Polygraph Section during the 1990's, a polygraph examination regarding a sexual abuse investigation was administered. The investigation involved multiple Spanish speaking offenders in which the sexual abuse had been on-going for several years. A Spanish interpreter was used and positioned in the conventional Interpreter Protocol for polygraph examinations, criminal investigative interviews, and interrogations. The conventional Interpreter Protocol had the interpreter sitting in front of the subject and the examiner sitting to the side of both the subject and interpreter. The examiner would verbally relay a question to the interpreter and the interpreter would relay the question back to the subject and then relay to the examiner the subject's response. For the rest of this explanation, the examiner will be referred to as the inquisitor for the commonality of understanding as the Interpreter Protocol can be used in a variety of face-to-face interactions outside the administration of polygraph examinations.

During the interview session the inquisitor realized the interaction between the interpreter and subject was taking longer than normal for basic questions. As the conversation in Spanish continued on between the interpreter and subject, the inquisitor asked repeatedly what was the subject saying. Finally the inquisitor interjected and demanded what was being said. The interpreter stated, "He is telling me he is sorry and wants to tell the truth now," the inquisitor was concerned about any legal issues as well as allowing the subject an objective offering of fact and immediately repositioned the interpreter behind the inquisitor. Thus, a revised approach of Interpreter Protocol was established and identified as the Crime Lab Interpreter Protocol (CLIP), or simply Interpreter Protocol.

The inquisitor now sits in front of the subject with the interpreter sitting behind the inquisitor in a somewhat diagonal position with the interpreter's side almost touching the inquisitor's back area. The inquisitor will cue the interpreter by the slightly tilting back of the head, on this cue the interpreter will speak into the ear of the inquisitor as the subject speaks in their foreign tongue. The inquisitor will respond to the subject verbally as the interpreter speaks out loud in Spanish to the subject at the same time. This practice of both the inquisitor and interpreter speaking at the same time may appear unconventional or confusing for communications; but, if the interpreter and inquisitor are in-sync the conversation between subject and inquisitor will follow the same communicative path. Due to the consistent efforts of the inquisitor, the subject will begin to follow the same communicative path of tonality in voice, gestures; etc., as both progresses through the interaction. During the Interpreter Protocol, subjects may attempt to talk or look at the interpreter, but the inquisitor's natural body position and slight movements will counter these attempts, and the body of the inquisitor will naturally shield the interpreter from view of the subject. During the face-to-face interaction the subject and inquisitor will begin to interact with each other as in a normal face-to-face interaction on the same communicative path. Enhancement to the Interpreter Protocol can increase due to the inquisitor's having knowledge or insight into the subject's particular culture.

During debriefings of interpreters after the Interpreter Protocol was administered, two consistent responses were expressed by interpreters: (1) "it was the most intense interpreting I have ever done," and, (2) "it was like I was not in the room and looking through a two-way mirror watching the interview."

The inquisitor also experienced similar intuitive feelings to the Interpreter Protocol, as if no interpreter was in the room and subject and inquisitor were carrying on a viable conversation between each other. Both subject and inquisitor become aligned to the same communicative path in which speech, gestures, nuances of the behavioral and cognitive mechanism of communications are exchanged as in any normal face-toface interaction.

The positive results in positioning the interpreter behind the inquisitor and the interpreter is not looking at the subject, but only hears verbalization of the subject, speaks only those words used by subject and inquisitor, will minimizes any aversive outside influences affecting the interaction as well as allow the inquisitor to maintain direct control of the communicative processing. Another contribution of the Interpreter Protocol can be demonstrated in court, the inquisitor can exemplify a sincere concern for a fair and impartial interactive session without any physical coercive mannerisms between subject and inquisitor.

The Interpreter Protocol can be an intense interactive strategic session, as the interpreter and inquisitor become in-sync with each other, this session can bring a communicative efficiency not normally experienced during investigative interviews and interrogations.

Interpreter Protocol, In-Sync, Communicative Path

#### I30 Forensic Evaluation and Aggression Risk Assessment in Violence Against Women to Prevent New Aggressions Through GPS Devices

Lorente Miguel, PhD\*, University of Granada, Avda. Madrid, 11, Medicina Legal, Granada, 18012, SPAIN

The goal of this presentation is to discuss how violence is usually approached in terms of results. Most of the studies try to establish differences among the distinct types of violence considering the injuries, circumstances around the aggressions or the procedures used to commit the attack; but, it is not enough to know the real differences among them and how forensic evaluation can help to prevent new aggressions. Violence against women (VAW) has different features compared with other forms of violence. It is a "moral violence" used by men against women, especially in the context of a relationship, and that is constructed under cultural references. It means that there is a part of this violence that is considered "normal" and this feature affects the evolution of this violence, usually increasing the risk of new and more serious aggressions and, even, homicide. These features must be known for the forensic evaluation and to develop strategies to prevent these new possible attacks. VAW is known by its recidivism and by the difficulties to break with it and, in consequence, to protect women. Forensic risk assessment allows knowledge of the specific circumstances of the cases and helps form conclusions about the future possibilities of new aggressions. Under this assessment, new measures can be taken to protect victims of this violence, one of which is to know the relative positions between the aggressor and the woman through a GPS device that alerts if there is an approach between them. This way the police can react and arrest the perpetrator.

**Hypothesis:** If the forensic evaluation establishes an objective risk for new aggressions in the context of a relationship where men use violence against women, the measures to protect these women and prevent violence must be adapted to the circumstances. In Spain, a system has been developed with a GPS device carried by the perpetrator in a permanent way (bracelet) that allows knowledge of the location of his position and the victim's location. This system can be used under a judicial order if the risk for new aggressions is high. The correct forensic risk assessment guarantees the protection of women and the prevention of new aggressions and can reduce the number of serious aggressions.

**Brief Synopsis of The Content and Summary of the Results:** The aggressor in the context of a VAW relationship develops a strategy to try to isolate the woman victim from her sources of support, usually family, friends, and work mates. He considers her as a property and he uses violence to keep the relationship under these references. Under these circumstances violence follows an evolution with increasing intensity. When the victim reports the case to the police or the court, the aggressor considers it as an offense and, in consequence, the possibility for new and more serious aggressions increases.

Spain has developed a system that allows for locating the position of the perpetrator and victim through a GPS device. After the first year, there were 1987 alarms that made it possible to arrest the aggressors when they were approaching the victims. At the end of the year the number of homicides decreased by 27.6%.

Forensic risk evaluation is the procedure used to make the decision to use this protection instrument. The presentation will explain the procedure, features of the system and results after its implementation. **Violence Against Women, Protection of Women Through GPS Device, Forensic Risk Assessment** 

#### I31 The Phenomenon of Homicide-Suicide in Italy

Maurizio Chiesi, PhD\*, Piazza Washington 39, Pontassieve, 50065, ITALY; Cinzia Gimelli, PhD\*, Viale Montegrappa, 29/C, Reggio Emilia, 42100, ITALY; Luciano Garofano, PhD\*, Via G. D'Annunzio n.9, Parma, 43100, ITALY; Sara Spada, PhD\*, Strada Villabella 31, Giarole, 15036, ITALY; and Daniele Berto, PhD\*, National Health System, ULSS 16, Padova – I, Via E. degli Scrovegni, 14, Padova, 35131, ITALY

After attending this presentation, attendees will have a better understanding of a particular crime: the Homicide-Suicide phenomenon in Italy.

This presentation will impact the forensic science community by providing a better understanding of the misdeed excluding emotional impulses it arouses, discussing observations conducted through the quantitative and qualitative analysis of data, and sharing the description of the phenomenon's characteristics that forensic scientists can evaluate in order to establish the right to a prevention strategy, which could lead to a reduction of the phenomenon.

This research has been focused on the classification of a single event, which allows the identification of five groups at risk following the Cluster Analysis.

These groups are: Alpha, Sierra, Tango, Golf, and India. The labels have fancy names but helped us to better identify individual clusters with particular characteristics. It has not been possible to assess whether, or not, the perpetrator was suffering from psychopathology because the detection of this condition was not conducted by mental health experts.

To date, there has been little research on this phenomenon in Italy, hence a study was conducted. Existing relevant literature will be reviewed. Using data from all information available taken coming from media, Internet, as well as offender data, data was extracted from both investigations and trial documentation.

Data will be presented using 578 cases, from January 1, 1997 to December 31, 2009. Some cases were not included, where the will of the victim's death was evident, as it became an active part in the criminal event.

The most relevant cases in order to better identify the factors that determine these particular events and try to find a way to set up a prevention policy will be reviewed and discussed.

Homicide-Suicide, Psychopathology, Prevention

#### I32 MAOA, the Warrior Gene: Skirmishes, Battles, and Truce

James S. Walker, PhD\*, and Stephen A. Montgomery, MD\*, Vanderiblt University School of Medicine, 1601 23rd Avenue South, Nashville, TN 37212; and William Bernet, MD\*, Vanderbilt Psychiatric Hospital, 1601 23rd Avenue South, Suite 3050, Nashville, TN 37212-3182

After attending this presentation, attendees will learn an evidence based and ethical approach to testifying about behavioral genomics at criminal trials.

This presentation will impact the forensic science community by improving the relevance, reliability, and validity of testimony regarding behavioral genomics at criminal trials.

Considerable research suggests that a gene x environment (G x E) interaction occurs between the *MAOA* gene (sometimes called the "warrior gene") and childhood maltreatment. Individuals with the low activity *MAOA* allele and a history of serious child abuse are reportedly much more likely to be antisocial and commit violent crimes than individuals with neither of these risk factors. This presentation – which is particularly relevant to the theme of this Annual Meeting, "Relevant, Reliable, and Valid Forensic Science" – describes the proper use of testimony regarding this G x E interaction at criminal trials.

James S. Walker, PhD, will present, "*Skirmishes between Law and Neuroscience: A Brief History of the MAOA Gene and Its Implications for Criminal Responsibility.*" Adult behavior is presumably the result of interaction among one's genetic constitution, the person's life experiences, and each individual's personal choices. As neuroscientists have rapidly advanced our understanding of the human mind through the study of behavioral genomics and brain imaging, challenging questions have arisen. What is the neuroscientific basis for our experience of "free will?" Should jurisprudence take neuroscience into consideration when addressing criminal responsibility? When sentencing occurs, should some G x E interactions be considered mitigating factors, while others are aggravating factors? How have the courts addressed neuroscientific explanations of criminal behavior?

Stephen A. Montgomery, MD, will present, "*Battle Lines: Research For and Against the Hypothesis that a Gene x Environment Interaction Is a Risk Factor for Future Violence.*" The theory that a G x E interaction is a risk factor for future violence was first proposed in an important paper by Avshalom Caspi and his colleagues in 2002. Since then, approximately thirty research teams have attempted to replicate Caspi's findings. In this presentation, the meta-analyses and the literature reviews that create the bases for testimony regarding this G x E interaction at criminal trials will be summarized.

William Bernet, MD, will present "*Time for a Truce: Collaboration among Science, Ethics, and Professionalism.*" The authors have testified regarding this G x E interaction at several criminal trials in a manner that they consider evidence-based and ethically sound. The testimony that

was presented at a murder trial in Tennessee will be summarized, in which the jury took testimony regarding behavioral genomics into consideration when addressing the defendant's criminal responsibility. Principles for testifying regarding these findings will be presented in a scientific and ethical manner: a genetic test all by itself means very little; assessment of behavioral genomics is only one part of a comprehensive forensic evaluation; this G x E interaction is only a risk factor, not a direct cause of violence.

Behavioral Genomics, MAOA Gene, Child Maltreatment

#### I33 Post-Traumatic Stress Disorder — Protective and Risk Factors: A Study of 18 Survivors of a Plane Crash

Felice Carabellese, MD\*, Roberto Catanesi, MD, Vito Martino, MD, Ignazio Grattagliano, PsyD, and Chiara Candelli, PhD, Section of Forensic Psychiatry, University of Bari, Piazza Giulio Cesare, Bari, 70124, ITALY; Giuseppe Troccoli, MD, Largo Giordano Bruno 65, Bari, 70121, ITALY; and Giancarlo Di Vella, PhD, Sezione di Medicina Legale, DIMIMP, University of Bari, Policlinico, piazza G. Cesare, Bari, 70121, ITALY

After attending this presentation, attendees will have a better understanding of how an individual's characteristics may constitute vulnerability or protective factors related to the development of psychopathological symptoms of Post-Traumatic Stress Disorder (PTSD).

This presentation will impact the forensic science community by adding data regarding PTSD risk factors.

**Objective:** Many retrospective studies on the risk factors for developing post-traumatic stress disorder have been published in the literature, however their results are equivocal. The mechanism by which only some subjects who are exposed to an intense traumatic event develop PTSD is still not completely clear (Shear, 2002). The study presented here proposes to identify predictive risk factors related to developing PTSD through data gathered from the survivors of an air disaster in which 16 people died and 23 were wounded.

**Method:** The investigations were conducted six months following the traumatic event by a team that consisted of four psychiatrists specialized in clinical and forensic psychiatry, two forensic psychologists, and two medical legal doctors.

In order to increase diagnostic accuracy and to avert any attempts at feigning harm (Hall, 2007), two expert psychiatrists, randomly chosen from among the four examiners, jointly conducted psychiatric observations of each of the survivors using the Clinician-Administered PTSD Scale-Diagnostic Version (CAPS-DX) to diagnose post-traumatic stress disorder. Each examiner calculated individual scores and the average weighted scores of each of them were used in the final legal medical diagnoses. Other diagnostic instruments used include the Zung Self-Rated Anxiety Scale (SAS); Zung Self-Rating Depression Scale (SDS); and the Profile of Mood States (POMS) by McNair, Lorr, and Droppleman.<sup>1</sup> With the goal of obtaining an evaluation of personality structure, the Rorschach projective test, using the Exner scoring system, was used. The subjects also underwent careful anamnestic and clinical examinations to ascertain any prior personal or family psychiatric histories, as well as to evaluate personality structure and cognitive capacity. Particular attention was paid to the behavior of each subject in the periods preceding and following the crash. The investigators compared personal declarations given during interviews with those of the other survivors, and recorded emotional/affective reactions. The subjects' physical injuries were also evaluated by medical legal doctors using the Patient Health Questionnaire 15-Item Somatic Symptom Severity Scale (PHQ-15) (Kroenke, 2002). This 15-item selfadministered diagnostic instrument was used to measure the severity of

\* Presenting Author

problems that arose from the injuries. It consists of seven questions related to regional pain, and eight questions related to general physical discomfort. For each symptom, the subjects' responses were recorded as follows: 0 ("not bothered at all"); 1 ("bothered a little"); or, 2 ("bothered a lot").

**Results:** Six-months following the traumatic event, only four of the survivors (22.2%) showed no psychopathological symptomatology. Fourteen survivors (77.8%) exhibited emotional/affective symptoms related to the event. Seven (38.9% of the entire sample) presented with all of the symptoms of PTSD; two had co-morbid depressive disorder; and seven presented with subsyndromal psychopathological symptoms for PTSD, which mostly involved increased *arousal* (i.e., hypervigilance, exaggerated startle reaction, and difficulty sleeping).

In addition to the severity of the traumatic event itself, other risk factors identified that correlated to the development of PTSD were the loss of a relative, the manifestation of depressive symptoms, and the severity of physical injuries sustained. Conversely, low levels of hostility and high levels of *self-efficacy* represented protective factors against developing PTSD.

**Conclusions:** The results demonstrate that, in a similar traumatic event, individual characteristics may constitute risk factors for the development of symptoms of PTSD. Conversely, maintaining cognitive, emotional, and physical efficiency while actively coping with the traumatic event (*self-efficacy*) is shown to be a protective factor. **Reference:** 

<sup>1</sup> McNair, D., Lorr, M., Droppleman, L.F. (1991) POMS, Profile of Mood States, Italian adaptation by M. Farnè, A. Sebellico, D. Gnugnoli, A. Corallo, Organizzazioni Speciali, Florence, Italy

Air Disaster, Posttraumatic Stress Disorder (PTSD), PTSD Risk Factors

#### I34 Honor Killing and Psychiatry

Rupali Chadha, MD\*, PO Box 2043, Beverly Hills, CA 90213; Park E. Dietz, PhD, 2906 Lafayette Road, Newport Beach, CA 92663

After attending this presentation, attendees will gain knowledge of the emerging phenomena of "honor killing" in the United States and learn how forensic psychiatrists may be called upon to examine defendants who commit these crimes.

This presentation will impact the forensic science community by increasing awareness of the phenomenon of "honor killing," its origins, recent cases, and how the forensic psychiatrist may be called upon in these cases.

Honor killings have become increasingly visible to the Western world in the last several years and appear also to be more prevalent in the West. A phenomenon previously occurring only in parts of the Middle East, South Asia, and Africa, now occurs in the Western world. Europe, the United Kingdom, and Canada reported the first cases, but in the last ten years, honor killings have increased in the United States. This presentation provides forensic scientists with a basic understanding of honor killings. The authors examine some of the cultural roots of honor killings, describe five cases occurring in the United States, and discuss policies toward immigrants, cultural defenses, and psychiatric aspects of the phenomenon. The careful psychiatric and cultural study of defendants will be needed to gain additional insight into any relationship that may exist between psychopathology and cultural determinants of the killing of a family member for "honor."

Honor Killing, Homicide, Family Violence

### I35 Family Murders: An Explorative Analysis of Psychological Risks Factors

Laura Volpini, PhysD\*, University of Rome "Sapienza", via dei marsi 78, Rome, 00185, ITALY; and Luciano Garofano, PhD\*, Via G. D'Annunzio n.9, Parma, 43100, ITALY

After attending this presentation, attendees will better understand what the psychological risk factors are that could help the family murder cases.

This presentation will impact the forensic science community by preventing family murder cases.

The goal of this research is to analyze the system of relationship and interaction of murder and victim, before and during the crime.

The goal, therefore, is to identify where within the control strategies of relational dyad murder-victim risk factors for homicide can be traced. In order to find them, are examined eleven trial files taken from the Court of Rome. These files relate to family and close murders occurred from 1992 to 2004.

For the quantitative and qualitative text analysis are created some categories. These are used to describe and make clear the relationship among the examined subjects. These categories are also useful to shed light on the critical situations emerged into such relationship. Trial files were examined by two judges, and the data analysis highlights some risk factors. In particular, the following results emerge: most of the examined subjects are married; they have a five-years or longer relationship and they used to live together every day. Moreover, most of the subjects have not had any psychological/psychiatric counseling relating to the problems the couple had experienced; many of them live in stressing conditions (i.e., changes in the social-economical status, modifications in the relationship with the partner, etc.). The stressing environment influences the relationship, which made them more vulnerable and aggressive. The relationship is usually of the rigidcomplementary kind: in absence of critical events, one subject always tends to prevail over the other, in an unbalanced division of powers and roles. The relation script most frequent, in absence of critical events, is getting hold (murderer) - protecting (victim). This means that in absence of critical events the murderer usually gets hold of the victim and look for the protection of the victim. The majority of the victims do not ask for help or the family or the social support services, tending to close in themselves. Many have no contact with the police or with the judicial authorities.

In most relationships, there are critical events which break the balance between the subjects. The relation pattern relevant to the critical event is *attack* (murderer) – *submit* (victim). This means that during the critical event the murderer tends to attack the victim, which is dominated by the murderer. The most relevant conflict is *care versus self-sufficiency*, which means that the conflicts within the relationship are mostly caused by a need of care and love. The traditional, more frequent pattern concerning the murder behavior are the following: *controlling* (murderer) – *asserting one's authority* (victim), *asserting one's authority* (murderer) – *asserting one's authority* (victim). Almost all the subjects look for some change in the relationship. In almost all the examined cases, the murderer's expectations in the relationship are disappointed.

Four created categories are considered particularly important. These categories were compared among themselves and showed that often the patterns that are found during the murder are already present during critical events. The murder is often due to the exacerbation of patterns are already in the murderer-victim relationship. The individualization of such risk factors could be extremely important for the forensic community for prevention of family murders.

Family and Murders, Psychological Risks Factors, Relationship Murderer - Victim

#### I36 Psychotic Parricides and Psychopathy

Kenan Osmanovic, MD, and Marianne Gobrial, DO, UMDNJ-RWJ Department of Psychiatry, 671 Hoes Lane West, D325, Piscataway, NJ 08854; Wade C. Myers, MD, Rhode Island Hospital, Department of Psychiatry, 593 Eddy Street, Providence, RI 02903; and Eleanor B. Vo, MD\*, Oma Desala Psychiatric Services, 200 Centennial Avenue, Suite 200, Piscataway, NJ 08854

After attending this presentation, attendees will have a better understanding of psychotic parricides and level of psychopathy.

This presentation will impact the forensic science community by discussing the unanswered questions in psychotic parricides: if there is a level of psychopathy or are the offenders mentally ill.

While parricide is a sensational crime, substantial research on the malfeasance is lacking. Case studies or small care series have yielded minimal research on the matter, while research that is at hand focuses on abuse as the major contributing factor. Some studies have examined how matricide and patricide counter one another; revealing that sons are predominately the offenders of pure matricides. Subsequently, sons suffer a great deal of mental illness in these distinct cases. The limited literature presented on this grave matter has yet to distinguish parricides where mental illness has contributed, from parricides as a result of sociopathy alone. For this small case-control study, six cases of psychotic parricides were compared to seven cases of non-psychotic parricides, in an attempt to delineate the differences that contribute to predicting and assessing risks within these two distinct populations. The sample was collected from private practice records as a retrospective chart review. Data was blinded, as to protect the cases anonymity. All parents, siblings and grandparents were not differentiated as step, half, or maternal/paternal to blind the data as much as possible. While such categorical accord lends itself to generalizations as combined families have more dynamics to be addressed this was the only way to blind the data. Parricide, Psychopathy, Psychotic

# **QUESTIONED DOCUMENTS**



#### J1 Reliability of Paper Brightness in Authenticating Documents

#### James A. Green, BS\*, PO Box 5379, Eugene, OR 97405

After attending this presentation, attendees will understand why inconsistencies in paper brightness may occur. The attendee will learn how paper brightness differs from paper whiteness, how brightness is achieved in the manufacturing process, and if paper brightness differences of individual pages within a ream are random or patterned.

The presentation will impact the forensic science community through increasing awareness by detailing the quality control issues related to brightness in paper during the manufacturing process.

The evaluation of paper brightness in multi-page documents is a common practice of forensic document examiners. Brightness is a measurement of light reflectance of a specific wavelength of blue light (457 nanometers, 44 nm wide, at a 45 degree angle). Foreign readers will recognize a paper whiteness index, commonly used outside of the United States, based upon light reflectance across all wavelengths of light comprising the full visible spectrum.

Although more sophisticated instruments are used in the manufacturing process, a long wave (approx. 300-400nm), ultraviolet light source is commonly used by document examiners to compare the consistency, or inconsistency, of the brightness in the paper specimens at issue.

Most common multipurpose paper will have the paper brightness number displayed on the ream packaging. There is an expectation that paper manufacturers will market their product with a consistent quality of paper size, weight, and brightness. However, research has established that quality control varies among paper companies and the stated paper brightness of sheets within a ream can differ.

Multiple reams of standard multipurpose paper from various manufacturers were examined with long wave ultraviolet light. Although several of the reams had sheets with a brightness quality that could not be differentiated one from another, some reams were found to have clearly identifiable differences in the degree of brightness among the pages. Technical information was obtained from professionals in the pulp/paper industry as well as during a tour of a paper manufacturing site.

During case examinations of a multi-page document (e.g., contract or will) the inconsistency of paper brightness in one page from surrounding pages may or may not provide an adequate basis in determining whether the document is a fabrication or forgery. Therefore, the examiner is cautioned to utilize other characteristics/discrepancies in conjunction with differences in paper brightness, before asserting a document is the product of a fabrication.

Paper, Brightness, Document

#### J2 Multivariate Statistical Procedures for the Analysis of Questioned Documents

Emily G. Riddell\*, Michigan State University, 560 Baker Hall, East Lansing, MI 48824; Christine E. Hay, BS, Forensic Science Program, Michigan State University, 560 Baker Hall, East Lansing, MI 48824; Johanna M. Smeekens, Department of Chemistry, Michigan State University, East Lansing, MI; and Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824

The goal of this presentation is to demonstrate the application of multivariate statistical procedures for the association and discrimination of different paper types based on elemental profiles that were generated using inductively coupled plasma mass spectrometry.

This research will impact the forensic science community by: (1) demonstrating an enhanced methodology for the analysis of paper samples; and, (2) addressing the need for statistical evaluation of comparisons involving forensic evidence.

Forensic examinations of questioned documents can involve numerous types of analysis, for example, handwriting, ink, and paper analysis. Historically, the comparison of paper has been based on physical properties such as dimensions, color, and weight. More recently, research has focused on differentiating papers based on the elements present as a result of the manufacturing and handling processes, as well as impurities in the starting materials. However, much of this research used older, less sensitive instrumental techniques, in which elements are analyzed one at a time. Such procedures are timeconsuming and therefore not attractive for practical applications in forensic laboratories.

Inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive, multi-element technique that is widely used for elemental profiling purposes. In the context of questioned documents, the technique can be used to generate an elemental "fingerprint" or "profile" of paper samples, which can then be compared, using multivariate statistical procedures, to determine similarities and differences based on the identity and levels of elements present.

In this work, a variety of paper samples were analyzed by ICP-MS to generate elemental profiles and statistical procedures were then applied to associate or discriminate the samples based on the elemental profiles. Reams of different types of paper (e.g., recycled paper, copier paper, and multi-purpose paper) produced by different manufacturers were collected from local office supply stores. Three sheets were selected from each ream and five samples were analyzed per sheet, allowing an assessment of element variability both within and between each ream. Each paper sample was microwave-digested in nitric acid and hydrogen peroxide and the resulting digests were analyzed by ICP-MS.

The first step in the analysis was to identify a suite of potentially useful elements for the discrimination of paper samples. A subset of digests was analyzed in the full scan mode to generate elemental profiles. Any elements that: (1) were present at significant levels in the procedural blanks; (2) varied significantly in concentration within a sheet; or, (3) were present at levels below the instrument's limit of quantitation were eliminated from the elemental suite. The remaining paper digests were then analyzed by ICP-MS using the selected ion monitoring mode for the remaining elements of interest. Elemental concentrations in each sample were quantified and expressed as  $\mu g$  element/g paper.

The resulting elemental profiles were then assessed using various statistical procedures. Variation in elemental concentration within and among reams of each type of paper was assessed using two-way analysis of variance (ANOVA), testing the null hypotheses that: (1) there was no difference in mean element concentration among the sheets (i.e., within ream); and (2) there was no difference in mean element concentration among the reams. Principal component analysis (PCA) was then used to associate or discriminate the paper samples, based on elemental profiles. Reams of the same type of paper were closely associated, while reams of different types of paper could be differentiated. Additionally, elements contributing most to the variance in the data set were identified, with the result that paper samples could be associated or differentiated using fewer elements than in the original suite.

Questioned Documents, Trace Elements, Inductively Coupled Plasma Mass Spectrometry

# J3 Printer Problems Defined by Industry Experts

Jacqueline J. Bonn, BS\*, 2025 Ide Street, Maplewood, MN 55109

The goal of this research is to compile information concerning individual characteristics on documents from the printing process and defined defects in printers. With this information, a forensic document examiner may know the cause of characteristics they observe on a document and may use such characteristics to assist in identifying or eliminating a source printer.

Knowledgeable resources such as industry repair and sales personnel can provide valuable information related to observed characteristics in common document examination problems. This presentation will impact the forensic science community by assisting in the accumulation of greater knowledge from industry repair experts who can assist to make more reliable determinations in forensic document cases.

To the trained observer, recognition of features in a printed document yields an array of information. A trained observer may be the person who repairs the machine that document evidence is printed on. The repairman's knowledge of printer technology and the possible cause of characteristics or defects observed assist the forensic document examiner to glean greater and more accurate information in cases involving documents printed via personal printing devices. This information can include manufacturing considerations versus characteristics caused by individual usage and may assist the examiner recognize the limitations of statements of individuality.

Through a series of interviews with printer repairmen around Minneapolis, a list of technology imperfections and defects of laser printers that cause or contribute to printing problems has been compiled. Each technician interviewed has experience in the field that allows them to describe in detail the technology imperfections, describe how the determination of the problem is made, and what a repair may mean to the individuality of future printed documents on the repaired machine. These imperfections are manifested in unique ways by the technology and may become unique and identifiable to that printer. This resource information can assist the forensic document examiner in a greater understanding of printer technology and the value of various characteristics found in a printed document.

**Printers, Defects, Repairmen** 

#### J4 Handwritten Documents as the Only Remaining Physical Evidence Linking a Person to Five Homicides

Gary A. Licht, MS\*, Iowa DCI Crime Lab, 2240 South Ankeny Boulevard, Ankeny, IA 50023-9093

After attending this presentation, attendees will be able to see the relevance of forensic document examination in criminal investigations.

This presentation will impact the forensic science community by reinforcing the knowledge that a broad spectrum approach to the use of forensic fields gives the greatest chance of success in developing physical evidence which can either corroborate or refute witness statements.

A broad spectrum approach to forensic evidence is used by many investigators. It matters not to these investigators where the focus of financing is in forensic laboratories, nor where criticism is directed. Government-funded forensic laboratories provide multiple areas of forensic examinations to meet these needs.

Questioned document evidence in any form is the only type of physical evidence which conveys thoughts or statements. Handwritten information, identified to a person, is the only line of physical evidence which conveys thoughts and statements that are definitively linked to a person. In the case presented here, there was a "tsunami" of verbal evidence; however, the handwritten documents were the only physical evidence linking either defendant to five murders.

In 1993, a federal witness, his girlfriend and her two young daughters, and another potential federal witness, went missing. The two witnesses had been two Iowa-based buyers and distributors of high purity methamphetamine manufactured in Arizona by one of the defendants. As investigators put pressure on the criminal enterprise, potential witnesses began cooperating with law enforcement agents. A few days before one defendant was scheduled to plead guilty to drug charges, four people disappeared. The defendant backed out of his plea arrangement. A few months later, a fifth person disappeared. In 1996, a poor quality copy of a permit to acquire a handgun was submitted for comparisons of the handwritten information and signature to the known writings of the defendants. In February of 1997, following the arrest of one defendant, a deteriorated scrap of paper was submitted to attempt to make the handwriting more readable. The note contained names and phone numbers of federal witnesses in the case. The second defendant was arrested in 2000. This second defendant confided in two inmates regarding her involvement in the five murders. Shortly thereafter, one inmate provided investigators with five scraps of white paper bearing very faint pencil writing. The notes provided information about the five murders and two maps to where the bodies were buried. The information was used to locate the five bodies. The five pieces of paper were eventually submitted to the Iowa DCI Crime Laboratory in order to make permanent readable images of the entries and to process the papers for latent prints. At about that same time, a note from one decedent's home was submitted for comparison with earlier known writings. A request was made for normal daily writings of anyone suspected of involvement with any of the notes. Eventually, in 2003, adequate known writing was submitted for comparison to allow an identification of the writer of the notes about the murders. One of the two defendants was identified as the writer of the notes.

This presentation will cover document evidence associated with: (1) the drug manufacturing; (2) purchase of a handgun; (3) conspiracy to destroy evidence and the state crime lab; (4) conspiracy to kill law enforcement officers and others in the judicial system, other witnesses; and, (5) the eventual information about the five murders and the locations of the bodies. Lines of forensic evidence examination included: forensic archaeology, firearms, drug identification, and questioned documents. At the time of each trial, the only physical evidence connecting either defendant to the five murders was the five

small pieces of paper describing the murders and showing maps of the locations of the bodies. In separate trials, each defendant was convicted, leading to the one defendant becoming the first female on the federal death row since 1953. In retrospect, the broad spectrum approach to forensic examination of physical evidence was nearly stymied by the determined criminals.

Forensic Document Examination, Handwriting Comparisons, Homicide

#### J5 Computer Vision Methods for Automated Writer Recognition

Jeffrey P. Woodard, PhD\*, MITRE Corporation, 7515 Colshire Drive, McLean, VA 22102-7508; and Mark J. Lancaster, PhD\*, 1320 Old Chain Bridge Road, Suite 130/204, McLean, VA 22101

After attending this presentation, attendees will understand how current computer vision techniques can be applied to images taken of handwritten text documents, and in particular, how the techniques can automate the recognition of a writer. Several related automated methods, all of which are novel to this application, will be presented with corresponding results discussed for databases of Arabic and Dutch handwritten text.

This presentation will impact the forensic science community by demonstrating how the automated tools developed under this research can facilitate automatically identifying individuals using less time and manpower than can be obtained by expert opinion, and whose expertise may not be readily available.

Unlike most competing automated writer recognition computer vision methods, those presented here based on so-called *Bag-of-Words* models require no human involvement, such as explicit segmentation of linguistic units, manual preprocessing, or supervised training. The statistical *Bag-of-Words* approach name has its origin because of its text documents retrieval roots, and also because in computer vision applications spatial information is discarded, as if one were throwing visual features into a "bag." In this model, signal processing represents images by local feature vectors that are distinctive and also reasonably resistant to moderate sources of image variation like rotations and scale. The local vectors are then quantized using an unsupervised clustering

method. Finally, unsupervised training and classification is performed based on a generative technique that originated from text document retrieval, called *Probabilistic Latent Semantic Analysis*.

These methods have been found to provide over 98.0% correct recognition for databases consisting of a total of 153 cursive documents from 51 Arabic writers. Results will also be presented on a Dutch document database with 251 writers. Although the text for each document was the same for the Arabic database, it varies for the Dutch, which can thus help assess the degree of text independence of the approach. Furthermore, the Dutch database includes both printed and cursively written documents.

