Northeastern Association
of
Forensic Scientists

Proceedings
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October 2022 Annual Meeting

Contents

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Forensic Toxicology Abstracts

Standardization, Impaired Driving, and the Regional Toxicology Liaison Demonstration Project

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Background/Introduction: There has been a sharp increase in drug-impaired driving across the country. A recent National Highway Traffic Safety Administration report on 2020 Traffic Fatality Data found 38,824 people died on US roadways, with a 6.8% increase in fatal crashes. Of that, 45% of fatal crashes involve risky behavior such as driving impaired by alcohol, speeding, or not wearing a seatbelt. Alarmingly, alcohol-impaired fatalities increased by 14% compared to 2019 data, even with an 11% decline in vehicle miles traveled. The National Safety Council recently published that the traffic death rates in 2021 exceeded the rate of 2019 by 19%.

Objectives: To more fully understand and address the issue of drug-impaired driving, the Regional Toxicology Liaison (RTL) Demonstration Project aims to benefit state toxicology programs through increased support, communications, resources, and criminal justice system coordination; decreased processing time of toxicology samples; and better data reporting. In 2022, the Project established Toxicology Liaisons that support states in NHTSA regions 5, 7, and 9 (https://www.nhtsa.gov/about-nhtsa), to assist with training, collaboration, and the standardization of testing across state laboratories as well as improving the reporting of data to understand the scope of the drug-impaired driving problem.

Methods: The Regional Toxicology Liaisons work within and collaborate between their respective regions identifying stakeholders within each state, laboratory engagement, collaboration, and evaluation of training requests. The RTLs work together to ensure consistency within the program and share information and resources. Additionally, the Project provides a quarterly report to the SOFT Board of Directors and periodic updates regarding activities, progress, and needs assessments.

Results: Through SOFT, Regional Toxicology Liaisons are involved in various committees to understand current trends in drugged driving, laboratory testing, and laboratory needs. The RTLs are engaged in meetings with stakeholders in each state, including NHTSA regional offices, State Impaired Driving Task Forces, State Traffic Safety Resource Prosecutors, and other regional liaisons, including Judicial Outreach Liaisons and Law Enforcement Liaisons.

Conclusion/Discussion: This presentation will provide an overview of the Project and activities accomplished within the first ten months of the program.

Antihistamines in Driving Under the Influence of Drugs Investigations

Jolene Bierly, NMS Labs

Intro: First generation-antihistamines with H1-antagonist action are known to produce CNS depressant side effects, such as drowsiness and fatigue. While these side effects are known to be incompatible with safe driving, few publications are available regarding the impacts of these drugs in DUID investigations. This study was performed to investigate the prevalence of first-generation antihistamines in the driving population and to provide case histories demonstrating their effects on driving.

Methods: First-generation antihistamines are included in the scope of Tier II DUID testing at NMS Labs. Diphenhydramine, hydroxyzine, chlorpheniramine, doxylamine, and promethazine are screened via LC-TOF/MS and confirmed via LC-MS/MS or GC-NPD at the reporting limits listed in Table I. Antemortem blood specimens requesting the Tier II DUID expanded panel submitted between January 1, 2017 and May 31, 2022 were reviewed.

Table I. Screening and Confirmation Reporting Limits and Confirmation Instrumentation for First- Generation Antihistamines

amine	ng Reporting Limi)	nation Reporting Limit)	nation Instrumentation
hydramine			/MS
yzine			/MS
nazine			/MS
eniramine			/MS
mine			D

Results: More than 10,500 Tier II expanded panels were ordered during the study period. Positivity and concentration information for each antihistamine in the Tier II panel is provided below (Table II).