Significantly, the statistically based *Bag-of-Words* approach does not explicitly incorporate linguistic knowledge. Therefore, this automated approach to writer recognition can work on databases of varying languages and corpuses without extensive and time-consuming re-engineering. In conclusion, an automated approach to writer recognition based on current computer vision methods performs at a high level of reliability across several languages and independently of the text being written.

Computer Vision, Writer Recognition, Handwriting

#### J6 A Study of Modified Genuine Signatures by Teenage Writers

Ellen M. Schuetzner, BA\*, 6348 North Milwaukee Avenue, #161, Chicago, IL 60646-3728

After attending this presentation, attendees will gain a better understanding of the forensic handwriting examination of modified genuine signatures and the handwriting characteristics observed and evaluated in these writings.

This presentation will impact the forensic science community by providing information relating to a specific problem addressed when assessing a particular type of disguise (modified genuine signatures) in forensic handwriting examination and also providing information regarding the characteristics found in the natural signatures of individuals. Modified genuine signature is a term used to describe a type of handwriting in which a writer attempts to sign his/her name with the intention of later denying authorship. In some literature, this type of signature has been referred to as an autoforgery. It is believed that modified genuine signature is a more correct term to describe these signatures, and therefore, the term autoforgery will not be used.

The original idea to conduct research in the forensic handwriting examination of modified genuine signatures came as a result of a search in the forensic document examination literature. As reported in the literature, the writer would attempt to alter some of the natural handwriting characteristics that would be present in a naturally written signature. Some suggest that modified genuine signatures, formerly called autoforgeries, merely bear the characteristics of disguise while others s write that there are additional characteristics. In recent years, disguise in handwriting, simulated written signatures, traced signatures, and modified genuine signatures have been popular areas of forensic handwriting examination discussion. As additional research studies are needed, this study proposes to provide information on the occurrence of characteristics that were found in the comparison of attempt modified genuine signatures by teenage writers.

Handwriting samples from a group of one hundred teenage writers were collected. These writers, both male and female, were enrolled in one high school and were between the ages of sixteen and eighteen years. Among the writings supplied by the core group, there were samples of naturally written signatures and samples of modified genuine signatures. Along with requested naturally written signatures, the writers were requested to produce samples of their signatures modified in a manner that made the signatures suitable to be denied as being their genuine signatures at a later date. The manner in which the writer chooses to perform this type of signature modification will be examined and discussed.

The collected sample signatures and the examination and evaluation conducted will be discussed. The literature regarding modified genuine signatures will also be discussed. The collected samples were examined for characteristics that are indicative of the methods of production when compared to naturally written signatures. Various characteristics of disguise were also assessed. Information drawn from the conclusions of this study of modified genuine signatures by teenage writers will be presented, and hopefully, the data will assist in the forensic examination of signatures.

Forensic Handwriting Examinations, Signatures, Disguise

# J7 Signature Frequency and Classification in the Military

Daniel M.T. Nguyen\*, PO Box 3845, United States Air Force Academy, CO 80840; Derek L. Hammond, BA\*, United States Army, Criminal Investigation Lab, 4930 North 31st Street, Forest Park, GA 30297-5205; and Michael J. Salyards, PhD, United States Army Criminal Investigation Laboratory, 45 High Street, Sharpsburg, GA 30277

After attending this presentation, attendees will understand the classification of signature styles in a large population into three main structural types and what characteristics determine their classification. Attendees will also learn about trends in the frequency of signature styles in certain ethnic groups as well as trends based on gender and age.

This presentation will impact the forensic science community by providing additional empirical data on the frequency of signature styles based upon a relatively new objectively based system for the classification of signatures.

Forensic Document Examiners (FDEs) have been identifying and comparing signatures for over 100 years. Prior to 2008, signatures were typically classified as either "formal," "informal," or "receipt/illegible" style signatures. While the classification of signatures in this manner was generally well accepted within the FDE community, this system of classification was not well defined and lacked clear objective criteria. In 2008, Linton Mohammed's article, "*Frequency of Signature Styles in San Diego County*" introduced FDEs to a new objective system of signature classification based upon the number of legible allographs present in a signature.

In this study 1,500 signatures from U.S. military personnel were examined and classified following Mohammed's scheme as one of three types of signatures: text based, mixed, or stylized. Text based signatures are signatures where each allograph of the name is clearly written. A mixed signature is defined as a signature in which two or more (but not all) allographs are legible, and a stylized signature is defined as a signature in which there are no discernable allographs.

The demographic of the signatures taken reflected the demographic of America fairly well. Results show that female signatures are vastly different than male signatures (chi squared = 68.4 p < .001). As for ethnicity, the data shows that Asians produce fewer text based signatures and more stylized signatures (chi squared = 7.11, p<.01) compared to non-Asians. In contrast, among all ethnic groups, African-Americans were found to produce the highest percentage of text based signatures. In addition, African-Americans were also found to produce the lowest percentage of stylized signatures (chi squared = 12.8, p<.001) compared to non-African Americans. All genders and ethnicities have about the same percentage of people who have a mixed signature.

This research could be the foundation for further research regarding signature styles of genders or ethnicities. Additional research should be conducted to investigate the basis behind the observed differences in signature styles between males and females and to establish why different ethnicities appear to produce different types of signatures. **Signature, Classification, Demographics** 

## J8 Determination of Variations in the Writings of Rural and Urban People From Their Letter Characteristics in Roman Script

#### Jassy Anand, PhD\*, 2192, Sector 35-C, Chandigarh, INDIA

The goal of this presentation is to narrow down the investigation when a sample of handwriting is found at the scene of crime.

This presentation will impact the forensic science community by demonstrating features that differentiate skilled rural writers in India from urban writers.

In the present study, a comparison of letter characteristics in samples of handwriting written by "ruralites" and "urbanites" coming from different educational backgrounds has been made. This study was performed to examine the handwriting characteristics of writers from different educational backgrounds. This study will provide information regarding the effect of the educational background on handwriting. It is estimated that the study will provide useful information and help in the investigation in the field of forensic science and document examination.

Two hundred samples of writing written by individuals coming from different educational backgrounds (rural and urban) written in Roman script were collected in order to examine letter characteristics. It has been observed that some letters in Roman script show marked differences and variations in their form between the rural and the urban writer. On the average, it was possible to differentiate the writings between the rural and urban writers 90% of the time. The observations could not be made in 10% of the cases, where the letters and strokes were made in an illegible manner. Hence, the results have been found to be highly reliable and informative as to the variations in writing characteristics between urban and rural writers. This information may be useful in assessing the possible background of an individual, particularly a writer of an anonymous letter. It can be derived from the present study that the educational background of the individual influences the development of handwriting characteristics. The details of the results will be presented.

**Roman Script, Letter Characteristics, Marked Variations** 

#### J9 Examination of Signatures Written by Japanese

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After attending this presentation, attendees will understand the uniqueness of the Japanese idea of a signature and the common characteristics of the character arrangement observed in a genuine signature and a handwritten false name.

This presentation will impact the forensic science community by demonstrating the uniqueness of the Japanese idea of a signature and the writing behavior of a Japanese writer.

One of the characteristics of a signature written by a Japanese writer is the readability of the signature. There are many cases in Japan where one is required to write one's name in a clear and square style. The importance of a seal stamp explains this: a seal stamp is expected to identify a person together with his handwritten name in a clear and square style. Does a Japanese writer have his own way of signing his name? Do Japanese writers write another's name in a different manner than their own? Research was conducted to answer these questions.

One hundred and thirty subjects, 112 male and 18 female, were asked to write their own name and an assigned name twenty times, respectively. The assigned name was a false male name and all the subjects wrote the same assigned name. No subjects had the same family name or given name as the assigned name. The assigned name was written in four kanji characters, two were for the family name and other two were for the given name, the combination of which being the most popular in Japan. All the male subjects' names and 12 female subjects' names had the same number of kanji characters as the assigned name in both the family and the given names. So, the experiments were conducted using 124 subjects' samples. The space given for writing the respective name was an 8 mm by 75 mm space which was surrounded by ruled lines. The following distances were measured: (1) the distance between the far left of the space and the far left of the first character; (2) the distance between the right edge of the space and the right extremity of the last character; and, (3) the distance between two characters. After the measurement, intra individual variance and the inter-individual difference were examined: (1) Intra-individual variance: Intraindividual variance was examined as for the character arrangement of the writer's own name and the assigned name respectively. Then, the difference between the character arrangement of the writer's own name and the assigned name was examined; and, (2) Inter-individual difference: Inter-individual difference was examined as for the writer's own name and the assigned name respectively.

Intra-individual variance was not statistically significant either for the subject's own name, assigned name or the difference between the subject's own name and the assigned name. The distance between the second character of the family name and the first character of the given name was significantly larger than the distance between the other two characters both on the subject's name and on the assigned name. Interindividual difference was statistically significant both on the subject's own name and the assigned name.

Suggestions obtained from the results are as follows: (1) the Japanese idea of a signature is different from that of the western writer; (2) Japanese people write their own names in a square style and do not have a special writing manner peculiar to their signature; (3) Japanese writers regard signing to be the same task as locating the characters in a given space; (4) Japanese writers arrange characters following their own way when they are asked to write their name. Japanese writers apply the same manner as writing their name to writing another person's name when they are asked to write other person's name which has the same number of characters as theirs; (5) a large number of Japanese writers separate their family name and given name by a larger space between them. That is true of the case where they write other person's name; and, (6) In the case of the examination of a handwritten fraud name, character arrangement gives a clue as to the genuine writer to the examiner.

Handwriting Identification, Signature, Arrangement

# J10 Scanned Images: How Well Do They Depict the Subtle Features in Handwriting?

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After attending this presentation, attendees will be able to understand and anticipate the limitations inherent in examination of handwriting from scanned images and should be better prepared to conduct such examinations.

The presentation will impact the forensic science community by educating practitioners about increasingly common examinations of handwriting from digital images.

It is increasingly common for companies and governmental agencies to retain scanned images of documents in lieu of originals. While in some cases original documents are also retained, in many instances the originals are destroyed after imaging. As a result, the best image available for examination by a forensic document examiner may, in fact, be a digital image acquired by scanning the document.

Document examiners always want to examine original documents. However, because digital images are sometimes all that exist, forensic document examiners are increasingly called on to make examinations from these images. Furthermore, because of the ease of sending scanned images as attachments to e-mail messages, attorneys at times send these digital images to document examiners for review even when the originals are ultimately available.

This study will address which features in handwriting are reliably and accurately depicted in scanned images and will concentrate on subtle features such as line quality, natural breaks in the writing line, stroke directions, and lightly-written strokes. The image quality obtained from using various scanning parameters and transmission methods will be studied. How and to what extent print-out equipment affects the examination of handwriting from scanned images will also be considered.

Scanned Images, Handwriting, Digital Images

#### J11 Characteristics of Gel Pen Inks by Microscopy and VOCs Using HS-SPME GC/MS

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After attending this presentation attendees will learn about two common analytical methods that were used to attempt to differentiate 16 gel pen ink specimens.

This presentation will impact the forensic science community by presenting the results of this study, which indicate that distinct characteristics about manufacture and brand could be a useful tool for discriminating between gel pen inks.

Recently, gel pens have become a popular type of writing instrument due to their smooth writing characteristics, vivid color, and environmentally friendly properties. However, up to now, ink analysis for forensic purposes has mainly focused on ball-point pen inks. As the composition of gel pen inks are largely different from that of ball-point pen inks, it is difficult to identify gel pen ink using the methods for ballpoint pen analysis. In this study, 16 different brands of gel pen were analyzed by Scanning Electron Microscopy (SEM) as a relatively nondestructive method of analysis for confirming the surface morphology of their dyes and pigments.

In addition, black gel pen inks were analyzed for their volatile organic compounds (VOCs), which served as an important classifying characteristic, using Headspace Solid Phase Microextraction (HS-SPME). Twenty different VOCs were detected among the pens analyzed (namely, isopropyl alcohol; 2-methyl-2-propanol; 2-butanone; hydrazinecarbothioamide; benzeneacetic acid (ethyl ester); benzeneacetic acid; dimethoxymethy-silane; 2,2-dimethoxybutane; tetrahydro-2-methyl-furan; 1,2-ethanediol; silicic acid (tetramethyl ester); 1,2-propanediol; propylene glycol; 3-ethyl-3-hexanol; 1,1dipropoxy-propane; 2-butoxy-ethanol; 2,2'-oxybisethanol; 1-butylbenzene; 2-pyrrolidinone; and 2-(2-butoxyethoxy)-ethanol). The three most prevalent were 2,2-dimethoxybutane (3.02~47 % ratio); tetrahydro-2-methyl-furan (1.19~52.19 % ratio); and 1,2-ethanediol (52.83~95.84 % ratio).

It was also possible to discriminate between inks made in Japan and Korea through detecting the presence of two VOCs (Japanese inks contained 1,2-ethanediol, 52.83~95.84 %, while Korean inks contained 1,2-propanediol, 76.17~93.51 %).

Gel Pen Inks, HS-SPME, SEM

#### J12 Development and Utilization of a Mixture of Dyes to be Used as a Standard in the Examination of Writing Inks Via Thin Layer Chromatography

Alisa Skinner, BS\*, Internal Revenue Service, National Forensic Laboratory, 29 North Wacker Drive, 3rd Floor, Chicago, IL 60606

The goal of this presentation is to illustrate the benefits of using a standard mixture of dyes to evaluate the overall performance of the Thin Layer Chromatography (TLC) system used in the examination and comparison of questioned writing inks.

This presentation will impact the forensic science community by providing a procedure that will aid in quality assurance of TLC examinations of unknown writing inks in casework. One of the techniques used in the examination of writing inks is TLC. This technique is often used to easily differentiate inks found on a questioned document. In addition, over the years, inks have been collected from ink manufacturers and compiled into an ink library. Inks in the library have been run on TLC plates. The TLC plates are filed based on how the components in the ink formulas separate on the plate. A questioned ink is run on a TLC plate which is then compared to the TLC plates filed in the library (with the hope of finding a matching reference sample with a known manufacturing date/history). When similar inks are found, information about those inks is used to aid in determining the date a particular ink formula was introduced.

Questioned inks that are run today are being compared to the TLC plates of library inks that may have been run many years previously. Without reproducible ink separations, potentially matching inks may be missed and not compared to the questioned ink. Therefore, it is important that the TLC plates are run under consistent conditions to ensure reproducibility from year-to-year.

It is well known that there are many variables that may affect the results of a TLC separation. Some of those variables include environmental factors (such as temperature and humidity); the extraction solvent and solvent system used; the extent of developing chamber saturation; TLC plate uniformity; and plate spotting technique. Development of a standard that has demonstrated a reproducible separation under optimum conditions for TLC could be used to evaluate the quality of a TLC run.

A mixture of known dyes that elute at different Rf values will be developed. Numerous TLC plates of the dye combination will be run under optimum conditions to verify the reproducibility of the separation of the dyes in the standard. This mixture of dyes will be used as a standard to be run with unknown writing inks. In the event that a separation of the standard on a TLC plate is not the same as the separation that was produced under optimum conditions, the examiner may evaluate their procedure and run an additional plate.

Ink Chemistry, Thin Layer Chromatography, Quality Control

#### J13 Raman Spectroscopy of Blue Ballpoint Pen Inks and Dyes Found in Inks: Investigation of the Effects of Varying Laser Wavelength

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After attending this presentation, attendees will understand the main dyes used in blue ballpoint pens and their spectra using different excitation lasers. The Raman spectra of blue ballpoint inks mainly result from one dye or a mixture of several of dyes. The reason why the Raman spectra at 514nm was not influenced by fluorescence but the discriminative power was poor will be explained clearly. A 633nm laser was chosen as a balance between the fluorescence and discriminative power. A 785nm laser can also be used as a complement to differentiate inks. Dyes in inks can be revealed by an analysis of their Raman spectra obtained using different wavelengths.

This presentation will impact the forensic science community by demonstrating how blue ballpoint pen inks and dyes found in inks should be analyzed by Raman spectra obtained using lasers of three different wavelengths in a systematic manner.

Raman spectroscopy can be helpful for characterizing and discriminating inks based on their composition. However, fluorescence interference is a problem that is sometimes encountered and may result in poor analytical performance. The applicability of Raman spectroscopy to the analysis of 50 blue ballpoints and 8 synthetic dyes commonly found in blue ballpoint inks was investigated in this study. Excitation wavelengths varying from 514nm, 633nm, and 785nm were

used to identify the dyes and caution of fluorescence observed in spectra of inks. Methyl violet derivatives are commonly used in blue ballpoint inks, which include crystal violet, methyl violet, pararosaniline, and so on. Copper phthalocyanine is another kind of dye found in many blue ballpoint inks. Basic blue 7, Victoria blue, acid blue, aniline blue, and pigment blue 7 are also added by some manufacturers.

All dyes were measured by focusing through test drops deposited on the surface of the microscope slides and inks were detected by focusing on ink lines on paper. The spectra of Methyl violet derivatives obtained at 514nm are almost identical, which are similar to basic blue 7 and stronger than Victoria blue. Other dyes produced intense Raman scattering. For seven dyes, fluorescence and weak bands were observed in the 633 nm spectra and high levels of fluorescence were in the 785 nm spectra. Only copper phthalocyanine responded well to both laser wavelengths showing consistent peaks of high intensity.

For 50 blue ballpoint inks, the Raman spectra at 514nm did not provide adequate discriminative power and the tested inks could be divided into only eight groups. Spectra of 35 blue ballpoint inks dominated by methyl violet derivatives whose main peaks were at 1619cm-1 1584 cm-1 1540 cm-1 1372 cm-1 1173 cm-1 911 cm-1 and 801cm-1 were included in the MV group. Spectra of four inks were similar with the MV group (two of them whose Raman signal near 1175cm-1 split to 1362 cm-1 and 1389 cm-1, and the other two had different peaks at 692cm-1 and 1647cm-1 respectively). According to peaks at 1070 cm-1, 1157 cm-1, 1175 cm-1, 1197 cm-1, the spectra of nine blue ballpoints were dominated by Basic blue 7, which could be divided into one group with two inks and one group with seven inks because of the different bands at 1175 cm-1 and 1197 cm-1. There was one ballpoint whose spectrogram was matched with that of pigment blue 15. And the last spectrogram was dominated by a fluorescent background and weak Raman shifts at 1645 cm-1 and 1615 cm-1. The discriminative power by 514nm laser was 0.50 because 70% of spectra were methyl violet derivatives.

Upon excitation at 633 nm, the inks were separated into 11 groups. One group contained ten inks which belonged to the MV group whose Raman signal at 514nm was submerged by inflorescence. One ink had a weak peak at 1616cm-1 and another one had weak peaks at 1616cm-1 and 796cm-1 on the strong inflorescence background. The remaining 37 inks produce abundant Raman shifts, the different ratio of height 1616cm-1 versus 1539cm-1 suggested the different ratio of methyl violet and copper phthalocyanine in ink. Twenty-eight inks with higher 1616cm-1 peaks were divided into five groups by slight peak difference. Ten inks with lower 1616cm-1 peaks were divided into two groups by the weak and stable peak at 681cm-1. The ink matched pigment 15 upon 514nm laser showed both Raman signals of pigment blue 15 and copper phthalocyanine. The discriminative power by the 633nm laser was 0.84, which was higher than that of the 514nm laser.

The Raman spectra obtained using the 785 nm laser allowed division of the inks into seven groups and resulted in a lower discriminative power (0.77) than that of the 633nm laser. Fluorescence played stronger influence on spectra and 16 inks were dominated by it. The other 22 inks generated main Raman scattering bands at 1544cm-1, 1341cm-1 and 749cm-1 from the influence background and certain shifts classified inks into three3 groups. Only eight inks produced a strong Raman signal and could be separated into three types, one of them was almost the same with copper phthalocyanine.

**Conclusions:** Raman spectra at 514nm were not influenced by fluorescence, whereas the discriminative power was not good since the widely used dye methyl violet derivatives generated strong Raman scattering at 514nm. Raman spectra at 633nm showed the fluorescence of basic blue 7 or acid blue as well as the mixture of methyl violet and copper phthalocyanine and gave the best discriminative power of the three lasers. The weak bands at 785nm could distinguish more inks than 514nm excitation although the spectra were susceptible by fluorescence.

The main dyes used in blue ballpoint pen inks could be inferred by Raman spectra. Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) could be considered for further research to identify the exact dyes.

Blue Ballpoint Pen Inks, Dyes, Raman Spectroscopy

#### J14 Optimal Methods for Developing Machine-Made Indentations on Paper

# Larry A. Olson, MFS\*, Internal Revenue Service, National Forensic Lab, 29 North Wacker Drive, Chicago, IL 60606

After attending this presentation, attendees will learn various optimal techniques for developing the indentations on paper documents caused by modern business machines.

This presentation will impact the forensic science community by providing a guide for developing the best results of indentations on paper caused by business machines (printers, fax machines, and/or photocopiers).

For at least 30 years, document examiners have developed indentations of various types of paper fiber disturbances using an instrument known as an electrostatic detection device (EDD). An EDD is used most often to attempt to visualize impressions from handwriting, but it can also detect erasures, as well as other impressions in the paper. It has only been in the past several years that papers have discussed that an EDD can also develop indentations caused when a document comes in contact with the working parts of business machines (e.g., printers, fax machines, and photocopiers).

Inspired by a case in which distinctive machine indentations were found on a number of questioned documents, research was started to determine what indentations could be caused by a pair of photocopiers of the same make and model. A document can receive indentations from a photocopier in one of two ways: either as (1) as an original document being copied, if it is processed by an automated feeder mechanism; or (2) as a photocopy passing from a paper tray through the machine to the print and fixing mechanisms. Exemplars were created from the subject copiers by the two means listed above. The specimens were processed with an EDD known as an ESDA2 (manufactured by Foster + Freeman, Ltd.), in accordance with recommended operating instructions, using the cascade method of development.

The research revealed that although there were parts of the subject copiers that did come into contact with the paper, the development of indentations from this contact could not be guaranteed, and the extent of the indentations visualized was not consistent from specimen to specimen, despite the use of the same type of paper.

The purpose of this current research was to improve the quality and quantity of machine-made indentations on paper by attempting to determine the optimum conditions for their development.

Specimens were created using a single type of paper and were passed through a photocopier known to create indentations. Two different EDDs were used for the processing, an ESDA and an ESDA2 machine. All possible means of image development were attempted: (1) development by the aerosol application of toner powder; (2) development by the application of toner covered cascade beads; and, (3) development by the use of a toner activation device (TAD). Different orientations of holding the corona wand were tried to vary the distribution of an electrostatic charge on the document's surface. Specimens were developed both with and without humidification, and they were processed at different times of the year, with different levels of relative humidity in the processing room.

Indentations, Business Machines, Questioned Documents

#### J15 Dating Documents and Photographs Based Upon Atomic-Bomb Derived Radiocarbon Content

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After attending this presentation, attendees will understand how radiocarbon measurement can be applied to the authentication of documents and photographs.

This presentation will impact the forensic science community by highlighting the development of a new tool for this purpose.

The radiocarbon content of documents and photographs indicates when these media were manufactured, and thus may provide information relevant to the determination of authenticity. Since 1955, the earth's atmosphere has contained elevated levels of radiocarbon due to aboveground nuclear testing that occurred up until 1963. The elevated radiocarbon levels have been circulating through the atmosphere, the oceans, and the biosphere ever since. When detected in plant or animal tissues, elevated radiocarbon levels provide a time-stamp for organisms living in the Nuclear Age. This has been exploited for a variety of purposes including forensic dating of human remains. Paper and photographic materials are derived from plant and animal tissues. Consequently, they should also contain elevated levels of radiocarbon if produced after 1955.

The uptake of radiocarbons into known-age samples of paper and photographs has been examined in order to understand the dynamics of radiocarbon uptake. Preliminary results indicate that unambiguously elevated radiocarbon levels do not appear in paper products until around 1965. This is most likely a consequence of multiple factors: (1) the pattern of accumulation of new tissues in trees used for paper production; (2) temporal patterns of pulpwood growth and harvest; and, (3) temporal patterns of paper production, distribution and use; and other phenomena. In spite of these uncertainties, the uptake of bomb-derived radiocarbon into paper products can be applied to the authentication of dated documents. The method has been successfully used to unambiguously detect late 20<sup>th</sup> Century forgeries of early 20<sup>th</sup> Century documents and a few case studies will be presented. Establishing a higher resolution is the objective of ongoing research.

Current research is focusing on the differential measurement of radiocarbon in photographic paper and photographic emulsions. The dynamics of atmospheric radiocarbon uptake into these different components of a photograph may help to more precisely identify when the photographic paper was produced. Whereas photographic paper is derived from pulp wood (and thus from relatively long-lived trees), photographic emulsion is derived from animal gelatin, which is, conversely, produced from relatively short-lived animal species. These differences in lifespan influence the pattern of bomb-radiocarbon uptake from the environment. The uptake of bomb-derived radiocarbon into emulsion would theoretically be faster and could achieve higher levels than those found in paper, and the differential levels might improve estimates of the manufacturing date of photo paper. Preliminary measurements made on photographic papers of known age produced in the middle decades of the 20<sup>th</sup> Century bear this hypothesis out.

Clearly there are uncontrollable factors that affect the utility of this approach in real-world situations. The method indicates when paper and photographic materials were manufactured, or more precisely, when the organisms used as raw materials for them were living. It does not identify when a piece of paper was printed or written upon, or when a photographic image was produced. Nevertheless, the information can be used to answer certain types of questions regarding document or photograph authenticity. The method has a potentially broad application in forensic science, and parallels a larger research project examining bomb-carbon uptake into human tissues for purposes of post-mortem determination of a subject's year of birth and year of death.

Atomic Bomb-Derived Radiocarbon, Photograph, Dating

#### J16 SWGDOC – Where We've Been, Where We Are, and Where We're Going

# Ted M. Burkes, BS\*, FBI Laboratory, 2501 Investigation Parkway, Room 2158, Quantico, VA 22135

After attending this presentation, attendees will understand the history, accomplishments, and future of SWGDOC.

This presentation will impact the forensic science community by educating attendees about the direction of this Scientific Working Group.

SWGDOC began in 1997 as TWGDOC (Technical Working Group for Forensic Document Examination), was renamed SWGDOC in 1999, and was reorganized in 2001 to its current form of operation.

SWGDOC is composed of the chairperson and numerous government and private forensic document examiners from throughout the United States. The government examiners are from federal, state, and county laboratories.

The mission of SWGDOC is to assemble representatives from the forensic document examination community in order to: (1) define the scope and practice areas of the profession; (2) standardize operating procedures, protocols, and terminology; (3) consolidate and enhance the profession of forensic document examination; and, (4) promote self-regulation, documentation, training, continuing education, and research in the area of forensic document examination.

The field of questioned documents was one of the first to receive repeated challenges in court by a group of law school professors who claimed to be "experts on experts." As a result, the discipline was motivated to begin writing and publishing sub-discipline specific operating procedures for use during questioned document examinations (e.g., handwriting, typewriting, indentations, etc.). It was determined that SWGDOC would publish through ASTM International, as draft documents are reviewed by practitioners in many different forensic disciplines through that organization.

SWGDOC operates using sub-groups with a population of generally five to seven individuals. These sub-groups draft or update operating procedures for specific sub-disciplines within the forensic document expertise. These drafts are vetted through the other sub-groups and then submitted to ASTM International (a consensus-based standards publishing organization) for balloting and eventual publication. SWGDOC has either written and/or updated eighteen standards published through ASTM International. There are also fifteen additional draft standards that have been prepared for balloting.

SWGDOC's current goals are to: (1) strengthen the content and the enforcement of published performance standards; (2) continue to write and foster the publication of performance standards for sub-discipline examinations; (3) publish and maintain the *Daubert Factors for Attorneys and Daubert Factors for Forensic Document Examiners* presentations (as they relate to forensic document examination); (4) participate in and support a Human Factors Working Group for Forensic Document Examination; and, (5) expand the participant pool to include academicians, statisticians, legal professionals, and practitioners from other forensic disciplines.

SWG, Scientific Working Group, Questioned Documents

#### J17 Guide for the Development of Forensic Document Examination Capacity

Meredith DeKalb Miller, MFS\*, 2915 Middle Road, Davenport, IA 52803; Magali Bernard, MS, and Barbara Remberg, MS, United Nations Office of Drugs and Crime, Vienna International Center, Wagramer Strasse 5, A 1400, Vienna, AUSTRIA

After attending this presentation, attendees will learn about a guide developed by an international panel to provide practical assistance for the establishment or upgrading of forensic document examination capacities in two categories of service providers: (1) immigration and border control agencies; and (2) forensic science laboratories.

This presentation will impact the forensic science community by presenting a holistic approach to document examinations encompassing identity, security, and other types of documents without security features.

This guide will assist both donor and beneficiary countries in their efforts to design, build, and strengthen forensic document examination and intelligence dissemination capacities.

Fraudulent identity and security documents are integral prerequisites for the smuggling of migrants, trafficking in persons, terrorist mobility, facilitating the smuggling of drugs, weapons and other goods, and committing fraud. Fraudulent documents are the grease that eases cross-border crime of all types. They include fraudulently obtained, illegally issued, forged, and counterfeit documents.

Security documents refer to documents containing incorporated security features within the document to protect the value of the document. Many identity documents contain security features such as passports, identity cards, and driving licenses. Other examples of security documents include currency, social security cards, travel visas, and lottery tickets, etc.

Identity documents are any documents which may be used to verify aspects of a person's personal identity. Some countries require individuals to carry a government issued identity card, while other countries may accept a driver's license as an effective means of proof of identity.

Many countries in the world recognize that forensic document examination is vital to immigration and border control security and have a forensic document examination facility. Although the ability to detect and disseminate intelligence about fraudulent documents is vital to border security, there are still countries lacking this capacity. Moreover, there is a lack of awareness among relevant criminal justice practitioners of the benefits that forensic document examinations may provide to assist border control security and immigration facilities.

Traditionally, forensic document examination units are part of a forensic science laboratory. These units examine and compare handwriting, typewriting, printing processes, inks, and other document characteristics which may or may not include document security features. To respond to the detection of fraudulent identity and security documents specifically, many countries have established additional specialized structures. These structures are often under the immigration service and border control agencies and are housed in port-of-entry facilities.

Some countries have a sophisticated forensic document examination capacity both in their national forensic science laboratory and under immigration or border control services. Other countries have a more limited forensic capacity under their immigration and border control services and more advanced document examinations are done at the national forensic science laboratory. Still others rely entirely on their national forensic science laboratory for the examination and analysis of all documents.

Several levels of infrastructure development ranging from basic to advanced capacity are covered. The focus is on staff skill and educational requirements needed to perform forensic document examinations and to provide court testimony, intelligence alerts and training. Recommendations on forensic equipment, reference collections and databases as well as general guidance for designing, establishing, and maintaining a forensic document examination facility are included. This guide should not be used as a simple checklist of equipment and materials to be obtained but rather as an aid for developing capacity in the area of document examinations.

**Document Examination, Intelligence, Capacity Building** 

### J18 Enhancing the Subjective Decision Making Process in Non-Destructive Differentiation of Writing Inks: Calibrating the Forensic Document Examiner

#### Derek L. Hammond, BA\*, United States Army, Criminal Investigations Lab, 4930 North 31st Street, Forest Park, GA 30297-5205

After attending this presentation attendees will gain an understanding of the potential for inter-examiner variation in results stemming from the non-destructive examination of writing inks. In addition, attendees will be able to assess the potential benefits of a proposed change to the manner in which forensic document examiners (FDEs) are trained to non-destructively differentiate writing inks.

This presentation will impact the forensic science community by providing empirical data relating to the potential existence and frequency of inter-examiner variation in the results obtained through the nondestructive examination of writing inks. Furthermore, a modification to current training methods relating to non-destructive ink differentiation tasks is proposed along with data which suggests that this training modification may improve FDEs' results and provide FDEs' with a method to further calibrate their subjective decision making processes for this task.

Per ASTM, FDE trainees are required to receive training in the nondestructive analyses of inks. Traditionally, this training has included: (1) an overview of the history of writing inks; their composition, and manufacture; (2) the methods of examining and differentiating inks, and the application of these methods to specific forensic problems; and (3) the operation and maintenance of the relevant instruments available in the laboratory. At the conclusion of this training the student is typically required to pass a knowledge-based written test and/or a practical exercise. Although the examination of writing inks often involves instrumental analysis, the examiner must still employ subjective decision making in determining whether two or more ink samples are different. Given that subjective judgments are required; does the current training format ensure that the FDE has acquired the knowledge and experience to make accurate and reliable judgments about when the instrumental result supports differentiation and when it does not?

This presentation will focus on an intra-laboratory pilot study assessing inter-examiner variability in the differentiation of black ballpoint ink samples using hyperspectral imaging technology. In this study, five similarly trained FDEs were tasked with examining 25 penpair samples to determine whether the two inks present on each pen-pair sample were created using different inks or if they were unable to say. Three of the pen-pair samples were created using the same ink and the remaining 22 pen-pairs were created using different inks. The majority of the pen-pair samples created using different inks could be classified as "close non-matches" (i.e., visually different but not obviously so). High inter-examiner variation in results and several "false negatives" were observed. In fact, the participants were unanimous in their opinions on only five of the 25 pen-pair samples. At the conclusion of the analysis phase of the experiment FDEs were informed of the "ground truth" answer for each pen-pair sample and were given an opportunity to review their answers as well as the digital image files captured during analysis. Afterwards, ten of the previously examined samples were reexamined by four out of the five original participants, after being reblinded to each of the examiners, and the examiners' opinions were reevaluated to assess whether evidence of a learning effect (i.e., calibration of the examiner's decision making process) existed.

As a result of this pilot study, it is believed that there is evidence to support the contention that current training procedures for the nondestructive analyses of writing inks need to be modified. A low cost training mechanism will be proposed which may provide a basis for FDEs to calibrate their decision making threshold for the non-destructive examination of inks thereby promoting higher FDE correct call rates and enhancements to the reliability and skill of the expert.

Calibration, Subjectivity, Training

### J19 The Current Status and Future of Ink Dating Methods

Gerald M. LaPorte, MSFS\*, National Institute of Justice, Investigation & Forensic Science Division, 810 Seventh Street, Northwest, Washington, DC 20531

After attending this presentation, attendees will gain greater understanding about ink dating methods and be updated on the current status.

This presentation will impact the forensic science community by providing a comprehensive retrospective on ink dating and a look toward its future.

Requests to determine the age of a writing ink used on a document are frequently encountered by forensic document examiners. Dr. Antonio Cantu formerly outlined two analytical approaches for determining the age of an ink on a questioned document: static and dynamic. The static approach to ink dating generally applies to methods that are based on comparisons with a standard reference collection of inks to determine the first date of production. The dynamic approach includes methods that incorporate procedures for the purpose of measuring the physical and/or chemical properties of an ink that change with time. The basic principle is that when ink is placed on a piece of paper, it undergoes an aging process due to evaporation of solvents and complex interactions that result from oxidation and polymerization of resins or other components. The changes that occur over a given period of time can generally be referred to as aging characteristics. Different approaches to measuring the age of an ink, once it has been placed on a document, have been discussed in the literature over the past two decades, but there still exists significant controversy about the accuracy, reliability, and validity of the dynamic procedures.

This presentation will provide an historical perspective of ink dating methods and an update regarding the current status. A synopsis of the research that has been published on this topic and a review of court rulings will be provided. Specifically, how these methods have fared when subjected to *Frye* and *Daubert* hearings will be highlighted. There is a very small number of forensic ink dating specialists worldwide, so attendees will benefit from a comprehensive retrospective on ink dating and a look toward its future.

Ink Dating, Ink Analysis, Ink Aging

#### J20 Differentiation of Black Permanent Marker Inks by Thin-Layer Chromatography and Gas Chromatography-Mass Spectrometry

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After attending this presentation, attendees will understand how brands of permanent marker ink can be differentiated by thin-layer chromatography and gas chromatography-mass spectrometry. This presentation will impact the forensic science community by demonstrating how thin-layer chromatography and gas chromatographymass spectrometry can aid document examiners in the differentiation of brands of permanent markers.

Identifying the formulation of ink can be important for questioned document examination. Knowledge of ink formulations can help determine the authenticity of a document, including its age and the presence of any alterations of the document. Permanent markers are commonly available and widely used writing instruments; they are used particularly for labeling items made from non-porous materials, such as glass and plastic. Thin layer chromatography and gas chromatographymass spectrometry are well established methods for determining the composition of ball pen and gel pen inks. It is believed that thin layer chromatography and gas chromatography-mass spectrometry would also have significant value in the analysis of permanent marker inks.

Fifteen brands of black permanent marker were purchases in Virginia, Ohio, and the District of Columbia. For brands that had more than one marker per pack, all markers in the pack were tested separately to ensure consistency within the brand. This gave a total of forty-eight markers. Scribble sheets were created with each marker using 1-inch squares of filter paper. The ink was then extracted from a 7mm circle of the scribble sheet using 40µL methanol in a small glass vial. This extract was used for both thin-layer chromatography and gas chromatographymass spectrometry. The inks were examined by thin-layer chromatography by spotting approximately 4µL extract on silica gel plates without fluorescent indicator. A mobile phase consisting of ethyl acetate, ethanol, and water (75:35:30) was used. The solvent front was allowed to migrate 4 cm before the plate was removed from the mobile phase. For gas chromatography-mass spectrometry a non-polar 30-meter capillary column was used. The oven temperature program had an initial hold at 50°C for one-minute, followed by a ramp to 200°C at a rate of 10°C per minute with a two-minute hold. Finally the oven temperature was increased to 300°C at a rate of 25°C per minute with a final hold for two-minutes. The mass analyzer was an ion trap operating in electron impact mode with a scan range from 40 m/z to 650 m/z. The samples analyzed by thin-layer chromatography were run in duplicate, and those analyzed by gas chromatography-mass spectrometry were run in triplicate to ensure reproducibility. Splitless injection was used for the samples analyzed by gas chromatography-mass spectrometry to make sure that all volatile organic ink components were detected.

Thin-layer chromatography separates the dyes used in the ink, while gas chromatography-mass spectrometry appears to identify mainly other ink components. These two analytical methods are therefore complementary. It was determined that one group of three brands and one group of two brands could not be differentiated by thin-layer chromatography. The Discriminating Power (DP) for thin-layer chromatography was determined to be 0.962 with 105 total possible pairs of permanent marker brands. All brands of permanent marker tested could be differentiated by gas chromatography-mass spectrometry based on the presence or absence of specific peaks in the chromatograms. Gas chromatography without mass spectrometry would therefore also appear to be a highly discriminating method for the analysis of permanent markers. Further work is underway to identify as many of the volatile organic components detected by gas chromatography-mass spectrometry as possible. The effects of aging of permanent marker inks are also being examined. These methods can aid document examiners in the differentiation of brands of permanent markers.

Questioned Documents, Thin-Layer Chromatography, Gas Chromatography-Mass Spectrometry

### J21 Differentiation of Black Permanent Marker Inks by Ultraviolet-Visible-Near Infrared Spectrophotometry and Fourier Transform Infrared Spectrometry

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After attending this presentation, attendees will understand how Ultraviolet-Visible-Near Infrared Spectrophotometry (UV-VIS-NIR) and Fourier Transform Infrared Spectrometry (FTIR) may be used to differentiate brands of black permanent markers.

This presentation will impact the forensic science community by showing the high level of discrimination of different brands of black permanent marker that can be obtained through the use of UV-VIS-NIR spectrophotometry and FTIR spectrometry.

Identifying the formulation of an ink can be important for questioned document examinations. Knowledge of ink formulations can help determine the authenticity of a document, including its age and the presence of any alterations of the document. Permanent markers are commonly available and widely used writing instruments; they are used particularly for labeling items made from non-porous materials, such as glass and plastic. Ultraviolet-visible near-infrared spectrophotometry (UV-VIS-NIR) has been used to analyze ball pen and gel pen inks with some degree of success. Several studies have been conducted on the differentiation of ball pen inks using Fourier Transform Infrared (FTIR) spectrometry. The application of these two spectroscopic methods of analysis to black permanent marker inks in an effort to determine their utility for the differentiation of black permanent marker inks have been examined.

Fifteen brands of black permanent marker were purchased in Virginia, Ohio, and the District of Columbia. For brands that had more than one marker per pack, all markers in the pack were tested separately to ensure consistency within the brand. This gave a total of forty-eight markers. Scribble sheets were created with each marker using 1-inch squares of filter paper. The UV-VIS-NIR spectra of the scribble sheets were recorded in reflection mode using a UV-VIS-NIR spectrophotometer equipped with an integrating sphere. The reflectance spectra were scanned from 300 nm to 2000 nm. The infrared spectra were scanned in reflectance mode from 700 cm-1 to 4000 cm-1 using a Fourier Transform Infrared spectrometer equipped with an infrared microscope. Samples for infrared analysis were prepared by extracting ink from a 7-mm disk punched from the scribble sheets. Each disk was extracted with 40 µL of methanol; extracts were then spotted on the dull side of a clean sheet of aluminum foil and allowed to air dry. Care was taken to obtain spectra only from regions of the spotted samples where no dye separation was evident. The infrared spectra of the permanent marker inks showed the characteristic absorption features of triaryl methane dyes; in general the infrared spectra of the permanent markers were similar to those of ball pen inks.

The UV-VIS-NIR and FTIR spectral data were scaled and then subjected to principal components analysis (PCA). The UV-VIS-NIR spectral range from 400 nm to 1300 nm was used for PCA to avoid fluorescence and NIR absorptions of the filter paper substrate. Only the infrared spectral range from 800 cm-1 to 1800 cm-1 of the FTIR spectra was used. The truncation of the infrared spectra was carried out to reduce the number of variables in the PCA; the spectral region selected was also observed to contain the most significant spectral variation among the permanent marker samples. The latent factors extracted by PCA were used to carry out agglomerative hierarchical clustering (AHC) and linear discriminant analysis (LDA). Based on their UV-VIS-NIR spectra the fifteen brands of black permanent markers could be placed in nine groups. UV-VIS-NIR spectrophotometry was found to have a discriminating power (DP) of 0.848. Based on their infrared spectra, the markers could be placed in four groups. FTIR was found to have a DP of 0.714. Combining FTIR with UV-VIS-NIR spectrophotometry did not provide any additional discrimination among brands.

UV-VIS-NIR and FTIR analyses are minimally non-destructive tests and can be conducted on inks *in situ* on a substrate. While they do not provide complete discrimination of brands of permanent markers, they do provide a degree of discrimination that may be useful in many forensic investigations.

Questioned Documents, UV-VIS-NIR Spectrophotometer, FTIR

#### J22 The Application of Raman Spectroscopy to the Analysis of Blue, Red, and Black Gel Pen Writing Inks

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After attending this presentation, attendees will understand the basic principles of Raman Spectroscopy and how it can be applied to ink analysis for questioned document casework, as well as the benefits and drawbacks of the technique.

This presentation will impact on the forensic science community by providing practitioners with an overview of the most suitable excitation wavelengths and ideal operating conditions for the analysis of common colours of gel ink on white office paper. It will extend the existing body of research which has already demonstrated the potential of Raman Spectroscopy for the analysis of gel pen writing inks where traditional techniques have been found to not be as effective. It will also demonstrate for the first time, to the best of the authors' knowledge and belief, the use of a multivariate statistical approach to discriminate between different brand and model combinations of gel pens.

During the past 15 years, the gel pen has become an increasingly popular choice of writing instrument around the globe, primarily due to its relatively low cost, long writing life, and environmentally friendly ink composition. The gel pen utilizes water based ink composed of either pigments or dyestuffs, giving a wide selection of colors. With this increased popularity, comes a need within the forensic community to find a suitable analytical approach by which to identify and classify different brands and/or models of gel pen ink. Among other analytical techniques investigated for this purpose, Raman Spectroscopy has been shown to provide a good ability to discriminate between different pigment-based gel pen inks. The technique can be performed in situ and involves directing a laser of specific excitation wavelength onto an ink sample in order to detect scattered light at longer wavelength to generate a Raman spectrum characteristic of its molecular structure, thus providing a molecular fingerprint for comparison. Different spectra of the same ink sample can be produced by using different excitation wavelengths providing additional discriminating ability.

A selection of over 450 gel ink pens in blue, red, and black colors, representing a variety of different brand/model combinations available on the worldwide market were analyzed using different Raman Spectrometer systems and several different excitation wavelengths (514nm; 633nm; 685nm; 785nm and 830nm). Resulting spectra were first grouped according to visual pattern recognition. Then, the application of a multivariate statistical approach in the form of hierarchical cluster analysis (HCA) was used in an attempt to

discriminate between different brand/model combinations. The data presented here discusses some of the findings from this investigation and makes some recommendations as to the use of Raman Spectroscopy in gel ink analysis.

This work forms part of a wider investigation into the ability of other analytical techniques to discriminate within and between batches of different gel pen inks, focusing in particular on the potential of stable isotope analysis at a natural abundance level of the elements C, N, H, and O by Isotope Ratio Mass Spectrometry (IRMS) for this purpose. It is envisaged that this will lead to the development and validation of a reliable analytical methodology for the analysis of gel pens inks within forensic casework.

Questioned Documents, Raman Spectroscopy, Gel Pen Inks

#### J23 The Use of Hyperspectral Imaging for Ballpoint Pen Ink Differentiation

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After attending the presentation, attendees will have an understanding of the discriminating power of hyperspectral imaging (HSI) methodologies, as related to ballpoint pen ink differentiation.

This presentation will impact the forensic science community by providing guidelines for HSI reflectance and luminescence data collection, as both methods have been shown to provide increased discrimination capabilities as compared to other imaging technologies currently available to forensic document examiners.

Ink discrimination can be further enhanced by employing more sensitive techniques and equipment. Visible/near-infrared (NIR) reflectance and luminescence can be a major asset to the forensic document examiner, providing the means for both nondestructive and chemistry-based analysis. HSI combines both spectroscopic and digital imaging information by recording images of the samples as a function of wavelength through the use of a highly efficient electro-optic imaging spectrometer. When liquid crystal tunable filters (LCTF) are employed during data collection, one is able to achieve a finer spectral resolution as well as more detailed spectra to reveal small spectroscopic variations within a given sample. HSI provides visible and NIR data as well as high resolution images that equip examiners with the tools to detect small chemical variations amongst a set of given inks.

In this study 44 black ballpoint pens were paired in various combinations and then were analyzed with the goal of determining HSI's ability to detect, discriminate, and provide satisfactory sample to sample (as well as sample to substrate) contrast. The samples were also previously examined using VSA (video spectral analysis) and LAB Color Mode. Over 500 samples were studied using reflectance/absorbance and luminescence modes, to exploit several spectroscopic properties for each pen pair. The overall results from the study will be discussed, including potential sources of error, as well as a general comparison of the performance of HSI to other techniques used to analyze the pen pair samples. The research aims to demonstrate how HSI can be utilized by document examiners in daily casework as well as ultimately show the analytical capabilities HSI has for discriminating black ballpoint pen inks.

Hyperspectral Imaging, Ballpoint Pen, Ink Discrimination

#### J24 Sixty Years of Milestones in Forensic Document Examination

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After attending this presentation, attendees will gain a brief historical insight into the establishment of the American Academy of Forensic Sciences (AAFS) and its Questioned Document Section and gain knowledge of the progression of Forensic Document Examination (FDE) over a period of sixty years.

This presentation will impact the forensic science community by providing specific historical information relating to the evolutionary developments in FDE. Sixty years of milestones will be offered in a brief and simple format. This presentation will demonstrate how the continuing advances and challenges of technological progression have historically influenced the development and evolution of the science. New technology creates new questions to answer and new problems to solve.

The original idea to research milestones in the forensic sciences was developed by President Bruce A. Goldberger, PhD, who was president during the sixtieth anniversary year of the AAFS. The section officers of each section in the AAFS were encouraged to develop a "sixty years of milestones" text regarding their specific sections. Motivated by this wonderful idea, the research for this project, sixty years of milestones in FDE, was conducted and the milestones were documented. This presentation, which is designed to be concise, will feature the evolution of sixty years of milestones in FDE and address areas including forensic organizations, certification, establishing standards, tools and techniques, texts, writing instruments, typewriters, ink, copiers, desktop publishing, and handwriting.

This presenter has personally experienced much of the historical evolution in FDE through decades of participation in the Questioned Document Section of the AAFS. The historical information contained in the milestones is the result of experience in the field, communications with knowledgeable members of the AAFS Questioned Document Section and researching FDE history contained in published reference materials which were written by Questioned Document Section members.

It is especially important to have knowledge of the evolutionary milestones of one's forensic discipline. Along with the rapid technological advances in society, forensic science will continue to evolve, and new milestones will be documented. Future milestones will occur in an exceedingly rapid manner when compared to the milestones of the past sixty years. Court rulings directly affecting the forensic sciences will progress, challenges to forensic science disciplines will continue, scientific research are on-going and there will be new problems to solve and new questions to answer.

**Evolution, Milestone, Forensic Document Examination** 

### J25 Trends in Handwriting Instruction

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After attending this presentation, attendees will be made aware of recent trends and theories in handwriting instruction and the spread of newer and revised handwriting systems in the United States. Class characteristics of notable new and revised systems will be highlighted. Information from a survey of school systems will be presented.

This presentation will impact the forensic science community by increasing awareness of newer handwriting systems and their class characteristics, which will aid in the evaluation of handwriting in the practice of forensic document examination. Knowledge of the forms in prescribed writing systems is important in forensic document examination in evaluating the uniqueness and significance of handwriting characteristics. Forensic document examiners must be aware of characteristics which may have been influenced by a system and thus are common to a large class of writers, and those which are individual and serve to uniquely characterize writing.

Previous surveys of handwriting systems have shown that the Palmer and Zaner-Bloser systems were dominant through much of the 20th century, with Zaner-Bloser eventually becoming the more widelyused system in public schools.

Toward the end of the century and into the present, emphasis in schools has gradually shifted away from handwriting instruction to more time spent on keyboarding and computer skills. Students also write less in school and in their home lives, choosing computers, text messaging, and electronic media over handwriting as the preferred method of communication. Educators have noted that by the time students reach junior high and high school age, their handwriting is often difficult to read and partly illegible. The result of this is that educators have sought handwriting systems that are easier and quicker to learn and that emphasize legibility and speed.

The D'Nealian system of handwriting was created in part as a response to this need. By the 1990s, studies of portions of the United States showed that the D'Nealian system had surpassed Zaner-Bloser as the most popular system in public schools in the majority of the states surveyed.

Other simplified systems have also been developed based on different theories as to which forms and methods of letter construction are easier to learn, retain, and more likely to remain legible after initial instruction. The Handwriting Without Tears (HWT) system has become popular and is used by a growing number of school systems. Zaner-Bloser has moved beyond their traditional system to offer a simplified system; recent information indicates that Zaner-Bloser again shares the industry lead alongside D'Nealian. There has also been discussion in some schools of teaching only printing, and dropping cursive writing instruction altogether. Another movement supports the idea of teaching only one italic-based system rather than the current two system approach of teaching cursive and printing.

Information for this presentation is based on a literature survey and interviews with personnel at handwriting system companies, and on an ongoing survey of schools; data presented will include results from the Chicago metropolitan area and other regions as available.

Questioned Documents, Handwriting Systems, Writing Instruction

#### J26 Reducing Error: The Benefits of Checklists in Forensic Document Examination

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After attending this presentation, attendees will recognize the complexity of forensic document examination and the manner in which error can occur due to the numerous tasks that can be required within an examination. Is expertise enough to fully prevent error in forensic science? A simple mechanism proven as a quality-enhancing tool in high risk and complicated industries will be suggested and direct application will be made to the forensic document examination profession.

As added technology and knowledge levels have increased the intricacy and core competencies in forensic science and the forensic examination of documents, by instituting the use of checklists for various document examination procedures, improved reliability and consistency will occur in examinations, thus aiding and impacting the forensic community and society.

Research by Kam et al. and Found, Sita, & Rogers has shown that Forensic Document Examiners (FDEs) have significantly more expertise than laypersons for the task of determining the authenticity of signatures and handwriting. In these studies; however, the FDEs were found to have error rates as high as 7% for some tasks. Beck in a 1995 article cited three possible main sources of error: (1) failure to properly evaluate differences; (2) failure to detect significant movement characteristics; and, (3) the use of self-serving exemplars. Other contributions known to lead to error will be discussed.

A taxonomy of FDE tasks finds examination categories (materials analysis, pattern classification, pattern analysis, restoration, visualization) with numerous sub-categories of examinations structured within each category (signatures, handwriting, hand printing, physical ink examination, chemical ink comparisons, alteration, substitution). The decision and evaluation process for multiple aggregated examinations is complex and can be daunting. For these reasons, the training of a FDE takes between two to three years of formal study with systematic practical exercises. The vast sum of techniques, knowledge and limitations to remember and apply is significant, and becomes even more so when a case is multi-faceted requiring the application of a combination of examinations conducted in an appropriate sequence.

Many examiners have developed their own way of designing the sequence of examination. They rely on their training and experience to do this. However, because of the complexity of the tasks involved, it is possible to overlook a simple examination or consideration within an examination or at the evaluation phase which may then lead to an error in the conclusion or result.

The use of a checklist may be one way to reduce this possible source of error. This is not a novel idea in forensic document examination. Historic texts describe lists of principal points for consideration or caution (Osborn) or for "document consciousness" (Conway). These were intended to provide a "thoughtful, reasoning approach" to the examination of a document, and to remind the thorough examiner of problems or issues to consider.

The development, design and use of checklists in non-forensic industries and the resulting effects will be discussed, including the research of Dr. Peter Pronovost in one specific medical issue at Johns Hopkins Hospital that led to more open discussion and implementation of checklists within some medical circles. Checklist advantages for transparency and application of science endeavors will be reviewed. Checklists designed for FDEs will be presented and discussed. These checklists will range from one for a simple signature examination to another for the analysis of a multi-faceted business or medical record.

As added technology and knowledge levels have increased the intricacy and core competencies in forensic science and the forensic examination of documents, by instituting the use of checklists for various document examination procedures, improved reliability and consistency will occur in examinations, thus aiding and impacting the forensic community and society.

Forensic Document Examination, Checklists, Error Rates

## J27 "I am 99% Certain That Mr X Wrote This Document!" – An Introduction to Handling Uncertainty in Conclusions

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After attending this presentation, attendees will have a better understanding of the meaning, implications and requirements of the "probabilistic language" used in the ASTM standard E1658 on Standard Terminology for Expressing Conclusion of Forensic Document Examiners. This presentation is aimed to address these challenges and provide the audience with some background knowledge and examples to be able to answer questions relative to error and uncertainty in their field.

This presentation will impact the forensic science community by increasing the level of understanding and readiness when addressing error and probabilistic questions from customers.

ASTM Standard E1658-2009 proposes a specific terminology for expressing conclusions of forensic examinations performed on questioned documents. The range of conclusions proposed in this standard certainly appeals to common sense. In addition, it is likely to be easily understood by the various actors of the criminal justice system and other customers of questioned document examiners.

Nevertheless, the proposed terminology has a specific meaning, and particular requirements and implications. These need to be fully understood in order to correctly handle and report the uncertainty underlying all forensic examinations (not to mention, defending it in Court...).

Indeed, are less-than-certain conclusions opening the door for questions on "errors?" And if so, what kind of "errors?" How should questioned document examiners express probability in the absence of reliable or validated statistics in their field? And in fact, what are these statistics really measuring, and how are they related to E1658?

This presentation will review the exact sense, the requirements and the implications underlying the terminology proposed in the ASTM standard E1658. Through the use of examples, the assignment and meaning of probabilities in conclusion statements will be investigated. And finally, how to address questions on errors and contextual bias will be presented.

Uncertainty, Probability, Conclusion



# TOXICOLOGY



### K1 Analysis of Amphetamine on Swabs and Oral Fluid Sampling Device: A SPE Approach

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After attending this presentation, attendees will learn about the extraction of amphetamine from an oral swab and an oral fluid sampling device using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to compare results from both types of sampling methods.

This presentation will impact the forensic science community by offering analysts in forensic toxicology data from methods that permit samples of oral fluid material to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both SPE and LC-MS/MS.

Method: Extraction (SPE) was performed on mixed mode column (C<sub>8</sub>/SCX) conditioned with methanol, deionized water, and pH 6 buffer (3 mL, 3 mL, and 1 mL, respectively) prior to sample loading. Oral samples (swabs/ fluid sampling device) were taken 1 hour after administration of prescribed amphetamine. The swabs were extracted with methanol and adjusted to pH 6 with 0.1 M phosphate buffer (5 mL). The samples from the sampling device were extracted into 3 mL of a proprietary formulated buffer (pH 7) containing a non-azide preservative. A 1 mL aliquot was buffered with 5 mL of 0.1 M phosphate buffer. To both sets of sample an internal standard was added (amphetamine-d<sub>5</sub>). After loading the sample, the sorbent was washed with deionized water, acetic acid, and methanol (3 mL of each, respectively). Each SPE column was eluted with 3 mL of a solvent consisting of dichloromethane-isopropanol-ammonium hydroxide (78:20:2). An aliquot of this solvent was treated (details presented) with the mobile phase and analyzed by LC-MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of amphetamine and the internal standard, respectively.

Liquid chromatography was performed in gradient mode employing a 50 mm x 2.0 mm  $C_{18}$  analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% for re-injection. The total run time for each analysis was less than 5 minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis.

**Results:** The limits of detection/quantification for this method were determined to be 5 ng/ mL and 10 ng/ mL, respectively for amphetamine. The method was found to be linear from 10 ng/ mL to 500 ng/ mL ( $r^2>0.999$ ). Data is presented to show that recovery of amphetamine was found to be > 94 %. Interday and Intraday analysis of amphetamine were found to be < 4% and < 6%, respectively. Matrix effects were determined to be < 4%. Analysis of the subject swab concentrations ranged from 16 to 129 ng/ mL (mean: 46 ng/ mL (sample size =10)), while the oral sampling device ranged from 52 to 846 ng/ mL (mean: 132 ng/ mL (sample size =10)).

**Conclusion:** The use of this procedure for the analysis of amphetamine adds to the body of knowledge regarding the analysis of amphetamine. The data should be of great use to analysts in the field of forensic toxicology employing oral fluid analysis, as it demonstrates how far the horizons of oral fluid sampling can be expanded as it permits a direct comparison between oral swabs and oral sampling devices in relation to the analysis of amphetamine after oral administration. The

novelty of this study is the originality of the compare and contrast approach (as demonstrated by the presented data) to the analysis of amphetamine using readily available swabs and commercially available oral fluid devices. This limited study indicates the range of concentrations of the drug that can be achieved using either system. Amphetamine, SPE, LC-MS/MS

#### K2 The Effect of Drugs and Alcohol on Autopsy Cases Performed at the William L. Jenkins Forensic Center From 2003-2009

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After attending this presentation, attendees will have an appreciation of the effect of drugs and alcohol in the autopsy cases for Upper East Tennessee performed at the William L. Jenkins Forensic Center from 2003 through 2009.

This presentation will impact the forensic science community by providing descriptive statistics on the impact of alcohol and/or drugs and determining whether any exist in the autopsies performed from 2003 through 2009.

The William L. Jenkins Forensic Center has performed autopsies on questionable and medico-legal deaths which occurred in the eight counties of the First Tennessee Development District from 2003 through 2009. The purpose of this research was to compile descriptive statistics on the impact of alcohol and/or drugs, and determine whether any trends exist in the autopsies performed from 2003 through 2009. Toxicological evaluations of specimens collected at autopsy were used to determine if drugs and/or alcohol were involved in the deaths. A descriptive database was established defining all parameters and data pertinent in each case (age, sex, cause/manner of death, and toxicological results). Specimens (blood, gastric contents, urine, and vitreous humor) from the autopsies were analyzed for drugs and alcohol using multiple analytical toxicological procedures including: colorimetric, thin layer chromatography (TLC), immunochemistry, gas chromatography (GC), gas chromatography mass spectroscopy (GCMS), and liquid chromatography mass spectroscopy (LCMS). Toxicological results were compiled in an electronic database to allow for analysis and interpretation. Case number per year ranged from a minimum of 226 (2004) to a maximum of 306 (2009) with a general increase in the number of cases per year over the period. Results indicate that the impact of alcohol and drugs as a percentage of cases ranged as follows: positive for drugs from 76% (2009) to 87% (2003), positive for drugs and alcohol from 19% (2009) to 33% (2003), and positive for alcohol alone from 22% (2009) to 36% (2004). Acute drug overdose was the cause of death in 22% (2009) to 35% (2007) of cases per year. While the percentages of cases with drugs, drugs and alcohol, and alcohol alone varied from year to year, the proportionality of these groups to one another remained relatively constant over the years analyzed. In the

range of years studied, drugs appeared in a greater percentage of cases than drugs and alcohol, which appeared in a greater percentage of cases than alcohol alone. The most prevalent groups of drugs present at autopsy, other than alcohol, were opiates and benzodiazepines. These drugs were present in ranges from 14% (2005) to 26% (2007). Other major drugs, or classes of drugs present (as a percentage of case per year) were: cocaine from 6% (2009) to 15% (2005), methadone from 6% (2003 and 2009) to 12% (2006 and 2007), stimulants from 7% (2003 and 2009) to 16% (2005 and 2006), and sedatives from 5% (2004) to 12% (2003 and 2006). There was an increased prevalence of opiates and benzodiazepines in our forensic cases from 2005 through 2009, as well as an increase in the number of autopsy cases in which these drugs were found in combination. This increase may reflect the amplified clinical use of these drugs in our region, misuse of prescription drugs, or increased diversion of prescription medications.

Toxicology, Alcohol, Autopsy

#### K3 Measurement Uncertainty of GC Method in Determining Ethanol Concentration of In-House Prepared Aqueous Standards for use with Evidentiary Breath Test Instruments

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After attending this presentation, attendees will better understand the principles of ISO "Guide to the Expression of Uncertainty in Measurement" as applied to a specific gas chromatography (GC) method for ethanol chemical measurement. This paper defines the measurement problem, describes the evaluation steps involved, shows the largest sources of uncertainty, and demonstrates how the formal process led to changes to the method that resulted in achieving a combined uncertainty goal.

This presentation will impact the forensic science community by giving an example of how to determine and express the confidence of results as a combined uncertainty based on traceability to a certified reference material (CRM).

The objective of this work was to establish the uncertainty of the concentration of ethanol in aqueous solution standards prepared by our laboratory. The principle goals of this study were: (1) to estimate the combined standard uncertainty of in-house standard solution using a GC method; and (2) to see if it was possible to develop a new GC method with tighter quality controls than available with the present method.

The measurement was the mass concentration of ethanol in water in which the concentration is a function of the uncertainty sources of the batch sampling, the GC method, which included the entire sequence of steps from sample auto-dilution through final GC analysis, the uncertainty of the certified reference material used in determining the linear calibration slope and the uncertainty of the calibration least squares fit process.

The measurement problem was a concentration of ethanol analyte in single sample matrix with a range of analyte concentrations. The GC measurement is calibrated against traceable CRMs. Because the method has been under statistical control, the precision information from previous runs includes the *combined* effect of nearly all of the potential sources of uncertainty. Precision estimates used were over an extended period of time, by different analysts using different equipment and the replicate analysis of several samples was the choice for precision data.

The precision of the measurement was found to vary both proportionally with analyte concentration level (level dependant contributions of analyte) and a constant related to the calibration regression method that is independent of analyte concentration. The three largest sources of uncertainty were determined to be Calibration Slope (1.2%), GC Analytical (0.6%), and CRM (0.7%). The Combined Standard Uncertainty was calculated as U = 1.6% (95% C.L., K=2).

The results show that the proposed method is suitable to expect GC calibrators (ones above 0.098 g/100ml) to be within 2% (k=2) of their target value vs. the present method of 5%; and the sample solution measurement to be within 2% (k=2) of target vs. the present method of 10% of QCs. Below 0.098 g/100ml concentration, the acceptance criteria will need to be established that is not based on a percentage since the uncertainty is not linearly proportionally to concentration in the extremes.

Based on this study, routine 0.080 g/210L and 0.160 g/210L breath alcohol standard solutions are estimated to have a combined standard uncertainty of < 2 % at a 95% confidence limit.

Measurement Uncertainty, Standards, Gas Chromatography

#### K4 Prevalence of Norhydrocodone in Authentic Hydrocodone Urine Specimens

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The goal of this presentation is to evaluate the incidence and disposition of norhydrocodone in authentic urine specimens of pain patients prescribed hydrocodone.

This presentation will impact the forensic science community by demonstrating applicability of norhydrocodone for the reliable confirmation of hydrocodone positive urine samples.

Hydrocodone is a common semi-synthetic opiate used as an antitussive and an analgesic.1 Hydrocodone is excreted in urine as unchanged drug (49.8%) and metabolites: norhydrocodone (20.7%), conjugated hydromorphone (16.6%), 6-hydrocodol (12.4%), and conjugated 6-hydromorphol (0.4%).<sup>1</sup> The high potential for hydrocodone abuse may be due to the relative ease of purchase and the prevalence of use among chronic pain patients. Urine drug testing of pain patients for such drugs plays a pivotal role in the management of their prescribed medication. Monitoring of drug adherence, possible drug abuse, and diversion of prescribed drugs should be considered. Opiate urinalysis has been challenged by complex biotransformation of parent drugs and the commercial availability of the metabolites, such as hydromorphone.<sup>2</sup> Therefore, it is necessary to detect distinctive biomarkers for more accurate interpretation in the absence of parent drug. Normetabolites are considered unique metabolites indicating the ingestion of parent drugs. This study aims to detect norhydrocodone in urine specimens of pain management patients prescribed hydrocodone.

Authentic urine specimens (n=101) from pain management patients prescribed hydrocodone were obtained. Norhydrocodone was incorporated into the current opiate assay, which includes codeine, hydrocodone, hydromorphone, morphine, noroxycodone, oxycodone, and oxymorphone. The absence of co-elution effects between norhydrocodone and the other opiates was confirmed. The new method was validated to verify the reliability of norhydrocodone detection and quantification. The concentrations of norhydrocodone, hydrocodone, and hydromorphone in urine samples were measured. Urine specimens were treated with acid for the hydrolysis of conjugated glucuronide moiety and then injected into a liquid chromatograph tandem mass spectrometer (LC/MS/MS) equipped with columns utilizing turbulent flow technology.

The limits of detection and quantification (LOD and LOQ) and the upper limit of linearity (ULOL) for norhydrocodone were 100ng/mL,

250ng/mL and 100,000ng/mL, respectively. Intraday and interday precision and accuracy were conducted at 300, 3000, 30000ng/mL and showed <14.4% coefficient of variation and < $\pm$ 14.7% deviation from the target concentrations. Of the total urine specimens, 90.1% were positive for norhydrocodone, demonstrating that it is a common metabolite in hydrocodone urine specimens. Urine specimens containing norhydrocodone alone totaled 3%. The mean relative abundances of hydrocodone, norhydrocodone, and hydromorphone in the urine samples were 31.1%, 62.4% and 11.8%, respectively. This is inconsistent with previous reports showing unchanged parent drug as the major analyte present in urine after metabolism of hydrocodone. The results imply that the chronic use of hydrocodone increases the abundance of norhydrocodone metabolite in urine compared to single-dose usage.

Norhydrocodone is a prevalent and dominant metabolite in urine following the consumption of hydrocodone by chronic pain patients. It is a unique biomarker that can provide more conclusive confirmation and to a lesser extent reduce false negatives in urine drug testing for hydrocodone.

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Norhydrocodone, LC/MS/MS, Prevalence

### K5 Self-Administration of Anesthetic (Propofol and Midazolam) and Psychotropic (Amitriptyline and Zolpidem) Drugs: Recreational Abuse and Suicidal Manner in an Anesthetist

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After attending this presentation, attendees will understand that forensic investigations are based on a multidisciplinary approach in which autopsy findings and toxicological results often result in association with circumstances and crime scene investigations.

This presentation will impact the forensic science community by demonstrating how recreational abuse of anesthetic and sedative agents in health care practitioners, especially anesthesiologists is an increasing event. This presentation will also show an unusual case of suicide in which the manner and means of suicide was dependent upon the occupation of the victim.

Presented is a case of a 50-year-old man, anesthetist at the main local hospital, who was found dead in the house where he lived alone since separating from his wife. On the previous evening he was found on the landing's floor with an occipital bruise injury and treated in the Emergency Department. The next day his brother, alerted by his colleagues that tried in vain to contact him, went to his house. He found that the front door had been left ajar, with a piece of furniture behind it. When he entered the flat, he noticed the corpse of the brother, supine on the living room's floor near a piece of furniture. There were two drips with intravenous tubes almost empty (approximately 1 ml). One drip was still inserted in the dorsum of the victim's right hand with tube for intravenous drip totally open. On the glass of this drip there was written "Miclela Caput" (meaning "Caput Mixture," written incorrectly). On the glass of the other drip there was written "500 TPS+200 DIPR" (meaning Sodium Thiopental+Diprivan). In the house there were some empty blisters of Zolpidem, more than 20 packs of different drugs (some of them empty), an ash-tray containing white liquid, several empty ampoules of Propofol, Midazolam and Thiopental, and several new and used syringes. In the bedroom there were two knapsacks containing pornographic materials and four plastic phalli.

External examination revealed abundant livor mortis, numerous recent needle marks with fresh and older hemorrhages in both arms, and a sutured occipital injury.

Autopsy and histological findings were pulmonary and brain oedema, moderate fatty liver, acute poly-visceral congestion, hemorrhagic pancreatitis.

Systematic toxicological analysis was performed on biological and non biological samples for alcohol, drugs of abuse and pharmaceuticals.

Blood toxicological examination by GC/MS revealed lethal concentration of Zolpidem (0.86  $\mu$ g/ml) and high therapeutic blood concentrations of Propofol (0.30  $\mu$ g/ml), Midazolam (0.08  $\mu$ g/ml), Amitriptyline (0.07  $\mu$ g/ml), and low concentration of Thiopental (0.03  $\mu$ g/ml). Zolpidem was also found in gastric content while Thiopental was found in urine. Hair segment analysis (0 – 2 cm) revealed Propofol (4,7  $\mu$ g/mg) and the presence of Zolpidem, Amitriptyline and Ketoprofen.

Residual's toxicological analysis of the inserted drip ("Caput mixture") revealed Propofol and Midazolam (approximately 1,9 and 0,08 mg/ml). Analysis of the non-inserted drip, showed Propofol and Thiopental (approximately 2 and 5 mg/ml). The low blood concentration of Thiopental suggests a self administration of the non-inserted drip at least 12 hours before death.

The blood Propofol level was lower than or within the commonly accepted therapeutic range of  $1.3-6.8 \ \mu g/ml$  after a standard anesthetic induction dose. Published reports indicate that in most cases, the postmortem Propofol concentrations were at therapeutic levels. It should be pointed out that especially for those agents used in anesthesia; the therapeutic concentrations refer to patients being supported respiratorywise, while in non-supported or non-intubated patients such concentrations may be lethal. Most of those deaths are thought to have occurred because of the rapidity of Propofol's injection which led to apnea and death. A mere interpretation of the blood and tissue concentrations of Propofol in the toxicological analysis may be of limited diagnostic significance without taking into account the before mentioned reports. Toxicological analysis of hair confirmed the recreational abuse of Propofol.

These anesthetic and sedative drugs are often used in combination for anesthesia's induction. All of these act synergistically in combination and may induce respiratory depression. This effect depends on individual susceptibility, on dose used and, especially for Propofol and Midazolam, infusion's rapidity.

In conclusion, the victim was administered a solution of anesthetic drugs, rapidly infused in a lethal combination and simultaneously a hypnotic drug in lethal dose.

Anesthetic Drugs, Propofol, Suicide

#### K6 Simultaneous Detection of Psychedelic Amphetamines in Urine By LC/MS/MS

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After attending this presentation, attendees will be familiar with a technique for the simultaneous detection of eleven designer amphetamines in urine by liquid chromatography/tandem mass spectrometry (LC/MS/MS).

This presentation will impact the forensic science community by providing a new method for the simultaneous detection and quantitation of emerging drugs of abuse in urine.

Psychedelic amphetamines are a relatively new class of designer drug in the United States. These drugs were initially popular in Europe and Asia, but the 2C-, 2CT- and DO- series of amphetamines are now routinely seized throughout the United States. A number of these substances are not scheduled in the Federal Controlled Substances Act, offering users a legal alternative to the more traditional designer amphetamines like 3,4-methylenedioxymethamphetamine (MDMA). Many of these newer designer amphetamines produce profound hallucinogenic effects due to their structural similarity towards both mescaline and amphetamine. The pharmacology and toxicology of these drugs are considerably less studied than their conventional counterparts. Their prevalence among toxicological casework in the U.S. is not

known, but many toxicology laboratories do not screen for these substances.

The drugs included in this study were 4-bromo-2,5dimethoxyphenethylamine (2C-B), 4-methylthioamphetamine (4-MTA), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4iodoamphetamine (DOI), 2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethyoxy-4-ethylthiophenethylamine (2C-T-2), 2,5-dimethyoxy-4-(i)-propylthiophenethylamine (2C-T-4), 2,5-dimethoxy-4-(n)propylthiophenethylamine (2C-T-7), 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxy-4-iodophenethylamine (2C-I), and 2,5dimethoxy-4-bromoamphetamine (DOB). A positive electrospray ionization (ESI) LC/MS/MS procedure was developed to allow simultaneous detection and quantitation of these substances following solid phase extraction (SPE).

Negative urine was fortified with the drugs of interest and extracted using SPE. In the absence of commercially available deuterated analogs, mescaline-d9 was chosen as the internal standard. An alkaline extraction using a copolymeric mixed-mode SPE column was used to isolate the drugs. Optimal drug recoveries were achieved using 2% ammonium hydroxide in 95:5 v/v methylene chloride:isopropanol. Separation was achieved using a C18 LC column and gradient elution (5% methanol in 50mM ammonium acetate and 100% acetonitrile in 50mM ammonium acetate). The total run time was approximately 5 minutes. Data was acquired using the following ions (precursor ions are underlined): m/z 262, 245, 230 for 2C-B; m/z 182, 165, 117 for 4-MTA; m/z 224, 207, 179 for DOET; m/z 322, 305, 105 for DOI; m/z 210, 193, 178 for DOM; m/z 242, 225, 134 for 2C-T-2; m/z 256, 239, 197 for 2C-T-4; m/z 256, 239, 197 for 2C-T-7; m/z 182, 165, 150 for 2C-H; m/z 308, 291, 276 for 2C-I; m/z 276, 259, 231 for DOB. Limits of detection for all target analytes were 1-2 ng/mL and limits of quantitation were 1-6 ng/mL. Precision and accuracy was evaluated at 20 and 75 ng/mL. For all drugs, accuracy at 20 ng/mL was 95-115% and CVs were 4.3-10.0% (n=4). At 75 ng/mL accuracy was measured in the range 83-120% and CVs were 3.7-12.0 (n=4). Ion suppression and interferences from common amphetamines and endogenous phenethylamines were included in the study.

The technique allows for the simultaneous low-level detection of eleven psychedelic amphetamines in urine samples. The method will be

further developed to determine the prevalence of these drugs in toxicological casework and to further develop a confirmation and quantitation procedure for these and other designer drugs in blood samples.

Designer Amphetamines, LC/MS/MS, Solid Phase Extraction

#### K7 Study of L-2-Aminothiazoline-4-Carboxylic Acid as a Biomarker for Cyanide Poisoning by LC-MS/MS Analysis

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After attending this presentation, attendees will understand how cyanide poisoning can be detected by measuring the amount of a derivative of cyanide, L-2-Aminothiazoline-4-Carboxylic Acid (ATCA) in biological fluids. ATCA can be easily measured in blood, urine, and organs from a subject.

This presentation will impact the forensic science community, as well as the Army, by improving the detection of cyanide from poisoning in various ways.

One threat of cyanide poisoning is the use of cyanide as a chemical warfare agent (CWA). Once exposure is identified, the amount of poison can be quantified and a more accurate treatment distributed. Identification of cyanide or its metabolites in biological fluids is necessary for many purposes in forensic, clinical, military, research, and veterinary fields. However, because of the volatility of cyanide and the difficulty of establishing steady-state cyanide levels with time, methods of directly evaluating cyanide levels are limited.

These studies focus on a chemically stable urinary metabolite of cyanide, 2-aminothiazoline-4-carboxylic acid (ATCA), which is an effective biomarker for cyanide exposure, specifically in mice liver samples. ATCA was used because it is stable over time, unlike cyanide, and its concentration level is directly proportional to the amount of cyanide from exposure. After using a method previously developed to dissect, preserve organs, and homogenize the livers, the organs were spiked with an internal standard, 2-aminothiazole-4-carboxylic acid (ATZA). The similarity between ATCA and ATZA is advantageous because ATZA is co-eluted with ATCA and detected at the same time by LC-MS/MS, therefore experiencing the same magnitude of ion suppression. ATCA was then extracted by solid phase extraction (SPE). Endogenous levels of ATCA were determined by comparing the non-exposed livers to calibrators containing known concentrations of ATCA, both of which were evaluated by the LC-MS/MS.

Mice were later exposed to various doses of cyanide and liver ATCA contents were compared to the dose of cyanide mice were given. An optimal method was developed to detect ATCA, with a recovery of 40-50%. Endogenous levels of ATCA in liver were found to be at least 100 ng/ml and were measured multiple times. This study indicates that this method can continue to be used for other organs, such as kidney, lung, and heart, to detect endogenous ATCA. Future studies will also concentrate on determining the concentration of ATCA in organs obtained from mice that were previously exposed to cyanide and compare the differences between endogenous ATCA levels and levels after exposure.

These studies were supported by the Army Medical Research Institute of Chemical Defense (Delivery Order 0878, Contract No. DAAD19-02-D-0001, TCN 06-170 and 08284) and the Robert A. Welch Foundation (x-0011) at Sam Houston State University, Huntsville, TX. **Cyanide, L-2-aminothiazoline-4-carboxylic acid (ATCA), Trace Evidence** 

#### K8 Purity of Street Ketamine Preparations Retrieved From Night Club Amnesty Bins in London

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The goals of this presentation are to describe the analysis of street ketamine in order to determine the purity of samples commonly available and to identify what impurities might be present.

This presentation will impact the forensic science community by showing how the majority of street ketamine samples analyzed were of high percentage purity suggesting that ketamine may be responsible for effects on the urogenital system. This also supports the observation that a number of patients undergoing clinical therapy with ketamine have reported similar symptoms.

Introduction: Ketamine has been widely used in medicine and veterinary practice for its anesthetic and analgesic properties linked with minimal respiratory depression. More recently the drug has gained popularity as a recreational substance amongst young people. Street prices of the drug vary between £10 and £20 per gram in the UK. The UK club magazine Mixmag survey of its readers in 2009 shows 51% used ketamine in last year, 32% in last month and 18% use it weekly. 30% experienced stomach pains after taking ketamine and 20% experienced urinary tract problems (more in women). A number of reports have appeared in the medical literature suggesting a possible link between ketamine misuse and kidney and bladder disorders. The pathological cause of the bladder related problems is at present unknown and it is uncertain whether they are attributable to ketamine or to impurities that may be present in street preparations. Little information is available concerning the purity of street ketamine hence analysis was undertaken on street preparations of the drug retrieved from amnesty bins in London night clubs. In this paper, the analysis of street ketamine is described to determine the purity of samples commonly available and to identify what impurities might be present.

**Method:** Street ketamine samples were analyzed using HPLC with diode-array in order to determine the percentage of ketamine present in the sample and identify any impurities. The system was equipped with a  $C_{18}$  reversed phase column which was maintained at 50°C. The mobile phase was a mixture of 5 mM SDS in 20 mM KH<sub>2</sub>PO<sub>4</sub>:acetonitrile (65:35, v:v) at a flow rate of 1.0 mL/min. In addition to HPLC analysis, samples were also analyzed using electron microscopy, color tests, FTIR with golden gate, GC-MS and TLC in an attempt to determine the nature of any impurities present.

**Results:** The purity of samples containing Ketamine only ranged between 65%—100% (mean = 87.9%; SD = 11.66%). Benzocaine was the principal impurity detected and ranged between 2.75%—16.60% (mean = of 7.27%; SD = 3.96%). Ketamine in samples containing Benzocaine ranged between 49.9% - 84% (mean = 67.21%; SD = 9.71%).

**Conclusion:** The majority of street ketamine samples were of high percentage purity suggesting that ketamine may be responsible for effects on the urogenital system. This also supports the observation that a number of patients undergoing clinical therapy with ketamine have reported similar symptoms.

Ketamine use is increasing rapidly worldwide and knowledge concerning the availability, purity, and trends in drug use can be of assistance to drug enforcement/legislation agencies as well as healthcare workers who may be involved in the provision of care to individuals following drug use. The results of this survey would support a hypothesis that bladder related diseases observed in ketamine users is likely to be attributable to ketamine rather than impurities or cutting agents.

Ketamine, Purity, Bladder Disorders

#### K9 Confirmation of Oleander Poisoning by LC/MS

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After attending this presentation, attendees will understand how to confirm oleander poisoning cases from blood and urine specimens. This presentation will impact the forensic science community by providing the toxicological data necessary to make diagnostic decision about the patient when oleandrin is detected by toxicological screening.

In this case a 60-year-old woman was brought to emergency room with initial symptoms of vomiting, diarrhea, and abdominal pain. The patient's heart beat was normal at the beginning but then sinus bradycardia was observed gradually. Information obtained from her indicated that she is a cancer patient and that she drank the juice of some leaves of the oleander plant (*Nerium oleander L.* - Apocynaceae) for herbal self treatment. *Nerium oleander L.* is a member of Apocynaceae family. Leaves from *Nerium oleander* were shown to contain 0.018 to 0.425% oleandrin (weight/wet weight). Oleander extracts have been used for the treatment of indigestion, malaria, leprosy, mental or venereal diseases but the unconscious usage may cause toxicity.

Blood and urine sample on admission was assayed for oleandrin, the major cardiac glycoside of *N. oleander*, which has a wide geographical range and ecological distribution throughout the world and also in Turkey. Both specimens were extracted with ethylacetate: n-heptan (1:1) solvent mixture at 9.5 pH. Additionally, some parts of the oleander plant such as one flower, two leaves and one bark were chosen for extraction. These parts were cut and crushed in a 50 mL flacon to obtain about 2 mL sticky juice and then this was diluted with 3 mL water and extracted with the same solvent mixture.

All separated specimens were performed on a highly specific LC-MS procedure with gradient elution. Using this analytical setting, the average retention time for oleandrin was 0.9 min. The major ions monitored for oleandrin were m/z **577** and **433** indicating total molecular weight and without glycosides form, respectively. The highest sensitivity for this assay was obtained with 70 eV.

Qualitative results of the blood and urine samples on admission were compared with the plant extracts. Also qualitative result of the blood sample with urine sample was compared with each other. The most important thing was that the patient recovered without using any digoxin antibody such as Digifab or Digibind.

This procedure provided the toxicological data necessary to make diagnostic decisions about the patient when oleandrin was detected by toxicological screening. Also LC-MS appears to be the method of choice for the forensic-toxicological investigation of poisonings by cardiac glycosides.

**Oleandrin, Poisoning, LC/MS** 

### K10 Analysis of Hydrocodone, Hydromorphone, and Norhydrocodone in Urine Using Liquid Chromatography - Tandem Mass Spectrometry (LC/MS/MS)

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The goal of this presentation is to present a validated LC/MS/MS method for quantitative analysis of hydrocodone (HC) and metabolites and present data from human subjects administered HC.

The presentation will impact the forensic science community by providing data obtained from a method valdiation study of urinary HC and its metabolites.

Measurement of HC, a semi-synthetic opioid analgesic used for moderate and severe pain relief, can be used to monitor pain management compliance; however, HC levels can also be useful in drug testing cases to determine abuse or misuse of this commonly abused opioid. Hydrocodone is metabolized to its major metabolite, HM, and to a lesser extent to minor metabolites, NHC, and  $6-\alpha$ - and  $6-\beta$ hydroxymetabolites. Knowledge of metabolism and excretion profiles of administered HC can help in determining dose, time since last dose, and expected peak concentrations in subjects whose specific drug use is unknown. To effectively monitor and evaluate metabolism and excretion profiles, a sensitive and specific drug test is needed to ensure that the drug and its metabolites can be measured to the lowest detectable amount.

Standards spiked with concentrations of HC, HM, and NHC ranging from 1 - 10,000 ng/mL were prepared in opioid negative urine. Urine samples collected from subjects following HC administration were also evaluated. The LC gradient mobile phase consisted of (A) 0.1% formic acid and (B) acetonitrile; flow rate was set at 0.5 mL/minute. The internal standard solution contained 1µg/mL HC-D3, HM-D3 and NHC-D3 in methanol. A 250 µL aliquot of standard or urine was mixed with 25 µL of internal standard solution. Urine samples were hydrolyzed with  $\beta$ -glucuronidase, solid phase extraction (SPE) performed, followed by 10 µL injection on the LC/MS/MS system. The mass spectrometer was set in the ESI positive mode and analysis was performed using two multiple reaction monitoring (MRM) transitions per analyte. The MS/MS ion transitions monitored were m/z 300.2 $\rightarrow$ 199.1 and 300.2 $\rightarrow$ 171.0 for HC; m/z 286.1 $\rightarrow$ 185.0 and 286.1 $\rightarrow$ 157.0 for HM; m/z 286.2 $\rightarrow$ 199.1 and 286.2→241.1 for NHC; *m/z* 303.2→199.0 for HC-D3, 289.2→185.2 for HM-D3 and m/z 289.0 $\rightarrow$ 202.0 for NHC-D3.

The linear range was determined for this procedure by analysis on six different runs on concentrations ranging from 1 to 10,000 ng/mL of each analyte prepared in urine. The linear range was shown to be 5 to 10,000 ng/mL for HC and HM and 5 - 5,000 ng/mL for NHC with r value > 0.99 for all compounds. The limit of detection (LOD) was 2.5 ng/mL for HC and NHC and 5 ng/mL for HM. The limit of quantitation (LOQ) for all analytes in urine was 5 ng/mL. The method yielded good precision with RSDs of < 10% at 100 ng/mL HC, HM, and NHC. Based on this procedure, measurable amounts of HC, HM, and NHC were detected in human urine for up to at least 9 hours post dose HC.

The present study will provide a validated LC/MS/MS method for quantitation of HC, HM and NHC in urine and will also provide evaluation of urine samples obtained from individuals administered HC. **Hydrocodone, Metabolism, LC/MS/MS** 

### K11 Determination of Titanium Element in Gingival Biopsies of Patients Treated With Dental Implants by Laser Ablation – Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS)

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After attending this presentation, attendees will understand the transition of the titanium element into gingival biopsies which determined using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS).

This presentation will impact the forensic science community by determining low amount of biopsy materials for any titanium element with suitable Laser Ablation method equipped by ICP-MS.

**Introduction:** Titanium element is widely used material as an implant in medical applications especially in dentistry. The use of dental implants in the treatment of partial and complete edentulism has become a successful treatment modality in modern dentistry. Dental implants and their prosthetic parts are made of biocompatible materials. Today titanium and its alloys are the first choice to fabricate implant materials. Although titanium is a very inert material, it may corrode when in contact with the oral cavity. If titanium corrodes it releases ions which can cause local reactions such as pain and swelling or activate immune response.

**Materials and Methods:** The study was carried out in the Clinic of the Department of Oral Implantology at the Faculty of Dentistry and Institute of Forensic Science, Forensic Toxicology Laboratory in Istanbul University. The study group comprised 20 two-staged dental implants. Osteotomy and implant installation were performed according to the manufacturer's surgical protocol. The implants were exposed (second stage surgery) after three months and gingival biopsies were collected at each site. The biopsies were stored at -18°C until use. Samples were fixed to a lamina by an adhesive and dried in an oven at 90°C for 2 hours.

For comparison and prediction the change of elemental composition of gum tissues, sheep gum was used as a control matrix and confirmed that the sheep gum had no titanium element. An adhesive material fixed to a lamina with no sample was also used as blank for samples. Certified Standard Material (CRM), NIST 612 glass matrix was used for quality control sample. All samples fixed to lamina were analyzed by LA-ICP-MS. Titanium element was detected and compared with sheep gum and also with blank lamina.

**Results and Discussion:** Sheep gums were repeated five times and the mean value was accepted as the lowest amount for Titanium element. According to the results, some of samples showed titanium element significantly more than sheep samples. NIST 612 glass matrix showed that LA-ICP-MS system analyzed the titanium element close to certified amount. Moreover, there was no response to titanium in blank lamina which had no tissue. It can be concluded that adhesive didn't contain any contamination for titanium, and this may be suitable sample preparation process for biological tissues when they are studied using Laser Ablation.

**Conclusion:** Although all patients were exposed to titanium implant for three months, elemental quantitative results were variable. The best way to determine these kinds of patients might be monitoring

for their titanium level in biological samples such as urine or blood with specific time periods. Even if titanium is an inert material, these implants will be in contact with the oral cavity for a long time and may have toxic reactions in the body during the man's life. So, further studies will elucidate whether patients are under risk for titanium toxicity or not in time. Since gingival biopsy materials have very low amount, Laser Ablation may be the best method to determine the inorganic profile by ablating the tissue surface.

Dental Implant, Titanium, Laser Ablation

#### K12 Evaluation of Enzymatic Hydrolysis Efficiency for Buprenorphine Analysis

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After attending this presentation, attendees will understand how to apply Ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) to buprenorphine analysis and evaluate efficiency of three different recommended buprenorphine hydrolysis methods using authentic samples.

This presentation will impact the forensic science community by providing an alternative, accurate method for buprenorphine analysis, and if UPLC/MS/MS analysis is not available, it offers an acceptable hydrolysis method.

Buprenorphine is quickly becoming a commonly prescribed analgesic for pain management. It is metabolized to norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide and is extensively eliminated in conjugated form. The glucuronides are cleaved by hydrolysis for conventional buprenorphine analysis by gas chromatography/mass spectrometry (GC/MS). Ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) is an advanced technology that can measure intact glucuronides and allows reliable analysis of un-hydrolyzed buprenorphine samples. UPLC/MS/MS application can drastically reduce the cost and time associated with buprenorphine sample preparation. This study used UPLC/MS/MS to assess a non-hydrolysis method and compare the efficiency of the three most published buprenorphine hydrolysis methods.

Authentic buprenorphine positive samples (n=100) were separately analyzed by UPLC/MS/MS after sample pretreatment by dilution only or enzymatic hydrolysis using  $\beta$ -glucuronidase from *Helix pomatia* (*H. pomatia*), glusulase or *Escherichia coli* (*E coli*). *H. pomatia*-treated samples were incubated for four hours at 60°C. Glusulase-treated samples required one hour incubation at 60°C. The *E.coli* –treated samples were incubated at 37°C for two hours and sixteen hours for hydrolysis of buprenorphine glucuronide and norbuprenorphine glucuronide, respectively. Un-hydrolyzed samples were diluted only and then analyzed and used as references for hydrolysis efficiency. The UPLC/MS/MS gradient method for buprenorphine, norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide was previously validated with a linear range of 5-5000 ng/mL, precision < 6%, and coefficient of variation and accuracy ±18% of the target concentrations for all analytes.

The mean relative abundances of unconjugated buprenorphine, buprenorphine glucuronide, unconjugated norbuprenorphine, and norbuprenorphine glucuronide in the un-hydrolyzed urine samples were 0.2%, 19.2%, 8.6%, and 72.1%, respectively. This ratio is comparable with previously published distributions in plasma.<sup>1</sup> *H. pomatia* demonstrated excellent mean hydrolysis efficiency for both buprenorphine glucuronide and norbuprenorphine glucuronide (99.6% and 99.0%, respectively). Glusulase demonstrated very good mean

hydrolysis efficiency for both buprenorphine glucuronide and norbuprenorphine glucuronide (97.3% and 95.4%, respectively). E. coli demonstrated satisfactory overall mean hydrolysis efficiency; giving 99.1% hydrolysis for buprenorphine glucuronide and 85.9% hydrolysis for norbuprenorphine glucuronide. There was a noticeable decrease in the total buprenorphine and norbuprenorphine concentrations of the hydrolyzed samples compared to the un-hydrolyzed samples suggesting possible degradation during hydrolysis. Statistical analysis was performed on the data for the hydrolyzed and un-hydrolyzed samples at a 95% confidence interval. The percent loss for buprenorphine and norbuprenorphine in the H. pomatia samples was 13.33% and 11.65%, respectively, and was statistically significant for both analytes (P=0.0055, P=0.0208). The percent loss in the Glusulase samples was 23.12% and 21.97% for buprenorphine and norbuprenorphine, respectively and was highly significant (P=0.0001, P=0.0007). Percent loss was 10.75% for buprenorphine and 24.79% for norbuprenorphine in the E.Coli samples. The decrease was significant for buprenorphine (P=0.0158) and highly significant for norbuprenorphine (P=0.0001).

UPLC/MS/MS can be employed for buprenorphine analysis and its application eliminates the need for sample hydrolysis because both the conjugated and unconjugated forms of buprenorphine can be detected and quantified. It also improves accuracy by precluding sample degradation due to hydrolysis. However, if the mode of buprenorphine analysis requires hydrolysis then *H. pomatia* is recommended as the hydrolyzing agent because it is most efficient and results in less degradation compared to the other two methods. **Reference:** 

Concheiro M, Jones H, Johnson R, Shakleya D, Huestis M.

Confirmatory Analysis of Buprenorphine, Norbuprenorphine, and Glucuronide Metabolites in Plasma by LCMSMS. Application to Umbilical Cord Plasma from Buprenorphine-maintained Pregnant Women. Journal of Chromatography B, 2010; 878: 13–20.

Buprenorphine, UPLC/MS/MS, Hydrolysis

#### K13 Automated and Comprehensive Analysis of Drugs in Whole Blood Using Cleanup Tips and LC/MS/MS

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The goal of this presentation is to present the development of an automated sample preparation method for the analysis of drugs in whole blood using minimal manual labor. The method is comprehensive and combines protein precipitation and "cleanup" for the analysis of acidic, basic, and neutral drugs.

This presentation will impact the forensic science community by demonstrating how automated sample preparation allows forensic labs to improve throughput, minimize sample handling, and increase confidence of results.

Recently, protein precipitated blood specimens have been analyzed directly by LC/MS/MS without additional solid-phase extraction (SPE) or cleanup procedures. In this study a comparison of samples analyzed with and without cleanup is shown. The advantages of using a cleanup procedure are: (1) the method is rapid because it does not involve condition, wash and elution steps; (2) less maintenance issues are required for the LC and MS instrumentation; (3) better sensitivity due to elimination of ion suppression and matrix effects; (4) better reproducibility for qualitative and quantitative measurements; and (5) more confidence in the screening and confirmation of drugs and metabolites.

A cleanup tip was developed that is used to simultaneously filter the proteins precipitated from whole blood and extract the sample matrix components. The extractions are performed completely automated using a dual rail GERSTEL MPS-2 instrument interfaced to an AB SCIEX 3200 Q-Trap instrument. The automation allows the analysis to be non-tedious and improves sample integrity by minimizing manual sample handling. Use of the Q-Trap permits the ability to obtain full scan mass spectral data of drugs and metabolites, even at low concentrations. The full scan capabilities gives greater confidence in compound identification, and is a great resource for unknown screening that is common in forensic toxicology.

In this study, analyses of over 60 drugs and metabolites in whole blood are shown using the cleanup tips. The drugs and metabolites include opiates, opioids, benzodiazepines, analgesics, anticonvulsants, stimulants, and hallucinogens. Recoveries are greater than 70% and RSDs less than 10%, with most recoveries being approximately 90%. Direct comparisons are shown of samples treated with and without cleanup.

Sample Preparation, LC/MS/MS, Automation

#### K14 Analytical Methods Development for Identifying and Quantifying Synthetic Cannabinoid Substances

Vanessa Hobbs, MS\*, and Ngee-Sing Chong, PhD, Middle Tennessee State University, PO Box 68, Chemistry Department, Murfreesboro, TN 37132

After attending this presentation, attendees will learn about the details of analytical methods including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and infrared spectrometry (IR) for analyzing synthetic cannabinoid products containing the JWH-018 ingredient. The chemical characteristics of the K2 and K3 products along with the metabolic breakdown of the active compounds in the urine matrix will be discussed. The findings and methodologies will contribute to future development of a protocol for analyzing banned synthetic cannabinoid substances.

This presentation will be impact the forensic science community, as well as, the drug enforcement community by providing important information on the development of laboratory methods for analyzing synthetic cannabinoid substances that are different from natural marihuana products. The new laws banning these substances will require robust methods based on GC-MS, LC-MS, and IR spectrometry for characterizing products seized in police raids and assessing the levels of the metabolites in the urine samples of suspected users.

Recently, several states have outlawed the sale, use, and possession of synthetic cannabinoids. For instance, Tennessee has banned the sale of synthetic cannabinoids (more specifically JWH-018, JWH-073, HU-210, and HU-211) as of July 1, 2010. The popularity of these drugs is largely due to the lack of an analytical protocol for analyzing the synthetic cannabinoid substances for enforcement purposes. The use of these illegal drugs is often associated with trying to get "high" or intoxicated while avoiding the detection of the metabolites of these synthetic cannabinoids in order to pass the standard drug test for natural cannabinoids. The development of an analytical method for determining the characteristic metabolites of synthetic cannabinoids will provide a reliable means of identifying individuals using synthetic cannabinoids and studying the detection periods of these metabolites in urine samples. These drugs are commonly available under the name K2 or K3, each of which have the active ingredient of JWH-018.

Since the emergence of synthetic cannabinoids and their "herbal blends" in the markets, little research has been published on the urine metabolites of these products. Some synthetic cannabinoids are extremely potent and regulated by the U.S. Drug Enforcement Agency (DEA). For example, HU-210 is 100 times more potent than the active ingredient in marijuana,  $\Delta$ 9-tetrahydrocannabinol (THC), and is subsequently considered a Schedule I controlled substance.<sup>1</sup> Analysis of the current commercially available synthetic cannabinoids has shown the presence of JWH-018 and CP 47,497-C8, as well as the derivatives of these two compounds.<sup>2</sup> The allure of these compounds is that they act on the same receptors (CB1 and CB2) resulting in a "high" without triggering a positive drug test for marijuana use.<sup>3</sup> The most recent publication related to the metabolites of synthetic cannabinoids focused on the analysis of urine samples following the administration of "Tropical Synergy" obtained from Russian law enforcement. The study confirmed the presence of two synthetic cannabinoids, JWH-018 and CP47,497-C8. Following urinalysis via gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), several hydroxylated derivatives of JWH-018 and CP47,497-C8 were found.4

In this study, the techniques of GC-MS, LC-MS, and infrared spectrometry (IR) are used to analyze synthetic cannabinoid compounds present in the herbal products, smokes, and urine samples of test subjects. Attenuated total reflectance and long path-length gas cells are used with IR spectrometry for both herbal product and smoke analysis. Solid phase extraction as well as gas sampling bags and sorbent tubes are used for the mass spectrometric analysis of cannabinoid constituents. Herbal mixtures with synthetic cannabinoids differ from manufacturer to manufacturer and the components of their mixtures are rarely given in the package labels. The combined GC-MS, LC-MS, and IR analysis provide unambiguous identification of constituents in the K2 and K3 The development of methods for characterizing the products. commercially available herbal products, the constituents in the smoke and smoke residues, and their metabolites in the urine matrix would be a significant step for the enforcement of laws regarding these illegal synthetic cannabinoid substances.

#### **References:**

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- <sup>3.</sup> Huffman, J.W., et.al. 3-indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interaction with the CB1 cannabinoid receptor. Bioorg. Med. Chem. 11, 2003, 539 – 549.
- <sup>4.</sup> Sobolevsky, T. et. al. Detection of JWH-018 metabolites in smoking mixture post-administration urine. For. Sci. Intl., 2010. In press.

Illegal Drugs, Synthetic Cannabinoids, Marijuana

#### K15 Stability and Reproducibility Studies for Carbohydrate Deficient Transferrin Analysis Using Capillary Electrophoresis

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After attending this presentation, attendees will develop an understanding of the protein Transferrin, and gain an understanding as to its stability and reproducibility and therefore its credibility as an analytical biological marker.

This presentation will impact the forensic science community by introducing a technique that, because of its stability and reproducibility, can be used in routine toxicological analysis concerning questions of chronic alcohol abuse.

The present research addresses the quantitative analysis of Carbohydrate Deficient Transferrin (CDT) levels in biological samples using capillary electrophoresis for reproducibility and repeatability as well as the analyte's stability *in vitro*.

Transferrin is a glycoprotein responsible for binding iron and transporting it via blood throughout the body. Multiple transferrin isoforms have been observed based on the presence of oligosaccharide chains containing acetylglucosamine, galactose, mannose and sialic acid. The sialic acid residues are in terminal positions of these chains and are the only part of the chain with a negative charge. The number of sialic residues in a transferrin molecule expresses the degree of transferrin glycosylation in an individual, which is usually:

Tetrasialio-transferrin: 75% Pentasialio-transferrin: 15% Trisialio-transferrin: 5% Disialio-transferrin: 2% Hexasialio-transferrin: 2% A-, Mono-, Hepta-, Octa- sialo-transferrin: <1%

Carbohydrate Deficient Transferrin (CDT) refers to the sum of the disialic, monosialic, and asialic groups. Research has indicated that individuals with a pattern of consuming > 50-80 grams of alcohol (approximately > 5-8 drinks) a day for at least seven consecutive days will have an increased CDT value. This indicates sustained alcohol consumption and provides information about an individual's drinking habits (indicate a potential alcohol abuser).

One sensitive and specific instrumental method used to detect CDT is Capillary Electrophoresis (CE). CE technology employs a voltage potential to a narrow-bore silica capillary and separates components based on size and charge. CE technology can separate the different transferrin glycoforms and, by assessing peak area ratios, determine the percentage of CDT in human serum.

The control and one sample serum were run six different days, six injections per day to examine both the intra-day variability (repeatability) as well as the inter-day variability (reproducibility).

Three different storage conditions were utilized to examine the % CDT values and assess the stability of CDT in serum. Aliquot sets from four different sample sera were stored on a lab bench top at room temperature  $(25^{\circ}C)$  over a nine-week period, in the refrigerator (approximately 4°C) over a ten-week period, and in the freezer (approximately -20°C) over a seventeen-week period. The % CDT was also checked every two weeks. Each serum sample along with the control was injected twice. The sample aliquots were stored in the freezer and analyzed every two weeks over an eight-week period.

The data generated during these studies indicated that CDT remains stable for extended periods of time when stored under various conditions but will remain stable the longest when stored at either 4°C or -20°C. Even if other studies are required to check the stability of the CDT related glycoproteins in serum samples over a longer span of time, the assessment of CDT under standard laboratory conditions highly supported the adoption of CDT as an indicator of alcohol abuse in the clinical and forensic environments.

CE technology proved again to be a simple and automated analytical tool producing easy reproducible and repeatable determinations of CDT in human serum, suitable for application in the daily routine of a toxicology laboratory.

Alcohol Abuse, Carbohydrate Deficient Transferrin, Capillary Electrophoresis

#### K16 Amitriptyline and Morphine Determination in Larvae of *Lucilia Sericata* and Decomposed Liver Using LC-MS/MS

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After attending this presentation, attendees will understand the reliability of insect larvae as samples for toxicological investigations and the methods that were developed in analysis of drugs in larvae and liver samples.

This presentation will impact the forensic science community by providing the real toxicological evidence from corpse and larvae.

Analytical entomotoxicology is a basic new of forensic toxicology, where a few studies exist in literature. The goal of this study is to evaluate the use of insects as alternative specimens for toxicological evidence. For this purpose, larvae of Lucilia sericata were reared on samples of minced chicken liver treated with different concentrations of amitriptyline and morphine; regarding therapeutic, toxic, and potentially lethal doses. A method was developed for amitryptiline determination in larvae and liver and morphine detection in larvae. Amitryptiline and morphine was detected in all tested larvae samples, confirming the reliability of these specimens for qualitative toxicology analysis. Quantitative concentrations of amitriptyline measured in larvae were correlated with levels in liver tissue. The recoveries for morphine was not repeatable and the method could only be used to detect this drug qualitatively. These observations bring new elements regarding the potential use of drug analysis in larvae for estimation of drug levels in human tissues.

**Introduction:** Insect colonization patterns are the most common factors utilized for postmortem interval (PMI) estimation, especially when the discovery of the corpse is delayed and the soft tissues are decomposed. Diptera larvae feed on decomposed tissues containing chemical substances because of antemortem drug exposure. Because of this, the use of necrophagous insect specimens can be valuable as evidence for qualitative drug detection and sometimes quantitative drug determination, when the liver is almost completely decayed.

**Materials and Methods:** *Breeding* - Approximately 400 eggs of *Lucilia sericata* which is a common necrophage species of Diptera in Europe, were deposited on different concentrations of amitriptyline with homogenized tissues of chicken liver (250.0 g). Each chicken liver homogenate was spiked with different amitriptyline concentrations. C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> were 500.00, 3000.00, 7000.00, 10000.00 ng/g, respectively. The non-spiked blank liver was regarded as C<sub>0</sub>. *Sampling* - At the end of the 117(±0.5) hours period (beginning from the egg phase to third feeding larvae phase), the larvae were collected from each liver, along with the corresponding liver sample for analysis. *Extraction procedure* - Approximately 0.500 g larvae and liver were homogenized in a 0.9% NaCl solution and an original LLE extraction procedure is developed. Organic layer of each sample was evaporated to dryness under N<sub>2</sub>, reconstituted in methanol and analyzed by ESI LC-MS/MS.

**Results and Discussion:** Amitriptyline and morphine were analyzed in this study. MRM method was developed with determined R*t* and selected m/z values. The ions  $286.1 \rightarrow 201.1$  for morphine and  $278.0 \rightarrow 91.0$  for amitryptiline were monitored. The method was validated in terms of specificity, linearity, accuracy (recovery  $\geq 82\%$ ), and precision and LOD and LOQ values were determined using
Eurachem method. Validation results established that routine quantitative amitriptyline and qualitative morphine determination can be achieved in liver and larvae matrices. Also at this study; a formula was suggested for a back-calculation from the results that were obtained from the decomposed liver matrix and larvae collected at the end of the  $117(\pm 0.5)$  hours period, to the 0<sup>th</sup> hour liver concentration.

Entomotoxicology, Tricyclic Antidepressants, Opiates

# K17 An Application of Speciated Isotope Dilution Mass Spectrometry (SIDMS) for Simultaneous Drug Quantitation of Gamma-Hydroxybutyric Acid (GHB) and Gamma-Butyrolactone (GBL) in Urine and Blood Matrices

Joshua Z. Seither, BS\*, 3645 Spring Valley Road, Akron, OH 44333; and H.M. Kingston, PhD, and Timothy Fahrenholz, BS, Duquesne University, 700 Forbes Avenue, Pittsburgh, PA 15219

After attending this presentation, attendees will learn how Speciated Isotope Dilution Mass Spectrometry (SIDMS) can be applied to the forensic community. This presentation will provide an example of GHB and GBL to show how this method can be applied.

This presentation will impact the forensic science community by showing the community a quicker and more accurate way to quantitate drugs with the example of GHB and GBL.

There are currently two major problems in the forensic science community: scrutiny of analytical methods and a rapidly growing backlog of samples. An accurate, rapid and simultaneous measurement of GHB and GBL in urine and blood was developed to combat these issues. This legally defensible method for analyzing both gammahydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) simultaneously uses speciated isotope dilution mass spectrometry (SIDMS). Current methods use gas chromatography mass spectrometry (GC/MS) and are not able to quantitate both GHB and GBL simultaneously; therefore, multiple extractions are required in order to quantitatively analyze GHB and GBL. To perform SIDMS, deuterium labeled GHB and carbon labeled GBL were utilized to spike the samples for quantitation. Once the naturally occurring analyte is spiked with the isotopically enriched analyte, SIDMS can account for any interconversion that occurs between GHB and GBL during sample preparation or analysis. After spiking the samples, a mixed-mode (phenyl and propyl sulfonic acid) solid phase extraction column was used for the filtration extraction of GHB and GBL from urine and blood samples. Mass spectrometry studies were done using electrospray ionization. Method validation was completed with triplicate sample preparation and analyses (n =9) with a known concentration of GHB and GBL in standardized urine and blood. Significant values of GHB and GBL were chosen based on previous studies completed in the literature. Concentration values of 5 ppm, 10 ppm, 200 ppm, and 400 ppm were used. Endogenous levels of GHB average below 10 ppm. Some studies have reported endogenous cutoff levels of GHB should be 6 ppm in urine to avoid false negatives. GHB overdoses were reported at an average of 200 ppm and have been seen as high as 400 ppm. The experimental values and the standard values were in agreement with the 95% confidence interval. By using SIDMS, inter-conversions between GHB and GBL can be accounted for and the correct quantification of both analytes can be made. Temperature and pH levels were varied to stimulate conversion between the two analytes, GHB and GBL. The inter-conversion was accounted for in the SIDMS calculation, which demonstrates the benefit for the use of this method in the forensic science community. Calculations were made to account for the inter-conversion, which demonstrate the use of the SIDMS method for drug quantitation.

This method can help forensic scientists by providing a procedure that is legally defensible and quicker than other traditional methods of analyzing GHB and GBL. This method can be beneficial to the forensic science community.

**Quantitation, SIDMS, GHB** 

# K18 Validating Immunoassay ELISA Kits to Detect Eighteen Benzodiazepines at Low Levels

### Danielle C. Mata, MS\*, 320 North Flower Street, Santa Ana, CA 92703

After attending the presentation, attendees will understand the process of validating an immunoassay benzodiazepine ELISA kit for low concentration of drugs.

This presentation will impact the forensic science community by demonstrating how changing the experimental parameters for an ELISA kit will allow you to detect more drugs at needed concentrations.

Reports have shown that 30-40% of drivers take benzodiazepines and that the use of these drugs could have impairing effects. The Orange County Crime Lab (OCCL) recently validated an immunoassay benzodiazepine ELISA screen to detect the 22 benzodiazepines confirmed by an LC/MS/MS method at similar detection levels. The main benzodiazepines prevalent in casework are alprazolam, diazepam, lorazepam, and clonazepam and detection limits of 2, 10, 4, and 3 ng/mL, respectively are required. The validation process addressed limits of detection, blanks, sample volumes, possible interferences, and saturation curves for all detected benzodiazepines. The validation determined that Temazepam at 3 ng/mL is the best benzodiazepine to use for the limit of detection, and allows the OCCL to detect 18 of the 22 benzodiazepines seen via LC/MS/MS. Only four benzodiazepine metabolites yield false negative results when no other benzodiazepines are present.

**ELISA Validation, Benzodiazepines, Low Concentrations** 

### K19 Detection of Acute Diazinon Exposure in Postmortem Bone Samples

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After attending this presentation, attendees will understand the techniques of analyzing the acute diazinon exposure in postmortem bone samples in explaining the mechanism and cause of death.

This presentation will impact the forensic science community by the mechanism of diazinon exposure in understanding the cause of death and the importance of detecting it with the forensic toxicology lab techniques.

Forensic toxicological analyses have traditionally focused on the use of blood, body fluids, and certain organs in examinations of deaths due to intoxication. However, in some situations, putrefaction and contamination make proper sampling from tissues and blood impossible, such as in exceedingly degraded exhumation cases. In these cases, bone might be useful as an alternative specimen since it is a potential depot for pesticides and other chemical agents.

This third study is focused on the use of alternative specimens where putrefaction and contamination make proper sampling from tissues and blood impossible. The first study, regarding this issue, was the use of bone marrow in detection of Endosulfan and Diazinon. The second study dealt with use of adipose tissue in detecting chronic organochlorine exposure. As the following experimental research after the study by Akcan et al. in 2009 and Daglioglu et al. in 2010, the present study separately deals with the use of bone samples in detection of diazinon in a longer postmortem period. In order to find out the value of use of alternative biological samples in long period of postmortem cases, further series of experimental researches examining different alternative samples are currently designed.

Diazinon is widely applied to control agricultural pests in the Cukurova region which is the largest agricultural area in Turkey. In this region, diazinon takes place as the most common cause for organophosphate related intoxications. Most poisonings by diazinon are due to suicidal or accidental exposure and usually occurs by oral ingestion. Therefore, detection of diazinon in postmortem cases or putrefied corpses is of high importance in forensic toxicological analyses.

The goal of this study is to determine diazinon in bone samples of close term postmortem cases and putrefied corpses of pesticide treated rabbits, in order to show and emphasize the value of boney tissue, a potential depot for most chemical agents, as an alternative toxicological sample of long term after death. A 2500 mg/kg dose of diazinon was orally given to six rabbits through a gavages tool. One rabbit was not treated with anything and served as a blank control sample. The rabbits were buried in soil, after obtaining postmortem right femoral bones of each as first sample. All seven rabbits were exhumed three months later, and remaining left femoral bones were sampled. The bone specimens were cleaned of any overlying muscle and putrefied tissue using a scalpel. The samples were subsequently rinsed with deionized water until the wash was clear and free of debris and air-dried. The bone was weighed (2 g), cut into slivers, soaked in methanol and rotated for 16 hours. Solid-phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS) were used for the analysis of diazinon in bone samples. The methanol supernatant was removed and then loaded into sample extraction cartridge, the eluent was evaporated to dryness under a nitrogen stream, reconstituted with methanol, and then analyzed by GC/MS. Ethion was used as an internal standard. Limit of detection (LOD) for diazinon was 0.03 mg/kg and limit of quantification (LOQ) for diazinon was 0.10 mg/kg. Calibration curve was prepared with seven sample concentrations and correlation coefficient were (r) > 0.999, the values obtained for intra- and interday precision and accuracy were within the criteria usually accepted for bioanalytical method validation.

The mean concentrations of diazinon in bone taken just after death and bone samples of exhumed corpses were 11.52 and 7.97 mg/kg, respectively.

These results suggest that in pesticide intoxication related deaths when other specimens are unavailable due to degradation, bone samples should be considered as useful alternative specimen.

Diazinon, Bone, Forensic Toxicology

# K20 An Overview of Modern Chromatographic Methods for Analysis of Anesthetics

Pallavi Dubey, MSc\*, Central Forensic Science Laboratory, Sector 36-A, Plot Number 2, Dakshin Marg, Chandigarh, INDIA

After attending this presentation, attendees will be aware of the past and current trends of chromatographic analysis of general and local anesthetics, and understand the history of instrumental chromatographic analysis of anesthetics goes back to the 1950's, when the first anesthetic lidocaine was analyzed. The technique of chromatographic analysis has taken over all other methods of analysis due to their rapidity and ease of sample preparation. This presentation will impact the forensic science community by making forensic experts aware of what technique, pre-sampling requirements, and extraction methods should be used for the analysis of anesthetics. Also, the presence of modern detectors and capillary columns has enabled the difficult analytical part easier for the forensic analysts.

Several attempts have been made to analyze various classes and combinations of anesthetics by spectroscopic, electrophoretic, and chromatographic methods. Chromatographic methods have taken over other conventional methods for their high detection limits, high resolution, sample recovery, and no prerequisites of sample pre-analysis.

This review focuses on the development of the chromatographic methods which includes the pre-analytical aspects such as extraction from pharmaceutical samples, body fluids such as blood, serum, plasma, CSF, hair, viscera, etc., selection of appropriate chromatographic technique, and comparison of the output after analysis. The various parameters which judge the ambiguousness of a particular technique for a said drug were LOD, LOQ, RSD, and mean recovery. The three major chromatographic analytical methods for detection of trace amount of about 15 anesthetics from various sources are reviewed. In addition to this, methods for analysis of various anesthetic combinations are summarized. This review describes various developments taken place during the last twenty years on applications of chromatographic techniques in clinical measurement of various anesthetics.

Chromatographic Methods, Anesthetics, HPLC

# K21 Determination of Lignocaine Hydrochloride and Bupivacaine Hydrochloride in Pharmaceutical Samples Using Thermogravimetry, IR Spectrophotometry and Atomic Absorption Spectrophotometry

Pallavi Dubey, MSc\*, Central Forensic Science Laboratory, Sector 36-A, Plot Number 2, Dakshin Marg, Chandigarh, INDIA; Sudhir K. Shukla, DPhil, Central Forensic Science Laboratory, Dakshin Marg, Plot Number 2, Chandigarh, AB, INDIA; and Swati Vaishya, MSc, Central Forensic Science Laboratory, Sector 36- A,Dakshin Marg, Chandigarh, AB, INDIA

After attending this presentation, attendees will understand the analysis and shortcomings of the anesthetics analysis countered as exhibits in case of anesthesia overdose. The short half-life and rapid degradation of these drugs have puzzled analysts for a fairly long period. Hence there was a need of an analytical method which would ensure the presence of drug in pharmaceutical samples, leftover vials, and such exhibits.

This presentation will impact the forensic science community by serving as a process aspect for a simple and rapid quantitative analysis of anesthetic drug overdose in cases where even qualitative analysis would have been difficult due to short half life of anesthetic drugs.

Ion associate complexes of lignocaine hydrochloride and bupivacaine hydrochloride with five metal tetrathiocynates (i.e., nickel, chromium, zinc, cobalt, manganese, and phosphomolybdate) were prepared. The precipitated ion associates were subjected to elemental analysis and further spectroscopic studies were done to determine the metal content and the association and stability of the metal-oxygen bond between the drug and the metal thiocynate. Solubilities of these solid ion associate complexes were studied and their solubility products were determined at different temperatures at the optimum pH for their quantitative precipitation. The thermodynamic parameters  $\Delta H$ ,  $\Delta G$ ,  $\Delta S$ were calculated after the thermal studies for the dissolution of lidocaine and tetrathiocynates. The development of the AAS method was done by precipitating the drug in excess inorganic metal complex ions i.e. tetrathiocynates and further determining the amount of excess metal ions unprecipitated by the drug by AAS. The method was applied for five metal tetrathiocynates and phosphomolybdate complexes in pure and pharmaceutical samples. The spectroscopic data revealed association of drug with metal with prominent M-O bonds indicating the stability of the drug metal complex. Further solubility and thermal studies revealed the physical and chemical characteristics of the complexes. The AAS studies of the complexes showed high precision and accuracy and gave a mean recovery of 99%. This research overcomes the shortcomings of analysis of anesthetics due to their short half life and minimal shelf life. **TG-DSC, AAS, Anesthetics** 

## K22 Can Pharmacogenetic Studies Improve the Effectiveness of Methadone Maintenance Programs?

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After attending this presentation, attendees will better understand how the effectiveness of methadone maintenance program can be improved by pharmocogenetic studies.

This presentation will impact the forensic science community by explaining how the utilization of modern technologies, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and single nucleotide polymorphism (SNP) for studying genetic variants and gas chromatography-mass spectrometry (GC-MS) analysis of pharmacokinetic parameters, would help place the maintenance program on a higher scientific ground.

Following the implementation of the "harm reduction" policy, methadone (MTD) has now been widely adopted for "treating" heroin addicts in Taiwan. In humans, MTD is metabolized by N-demethylation to 2-ethylidene-1,5-di-methyl-3,3-diphenyl-pyrrolidine (EDDP) and 2ethyl-5-methyl-3,3-diphenyl- 1-pyrrolidine (EMDP). It has been reported that: (a) treatment effectiveness was highly affected by the prescribed dose; and (b) patients receiving the same dose responded differently. With this understanding, a study was conducted on pharmacogenetic parameters of patients in a local MTD maintenance program, focusing on understanding the relationships between patients' plasma level of MTD (and its metabolites) and their treatment dose and genetic polymorphisms of ABCB1 and CYP2C19. Gas chromatography-mass spectrometry was used for the determination of these analytes' concentrations in plasma, while polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and single nucleotide polymorphism (SNP) genotyping assay were used for the analysis of genetic variants. The concentration of MTD, EDDP, and EMDP found in 55 patients (prescribed dose = 10-165 mg/day) were 39.2-805 ng/ml, 1.18-127 ng/ml, and < 0.5-38.3 ng/ml, respectively. For the low-dose group (< 50 mg/day), correlations of MTD dosage and the observed plasma concentrations of MTD and EDDP were  $R^2 = 0.638$  and  $R^2 = 0.680$ , respectively. For the high-dose group ( $\ge 50 \text{ mg/day}$ ), the corresponding correlations were  $R^2 = 0.141$  and  $R^2 = 0.103$ . The latter finding suggests that the observed concentrations of MTD and EDDP

might be associated with these patients' pharmacogenetic characteristics. Findings derived from PCR-RFLP and SNP genotyping assays include: (a) patients with GT, GA, TT, TA, and AA variants in their ABCB1 G2677T/A were associated with high EDDP plasma level (p = 0.003); (b) patients with 681A and 990T in CYP2C19 were associated with low EDDP plasma level (p = 0.015, 0.010, respectively); and (c) no definite pattern of plasma drug concentration could be established (p > 0.05) for patients with SNP C1236T and C3435T (in ABCB1) variants. In conclusion, understanding pharmacokinetic and pharmacogenetic parameters can potentially improve the effectiveness and safety in the implementation of the maintenance program.

Methadone Maintenance Program, EDDP, Pharmacogenetics

### K23 Taking the High Road: A Look at San Diego Auto Accidents Involving Inhalant Abuse

Chelsea Carter, MFS\*, San Diego Police Department, 1401 Broadway, San Diego, CA 92101

After attending this presentation, attendees will have a greater knowledge of the toxicology and methodology associated with hydrofluorocarbon analysis as well as the symptoms associated with inhalant abuse, as two case studies will be discussed.

This presentation will impact the forensic science community by illustrating the catastrophic effects of car accidents caused by inhalant abuse and by illustrating the importance of an inhalant method in the laboratory. Included in the discussion will be a look at inhalant abuse demographics with a portion focusing on the prevalence of inhalant abuse in the military. Method creation and validation will also be discussed.

Inhalants can be found everywhere; in schools, homes, offices, bedrooms, garages, and supermarkets. Inhalant abuse is considered the "intentional or deliberate inhalation of chemical vapors to achieve intoxication," and this can be done with any product that produces vapors. According to the U.S. Consumer Product Safety Commission, there are more than 1,000 products that contain dangerous solvents that can be abused. Inhalants are often seen as less harmful when compared to other recreational drugs but often "can result in total unconsciousness and even death the first, tenth, or one-hundredth time." The mechanism of inhalant deaths will be discussed along with the definition of sudden sniffing death and delayed death.

In San Diego City, approximately 62% of the drivers under the legal limit BAC of 0.08 grams % have something other than ethanol in their system; and some of these drivers' test results come back negative for commonly used recreational drugs. Due to the lack of apparent cause of the intoxication, the Forensic Chemistry Unit at the San Diego Police Department was asked to develop and test a method for presumptively identifying commonly abused inhalants (or volatile substances) in blood samples. The method created focuses on hydrofluorocarbons, namely 1,1-Difluoroethane and 1,1,1,2-Tetrafluoroethane, along with Ethylene Dichloride, and Toluene. A need for this kind of analysis became a priority after a 9-year-old girl was fatally killed in a car accident involving a driver who was under the influence of a volatile substance. This case will be discussed in depth during this presentation.

Inhalant intoxication is very similar to alcohol intoxication producing symptoms such as slurred speech, lack of coordination, euphoria, lightheadedness, delusions, and dizziness. Higher levels of intoxication can also produce confusion, nystagmus, and decreased reflexes. In the driving cases used in this study, drivers who were high on volatile substances exhibited many dangerous driving traits such as straddling the center lane, stopping without cause in a traffic lane, braking erratically, as well as accelerating and decelerating rapidly.

Manufacturers have implemented various techniques to deter the abuse of their products; this is referred to as product modification. Product modification can be done in three ways, by removing the harmful component, by adding a deterrent, or by modifying the package so that it is less likely to be misused. Product modification in the United States, as well as in Australia, will be discussed.

Inhalant Abuse, Toxicology, Driving

# K24 Alcohol Analysis in the 21st Century: Analysis, Reporting, and Interpretation

Anna T. Kelly, PhD\*, Andre Salazar, BS, Patricia L. Small, BS, and Ashlyn Beard, MS, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will gain knowledge about conducting thorough alcohol analyses in DUI cases, from analysis to accurate reporting, as well as, interpretation of the data using BAC software.

This presentation will impact the forensic science community by demonstrating a very thorough approach to the analysis of blood alcohol concentration (BAC) in DUI cases. In addition to the traditional approach of Gas Chromatography (GC)-Headspace analysis, uncertainty of the analysis will be introduced as well as the use of BAC software to extrapolate what the blood alcohol level was at the time of the incident in question, as in the case of an accident, based on the measured BAC. The application of this analysis method to several DUI cases will be discussed.

The analyses were performed on an GC-Headspace instrument that had been validated to confirm its accuracy based on results obtained from another instrument. A dual column system was used, the first of which quantitated the concentration of ethanol, while the second column served to confirm the presence of ethanol. The uncertainty of the measurements is taken into consideration by addressing a variety of areas, including the uncertainty in the purity of the standards used, the accuracy of the pipettes used, and the accuracy of the aliquoting of samples. Once the measurement was made, the BAC software was then used to extrapolate the BAC at the time of the incident. Using this software, a range of levels can be calculated by averaging BAC levels obtained by several published equations for the estimation of BAC.

One of the cases analyzed was that of a 50-year-old white female who was involved in a car accident. Her measured BAC was 0.19 g/dL at 3:30 a.m., whereas the accident she was involved in took place at 11:30 p.m. She stated that she had two martinis, the first one at 7:30 p.m. and the second at 10:00 p.m. Using the BAC software, it was determined that she would have had to have consumed 5.13 oz of 100% alcohol/volume during the time period that she stated that she had been drinking. Using the extrapolation function of the software, it was determined that the BAC at the time of the accident would have been  $0.26 \pm 0.03$  g/dL.

In conclusion, this method provides a thorough approach to the determination of BAC levels in DUI cases, resulting in a range of measurements that analysts can feel confident about.

### **References:**

- <sup>1.</sup> BAC Tracker. http://www.bac-tracker.com/
- <sup>2.</sup> LeBeau, Marc A. "Uncertainty of Quantitative Measurements." Society of Forensic Toxicologists meeting. Austin, Texas. 2006

**DUI, GC-Headspace, Extrapolation** 

# K25 Development of an LC/MS/MS Method for the Analysis of Fatty Acids

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After attending this presentation, attendees will be introduced to a novel LC/MS/MS method that can be used in the analysis of fatty acids. This method was applied to the analysis of fish oil and other omega-3-supplements and should be applicable to the analysis of other samples as well.

This presentation will impact the forensic science community by supplying a method that can alleviate time and effort in the analysis of fatty acids.

A common method for the analysis of fatty acids utilizes Gas Chromatography/Mass Spectrometry. This process requires several time-consuming and complicated steps to prepare the sample. This process also includes working with hazardous chemicals in order to derivatize the fatty acids. The current research focused on the development of an LC/MS/MS method that can be utilized in the analysis of fatty acids. This method was applied to the analysis of fish oil and other omega-3-fatty acid supplements. This method includes using a Restek Ultra C8 (3µm, 50x2.1 mm) along with a 20x2.1 mm Ultra C8 Guard Cartridge, also from Restek. Solvent A of the mobile phase was 50 mM formic acid/ 2 mM ammonium formate and Solvent B was 95% acetonitrile/water containing 50 mM formic acid and 2 mM ammonium formate. The following gradient was used: 50% B for five minutes, 50 to 100% B in 28 minutes, and holding at 100% B for two minutes. Heptadecenoic Acid was used as the internal standard. The essential omega-3 acids, Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) were quantitated in the supplements. The limits of detection for DHA and EPA were 0.49 and 0.33 ug/mL, respectively. The limits of quantitation for DHA and EPA were 1.12 and 1.11 ug/mL, respectively. The linear range for DHA was up to 5 mg/mL and the linear range for EPA was up to 2 mg/mL. Supplements were analyzed before and after a base hydrolysis step. The before samples were simply diluted with 70/30 acetonitrile/chloroform and injected. Base hydrolysis samples were extracted with chloroform and then diluted with acetonitrile, keeping with the 70/30 ratio. At least 24 different brands of omega-3 supplements were examined using this method.

Laws and regulations surrounding dietary supplements may not be firm enough to cover the safety and quantity of the ingredients included in these products. Due to increasing production and use of these omega-3 supplements, some monitoring of the composition and safety of these products is warranted. The newly developed LC/MS/MS method simplifies the procedures involved in the analysis of fatty acids and provides a less time consuming and less hazardous method that can be applied to dietary supplements such as fish oil but should be applicable to other samples as well.

LC/MS/MS, Dietary Supplements, Fatty Acids

### K26 An Investigation Into the Cellular Cytotoxicity of Benzylpiperazine (BZP) and Its Derivatives

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After attending this presentation, attendees will have an understanding of the methods for testing drugs of abuse for *in-vitro* cytotoxicity. Attendees will also have acquired a knowledge of the toxicity of BZP and a number of its major impurities, as well as, be able to relate this to the clinical significance.

This presentation will impact the forensic science community by providing, for the first time, evidence of the toxicity of BZP and its impurities at a cellular level. This allows us to begin to elucidate the mechanism of toxicity and thereby the treatment for those poisoned by these drugs.

The market for clandestine designer drugs has been expanding exponentially over the last decade. Piperazines are a group of psychoactive stimulants including 1-benzylpiperazine (BZP), 1-(trifluoromethyl-phenyl)piperazine (TFMPP), 1-(chlorophenylpiperazine) (CPP) and 1-(methoxyphenyl)piperazine (MeOPP).<sup>1</sup> Of these BZP is the most commonly encountered derivative marketed as a "herbal high"/"legal high" with the street name A2. BZP was originally produced as an anti-helminthic agent for livestock in 1944.<sup>2</sup> Studies on rats have revealed that BZP exerts its effects by elevating levels of serotonin and dopamine by blocking the re-absorption of these at neurological synapses producing the positive psycho-active effect.<sup>1</sup>

Over the last two years, there have been an increasing number of clinical reports published concerning fatalities after ingestion of BZP alone or in combination with other psycho-active agents. BZP is now a controlled substance in many countries. It is a class D drug in New Zealand, class C drug in the United Kingdom, and it is controlled as a schedule 1 drug in the United States.<sup>3</sup> There is very little published in the public domain regarding the toxicity of these drugs at a cellular level. Although there are numerous reports on the renal toxicity and concerns over chronic abuse, the actual activity of the drug has not been studied in cells from sites of biological filtration within the body.

This study examines the effects of short term exposure on immortalized cells derived from the kidney (CAKI-2), the liver (HepG2) and fibroblasts (3T3) to BZP, it's precursor piperazine hexahydrate, and synthetic by-product 1,4-dibenzylpiperzine (DZP).

Cells were exposed to the drugs for 1 hour at concentrations ranging from 0.783mg/ml-3.13mg/ml, following which they were assessed morphologically for evidence of cell death, either programmed cell death (apoptosis), uncontrolled cell death (necrosis), or no effect at all. To assess for cell death, cells were then labeled with annexin V to evidence the presence of apoptosis and propidium iodide (PI) for evidence of general cell death. These samples were analyzed using a BD FACS Calibur Flow Cytometer. Results were expressed according to the degree of annexin V and PI labeling as the percentage of viable cells (Annexin V -/PI -) versus the percentage of non-viable cells (Annexin V +/PI +). This data was confirmed using fluorescence microscopy and immune-labeling of the annexin V *in- situ*.

DZP causes comparatively higher levels of cell death giving LD<sup>50</sup> values of 2.25mg/kg (HepG2), 1.50mg/kg (CAKI-2) and 1.20mg/kg (3T3). Piperazine hexahydrate resulted in minimal cytotoxicity, being most potent in its activity against HepG2 with an LD<sup>50</sup> of 1.50mg/kg. BZP was most cytotoxic producing an LD<sup>50</sup> value of 1.1mg/kg (HepG2), 1.57mg/kg (CAKI-2) and 1.40 mg/kg (3T3). Further to this it is shown that HepG2 cells display a lower threshold for sensitivity to these drugs than CAKI-2 or 3T3.

These data provide clear evidence of the cellular cytotoxicity of BZP and DZP and its synthetic by-products at levels likely to occur following the ingestion of these drugs. Data also indicate that in general the liver, site of primary biological filtration, is most sensitive to the actions of these drugs. This supports the clinical evidence that BZP produces a very real threat of causing hepatic toxicity. **References:** 

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- <sup>2</sup> Gee P, Richardson S, Woltersdorf W, Moore G. Toxic effects of BZP-based herbal party pills in humans: a prospective study in Christchurch, New Zealand. N Z Med J. 2005;118(1227):U1784.
- <sup>3.</sup> Gee P, Jerram T, Bowie D. Multiorgan failure from 1benzylpiperazine ingestion—legal high or lethal high? Clin Toxicol (Phila). 2010;48(3):230-3.

**BZP**, Phenylpiperazine, Toxicity

# K27 A Statistical Analysis of Urine:Blood Data, and Oxycodone Redistribution: A Simple Ratio Will Not Suffice

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After attending this presentation, attendees will understand concept of a meta-analysis and the importance of statistical analyses in general. Attendees will gain an appreciation of the mathematical and statistical implications of simple ratios and what they imply.

This presentation will impact the forensic science community by introducing the concept of a quantitative review of literature rather than a qualitative review, and the importance of a rigorous statistical and mathematical analysis.

Meta-analysis, odds ratios, confidence intervals, prediction intervals, tolerance intervals, and bounds are demonstrated and these concepts are applied to several alcohol data sets including both blood and first- and second-void urine data and another meta-analysis on oxycodone redistribution will be demonstrated. A meta-analysis is a mathematical summary of previously done studies that address the same research hypothesis. The first meta-analysis, usually credited to Karl Pearson, a noted statistician, was done in 1904 to overcome the problem of small sample sizes and their reduced statistical power. A prediction interval is used to predict a value from a calibration curve while a confidence interval is used to express the uncertainties in the parameter estimates. Tolerance intervals are meant to contain a proportion of the population with a specified probability. Subject-level data available from urine-alcohol studies in the medical literature was extracted. Using multivariate regression techniques, the limits for urine-alcohol levels were critically examined from a statistical viewpoint taking into account whether first- or second-void urine samples were used, the assay method, and whether the subject was alive or dead. Oxycodone redistribution and, again, the improper use of only a simple ratio was also reviewed. The difficulties of modeling such data via regression strategies were examined.

Based upon an analysis of the data and pharmacokinetic, mathematical, and statistical principles, this presentation will show why the use of only a simple ratio is incorrect and misleading. Using the methodology described, it is easy to see that the per se values some state legislatures have incorporated into their laws and statutes allow innocent people to be convicted of a crime. Also, simple ratios and/or linear regressions will not always solve the data analysis problem, and more complicated models will be required. This will require collaboration with a statistician and other specialists. It is concluded that the levels of urine alcohol specified by some states are set statistically improperly. Experts must incorporate statistical methods to properly summarize their data and report error intervals.

Meta-Analysis, Alcohol, Oxycodone

# K28 Development of a Web Accessible Cheminformatic Mass Spectral Database for Shared Utilization by Forensic Laboratories

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After attending this presentation, attendees will understand how to using a free web accessible library with multiple spectral methodologies will allow electronic searching and comparison of unknown spectra against verified reference spectra including nominal mass, accurate mass, NMR, and FTIR spectra which can be applied to forensic toxicology, drug chemistry, and trace evidence such as ink dyes and explosives.

This presentation will impact the forensic science community by providing a free, community driven web accessible mass spectral library cataloging a standardized collection of compounds of forensic interest analyzed by various spectral technologies, thus enabling a more definitive compound identification.

Introduction: Cheminformatic databases are used for searching unknown spectra against reference spectra and for retrieval of chemical data such as structural information. In addition to traditional mass spectra, NMR and FTIR are also included to improve compound identification in some databases. The forensic utility of these databases varied due to the existence of relevant compounds and spectral methods, data quality, accessibility, and ability to search against reference spectra. Forensic applications of these databases routinely contain spectra from traditional instrumentation, such as electron ionization (EI) mass spectrometers (MS) and do not allow for cross-searching of other spectral methods. Direct Analysis in Real Time (DART) is a novel ion source coupled to an accurate mass time-of-flight (AccuTOF<sup>TM</sup>) mass analyzer DART has been primarily employed for controlled substances identification by forensic laboratories. Currently, there are no public databases that incorporate DART spectra, requiring laboratories to create in-house discipline specific library resulting in unnecessary duplicity. These in-house libraries are not readily accessible to the public.

**Methods:** Currently, RTI International in collaboration with Virginia Division of Forensic Sciences (VDFS) have begun the development and mass spectral population of a forensic cheminformatic database containing mass spectra, NMR and FTIR which will be Webaccessible and free for anyone with internet access. Users upload spectra through a Web portal to an editorial review board where selected, external "collaborator reviewers" evaluate the spectra based on established criteria. RTI staff, as the "database curator," also review the data and the reviewers' recommendations on whether the spectra should be accepted, rejected, or accepted with revisions. If all criteria are met the spectra is approved and moved into the cheminformatic database for public accessibility. Otherwise, the spectra are either rejected or the contributing user may be contacted to determine if better spectra can be submitted. This multi-step, interactive forensic practitioner review process with established criteria of acceptance will help maintain the validity and reliability of the spectra. Duplicity of a compound within the cheminformatic databases can be limited or eliminated as appropriate. Inclusion of DART spectra into the database required spectral evaluation and comparison by RTI and VDFS laboratories. Several commonly altered DART parameters were investigated to determine whether enough spectral dissimilarity existed to cause a false identification in the developed database. Collection of reference drug standards at RTI using the same instrumental parameters as VDFS evaluated the inter-laboratory reproducibility. A form has been developed to systematically document and evaluate spectra under varying DART conditions and instrument parameters thus allowing the assessment of their affects on DART spectra and the matching quality within the database.

**Results:** The current public database consists of 2,400 EI mass spectra previously collected and maintained by the American Academy of Forensic Sciences Toxicology Section MS Database Committee and 224 compound records each with one to four DART spectra at different CID voltages collected simultaneously using function switching at VDFS. It appears that function switching sacrifices sensitivity for more spectral detail. All compounds have been analyzed and parameters documented for optimization and acceptable ranges by VDFS. Currently, the same procedures are being finalized for DART analysis at RTI.

**Conclusion:** Forensic laboratories that use mass spectral, NMR and FTIR technologies for forensic toxicology, drug chemistry, and trace evidence such as ink dyes and explosives may find this new Web-accessible Cheminformatic database to be a highly reliable and valid tool for identifying unknown compounds of interest.

Cheminformatics, Mass Spectral Database, Collection Standardization

# K29 Identification of Markers of JWH-018 and JWH-073 Use in Human Urine

Sherri L. Kacinko, PhD\*, Allan Xu, PhD, Matthew M. McMullin, MS, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090

The goals of this presentation are to inform the audience of the current knowledge regarding the urinary metabolites of JWH-018 and JWH-073 and to outline screening and confirmation methods for identifying the use of these compounds.

This presentation will impact the forensic science community by providing up-to-date information on the urinary metabolites of JWH-018 and JWH-073.

Recently, synthetic cannabinoids have garnered media attention as a legal alternative to cannabis. Sold as constituents of "herbal incense" under a wide variety of names including Spice, Yucatan Fire, Smoke, Sence, K2, Skunk, Space, K2 Citron, and K2 Blonde these compounds such as HU-210, JWH-018, CP 47,497, JWH-073, JWH-250, and JWH-200 are mixed with plant material and smoked. These synthetic analytes have a varying degree of selectivity and affinity for cannabinoids (CB<sub>1</sub> and CB<sub>2</sub>) receptors and thus have different therapeutic and abuse potentials. As the popularity of these drugs increases, there is a developing need for analytical methods to identify and quantify the parent compounds in the herbal incense products as well as in biological matrices. On-going research will help identify metabolites of these compounds which can be used as markers of use in humans.

New drugs offer a unique challenge to the forensic toxicology community. Without authentic standard material for the multiple metabolites innovative methods of identifying the use of these compounds must be explored. Due to the lipophilic nature of these analytes, the parent compound is not excreted in urine emphasizing the important of quickly identifying the metabolites as markers of use. Urine was collected from participants who smoked incense containing JWH-018 and JWH-073. These specimens were used to identify urine metabolites of these two compounds based on literature reports and LC-TOF analysis. Based on the literature and in-house analysis, JWH-018 and JWH-073 undergo mono-, di- and tri-hydroxylation followed by glucuronidation. Qualitative screen and confirmation methods for identifying exposure to JWH-018 and JWH-073 were developed and validated based on the presence of these urinary metabolites.

Specimens were screened for the monohydroxy glucuronide metabolites. Solid phase extraction was used to clean and concentrate unhydrolyzed urine specimens and extracts were analyzed on an LC/MS/MS for the detection of monohydroxy-glucuronide metabolites. The instrument was operated in positive ionization mode employing atmospheric pressure chemical ionization. Separation was achieved using gradient elution on a C18 HPLC analytical column. Source fragmentation of JWH-073-monohydroxy-glucuronide and JWH-018monohydroxy-glucuronide was employed and the transitions resulting from the loss of the glucuronide moiety were monitored. Further fragmentation was then induced in the collision cell and two transitions monitored for identification purposes. The confirmation method employed is based on the presence of multiple urinary metabolites. Urine specimens underwent enzymatic hydrolysis and a liquid-liquid extraction prior to analysis. LC-MS/MS with electrospray ionization was performed on an Applied Biosystems<sup>™</sup> API5000 system. Multiple transitions were monitored for each analyte. The following table summarizes the monitored transitions for the screening and confirmation methods:

Screening Method	Comments and	and the second second second
	Source Fragmentation	Collision Cell Fragmentation
JWH-012-monohydroxyglucuronde	534÷358	358→155 & 358→127
JWH-073-monohydroxyglucuronide	520-3344	344→155 and 344→127
Confirmation Method		
and the second s	Preenisor Ion	Product ions
IWH-012-monohydroxy	358	155 127 284 130
JWH-013-dihydroxy*	376 374	214 171 155 127
JWH-018-thhydroxy	374	189 171
JWH-073-monohydroxy	344	155 127
JWH-073-dihydioxy*	362 360	200 171 155 127
JWH-073-trihydroxy	375	189 171

"Infultiple dilydroxy metabolites identified

Synthetic Cannabinoids, JWH-018, JWH-073

### K30 Toxicological Analysis of Synthetic Cannabinomimetic Spice Drugs

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After attending this presentation, attendees will be informed of an LC/MS/MS method for determining the concentration of "spice" drugs in forensic blood and urine specimens.

This presentation will benefit the forensic science community by providing a method for qualitatively and quantitatively detecting seven emerging indole cannabinoid drugs of abuse using liquid chromatography-tandem mass spectrometry (LC/MS/MS). The lack of methods available to analyze these drugs makes detection difficult in suspected cases of "spice." Therefore, as the prevalence of use increases, the need for validated detection methods becomes important.

Synthetic cannabinomimetic drugs have been studied primarily for their activity as  $CB_1$  and  $CB_2$  cannabinoid receptor agonists. Additionally, their strong binding affinity for  $CB_1$  receptors has made

these synthetic drugs potent marijuana alternatives which have become increasingly popular in recent years. Many of these drugs are sold as herbal incense under the name "spice" or more commonly "K2" in the United States. JWH-018 is the most commonly found drug in these herbal blends. The drugs analyzed in this study include JWH-015, JWH-018, JWH-019, JWH-073, JWH-200, JWH-250, and WIN55212-2.

"Spice" toxicity can present itself in conflicting psychological states such as nausea, excitability, sedation, and panic. Physiological changes can include sweating, tachycardia, dyspnea, and xerostomia. Numerous hospitalizations as well as a suicide have been following reported acute doses of "spice." Furthermore, long term effects include panic attacks, blurred vision, muscle spasms, and a case of diagnosed dependence syndrome.

Liquid-liquid extractions were used to extract the drugs from blood and urine. Drug standards were spiked into negative blood and urine specimens. Various extraction conditions were compared in order to optimize extraction efficiencies of the drugs in both blood and urine. In the end, pH10 sodium borate buffer and ethyl acetate provided the best extraction efficiency. LC/MS/MS was used to analyze the extracted drugs and develop a method to qualitatively and quantitatively identify the drugs. Prior to LC/MS/MS analysis, drug optimization on the instrument was performed in order to select the appropriate qualifier and quantifier ions for each drug as well as the fragmentor and collision voltages. To ensure optimal chromatography, diazepam-D5 was chosen as the internal standard after comparison with hydrocodone-D3 and fentanyl-D5. Methanol with 0.1% formic acid was chosen as the mobile phase as it allowed for adequate separation of the compounds of interest.

The method was validated using the laboratory's validation guidelines. A five-point calibration curve was developed from 1-250 ng/mL. Linear ranges were from 1-100 ng/mL for all drugs except JWH-200 and WIN55212-2 which maintained linearity from 1-250 ng/mL with R<sup>2</sup> greater than 0.995 for all drugs. To validate the method, two extractions were performed on separate days. Accuracy and precision were calculated at 10 ng/mL and 100 ng/mL using three replicates for each concentration. LOQ for all drugs was 1ng/mL.

This presentation provides a rapid, sensitive method for determining the presence and concentration of several indole-based cannabinomimetic drugs in blood. The combination of chromatographic separation and ion monitoring with LC/MS/MS allows for multiple drugs to be accurately detected. This method can prove useful with the increasing rate of synthetic cannabinomimetic drug use in the population.

Spice, Cannabinoid, LC/MS/MS

## K31 Analysis and Stability Determination of Salvinorin A and B in Human Blood, Plasma, and Urine by Liquid Chromatography Tandem Mass Spectrometry

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After attending this presentation, attendees will be able to describe the origins of and effects associated with abuse of *Salvia divinorum*, optimum methods for its analysis by liquid chromatography/tandem mass spectrometry, and limitations on its analysis based on analyte stability.

This presentation will impact the forensic science community by identifying a novel analytical approach to detection of an emerging hallucinogenic drug of abuse.

Salvinorin-A is an hallucinogenic compound that has no approved medical use in the United States. It is a naturally occurring, nonnitrogenous kappa opioid receptor agonist, and is the active component of the plant, *Salvia divinorum*, belonging to the mint family. The leaves of the plant are typically dried, crushed, and smoked for their dissociative hallucinogenic effect. Plant concentrate or extract is also commercially available. Salvia is a potent hallucinogen with effects distinct from LSD, mescaline, and other hallucinogens. An effective dose in humans is reportedly in the 200 to 1,000 microgram range when smoked. Salvinorin A and Salvinorin B have both been identified in the leaf and leaf extract; however, Salvinorin B is present in much smaller amounts. The Salvinorin A and Salvinorin B contents have been determined to be in the range of 3.2-5.0/0.10-0.17 mcg/mg in the dried leaf products, and 4.1-38.9/0.26-2.42 in the "concentrated extract" products.

After smoking Salvia, subjects experience rapid onset of an intense hallucinatory dissociative effect, during which they cannot speak or recognize their surroundings, lose psychomotor coordination and are highly impaired. Acute symptoms resolve within 8 to 12 minutes; however, longer term and residual effects have not been studied.

A validated a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed for the identification and quantitation of Salvinorin A and B in human blood, plasma, and urine.

Salvinorin A and B were extracted from biological matrices treated and preserved with sodium fluoride by a single step liquid/liquid extraction. Salvinorin A was analyzed under positive mode ESI-LC/MS/MS (ABI 5000 Tandem Mass Spectrometer, Shimadzu SIL 20A, HPLC). Ions monitored for Salvinorin A and its internal standard Salvinorin A-d3 are: m/z 433/373; 436/373. Ions monitored for Salvinorin B and its internal standard are: m/z 389/313; 391/359. HPLC conditions included 2% methanol in water gradient, vs water, at 1mL/min, on a Phenomenex Luna C8(2) 150cm column.

The linear range for this assay was established as 1–40 ng/mL for whole blood, plasma and urine. Response was linear, and the LLOQ was established at 1 ng/mL for both analytes. LLOD was approximately 0.25ng/mL. Within-run precision at the LLOQ was 3.2 % for Salvinorin A and 2.5% for Salvinorin B. The within-run accuracy was determined as  $100\pm5\%$  for both Salvinorin A and B.

Following development, the assay was validated according to laboratory procedure including assessment of inter- and intra- batch precision and accuracy, storage, extraction and autosampler stability, freeze thaw stability, dilution integrity, and recovery.

The stability experiments indicated that Salvinorin A and B in unpreserved urine were stable for 28 days refrigerated and frozen, Salvinorin A was stable for less than nine days at room temperature. Both compounds were unstable in sodium fluoride/potassium oxalate preserved whole blood at room temperature and refrigerated, being undetectable after one day. Samples that were preserved with sodium fluoride and EDTA and frozen, were stable for at least 28 days.

Challenges resulting from limited stability and likely low concentrations in human subjects make this a challenging assay for medico-legal applications and require the use of LC/MS/MS techniques. Salvia Divinorum, Salvinorin A, LC/MS/MS

### K32 Quantitative Analysis of Salvinorin A: (Salvia) in Blood

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After attending this presentation, attendees will be familiar with a technique for the extraction and quantification of Salvinorin A from blood specimens using solid phase extraction (SPE) and gas chromatography/mass spectrometry (GC/MS).

This presentation will impact the forensic science community by providing a new procedure for both qualitative and quantitative determination of the potent hallucinogen, Salvinorin A, from whole blood samples using GC/MS.

Salvia divinorum is a naturally occurring herb found within the Lamiaceae (mint) family. Salvinorin A is the trans-neoclerodane diterpene contained within its leaves that produces the plant's psychotropic properties. The drug is a potent naturally occurring hallucinogen. The availability and psychotropic effects associated with Salvinorin A have led to an increase in its use within the past decade. Studies have shown a growing trend in the *Salvia divinorum*-related media and internet traffic, as well as the use of the drug in persons age 12 or older. Salvinorin A is listed on the United States Drug Enforcement Administration's Drugs and Chemicals of Concern List but is not currently scheduled under the Federal Controlled Substances Act. Many states and other countries have already scheduled the drug and some are currently in the process.

Despite growing concerns regarding the recreational use of Salvia divinorum, published scientific literature describing Salvinorin A identification in toxicological specimens is very limited. Liquid chromatography/mass spectrometry (LC/MS) and related techniques have been reported. The objective for this study was to develop and validate a method for qualitative and quantitative identification of Salvinorin A in whole blood using a technique that was universally available in forensic toxicology laboratories (i.e. GC/MS). The development of the procedure evaluated potential protein precipitants, wash solvents, and elution solvents. The assay involves protein precipitation with 0.2 M zinc sulfate/methanol (20/80, v/v), mixed-mode solid phase extraction, a double wash step using hexane followed by hexane/dichloromethane (90/10, (v/v) and dichloromethane/ethyl acetate (80/20, v/v) as the elution solvent. Testosterone-d3 was used as the internal standard, and quantification was performed in selective ion monitoring mode. The ions selected were m/z 432, 273, and 94 for Salvinorin A and m/z 291, 249, and 124 for testosterone-d3 (quantitation ions underlined). The total run time was 23 minutes and the retention times for Salvinorin A and testosterone-d3 were 10.655 and 5.479, respectively.

The optimized GC/MS assay was evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, analytical recovery, linearity, interference, and carryover. The LOD and LOQ for the assay were determined to be 2 ng/mL. Precision and accuracy were evaluated at 15 and 150 ng/mL in blood. Both intra- and inter-assay CVs were in the range 4.5 - 7.7%. The 95% confidence intervals (95% CI) at 50 and 1000 ng/mL were 55.8  $\pm$  5.3 and 1059.2  $\pm$ 43.5 ng/mL, respectively. Accuracy determined over a range of concentrations was 87-104% and analytical recovery of Salvinorin A was 88%. Calibrations were linear at concentrations as high as 5,000 ng/mL (the highest concentration tested). Carryover was evident at 2,000 ng/mL but this greatly exceeds concentrations expected in blood samples of forensic interest, which are typically one hundred-fold lower. The interference study included 27 commonly encountered drugs of abuse in addition to other her structurally related Salvinorins and divinatorins. No interferences were present, either qualitatively or quantitatively. This presentation provides a reliable and effective method for the detection and analysis of salvinorin A in whole blood by GC/MS at low concentrations of forensic interest.

Salvinorin A, Gas Chromatography/Mass Spectrometry (GC/MS), Blood

## K33 Detection of Various Performance Enhancing Substances in Specimens Collected From Race Horses in Illinois: A Five-Year Experience

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After attending this presentation, attendees will understand the principles of testing of equine athletes for illicit performance enhancing substances, allowed medications, the scale of equine doping, and the most frequently detected drugs and substances.

This presentation will impact the forensic science community by demonstrating the pattern of use of performance enhancing drugs and substances in horse racing.

The goal of this study was to compile all analytical findings from fiveyear period of time (2004-2009) to determine what substances are used most frequently and to study drug use trends in general.

The Association of Racing Commissioners International classified all drugs having a potential of impacting the outcome from the race in the Uniform Classification Guidelines for Foreign Substances. All drugs and substances are categorized in five classes from Class 1 having the greatest potential for performance enhancing to Class 5, the least. All Illinois Racing Board rules and regulations regarding medication and testing for drugs are published in the General Assembly's Illinois Administrative Code, Part 603 (Medication). The rule lists thresholds of allowed medications in blood (serum) such as phenylbutazone, furosemide, flunixin, ketoprofen, thresholds of selected medications in urine (e.g., isoxsuprine, DMSO, selected anabolic steroids), as well as Odesmethylpyrilamine (pyrilamine metabolite) and benzoyloecgonine (cocaine metabolite). There is a "zero tolerance" established for Class 1-3 drugs. If the Class 4 or 5 drugs are found in the specimen, the quantification is required to be reported.

Testing protocol for urine and blood samples collected post-race from winning horses and others collected for various reasons as determined by track personnel in Illinois includes preliminary screening, on 65+ ELISA plates. The laboratory also analyzes the specimens collected postmortem and special exhibits such as syringes, needles, neat drugs, etc., found on race courses. In some cases the instrumental screening is performed using triple quad or ion trap LC-MS (e.g. anabolic steroids) or GC-MS (DMSO). All presumptive positive samples were subsequently confirmed by GC-MS or LC-MS. The use of alkalinizing agents, such as sodium bicarbonate, commonly called "milkshaking," is revealed by measuring the total carbon dioxide (TCO<sub>2</sub>) level in plasma.

During the five-year period of time (2004-2009) 91,808 specimens were analyzed (45,210 urine and 46,598 blood samples) collected postrace from the winning thoroughbred and harness horses at eight race tracks in Illinois. The total number of violations reported was 413 (0.45%). The total number of violations reported in blood was 207 (0.44% of all blood specimens), and in urine 206 (0.45% of all urine specimens). The number of reported violations ranged from 123 (2006) to 40 (2008). The total of 220 violations was reported for harness horses, and 193 for thoroughbred. The most frequent violations include the following substances: phenylbutazone (111), flunixin (44), cocaine (34), TCO<sub>2</sub> (33), furosemide (25), ergonovine and DMSO (21 each), Odesmethylpyrilamine (13), cromolyn, diclofenac and indometacin (9 each), isoxsuprine (7), acepromazine, (6), methocarbamol and procaine (5 each), naproxen (4), ketorolac (3), etorphine, lidocaine, and morphine (2 each). One violation of each was reported for the following drugs: acetaminofen, buprenorphine, carprofen, chloropromazine, codeine, desipramine, fluoxetine, glycopyrolate, guaifenesin, hydromorphone, imipramine, meperidine, mepivacaine, methamphetamine, nalbuphine, nalorphine, oxazepam, oxymorphone, phenobarbital, phentermine, prednisolone, prednisone, promazine, tramadol, and verapamil.

In conclusion, this presentation has demonstrated that while only a very small number of violations of the total tested samples were reported, a greatly varied pattern of performance enhancing drugs and substances in Illinois horse racing was revealed.

Equine Doping, Drugs, Forensic Toxicology

# K34 Concentrations of Amphetamine and Morphine in Femoral Blood in Overdose Deaths Compared With Venous Blood From DUID Suspects

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After attending this presentation, attendees will learn about two of the major drugs of abuse in Sweden (amphetamine and heroin) and gain firsthand knowledge about the concentrations of these substances in blood in overdose deaths as well as in people arrested for driving under the influence of drugs (DUID suspects). This presentation compares and contrasts the concentrations of two major recreational drugs, namely amphetamine and morphine (derived as a metabolite of heroin), in peripheral blood samples from the living and the dead.

This presentation will impact the forensic science community by enabling medical examiners and toxicologists to compare the concentrations of amphetamine and morphine in blood. The large size of the present material, the sampling and analysis of drugs in peripheral blood (femoral or venous) and use of modern analytical methods are some of the major strengths of this research. The results will impact attendees when they are called upon to interpret drug concentrations in overdose deaths or to testify in court in cases of drug-impaired driving.

Interpreting the concentration of drugs determined in postmortem blood in terms of acute toxicity and whether an overdose and a drug poisoning was a likely cause of death is fraught with difficulties. The circumstances surrounding the death, the police reports, eye-witness statements, findings at the scene, and the autopsy all need careful consideration. People differ widely in their response to the same dose of a drug depending on factors such as absorption rate, dosage form, routes of administration, ethnicity, enzyme polymorphism, least previous experience with the drugs in question, and the development of central nervous tolerance.

Unlike in the United States where methamphetamine is the preferred central stimulant amine subjected to abuse, in Sweden it is the primary amine amphetamine that has topped the list of illicit drugs over many decades. Elevated blood-amphetamine is a common finding in postmortem (PM) toxicology as well as in apprehended drivers. Information about amphetamine concentrations in the living and the dead was retrieved from a forensic toxicology database (TOXBASE) using a cut-off concentration for positive results of 0.03 mg/L. Amphetamine was determined in blood by isotope-dilution GC-MS. The use of heroin was verified by identification of the unique metabolite 6-monoacetylmorphine (6-MAM) in blood or urine. The poisoning deaths were identified from ICD-9 codes assigned by the medical examiner and then sorted according to whether these were mono-intoxications or poly drug users.

The mean (median) and upper 95<sup>th</sup> percentile concentration were 2.0 mg/L (1.5 mg/L) and 4.2 mg/L respectively for N = 36 monointoxications involving amphetamine. These findings compare with 1.6 mg/L (0.4 mg/L) and 4.3 mg/L, respectively for N = 383 poly-drug amphetamine-related deaths. The victims of amphetamine poisoning were mainly men (72-86%) and those in single-drug deaths were 13 years older than poly-drug deaths (48 y vs. 35 y). The median concentration of amphetamine in mono-intoxication deaths was four time higher than that of poly-drug users. The median concentration of amphetamine in blood of impaired drivers as the only drug was 0.9 mg/L compared with 0.6 mg/L in poly-drug DUID suspects. The DUID suspects had higher median B-amphetamine concentrations compared with medical examiner cases who were poly-drug users (0.4 mg/L). Regular use of amphetamine leads to tolerance although unusually high concentrations of this stimulant can be tolerated without a fatal outcome.

When forensic toxicologists report morphine in submitted bloodsamples this is usually taken to mean abuse of heroin, arguably the most dangerous recreational drug. In this study the presence of 6-MAM in blood or urine was used as a biomarker for recent use of heroin. In the autopsy material, most victims of heroin-related deaths were men (88%) although there was no gender difference in their age (mean 35 y). In traffic cases, 91% of heroin users were men and they were on average two years younger than the women (33 y v 35 y). The use of heroin was identified using an analytical cut-off concentration for 6-MAM in blood of 0.005 mg/L the same as that for B-morphine. Both opiates were determined in blood and urine by isotope-dilution GC-MS.

In medical examiner cases (N = 766), the median B-morphine concentration in heroin-related deaths was 0.24 mg/L, which compares with a median of 0.15 mg/L (N = 124) in apprehended drivers with 6-MAM in blood. The concentration distributions of B-morphine in the living and the dead overlapped to a large extent. In medical examiner cases, 65% of the victims had a B-morphine concentration > 0.2 mg/L compared with 36% in the DUID cases. In traffic cases when 6-MAM was present in urine (N = 1950) but not in blood, the median B-morphine concentration was considerably lower (0.03 mg/L) and only 3.6 % had a concentration exceeding 0.2 mg/L. The presence of 6-MAM in urine but not in blood means that more time has elapsed after the last use of heroin and consequently the concentrations of morphine in blood decreases through metabolism. It was found that the concentrations of morphine in blood (median values) were remarkably similar in mono-intoxication deaths (0.25 mg/L, N = 63), poly-drug deaths (0.24 mg/L, N = 703), and in heroin overdose deaths (0.25 mg/L, N = 669); and also when the person died in some other way than drug poisoning (0.23 mg/L, N = 97).

Interpreting the concentrations of drugs in postmortem toxicology is complicated because the results do not reveal any information about the extent of prior exposure and the development of tolerance. When it comes to acute toxicity of opiates, the loss of tolerance is perhaps the most important determinant of an overdose death, especially when the drugs are administered intravenously. The results from this study show that the concentration of amphetamine and morphine in forensic blood samples cannot be used *per se* to conclude that death was the result of drug poisoning. Toxicology results should not be interpreted in a vacuum and the autopsy findings including histology, the police investigation, knowledge of the deceased person's drug habits, as well as witness statements all need to be considered when the cause and manner of death are assigned.

Amphetamine, Morphine, Blood

# K35 LC/MS/MS Determinations of Hydrocodone and Hydromorphone in Oral Fluid, Urine, and Hair After Short-Term Therapy

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After attending this presentation, attendees will learn about LC/MS/MS-based opiate confirmation method in specimens from a subject who took hydrocodone over a few days for post-surgical pain.

This presentation will impact the forensic science community by providing a clearer view for interpreting forensic toxicology casework by distinguishing the profile of a therapeutic user versus one who abuses their medication.

This presentation illustrates a controlled system of how therapeutic doses of hydrocodone is distributed and detected in oral fluid, urine, and hair specimens. Attendees will learn about an LC/MS/MS-based opiate confirmation method in specimens from a subject who took hydrocodone over a few days for postsurgical pain. A comparison and contrast of these amounts will be made with those observed in DUI and DFSA casework.

Hydrocodone is a semisynthetic opiate indicated for acute and chronic pain relief. The hepatic enzyme CYP2D6 transforms it into hydromorphone and other metabolites, which follows an average serum half-life of 3.8 hours. Due to its side effects such as euphoria, sedation, and availability, hydrocodone is now one of the most commonly abused prescription drugs. It has become a common analyte in forensic toxicology confirmations, as well as controlled substance submissions from diversion and illicit use.

Most opiates are easily detected by immunoassay screens in blood, oral fluid, or urine. Modern techniques such as LC/MS/MS are very sensitive and selective in distinguishing hydrocodone from other opiates and determining the concentration. Therapeutic hydrocodone concentrations in blood typically range from 0.01-0.03 mg/L, rise to 0.10-0.20 mg/L in abusers, and plateau around 0.30-0.40 mg/L in cases of acute fatal overdose. Urine levels can be more difficult to interpret due to the inherent influences of diet, excretion patterns, and other factors.

Data on a subject who took therapeutic amounts of hydrocodone over a span of a few days will be presented. The times of doses and specimen collections were recorded and reconciled with confirmations by LC/MS/MS without an initial immunoassay screen. The extraction method for urine is a solid-phase extraction, whereas our oral fluid and hair extractions are simply diluted and filtered samples. Each type of curve provides reportable concentrations between 10 and 2,000 ng/mL. The limit of quantitation is 10 ng/mL, while the limit of detection is an administrative cutoff at 5 ng/mL.

For the therapeutic user in this case, the concentrations of hydrocodone in oral fluid remained between 0.001-0.01 mg/L, while hydromorphone levels remained at an undetectable level. In urine, hydrocodone levels were 0.01-0.50 mg/L and hydromorphone levels were within the range of 0.002-0.003 mg/L. When normalized to dosing and time, the hydrocodone and hydromorphone levels displayed a consistent ratio of concentrations between each other. In distinction, a retrospective analysis of hydrocodone in DWI and DFSA urine specimens gave hydrocodone concentrations from 0.02-73 mg/L and hydromorphone levels of 0.005-0.69 mg/L.

Hair was also examined, where the therapeutic user began submitting haircut specimens after three weeks and continued to provide clippings each week thereafter. In a sense, it is a reverse segmental analysis because the most distal ends opposite the root were sampled each week for opiate contents. Results showed an initial 25 pg/mg concentration at 18 days, which gradually peaked at 71 pg/mg after 52 days and rapidly declined in the following weeks.

These results support this methods for analyzing opiates in oral fluid, urine, and hair by LC/MS/MS. The data also provides a clearer view for interpreting forensic toxicology casework by distinguishing the profile of a therapeutic user versus one who abuses their medication.

LC/MS/MS, Hydrocodone, Hydromorphone

### **K36** The Uncertainty of Hair Analysis for Drugs and Poisons

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After attending this presentation, attendees will appreciate the various factors that contribute to uncertainty in interpreting results of hair analyses for drugs and poisons.

This presentation will impact the forensic science community, as well as, the field of forensic toxicology by providing a better understanding of the significance that hair growth, collection, external contamination, and the uncertainty in quantitative measurements play in interpreting the results of hair analyses for drugs and poisons.

Analysis of hair for drugs, poisons, and their metabolites has been widely reported in the scientific literature over the past two decades. There are a number of fundamental assumptions in interpreting results of these analyses including: (1) an average linear growth rate of hair of 1 cm per month; (2) sample collections occur with the hair being cut directly next to the scalp; (3) external contamination can be differentiated from ingestion; and (4) differences in measured concentrations between hair segments indicate a change in exposure.

This presentation will evaluate each of the above assumptions and provide useful information to help the attendee fully appreciate how measurement uncertainty plays an important role in interpreting the results of hair analysis for drugs and poisons. The results of a thorough review of hair growth studies will be presented and a more realistic growth rate of  $1.4 \pm 0.5$  cm/month will be proposed. Separately, the results of a hair collection study will be discussed. The results of this study suggest that an average of  $0.8 \pm 0.2$  cm of hair may be left on the scalp after collection, corresponding to  $0.6 \pm 0.3$  months of hair growth. The current status of the effect that external contamination may have on positive findings in hair will be addressed. Finally, the role that measurement uncertainty of quantitative results will be addressed with examples provided that demonstrate the limitations that uncertainty presents in assessing concentration differences between hair segments.

Measurement Uncertainty, Hair, Segmental Analysis

### K37 Analysis of Cocaine Analytes in Human Hair: Ultrastructural Evaluation of Human Hair by Microscopy for the Determination of Morphological Differences Following Surface Contamination and Laboratory Decontamination

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After attending this presentation, attendees will understand the morphological structure of different human hairs, the permeability of hairs and potential variations in drug absorbance, and how processes and procedures used by hair drug testing laboratories may affect hair morphology and consequently drug analyte concentrations in hair.

This presentation will impact the forensic science community by providing a better understanding of the relationship between hair morphology and the permeability of hair to drugs.

Introduction: The factors affecting the permeability of hair to drugs are not fully understood. In order to improve the analytical tools used in hair drug testing and better interpret the meaning of test results from that testing, research that examines the deposition of drugs onto hair, the factors that can contribute to drug deposition onto hair, and the role of environmental drug contamination is needed. If it is shown that hair color and or structure influences drug permeability, the current drug testing methods and interpretations may need to be modified in order to take these variations into account and remove any potential for bias and or unjustified accusation. The goal of this research is to examine the permeability of different hair types (color and ethnicities) to cocaine analytes by utilizing microscopy to help understand the relationship between hair structure and the extent of drug absorption.

Methods: Hairs (Caucasian light and dark hair types, African American; n=12 each) were contaminated with cocaine HCl powder. The structural differences between the hairs of the different types and ethnicities were visually examined before and after contamination and washing. Hairs from each sample were examined employing a variety of microscopy techniques including scanning electron microscopy (SEM), freeze fracturing combined with SEM, fluorescence microscopy, and brightfield microscopy. During fluorescence and brightfield microscopy, hairs were stained with methylene blue and rhodamine B and the extent of stain penetration examined.

Results: Multiple images were taken of each sample during examination with each microscopy technique and compiled into individual portfolios for comparison. Rhodamine B and methylene blue produced similar staining patterns when observed with bright field and fluorescent microscopy. Due to variations in excitation wavelengths, Rhodamine B fluoresced significantly better than methylene blue when examined with fluorescence microscopy. Significant differences were observed not only between hairs of different ethnicities, but between hairs within a single ethnicity as well. Deposition of dye was largely associated with the cuticular scale edges. In hair with damage or missing cuticle, the cortex was strongly stained. The thickness and number of cuticular scale layers were also examined between individuals and between ethnic groups. The SEM examination revealed ultrastructural details of the relationship between the cuticle and cortex, and demonstrated a wide variability in cuticle forms and delamination from the main hair body.

**Conclusion:** These preliminary data suggest that the collection of structural information from microscopic examination of hair may allow for the observed differences in hair morphology to be applied to differences in the permeability of hair to drugs. The information from this study may be useful to improve laboratory procedures employed by hair drug testing laboratories.

Hair Morphology, Drug and Dye Incorporation, Microscopy

# K38 Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types Following Surface Contamination and Laboratory Decontamination

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After attending this presentation, attendees will understand: (1) the in vitro model of drug surface contamination used to investigate cocaine analyte concentrations and ratios in hair; (2) the permeability of hairs and potential variations in drug absorbance in different hair color and types; and (3) how processes and procedures used by hair drug testing laboratories may affect drug analyte concentrations in hair.

This presentation will impact the forensic science community by directly affecting policy implementation for forensic applications of hair testing, such as the investigation of drug facilitated crimes and workplace drug testing.

Introduction: The mechanism(s) of permeability of hair to drugs are not fully understood. Research data suggest that hair color may affect cocaine's incorporation into and retention in the hair matrix. The possibility that because of hair color one individual may be more likely to test positive for a drug than another, despite both having ingested or having been exposed to the same amount of a drug, greatly concerns policymakers and forensic practitioners. The potential for such bias must be understood to ensure the correct interpretation of results and the appropriate use of hair testing. If it is shown that hair color influences drug permeability, current drug testing methods may need to be improved in order to take these variations into account and remove any potential for bias and false-positive results. The goal of this study was to evaluate cocaine analytes in hair of different color (e.g., light, dark) and ethnic origin (e.g., Caucasian, African American) after the hair has been subjected to surface contamination with cocaine and subsequent laboratory decontamination.

**Methods:** The *in vitro* surface contamination study design was modified to a shorter collection time, but generally followed a previously published method by Stout et al. 2006. Briefly, verified drug-free head hair samples (Caucasian light and dark hair types, African American; n=12 each) were collected under IRB protocol, contaminated with cocaine HCl powder, shampooed daily for 8 weeks with aliquots removed weekly for decontamination (two washing protocols: methanol and extensive phosphate buffer) and cocaine analyte testing by LC/MS/MS. Quantitative analytical procedures for the determination of COC, BE, CE, and NCOC in hair were performed on an Agilent Technologies 1200 Series liquid chromatography system coupled to a 6410 triple quadrupole mass spectrometer, operated in positive ESI mode. For confirmation, two transitions were monitored and one ion ratio was determined which was acceptable if within 20% of the ratio of known calibration standards. The limits of quantitation (LOQ) for COC was 25 pg/mg and BE, CE, and NCOC were 2.5 pg/mg. The upper limit of linearity was 55,000 pg/mg for cocaine and 1,000 pg/mg for all other analytes. Between run imprecision for COC at 150 pg/mg was less than 3% and at 15 pg/mg for all other analytes was less than 8%.

**Results:** While previous cocaine surface contamination studies were designed to provide an estimate of interindividual variation, this study included sufficient samples to determine differences between ethnic groups or hair color with statistical significance. The preliminary data suggests there was no apparent simple relationship between concentration and hair color by this *in vitro* cocaine surface contamination model.

**Conclusion:** The results of this study along with continued studies may influence how hair testing results are interpreted, and could have a significant impact on whether national agencies use hair testing.

Hair, Cocaine Analytes, LC/MS/MS

# K39 Investigation of the Effect of Vinegar on Oral Fluid Drug Testing: Effects on Immunoassay Screening

Eva M. Reichardt, MSc\*, Bournemouth University, Talbot Campus, Fern Barrow, Poole, BH12 5BB, UNITED KINGDOM; Dene Baldwin, PhD, Concateno, 92 Milton Park, Oxfordshire, Abingdon, OX14 4RY, UNITED KINGDOM; and Michael D. Osselton, Bournemouth University, Fern Barrow, Talbot Campus, Poole, BH12 5BB, UNITED KINGDOM

After attending this presentation, attendees will learn about: (1) the effects of different types of vinegars on the Orasure Intercept<sup>®</sup>, and microplate screen; and (2) the new Concateno Certus<sup>TM</sup> oral fluid collection devices with homogenous immunoassay screen.

This presentation will impact the forensic science community deomonstrating how the Orasure Intercept® oral fluid collection device exhibited many oral fluid false positive after the consumption of various types of vinegar.

Introduction: Oral fluid (OF) drug testing has become increasingly popular during recent years as an alternative matrix for drugs of abuse (DOA) testing. OF is simple and easy to collect and offers a non-invasive means of sample collection that can be applied for use in the work place, hospitals, drug treatment centers, and roadside. Although numerous studies have been published in relation to OF drug detection and identification, little work has been undertaken to investigate the effects of substances. In a separate study, several different foods and beverages and the result from this indicative study inferred the possibility that vinegar could cause an effect on an immunoassay screen were evaluated. This study was conducted to look at this effect in greater detail. This study investigates the effects of different types of vinegars on the Orasure Intercept® and microplate screen and the new Concateno Certus™ OF collection devices with homogenous immunoassay screen.

**Method:** Non-drug using human volunteers were asked to swirl 5mL of selected vinegars around the mouth. These included malt, white distilled, balsamic, red wine, and white wine vinegar. After consumption, OF was collected using the Orasure Intercept® or the new Concateno Certus<sup>™</sup> OF collection devices a) immediately after mouth emptying and b) 10, 20 and 30 minutes after mouth emptying. Each volunteer provided samples using both devices for all vinegars tested. The volume, pH and time for collection of samples were recorded. OF samples were subsequently analyzed using two different immunoassays for Amphetamine, Methamphetamine, Cocaine, Methadone and Opiates. Intercept® samples were analyzed using the Concateno homogeneous assays to observe whether the substances affected the immunoassay screening systems.

**Results:** The Intercept<sup>®</sup> device collected an average of 0.55 mL in the 3 minutes recommended by the manufacturer. The Certus<sup>™</sup> device has a built-in indicator and we collected an average of 1.15 mL in an average time of 1.67 minutes. The OF pH was not affected for either collection device. Presumptive positive Intercept<sup>®</sup>/Orasure samples were observed for amphetamine, methamphetamine and cocaine following consumption of all types of vinegar. Most presumptive positives were seen at the early time points although a significant number we also observed out to 30 minutes. The screen positives were submitted for GC/MS confirmation and found to be confirmation negative – screen false positives. There was also a depression of the binding for Intercept<sup>®</sup>/Orasure samples for opiate and methadone assays although this was not enough to trigger a positive response against the kit cut off. By comparison the Certus<sup>™</sup> /Concateno samples were negative for all vinegar types and time points.

**Conclusion:** The Orasure Intercept® OF collection device exhibited many OF false positive after the consumption of various types of vinegar. There was no significant difference between collection times, pH, or volume collected. False positive samples out to 30 minutes were a surprising observation. The Concateno Certus<sup>TM</sup> OF collection device was shown to collect larger volumes of fluid, more consistently, in a shorter time frame. All of the Certus<sup>TM</sup> screens were negative.

Oral fluid drug testing is increasing in popularity in forensic, clinical, and workplace scenarios. In order to avoid potential miscarriages of justice or misinterpretation of results, it is essential that any tests employed for human oral fluid drug screening should provide results that are accurate. Personnel using oral fluid drug testing devices should be aware of possible interactions that could provide false positive results. This presentation highlights the potential for false positive screening results that may be observed following the collection of oral fluid after the use of certain food types.

Oral Fluid, Concateno, Orasure

## K40 Drugs and Driving Special Scientific Session: Current Research Related to Drug-Impaired Driving

Laura J. Liddicoat, BS, Wisconsin State Lab of Hygiene, Forensic Toxicology Section, 2601 Agriculture Drive, Madison, WI 53707-7996; Christine Moore, PhD\*, Immunalysis Corporation, 829 Towne Center Drive, Pomona, CA 91767; Alain Verstraete, MD\*, University Ghent, UZ Gent, De Pintelaan 185, Gent, B-9000, BELGIUM; James P. Zacny, PhD\*, University of Chicago, Department of Anesthesia & Critical Care MC4028, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637; and Amy K. Miles, BS\*, Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, PO Box 7996, Madison, WI 53707-7996

After attending this presentation, attendees will have a greater understanding of the prevalence of drug use in drivers and the latest research findings in the area of drug-impaired driving.

This presentation will impact the forensic science community by enhancing the understanding of the extent of the drug-impaired driving problem and providing specific research findings related to several drugs' effects on the skills required to safely operate a motor vehicle.

**Introduction:** A large proportion of the population habitually drives while taking medical and/or recreational drugs. This special session will provide information on several research aspects of drug impairment including surveys that reveal the prevalence of drugs in United States and European drivers, how drugs may be categorized by the impairment they cause, recent research findings for specific drugs using psychopharmacological tests and a drugged-driving case study involving Tizanidine with assessment by a drug recognition officer.

**Topics Include:** "<u>National Roadside Survey 2007: Results from</u> <u>Paired Specimens of Oral Fluid and Whole Blood," Christine Moore,</u> <u>*PhD.*</u> This presentation provides an overview of the results pertinent to drugs detected in paired oral fluid – blood specimens from the National Roadside Survey (2007) that was conducted at selected sites across the United States. From night-time drivers, 5,869 oral fluid samples (OF) and 3,276 blood samples were taken. Of the paired specimens, 559 pairs showed at least one matrix as drug positive; 326 pairs were positive in both matrices.

"DRUID Project: Epidemiology Studies of Drug Prevalence in Europe," Alain Verstraete, PhD. Recent epidemiology data from surveys carried out in the European DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) project to assess the prevalence of alcohol and other psychoactive substances in drivers in general traffic (13 countries) and drivers involved in injury accidents (6 countries) will be presented.

"DRUID Project: A Classification System for Impairing Drugs," Alain Verstraete, PhD.

The classification and labeling of medicinal drugs according to their influence on driving performance is one of the work goals for DRUID. Dr. Verstraete will present the latest progress on this difficult task.

"Psychomotor and Mood-Altering Effects of CNS-Active Adjuvant Drugs Used in Treatment of Chronic Nonmalignant Pain, Alone and in Combination with Oxycodone in Healthy Volunteers," James P. Zacny, PhD. Very few research studies have studied the effects of carisoprodol or pregabalin on psychomotor abilities. Research on the combination of different drugs is even harder to find. A battery of psychopharmacological tests will be detailed along with the results of those tests on healthy volunteers dosed with carisoprodol and pregabalin, with and without an oral opioid on board.

"Psychomotor and Mood-Altering Effects of Oxycodone and Ethanol, Alone and in Combination, in Healthy Volunteers," James P. Zacny, PhD. Opioid use in the general population has escalated dramatically in recent years, and the incidence of opioids detected in drivers has followed a similar pattern. Dr. Zacny will present the results of an interaction study involving oxycodone and alcohol in healthy volunteers.

"<u>Analytical and Interpretation Challenges in a Tizanidine DUID</u> <u>Case," Amy Miles, BS</u>. Tizanidine is a short-acting muscle relaxer used for the treatment of muscle spasticity. In this case history the subject was stopped for impaired driving. A Drug Recognition Expert (DRE) was called in approximately an hour later to perform the 12-step evaluation. Upon completion of the evaluation the DRE was unable to find any impairment but concluded the subject was impaired at the time of the stop. The case will be discussed in detail including a review of the analytical challenges posed by this short-acting drug.

Drugs and Driving, Impairment, Forensic Toxicology

### K41 Direct LC/MS/MS Quantification of Plasma Cannabinoids and Their Glucuronides

David M. Schwope, MS\*, National Institute of Heath, NIDA, 251 Bayview Boulevard, Suite 200, Room 05A721, Baltimore, MD 21224; Karl B. Scheidweiler, PhD, National Institute of Heath, NIDA/IRP, 251 Bayview Boulevard, Suite 200, Room 05A721, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, National Institute of Heath, NIDA, 251 Bayview Boulevard, Room 05A721, Baltimore, MD 21224

After attending this presentation, attendees will be able to describe an LC/MS/MS method for the simultaneous identification and quantification of THC, its Phase I metabolites, 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH), other cannabis constituents, cannabidiol (CBD) and cannabinol (CBN), and its Phase II metabolites, THC-gluc and THCCOOH-gluc in 0.5 mL human plasma.

This presentation will impact the forensic science community by offering a novel analytical method for sensitive and specific simultaneous quantification of both Phase I and II cannabinoid metabolites in a single plasma extract.

**Introduction**: Cannabis is the illicit substance most commonly detected in blood of driving under the influence of drugs (DUID) cases and in fatally injured drivers. Cannabinoid glucuronides have been proposed as potential markers of recent cannabis intake; however, to our knowledge, no method that directly detects and quantifies  $\Delta^{9}$ -tetrahydrocannabinol (THC) and its metabolites THC-1-glucuronide (THC-gluc) and 11-nor-9-carboxy-THC glucuronide (THCCOOH-gluc) in plasma has been reported.

Method: Cannabinoids were extracted from 0.5 mL human plasma following pH adjustment with 1.5 mL 2% ammonium hydroxide (v/v), with reversed-phase polymeric SPE cartridges. Samples were reconstituted in 150 uL mobile phase consisting of 70% A (10 mM ammonium acetate, pH 6.15) and 30% B (acetonitrile). 30 uL was injected onto a LCMSMS instrument consisting of a Shimadzu SIL-20ACXR auto-sampler, DGU-20A3 de-gasser, LC-20ADXR pumps, and CTO-20AC column oven interfaced with an Applied Biosystems 3200 Qtrap mass spectrometer equipped with a TurboV ion source operated in electrospray mode. Gradient chromatographic separation was achieved utilizing a Restek Ultra II Biphenyl HPLC column (100 x 2.1 mm, 3 µm particle size) with a 0.4 mL/min flow rate and an overall run time of 9 min. Detection and quantification were conducted in MRM mode with THC-gluc, THCCOOH-gluc, 11-OH-THC, THCCOOH and CBD ionized in negative polarity mode while CBN and THC were ionized in positive polarity mode.

Results: Limits of quantification (LOQ) were 0.5 ng/mL for THCgluc, 1.0 ng/mL for THC, CBD, CBN, 11-OH-THC and 5.0 ng/mL for THCCOOH and THCCOOH-gluc. Calibration curves were 0.5-50 ng/mL for THC-gluc, 1-100 ng/mL for THC, CBD, CBN, 11-OH-THC and 5.0-250 ng/mL for THCCOOH and THCCOOH-gluc ( $r^2 > 0.990$  and concentrations  $\pm 15\%$  of target, except at the LOQ where  $\pm 20\%$  was acceptable). Validation parameters were tested at three concentrations spanning the linear dynamic range. Inter-day recovery (bias) and imprecision (N=18) were 100.0-108.0% of target concentration and 2.3-8.0% relative standard deviation (RSD), respectively. Extraction efficiencies were 67.6 - 91.8%. Matrix effects ranged from -56.2 -89.9%, depending on the analyte, with negative values indicating ion suppression; matrix effects at each quality control concentration were similar for native and corresponding deuterated compounds enabling low QC quantification within 88.1-117% of target concentration (N=18). Similar matrix effects were observed for twelve different blank plasma sources fortified with low quality control concentrations. Analyte stability was assessed under the following conditions: 24 h at room temperature, 72 h at 4°C, three -20°C freeze-thaw cycles, and 24 h on the 4°C autosampler; losses of less than 17.9% were observed for each condition, except for THCCOOH-gluc that experienced losses up to 25.2% during storage for 24 h at room temperature. No quantifiable analyte carryover was observed at two times the upper LOO.

**Conclusions:** A chromatographic method for the identification and quantification of cannabinoid metabolites in human plasma is described. This method will be employed in ongoing cannabinoid administration studies and will be useful for in assessing plasma cannabinoid concentrations in clinical toxicology and DUID cases.

Supported by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health.

Cannabinoids, Glucuronides, LC/MS/MS

## K42 Cannabinoid Concentrations in Daily Cannabis Smokers' Oral Fluid During Prolonged Monitored Abstinence

Dayong Lee, MS, and Allan J. Barnes, BS, National Institute on Drug Abuse, 251 Bayview Boulevard, Baltimore, MD 21224; Robert S. Goodwin, PhD, NIDA Intramural Research Program, Chemistry & Drug Metabolism Section, 251 Bayview Boulevard, Baltimore, MD 21224; Jussi Hirvonen, PhD, National Institute of Mental Health, Building 31, Room B2B37, 31 Center Drive MSC 2035, Bethesda, MD 20892-2035; and Marilyn A. Huestis, PhD\*, National Institute on Drug Abuse, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Boulevard, Room 05A721, Baltimore, MD 21224

After attending this presentation, attendees will: (1) be able to characterize THC and metabolite oral fluid disposition in daily cannabis smokers during monitored abstinence; and, (2) will be able to understand cannabinoid oral fluid detection windows for interpretation of oral fluid cannabinoid results.

This presentation will impact the forensic science community by improving the interpretation of oral fluid test results from chronic, daily cannabis smokers.

**Introduction:** Oral fluid is an increasingly popular alternative matrix for drug treatment, workplace, and driving under the influence of drugs testing programs due to ease of collection and reduced opportunity for specimen adulteration. The National Highway and Safety Administration's 2007 National Roadside Survey reported that more than 14% of nighttime drivers' oral fluid tested positive for potentially impairing drugs;  $\Delta^9$ -tetrahydrocannabinol (THC), the primary psychoactive component of cannabis, was the most commonly detected drug at 6.1%. Drug presence does not necessarily imply impairment, as THC and metabolites were detected in chronic cannabis smokers' plasma for up to seven days during monitored abstinence.

**Methods:** Healthy male daily cannabis smokers provided written informed consent to participate in this IRB-approved study. Participants resided on a closed research unit with continuous monitoring for up to 31 days. Oral fluid specimens were collected once each 24 h with the Quantisal<sup>™</sup> collection device, and analyzed for THC, cannabidiol (CBD), cannabinol (CBN), and 11-nor-9-carboxy-THC (THCCOOH) by two-dimensional (2D) GCMS. Limits of quantification (LOQ) were 0.5 ng/mL for THC and CBD, 1 ng/mL for CBN (electron impact 2D-GCMS), and 7.5 pg/mL for THCCOOH (negative chemical ionization 2D-GCMS).

Results: Seventeen cannabis smokers (19-43 years old) reported current smoking of 1-18 joints/day (median 9 joints/day), and up to 45 joints/day during peak use. Participants resided on the closed residential unit for 5 to 31 days. Cannabinoids were quantified in 304 oral fluid specimens. Maximum THC, CBD, and CBN concentrations occurred upon admission, while THCCOOH concentrations generally peaked within the first two days of abstinence. All specimens from one subject who spent 30 days on the research unit were below LOQ; however, selfreport data indicated only 1 joint/day typical use. THC was quantifiable in only 26 specimens (8.6%) at concentrations <82.5 ng/mL, and was never present without concurrent THCCOOH. Daily THC detection rates in Quantisal<sup>™</sup> collected oral fluid decreased from 94 to 41 to 18% during the first, second and third days of abstinence, with no THC detectable after this time. Of the specimens 6.6% were THC-positive at the recommended DRUID confirmation cutoff of 1 ng/mL and 5.3% at the proposed Substance Abuse and Mental Health Services Administration 2 ng/mL cutoff. THCCOOH detection in oral fluid was prolonged, with concentrations up to 202.5 pg/mL in 141 specimens (46.4%). All 17 participants resided on the secure research unit for at least five days, with 81.6% of daily THCCOOH concentrations  $\geq$ LOQ. During the first, second and third weeks of abstinence, 74.6, 41.3, and 29.6% of specimens were THCCOOH positive. For the 61 specimens

collected on days 22-31, 14.8% remained  $\geq$ 7.5 pg/mL. CBD (initial concentrations 2.6 - 3.4 ng/mL) and CBN (1.1 - 5.2 ng/mL) were only detectable on the first day in oral fluid from two and eleven subjects, respectively.

**Conclusions:** THC and THCCOOH were detected in oral fluid of chronic, daily cannabis smokers during monitored abstinence for 3 and 30 days, respectively. The differences in detection windows may be attributed to the much lower LOQ for THCCOOH. For the first time, it is documented that detection of cannabinoids in oral fluid of daily cannabis smokers may not reflect recent use and may not indicate impairment in daily chronic cannabis smokers. Neurocognitive impairment in daily chronic cannabis smokers was demonstrated on some measures for 7 to 28 days. Additional research is needed to determine if cognitive and performance impairment persists during the detection window of cannabinoids in oral fluid. It is hypothesized that residual THC in the brain produces the performance impairment observed in chronic daily cannabis smokers. These novel cannabinoid oral fluid data are important for interpreting oral fluid test results.

This research was funded by the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health. Oral Fluid, Cannabinoids, Chronic Cannabis Smoking

# K43 Effects of Smoking the Synthetic Cannabinoids JWH-018 and JWH-073 on Human Performance and Behavior: Controlled Administration and DUID Case Reports

Amy Miles, BS, Wisconsin Laboratory of Hygeine, 465 Henry Mall, Madison, WI 53706; Barry K. Logan, PhD\*, and Allan Xu, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 18901; Robert Welsh, MS, University of Central Missouri, Missouri Safety Center, UCM Humphreys Building, Suite 200, Warrensburg, MO 64093; and Tracey Durbin, University of Central Missouri, UCM Humphreys Building, Suite 200, Warrensburg, MO 64093

After attending this presentation, attendees will be able to identify the drugs present in herbal "legal high" smoking blends, and describe the effects on subjects' behavior and performance following smoked administration.

This presentation will impact the forensic science community by describing the behavior of subjects under the influence of synthetic cannabinoids drugs, their performance in field sobriety tests, and observations of actual subjects arrested for DUI after using these products.

Herbal materials sold as "legal highs" containing synthetic cannabinoids agonists with purported effects similar to tetrahydrocannabinol (THC) have been appearing on the United States recreational drug market since 2008. The most popular of these products is the K2 brand, sold as a variety of blends including Blonde, Summit, Strawberry, and a variety of others. These products have been shown to contain the cannabinoid CB<sub>1</sub> agonists JWH-018, and JWH-073.

A variety of K2 blends were administered to human subjects in a controlled environment, and their clinical signs, symptoms, and performance in the Drug Recognition Expert (DRE) assessment matrix were evaluated. Additionally, driving performance and actual behavior of subjects arrested under suspicion of DUID after smoking K2 are reported and discussed.

Ethical review was provided by the University of Central Missouri Institutional Review Board. They approved an experiment involving the administration of low doses via smoked route of administration of K2 blends containing JWH-018 and JWH-073 to six subjects. Subjects were screened and determined to be drug free prior to administration. Subjects were administered either one or two inhalations of K2 from a

\* Presenting Author

water pipe. Their response and vital signs were monitored by medical personnel. Subjects completed the DRE evaluation before and after administration of the drug. Blood, urine, and oral fluid were collected from subjects for later analysis. Analysis was performed by liquid chromatography tandem mass spectrometry (LCTMS), with a limit of detection of 0.1ng/mL in blood for the parent compounds.

Subjects began to feel the effects of the drug within 2-3 minutes of administration. Effects included increased pulse and blood pressure, dry mouth, bloodshot, watery eyes, and lack of convergence. Subjects reported a variety of subjective effects, including lightheadedness, blurred vision, motor restlessness, some mild agitation, and temporal disorientation. Four of six subjects indicated some mild anxiety. Subjects indicated a preference to continue dosing, although this was not permitted. Those subjects who were experienced marijuana users described the experience as qualitatively inferior to marijuana. Subjects' performance in field sobriety and psychophysical tests was variable. Some subjects demonstrated loss of balance, impaired coordination, and difficulty following instructions. Others displayed negligible performance effects following this low dose. The acute effects diminished between two and four hours following administration. Three of six subjects indicated high levels of fatigue once the acute effects had worn off.

Blood and oral fluid collected within an hour of administration were positive for both JWH-018 and JWH-073. Urine was positive for glucuronidated monohydroxy-, dihydroxy-, and trihydroxy- metabolites of both parent compounds. In one subject urine continued to test positive for metabolites for up to 24 hours post-administration.

Data was reviewed for several subjects arrested for suspected DUI following use of K2. Subjects displayed similar symptoms including increased pulse and blood pressure, bloodshot eyes, lack of convergence, and poor performance in field sobriety tests.

The synthetic cannabinoids contained in K2 cause marijuana-like effects on subjects' psychophysical response and driving performance. **Synthetic Cannabinoids, K2, Driving** 

# K44 Corresponding Impairment With Hydrocodone and Driving Under the Influence Investigations

### Danielle C. Mata, MS\*, 320 North Flower Street, Santa Ana, CA 92703

After attending this presentation, attendees learn more information about the impairing effects of hydrocodone, alone, and in combination with illicit and legal drugs for the purposes of driving.

This presentation will impact the forensic science community by providing a possible tool for court when testifying in DUID cases.

There has extensive debate over whether opiates, especially Hydrocodone, impair driving. Publications have not yet examined driving behaviors, driving under the influence (DUI) cases, and drug recognition evaluations (DRE) where toxicological results indicated hydrocodone was present. A retrospective three year study of DUI and DRE investigations involving hydrocodone, in Orange County, CA, was conducted. Driving behavior, the number of cues missed on the validated Standard Field Sobriety Tests (SFST), the demeanor of the driver, speech pattern, eye response, and physical appearance were evaluated. From over 150 cases evaluated, five cases were observed with only hydrocodone present. However, there was a high correlation between the officer reported impairment and hydrocodone when combined with muscle relaxants (n=81) and/or benzodiazepines The most frequently occurring muscle relaxant was (n=103). carisoprodol with alprazolam and diazepam as the most prevalent benzodiazepines. It was also determined that poly-drug use concurrent with hydrocodone was not limited to prescription drugs. An increased rate of vehicular collisions, of poor performance on psychological tests

and necessitated additional DRE evaluation was noted when hydrocodone and a central nervous system depressant were present. Furthermore, the psychological tests administered by the investigating officer appear to be a reliable measure of impairment for poly-drug cases involving hydrocodone with and without other drugs present.

Hydrocodone, DUID, DRE

## K45 Fatal Intoxication Due to Trihexyphenidyl -A Case Report

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After attending this presentation, attendees will learn about trihexyphenidyl pharmacological effects, metabolism and poisoning, method of analytical detection, and autopsy findings.

This presentation will impact the forensic science community by noting that fatal poisoning with trihexyphenidyl is very rare, based on the literature data, especially when no other central nervous system depressants and/or significant pathological changes are taken into account.

Trihexyphenidyl (THP) is an anticholinergic agent with forensic toxicological interest due its frequent abuse and reported overdose, while fatal poisoning is rare. It is a potent anticholinergic drug used in the treatment of Parkinsonism and in the control of drug-induced extrapyramidal side effects. Its mode of action is preventing the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle, cardiac muscle, and gland cells, in peripheral ganglia, and in the central nervous system. Side-effects of THP include disturbance of recent memory, tachycardia, bradycardia, and can precipitate glaucoma in predisposed patients. THPhydrochloride is well absorbed from the gastrointestinal tract producing average peak plasma levels at 1.3 h after single oral dose of 2 and 15 mg and reaching Cmax of 0.01 and 0.05 mg/l, respectively. The half-life time varies from 3.6 h up to 33 h, following multi-compartmental kinetics. THP undergoes extensive metabolism and hydroxy-THP was reported as the major metabolite present in plasma and urine. Ethanol and other central nervous system (CNS) depressants, such as anxiolytics, sedatives, and hypnotics, can increase the sedative effects of THP.

This report presents a case of a 59-year-old female with a history of paranoid disorder being treated in an outpatient program and who was found dead in the house where she lived alone. External examination of the body yielded no evidence of external injuries or violence. Autopsy findings revealed no marked pathological changes. Femoral venous blood, urine, bile, and gastric content were collected for toxicological analyses. Toxicological analysis based on gas chromatography-mass spectrometry (GC-MS) analysis revealed THP and its major metabolite (hydroxy – THP) in blood and urine. Ethanol was analyzed in femoral venous blood and urine by head-space gas-chromatography with a flame ionization detector (GC/FID).

Qualitative GC/MS analysis confirmed the presence of THP in blood and urine, hydroxy-THP in blood, urine and bile. The presence of these substances and other xenobiotics wasn't confirmed in gastric content. GC/MS quantitative analysis revealed THP concentration of 0.053 mg/L in femoral venous blood and 0.560 mg/L in urine. The blood and urine ethanol concentrations were 0.096 g/L and 0.100 g/L, respectively.

Based on these results and literature data the cause of death was determined to be THP poisoning. It is suggested that rare case of death associated with THP overdosage should be taken in conjunction with central nervous system depressants (benzodiazepines, ethanol) and/or with other pathological disorders. Thus, this case could not be supportive for this allegation. The circumstances of the case exclude homicide; however, these data are not sufficient to determine neither suicide nor accident as a manner of death.

Trihexyphenidyl, Fatal, Poisoning

## K46 Postmortem Toxicological Investigation of Alcoholic Ketoacidosis

Ingrid Bosman, PhD\*, and Rianne Vincenten, PhD, Netherlands Forensic Institute, Laan van Ypenburg 6, Den Haag, 2497 GB, NETHERLANDS

After attending this presentation, attendees will understand the importance of performing toxicological investigations for alcoholic ketoacidosis to provide a possible cause of death in postmortem cases with no anatomical and toxicological cause of death where victim has a history of alcohol abuse.

This presentation will impact the forensic science community by providing recommendations how to establish postmortem toxicological investigations of alcoholic ketoacidosis.

Ketoacidosis is a biochemical disturbance in the body. If no glucose is available, the body will utilize fatty acids as an alternative fuel pathway and ketone bodies will be produced. The increase of ketone bodies (acetoacetate, acetone and betahydroxybutyrate (BHB) or 3-hydroxybutyrate) in the blood will lower the blood pH. Two particular forms of ketoacidosis exist, alcoholic ketoacidosis as a result of chronic alcohol abuse and diabetic ketoacidosis as a result of a reduction in insulin. In contrast to diabetic ketoacidosis in which hyperglycemia occurs, alcohol ketoacidosis produces usually a hypoglycemia although a slight hyperglycemia can exist. The symptoms for the two forms are very similar and include nausea, vomiting, abdominal pains, loss of appetite, lethargy, weakness, and unconsciousness.

In this presentation, the toxicological results of postmortem cases at the Netherlands Forensic Institute from January 2006 with no anatomical cause of death and the victim having a history of alcohol abuse were examined. The goal was to evaluate the importance of toxicological investigations for alcoholic ketoacidosis to provide a possible cause of death in such cases. Included were those cases with no anatomical cause of death, the victim having a history of alcohol abuse, and toxicological analysis of BHB. All cases were toxicologically screened for the presence of alcohol, drugs of abuse and prescription drugs.

In total six cases were included; four male and two female with age ranging between 39 and 59 years. In five cases, the bodies were found dead in their homes and in one case the victim was found by her husband at her home needing resuscitation and subsequently died in the hospital. At autopsy, the pathologist found no anatomical cause of death or clear cause of death. Toxicological analysis for the presence of alcohol, drugs of abuse, and prescription drugs resulted in no indications for a toxicological cause of death. Alcohol was detected in blood in three cases in concentrations of 0.003, 0.032, and 0.18 g/dL, respectively, and in urine in four cases in concentrations varying from 0.006 to 0.24 g/dL. In four cases, prescription drugs were found. In all cases, acetone was detected in blood, urine, or both in the standard alcohol analysis method.

Further analysis on BHB and acetoacetate in blood, urine or vitreous humor was performed to determine possible ketoacidosis and

concentrations of glucose and lactate in blood, urine, or vitreous humor were analyzed to determine possible hypo- or hyperglycemia. Based on the combined glucose and lactate levels in vitreous humor, in one case an indication for hypoglycemia was found (measured concentration lower than 7.5 mmol/L) and in another case hyperglycemia was concluded (measured concentration was 30 mmol/L). Acetoacetate could not be detected except in a low concentration in one case, because it spontaneously decarboxylates to acetone. Measured concentrations of BHB varied from 1 to 14 mmol/L in blood and from 1 to 11 mmol/L in vitreous humor. In literature, BHB concentrations are considered normal below 0.5 mmol/L, elevated up to 2.5 mmol/L, and high and pathologically significant over 2.5 mmol/L. The measured BHB concentrations in this study are all elevated or high. Because no anatomical cause of death and toxicological cause of death due to alcohol or drugs were found, it was concluded that alcoholic ketoacidosis could have contributed to death in five cases and ketoacidosis due to hyperglycemia in one case.

In conclusion, in cases with no anatomical and toxicological cause of death, a history of alcohol abuse, and the presence of acetone in blood or urine, analysis of BHB in blood and vitreous humor may provide a possible cause of death by alcoholic ketoacidosis.

Alcoholic Ketoacidosis, Beta-Hydroxybutyrate, Postmortem

## K47 A Decade of Deaths in the OC Where Drugs Were Detected

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After attending this presentation, attendees will understand the compilation of information assembled from the last 10-years of postmortem cases where drugs were present. The attendees will be introduced to the use of the compilation involving cases where drugs were both associated and not associated with the coroner cause of death.

This presentation will impact the forensic science community by introducing a supplemental set of data to the currently used texts and references. The data summarizes the recent decade (2000-2009) in Orange County, California, where drugs were present in postmortem cases. The compilation of drug levels and associated types of death will provide an additional resource for toxicologists during examination and comparison against related casework.

Many current references used by toxicologists have limited numbers of subjects in order to interpret drug levels associated with postmortem cases. These references include levels from clinical and hospital submissions involving relatively few subjects or cases. To supplement this data, a compilation of postmortem investigations where drugs were detected during the 2000 to 2009 period was initiated. The results will be presented from cases involving trauma, natural death, overdose of the drug of interest, and poly-drug deaths for 52 of the most common drugs in Orange County, California (population 3 million). Furthermore, information is included from central blood, peripheral blood, and where possible, liver and brain tissue. In some of the natural and trauma cases where the drug was not the cause of death, liver or brain tissue levels are included. This additional information can assist in cases where blood is not available. Not included as a specific drug are alcohol and carbon monoxide, but these were included in the classification of poly-drug cases. The database will include half-life, structure, and other reference material for convenience, when available from literature sources.

Toxicology, Drug Level, Database

### K48 Helium Detection in Postmortem Specimens

Anna Kelly, PhD\*, Shahriar Shahreza, BS, Terry Danielson, PhD, Fessessework Guale, DVM, and Ashraf Mozayani, PhD, PharmD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will gain knowledge about the detection of helium in postmortem specimens using headspace gas chromatography/thermal conductivity detection (GC/TCD) in cases where death by asphyxiation using helium is suspected.

This presentation will impact the forensic science community by providing a method for the detection of helium in a variety of different postmortem specimens, allowing for a toxicological confirmation for the cause of death.

Currently, in the majority of cases, helium toxicity is only listed as the cause of death based on scene investigation; common scene observations in cases of suicide by helium inhalation includes a plastic bag covering the head with a hose running from it to a helium tank.

Helium is the second lightest element and the second most abundant element in the universe after hydrogen. Its major use is in cryogenics to cool superconducting magnets, such as those used in MRI devices. It is also used as a lifting gas in balloons and airships, as well as in combination with oxygen and nitrogen for deep sea diving in order to reduce the effects of narcosis, an alteration in consciousness that can occur; the proportions of oxygen, nitrogen, and helium are adjusted depending on the circumstances. Because of its low density, it can be inhaled unconsciously, making it a potential asphyxiant under certain conditions. There have only been a few cases reported of accidental asphyxiation by helium, but over recent years, its use as a means of suicide has increased. The increase in the occurrence of suicidal asphyxiation by helium is believed to be, in part, due to certain groups and internet sites advocating helium as the preferred method of suicide because it is widely available and a quick, painless death. One of the most influential publications is "Final Exit - The Practicalities of Self-Deliverance and Assisted Suicide for the Dying," in several reported cases, this literature was found on the scene.

The typical signs of asphyxia include cerebral and pulmonary edema, congestion of internal organs, petechial hemorrhages, and frothy edema in the respiratory tract. These signs are sometimes present, although there are often no significant postmortem abnormalities. In cases where such a cause of death is suspected, a reliable detection method is needed.

Postmortem specimens of five cases in which helium asphyxiation was cited as the cause of death were analyzed for the presence of helium. At the time of autopsy, samples of lung, brain, and blood were collected and sealed in 22 mL headspace vials by the forensic pathologist. Three of the five blood samples analyzed were femoral, and the source of the other two samples is not known. Each specimen was analyzed using headspace GC/TCD, with separation performed at 50°C (isothermal) on an HP-Molesieve column using nitrogen as the carrier gas. The vials were incubated at 38°C for 2 minutes, and then 100  $\mu$ L of headspace was removed from the vial and injected into the GC.

Helium was detected in four of the five cases analyzed. In each of the positive cases, helium was detected in the lung. Two of the cases also tested positive for helium in the brain. The limit of detection for this technique was determined using the formula LOD = Xm + 3SD, where Xm is the mean value of the peak areas for blank samples and SD is the

standard deviation of the mean value. Using this approach, the limit of detection was calculated to be a peak area of  $3.13 \times 10^5$ . Samples of lung and brain from cases in which the cause of death was not related to helium or any other inhalants were also analyzed and found to be negative. Confirmatory analyses are being conducted using gas chromatography/mass spectrometry (GC/MS) in order to verify GC/TCD identification of helium.

In conclusion, this analysis provides a method for detection of helium that is easily conducted, both in the acquiring of the specimen and the toxicological analysis.

Helium, Gas Chromatography, Postmortem

### K49 In Vitro Adsorption of Carbon Monoxide and Hydrogen Cyanide in Pooled Blood

Patrick S. Cardona, BA\*, Federal Aviation Administration, AAM-610, CAMI Building, 6500 South MacArthur Boulevard, Oklahoma City, OK 73169-6901

After attending this presentation, attendees will be able to apply the findings of this study to the interpretation of results of blood carboxyhemoglobin (COHb) and cyanide ( $CN^{-}$ ) analyses.

This presentation will impact the forensic science community by informing those who investigate accidents associated with fires of the effect that an atmosphere containing primary combustion gases—that is, carbon monoxide (CO) and hydrogen cyanide (HCN)—will have on postmortem blood from open wounds of victims.

The Federal Aviation Administration's Civil Aerospace Medical Institute (CAMI) assists in the investigation of fatal aircraft accidents by conducting toxicological analyses of specimens received from victims of the accidents. One aspect of the analyses is the determination for the presence of primary combustion gases in blood specimens. Combined with the crash site investigation, autopsy and pathology findings, and toxicological results, the investigators could determine whether the crew members were incapacitated by engine CO leaks into the cabin area, whether they survived the crash and were overcome by inhaling CO and HCN from aircraft fires, whether and/or the victims died on impact or came to a rapid death from the intense heat of the fire without inhaling these gases.

Because of the violent impacts involved in crashes, victims quite often suffer large open wounds near sites on the body from where autopsy whole blood is collected. Many aircraft crashes result in fire, which in turn, fill the atmosphere of the victims with smoke (CO and HCN). It is important to determine whether pooled blood in those open wounds may have adsorbed CO and HCN after death and could erroneously lead investigators to determine that the presence of COHb and  $CN^{-}$  in whole blood was the result of breathing in primary combustion gases.

A chamber was set up in the CAMI laboratory to determine whether CO and HCN may be adsorbed in undisturbed, pooled whole blood. To determine in vitro CO adsorption, a large laboratory desiccator was used as the chamber. A light film of silicone grease was applied to the valve and the rim of the lid and chamber. A female Luer-Lok fitting was affixed to the arm of the valve by use of a small piece of Tygon tubing. To facilitate air movement in the chamber, a large cross-shaped magnetic stirring bar was placed at the bottom of the chamber, which was rotated with a magnetic stirring plate. A ceramic plate with numerous rows of holes was placed above the stirring bar. Setting on it was a shallow open dish containing 4 mL of whole human blood that had been treated with sodium heparin. A 100-cc valved Luer gas syringe was used to evacuate air from the chamber and introduce pure CO into it to achieve desired concentrations. Prior to the setup, the volume of the chamber was determined by measuring the amount of water required to displace all the air in the chamber and lid, after taking into account the volumes of the blood sample and the items used in the desiccator. The chamber volume was determined to be 9038 cc. Various concentrations and lengths of CO exposure to the pooled blood were conducted. COHb concentrations were determined spectrophotometrically.

The apparatus was modified slightly for the determination of in vitro HCN adsorption by using an additional open dish containing a 5-mL beaker having a weighed amount of sodium cyanide (NaCN). The Ideal Gas Law was used to determine the amount of NaCN required to achieve the desired concentrations of HCN in the chamber. To conduct the experiment, 4 mL of heparin-treated, whole human blood was used in the second dish. With the lid of the chamber partially opened, 1 mL of concentrated sulfuric acid was added to the beaker containing the NaCN; then the chamber lid was immediately closed. The volume of the chamber was determined to be 8981 cc, after taking into account the volumes of the blood sample, sulfuric acid, and the items used in the desiccator. Two concentrations and various lengths of HCN exposure to the pooled blood were conducted. CN<sup>-</sup> concentrations were determined colorometrically by microdiffusion; then, positives were quantitated spectrophotometrically.

No significant amount of COHb was detected in the whole blood of the experiment after exposure to CO at 5532, 8298, 11064, 22129, and 33193 ppm for 30- and 60-minute exposure times. However, CN concentrations in whole blood increased with exposure to an atmosphere containing HCN at 100 and 200 ppm each at 15, 30, 45, and 60 minutes of exposure times. The CN<sup>-</sup> concentration in blood ranged from 1.55 to  $5.01 \ \mu g/mL$ .

Therefore, there is a potential for blood  $CN^-$  levels to increase by the adsorption of atmospheric HCN present in the smoke. This study also demonstrated that the COHb in pooled blood exposed to an atmosphere containing CO within the parameters of this experiment would not alter the integrity of postmortem blood at an aircraft crash site. This selective adsorption is consistent with the solubility of HCN and insolubility of CO in water. These findings suggest that the COHb and  $CN^-$  levels should be carefully interpreted in view of the potential for selective presence of these primary combustion gases in blood.

Carbon Monoxide, Hydrogen Cyanide, Blood

### K50 Levetiracetam (Keppra®) and Suicide

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After attending this presentation attendees will be educated on the effects of the drug levetiracetam (Keppra®) and will have explored its potential risk for suicide.

This presentation will impact the forensic science community by providing a detailed description of an anticonvulsant drug with relatively unknown toxicity. Only one case of drug overdose has been presented in the literature where the individual recovered with respiratory support. The North Carolina Office of the Chief Medical Examiner has two deaths from 2010 that are noted to have suicidal drug concentrations of levetiracetam.

Levetiracetam (Keppra<sup>®</sup>) is among the new anticonvulsant drugs that are replacing drugs such as carbamazepine, phenytoin, Phenobarbital, and valproic acid. Along with drugs such as topiramate, lamotrigene, and oxcarbazepine, the new drugs have been reported to have a more tolerable side-effect profile, better efficacy and an easier therapeutic maintenance. While the side-effect profile for levetiracetam has been good overall in comparison to classical anticonvulsants, there have been recognized psychiatric effects. The FDA revised the labeling of this drug in 2007 to include warnings regarding these potential behaviors. Individuals with prior psychiatric difficulties may be most at risk for possible mood changes, agitation, and thoughts of suicide. A 48-year-old male was found dead on arrival, barricaded in his bedroom. Over thirty empty medication bottles surrounded the body. He was discovered with tape over his mouth and a suicide note in his pocket.

The decedent had a past history of seizure disorder and multiple, attempted-suicidal, drug overdoses. An alkaline liquid-liquid extraction detected therapeutic levels of citalopram and cyclobenzaprine. Benzoylecgonine was detected after SPE. An extraction for acid/neutral compounds revealed elevated levetiracetam. The aorta and vena cava levetiracetam concentrations were 190 mg/L and 232 mg/L respectively.

A 56-year-old female was found dead in bed. She was last seen alive earlier that evening by her boyfriend. The decedent had several medical problems including diabetes mellitus, chronic pain, depression, and congestive heart failure requiring oxygen therapy. She was found with a broken oxygen concentrator, a bowl filled with pills, and a suicide note. The decedent had multiple prescriptions and was found with multiple drugs in her system including an elevated level of levetiracetam in the aorta blood at 35 mg/L and metaxalone at 26 mg/L.

The two cases mentioned above have added to knowledge of suicides involving levetiracetam and associated drug concentrations. While it is a known antiepileptic drug (AED), it has been studied in open-label trials for its potential to treat neuropathic pain and anxiety disorders. Because of levetiracetam's possible adverse psychiatric effects, it may be important to keep in mind the benefit-risk ratio of each patient during treatment with this medication.

Levetiracetam, Keppra, Suicide

# K51 Ethylene Glycol Fatalities: A New Look at Interpretation

Ashraf Mozayani, PhD, PharmD, Jeffrey P. Walterscheid, PhD\*, and Terry Danielson, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will have learned from five interesting cases of fatal Ethylene Glycol (EG) poisonings, where levels were characterized in blood, clots, vitreous humor, and gastric contents.

This presentation will impact the forensic science community by offering unique aspects for understanding the complexities of acute ethylene glycol intoxication.

Analysis of several ethylene glycol (EG) fatalities that emphasize the key aspects of interpreting intoxication and death will be presented. Collateral data based on witnessed accounts, a suicide journal, vitreous electrolytes and chemistries, as well as pathological calcium oxalate crystal formation is discussed and compared with published accounts.

EG toxicity is expressed in three clinical phases during a suicidal poisoning. The first stage is characterized by central nervous system depression, which occurs shortly after ingestion and lasts for several hours. This period involves drowsiness, disorientation, and confusion, where affected individuals may appear drunk. Convulsion, stupor, and coma may develop in the next stage, when ethylene glycol metabolites cause severe metabolic acidosis, cardiopulmonary manifestations, and multisystem organ failure. In the third stage, a well-known pathological feature is the formation of microscopically visible calcium oxalate crystals from the metabolism of EG into oxalic acid and calcium oxalate.

<u>Case #1</u>: A 50-year-old white male who drank antifreeze and was found alive but unresponsive. He was transported to the hospital and received treatment for ethylene glycol toxicity, acidosis, and myocardial infarction. He survived three more days at the hospital, but ultimately died. Postmortem examinations on the hospital admission blood revealed 5622 mg/L EG. There was no evidence of EG in further characterizations of the postmortem vitreous humor. This finding is supportive of 150 mg/L/hr clearance rate while EG was metabolized and eliminated during the 60-70 hours before death. <u>Case #2</u>: A 21-year-old white male who was observed to be "drunk and sleepy" by his roommate. The roommate left for several hours and found him unresponsive on the floor upon returning. A note was found, where the decedent expressed his intent to commit suicide by drinking EG. Tests later confirmed 588 mg/L in femoral blood, 940 mg/L in vitreous humor, and 1612 mg/L in gastric contents. Increased vitreous creatinine and glucose levels support the renal failure that often accompanies EG toxicosis.

<u>Case #3</u>: A 25-year-old white male who had a history of two suicide attempts; once eight months earlier by heroin, and EG only 3 months before the latest ingestion. Toxicology analysis showed the presence of alprazolam, fluoxetine, and dextromethorphan/chlorpheniramine in therapeutic amounts. However, EG was 2701 mg/L in blood, 3597 mg/L in vitreous, and 5057 mg/L in gastric contents. Prior attempts at suicide by EG were affirmed by numerous calcium oxalate crystals found, which correlates with cumulative exposure.

<u>Case #4</u>: A 56-year-old white male who tried to commit suicide by doxylamine pills, but recovered on his own. He told a friend that he planned to kill himself by drinking EG. Several days later, he was found in a wooded area with a suicide note in his pocket. The toxicology analysis showed that EG had reached 7974 mg/L in femoral blood, 12446 mg/L in vitreous humor, and 23296 mg/L in his gastric contents. However, the pathological examination exposed only a slight number of calcium oxalate crystals in the kidneys.

<u>Case #5</u>: A 38-year-old white male who ingested a cocktail of Gatorade and EG. He had a history of depression and at least three failed suicidal attempts. Lab results revealed EG levels of 4200 mg/L in a blood clot and 5300 mg/L in vitreous humor. A "suicide journal" was present on the scene, where he recorded his experiences as the intoxication progressed over a few hours. This timeline was analyzed to correlate the behavioral and physical disturbances reported by the victim prior to his death.

These cases offer unique aspects for understanding the complexities of acute ethylene glycol intoxication. From strange investigative findings to comprehensive toxicology results, this report provides new insight into ethylene glycol poisoning at the time of ingestion to the autopsy examination and interpretation.

Ethylene Glycol, EG, Toxicity

# K52 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain 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 casespecific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 12<sup>th</sup> Annual Special Session, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information 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 including pharmacotoxicokinetic data and other relevant ancillary information followed by

audience participation to provide interpretive clarity around the casespecific impact of the toxicological findings.

Drs. Scott Denton and Tom Andrew will discuss similar cases involving comfort care of children and postmortem morphine findings. Dr. Andy Robinson will discuss a pediatric case involving methadone, Dr. Laureen Marinetti will describe a case involving the pesticide DEET, and Dr. Bruce Goldberger will present the results of a pediatric research study conducted in Afghanistan.

Pediatric, Toxicology, Postmortem

# LAST WORD SOCIETY





# LW1 The Mystery of the 1958 Floyd County, Kentucky, School Bus Crash

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After attending this presentation, attendees will have a better understanding of how the application of simple physical principles and knowledge of human factors can be applied to explain a horrific event whose cause has been considered a mystery for over fifty years.

The presentation will impact the forensic science community by answering questions surrounding the heretofore unexplained tragedy, lay to rest unwarranted speculation about its causes, and hopefully put at ease the minds of victims and survivors.

On the morning of February 28th, 1958, in Floyd County, Kentucky, USA, near Prestonsburg, a loaded school bus struck a rear corner of a wrecker, swerved across the highway, struck a parked automobile on the opposite shoulder, went down an embankment, and plunged into a flooded river.

Before the bus sank, twenty-two children escaped and survived. Twenty-six others and the driver drowned. It is the worst crash involving children in the history of the country.

The sequence of events began with Donald Horn driving a wrecker on a rural two lane highway about 100 feet in front of the school bus. Both were traveling between 35 and 40 miles per hour. Horn began slowing in order to pull a vehicle out of a ditch and probably just before he came to a stop had his vehicle struck by the bus. John DeRossett, driving the bus, had swerved to his left without braking and almost missed the wrecker. He apparently thought that a normal passing maneuver into the unoccupied oncoming lane would suffice. After impact he kept attempting to steer but was unable to regain control. There was no post impact braking.

The causes of traffic accidents are divided into three areas: environmental, mechanical, and human factors. Considering environmental factors first, an inquiry found that the weather on the morning of the crash was clear and dry. There was no fog or other impairment of sufficient sight distance for the bus driver. The wrecker had no flashing yellow light as was required of emergency vehicles nor were there flag people, reflective triangles, or fusees in place. There was no guardrail protecting the embankment.

There has been speculation that a mechanical failure must have been the cause. However, the bus had received regular maintenance only two days before the crash. The pre-crash status of the braking and steering systems could not be conclusively determined because the front undercarriage of the bus was pulled loose when it was winched from the river; however, an inspection of the vehicle showed no problems with its components.

The single tail lamp of the wrecker that was struck was seen to be illuminated as it slowed, so its failure can be ruled out.

Regarding human factors, there has also been much speculation that the bus driver suffered some sudden physical impairment. However, DeRossett was 27-years-old with no known history of health problems and his autopsy showed that he drowned in his seat and had not had a heart attack.

DeRossett swerved before impact and struggled to regain directional control afterwards, so he was conscious and had his vision before probably being knocked unconscious when the bus struck a small level area on the river bank at the bottom of the fifty foot embankment.

There could have been an onboard distraction, as is not unexpected on school buses, but testimony from several of the surviving children was that all was quiet and that no commotion had occurred. No one has ever disputed this.

Calculations based on estimates of normal driving activities reveal it was about five seconds between the time Horn began to slow and the impact occurred, and the time for the bus to swerve was approximately a second. This leaves about four seconds during which DeRossett perceived the situation, reacted to it, and took action. Perception and reaction times typically vary from about one to five seconds, so this was an above average time but well within the range of what would be considered normal. Either DeRossett required a longer than average perception and reaction time due to the complexities of the situation, was momentarily distracted by something outside the bus, misjudged the time and distance available, or a combination of these. We will never know for certain, but we can be sure there was nothing extraordinary about the causes of the crash and hope survivors find some comfort in this result. Bus Crash, School Bus, Kentucky Bus

# LW2 New Identifications in the Houston Mass Murder Cases of 1973

Sharon M. Derrick, PhD\*, Jennifer C. Love, PhD, Jason M. Wiersema, PhD, and Luis A. Sanchez, MD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will receive a brief overview of the first large-scale serial murder case in the United States followed by a detailed description of the process leading to the recent progress in identification.

This presentation will impact the forensic science community by describing the successful use of standardized procedures to obtain identities in cold cases.

Solving the identities of the teen victims of the Houston mass murders is an ongoing effort for the Forensic Anthropology Division (FAD) of the Harris County Institute of Forensic Sciences (HCIFS). An unanticipated DNA-based identification in 2009 resulted in the identification of a 28th victim to the former total of 27 cases. New information processed by the FAD in 2010 led to the discovery that an identification made in 1973 is likely incorrect. DNA analysis is in process to determine if the identity belongs to one of the remaining unidentified victims instead of the decedent released to family 37 years ago.

In August 1973, the Harris County Morgue was inundated over a three-day period with 27 companion cases representing 26 of 27 recovered adolescent victims of a serial murder spree as well as the alleged perpetrator. None of the adolescents were recognizable and the stage of decomposition ranged from moderate to advanced and skeletal. Dr. Joseph Jachimczyk and the small number of staff employed by the Morgue at that time worked around the clock to autopsy and identify these difficult cases while maintaining routine operations. The majority of the decedents were identified within three months by a forensically trained odontologist or through pathological comparison of antemortem to postmortem radiographs of the skeleton.

Three of the decedents remained unidentified in 2006. These cases were assigned to a FAD anthropologist for follow-up and they received a full anthropological examination, DNA sample submission, case file review, and internet database entry that are FAD protocol for cold cases. In addition, facial approximations were obtained and widely distributed. Archived Harris County Morgue files and law enforcement records were combed for new leads. Buccal swabs were collected from several individuals whose teenage relative disappeared in Houston in the early 1970s. In a stroke of success, one of the three decedents (ML73-3349) was identified and released to family in 2007.

In November 2009, the FAD received DNA results from buccal swabs collected for comparison with the two remaining unidentified decedents (ML73-3356, ML73-3378). Unexpectedly, the comparison was consistent with a sibling relationship with an unidentified decedent recovered in 1983 (ML83-6849). Further review of the case circumstances established the now-identified decedent as the 28<sup>th</sup> victim of the mass murders. Family was notified of the identification.

In April and May 2010, the FAD investigated possible discrepancies in the identification of a victim who was released to the family in 1973. After review of the case files, the FAD found that in contrast to the majority of the 1973 identifications, this decedent (ML73-3333) was identified based largely on circumstantial information. Anthropologic evaluation of the amount of decomposition and certain skeletal and dental characteristics visible in archived photographs of ML73-3333 supported a misidentification. In June, buccal swabs were collected from a sibling of the originally identified missing person. DNA comparisons of the swab profiles with bone specimen profiles obtained from ML73-3333 and one of the unidentified decedents, ML73-3378, are in process. Pending the DNA results, ML73-3378 may be identified as the missing person. If so, the DNA profile for ML73-3333 will be uploaded to CODIS-MP (Combined DNA Index System for Missing Persons) and the FAD will work to obtain identification for this decedent.

The FAD, in collaboration with the University of North Texas Center for Human Identification and the Louisiana State University FACES Laboratory, has successfully obtained identifications for at least two victims of the Houston mass murders since 2006. The effort to identify the remaining two victims continues.

Cold Case Identification, Forensic Anthropology, Houston Mass Murders

# LW3 The Basement Box of Bordentown: A Time Capsule Into the World of Fraternal Initiation Rites

Stuart E. Alexander, DMD\*, 19 North Main Street, Cranbury, NJ 08512; Ian C. Hood, MB, Medical Examiner's Office, 4 Academy Drive, Westampton, NJ 08060; and Donna A. Fontana, MS, New Jersey State Police, Office of Forensic Sciences, 1200 Negron Drive, Hamilton, NJ 08691

After attending this presentation, attendees will have a better appreciation of the possible initiation rites of the Knights of Pythias chapter of Bordentown, New Jersey. Attendees will also be able to observe several articles used in these rites.

This presentation will impact the forensic science community by shedding light on past initiation rites and artifacts so that, when discovered as part of a forensic investigation, they can be readily identified.

At approximately 4:00 p.m. on November 23, 2008, two homeowners were walking through their recently acquired property in Bordentown, NJ. In the basement, they came across a large, locked wooden box. They broke the lock on the box and inside they saw what appeared to be human remains inside a coffin, along with other undetermined artifacts. The local authorities were contacted and the Burlington County Medical Examiner agreed to take possession of all contents. The contents of the box included a human cranium, several shields, swords, three triangular "beds of nails," and a small coffin. The registers for membership and the history of The Knights of Pythias Phoenix Lodge #5 were found among the articles in the box.

Investigators noticed that on the outside of the Bordentown residence, engraved in the foundation, were the words "Masonic and K of P Hall Rebuilt 1924." The Knights of Pythias was a fraternal order founded in 1868 in Maryland by Justus Henry Rathbone and was the first fraternal order recognized by an act of Congress. At one point it was one of the largest fraternal orders in the United States.

Forensic examination of the cranium was performed by the Burlington County Medical Examiners Office and the New Jersey State Police Forensic Anthropology Laboratory. The dehydrated condition of the cranium with cortical bone cracking suggests that is was "clearly of some antiquity."

The other articles can all be traced to the initiation and advancement of the Knights of Pythias. For many fraternal orders, initiation rites were an integral part of recruiting or advancing of its members. Many of the initiation rites have been well documented. The phrase "riding the goat" is synonymous with Masonic culture and has been used since 1845; however, other rites of submission have not been as well publicized. Several companies, including DeMoulin Bros. & Co., Stiltz & Bros., and Pettibone Manufacturing Co. were known to have supplied the articles used in initiation rites but few have survived and many of those articles that remain today are hidden from public view.

This presentation will provide a history of the Knights of Pythias, the development of initiation rites in the United States and the possible uses of the articles found at this property as they relate to the Knights of Pythias.

**Initiation, Knights of Pythias, Rites** 

# LW4 Fort Pillow – A Review of the Evidence of a Civil War Massacre

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After attending this presentation, attendees will understand the legal and forensic aspects of the investigation of a massacre at the Battle of Fort Pillow in the American Civil War. Attendees will learn the basic legal principles of establishing whether a massacre occurred under the laws of war in existence at that time and hear the evidence in the form of eye witness statements and contemporaneous reports.

The presentation will impact the forensic science community by providing information on the legal and forensic aspects of a major historical event from the Civil War that remains controversial today.

The proposition presented is that a "massacre" did occur at Fort Pillow within the legal definition of that time and that responsible persons could have been prosecuted under the existing laws for intentional killing of soldiers who surrendered in the field. The presentation will include a summary of the evidence supporting this proposition including detailed eye witness accounts and modern photographs of the reconstructed scene.

The Battle at Fort Pillow, also referred to as the "Fort Pillow Massacre," occurred on April 12, 1864 at an isolated fort on the Mississippi River in Western Tennessee. Confederate troops under the command of General Nathan Bedford Forrest surrounded and attacked Fort Pillow and its garrison of African-American and Tennessee Unionist soldiers. Over 300 of the roughly 600 defenders were killed, most allegedly after they surrendered to the attacking force. Almost immediately after the incident, northern newspapers accused Forrest's troops of deliberately killing unarmed African-American soldiers after

they surrendered and asked for quarter. Moreover, some alleged Forrest's men committed even more heinous atrocities such as burning and burying men alive.

A massacre is typically defined as, "the wanton killing of a large number of especially unresisting human beings." The existing written and unwritten laws of warfare at the time of the Civil War required quarter be shown to soldiers attempting to surrender. This was codified in written form in what was known as General Orders Number 100 for the Union Army.

Despite rhetoric to the contrary at the time, the Federal Government never charged Nathan Bedford Forrest or any of his subordinates for actions committed at Fort Pillow. This left open the issue of their criminal culpability for the deaths of a large number of African-American and white soldiers.

While little actual forensic evidence of the battle survives, there are numerous eye witness accounts of the events at Fort Pillow which allow for a forensic and legal analysis. Some witnesses gave extant accounts describing the condition of the bodies of Union soldiers found at Fort Pillow. These include a description by one Union officer of what appeared to be close range gunshot wounds to the heads of many soldiers. Additionally, the official records contain accounts by physicians treating wounded soldiers from Fort Pillow, describing many of the wounds in graphic detail. Numerous eyewitness accounts from both Union and Confederate witnesses describe the deliberate killing of unarmed Union soldiers. Recent archeological excavations also uncovered valuable information.

Massacre, Civil War, Fort Pillow

# LW5 The Vampire of Venice: A Real Ancient Ancestor of Twilight Investigated By Modern Forensic Sciences

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After attending this presentation, attendees will learn what type of information it is possible to find from incomplete human skeletal remains and what kind of analyses can possibly be conducted.

This presentation will impact the forensic science community showing the possible applications of physical and forensic anthropology. The case selected describes ancient remains on which forensic analysis has been used to discover how the ancient belief in vampires originated.

During an archeological excavation in 2006 in an island of Venice's lagoon a partial human skeleton was found in a mass grave from the 16th century plague. The individual appeared particularly interesting because a piece of brick was found imbedded in the mouth, keeping the mouth wide open.

The first forensic examination of this skeleton was taphonomic analysis; seeking to understand when and why the body was dismembered and why the brick was put in its mouth.

The examination demonstrated that the body was dismembered after skeletonization (during a second utilization of the cemetery in the 17th century) while the brick appeared to have been put in the mouth while the flesh was still present. A bibliographical search of funerary practices during the occurrence of the plague showed an exact relationship between the plague and a well known folklore concerning vampires. A specific kind of vampire called nachtzerer was believed to be spreading the plague by chewing the funerary shroud. To kill this undead being, a brick or something similar was put in its mouth in place of the shroud that the creature was eating. A scientific review of the reports about the corpses of supposed vampires for the 17th and 18th centuries allowed us to understand why this superstition began. In the past the decomposition process was ignored and the postmortem changes (e.g., bloating abdomen, no rigor, skin slippage) were misinterpreted as evidence of vampirism.

After the discovery of the historical importance of this skeleton as a witness to the ancient belief in vampirism, it was decided to use forensic anthropology and related sciences to discover what might be known of this individual.

To begin, a biological profile was developed with sex estimation being first in order. The skeleton was preserved only from half the chest to the skull, so it was impossible to use the pelvic region, the most diagnostic of sexual dimorphism. Instead of the pelvis, the skull morphology and the measurements of the head, the humerus, and the clavicle were used, proving the "vampire" was a female.

Metric analysis of the skull has been used for ancestry estimation, but the database software did not have medieval samples so that test did not work. A first estimation of the human group has been made on a morphological basis and then supported by genetic analysis. The DNA study on this skeleton has been used to establish the geographic provenance of the "vampire's ancestor," but forensics knew that the DNA extraction can be useful on skeletonized remains also for a genetic match to confirm the first anthropological identification. In the present case, no comparative test was possible or necessary.

To establish the age of the "Venice vampire" it was impossible to use some of the most common and recommended methods: the pubic symphysis was absent and the 4th rib was found with a badly preserved sternal extremity. Only the skull could be useful to solve the age question, while rejecting the popular but untrustworthy cranial suture evaluation and the analysis of tooth erosion, a portable X-ray device was used on the teeth. The canine radiological image allowed the estimation of the age as between 61 to 71 years of age.

As in forensic cases, a morphometric evaluation of osteobiographical traits (stature, ancient trauma, pathological issues) was conducted by CT scan to increase the elements referable to this ancient woman. Antemortem trauma on the skull and arthritic deformation of the left shoulder was found.

To conclude the analysis on the "vampire" skeleton, a facial reconstruction of her skull was performed. Facial approximation is a useful *extrema ratio* for the identification when other investigative methods failed, but in the present case it has been used to see the hypothetical features of an ancient woman who was the victim of a plague as well as a terrible superstition.

A paradigmatic case will be presented of a partial human skeleton with such a biological profile and physical characteristics as are useful for personal identification under a forensic anthropological approach. The exemplar of this spooky archaeological skeleton allows the understanding of the applications of forensic science not only in modern identification cases but also to historical remains and ancient beliefs in "vampirism."

Forensic Anthropology, Skeletal Identification, Vampire



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