Table II. Summary of First-Generation Antihistamine Positivity from DUID Casework, 2017 to 2022

amine	Positivity	r of Positive nations	SD (ng/mL)	(ng/mL)	tration Range)
hydramine			10		00
yzine					0
nazine					0
eniramine			51		
mine			6		00

Diphenhydramine and hydroxyzine were the most prevalent antihistamines (78%). Polypharmacy was common with 94% of all antihistamine cases involving other drugs. Common drug combinations included antihistamines with benzodiazepines, opioids, and antidepressants. There were 13 cases where diphenhydramine was the only drug indicated and an additional 6 hydroxyzine only cases. Means ± SD, medians, and ranges for these cases were 772 ± 957 ng/mL, 320 ng/mL, (59 – 3200 ng/mL) and 30 ± 23 ng/mL, 19 ng/mL, (10-78 ng/mL), respectively. One case history involved a 27-year-old male who ran a stop sign. CNS depressant effects, such as swaying, inability to follow instructions, and poor balance were observed by law enforcement. Toxicology testing confirmed the presence of hydroxyzine at 16 ± 4 ng/mL with no other significant findings.

Conclusion: This review found the overall positivity of first-generation antihistamines in DUID cases to be <3% for each drug included in the scope. Most antihistamine confirmations involved diphenhydramine and hydroxyzine (78%). Significant impairment to driving and human performance was observed in case histories involving first-generation antihistamines. Approximately 94% of the cases involved antihistamines in combination with other drugs.

Validation of Drugs in Umbilical Cord Tissue by LCMSMS

Andrea Belec, Champlain Toxicology Lab

With the opiate epidemic still raging, the most helpless victims are infants born to mothers struggling with Substance Use Disorder. Depending on what specific drugs were being taken by the mother, infants may not show signs of withdrawal until five to ten days after their birth. The wide majority of babies are home and not under direct medical supervision when withdrawal symptoms begin. Drugs can deposit into umbilical cord tissue up to 20 weeks back in a pregnancy so the "look back" is dramatically longer when compared to other matrices. By identifying drugs in umbilical cord tissue within hours of their birth, hospitals can clinically manage these little patients and work to minimize effects. This presentation will discuss all aspects of validation and include detailed discussion of sample preparation challenges with this matrix compared to other matrices, the specific solid phase extraction process that was performed and the subsequent LCMSMS analysis.

Review of a Drug Facilitated Crime Involving Zolpidem

Celeste Wareing, MS, Boston University School of Medicine, Biomedical Forensic Sciences

The sedative-hypnotic and amnesic effects of Zolpidem make it an attractive drug used in drug facilitated crimes. This case involves a young female exchange student that was allegedly dosed with zolpidem by her host father under the guise of it being aspirin. Both blood and urine were collected during a sexual assault examination the morning after the alleged assault. Toxicological analysis showed the presence of zolpidem in both samples.

Chiral Analysis of Methamphetamine in Hair Samples

Kristen Payes[1], Damon Borg[1,2], Richard Stripp[1,2], Marta Concheiro-Guisan[1], [1]John Jay College of Criminal Justice, [2]Cordant Health Solutions

Methamphetamine (MAMP) is a highly addictive illicit drug typically abused for its nervous system stimulating effects. Conversely, methamphetamine has therapeutic use treating attention deficit hyperactivity disorder, controlling appetite and assisting with weight loss, and is available as the pure l- isomer in over-the-counter (OTC) nasal inhalers due to its decongestant activity. Because l- methamphetamine (l-MAMP) is available in OTC form, forensic guidelines require a sample to contain greater than 20% d-methamphetamine (d-MAMP) when classifying results as consistent with illicit MAMP use. Chiral chromatographic analysis is capable of distinguishing between l- MAMP and d-MAMP. This study was designed to develop and validate a method for the detection of d/l-MAMP in hair. Reverse phase liquid chromatography with tandem mass spectrometry (LC/MSMS) and a chiral derivatizing agent were used in this study. MAMP was extracted from hair specimens using mixed mode cation exchange solid phase extraction cartridges and extracts derivatized with Marfey's reagent. Chromatographic separation of the isomers was achieved using a standard reverse phase (C18) LC column. Linearity, accuracy and precision were all within acceptable criteria. Intraday accuracy ranged from 96.76% to 102.62% and a precision 0.31 to 2.80%. Previously tested hair samples that resulted in a positive result for methamphetamine using non-chiral analysis were analyzed using this validated method. It was found that 100% of all samples (n = 20) tested positive for d-MAMP at greater than 20%.

Keywords: l-methamphetamine, d-methamphetamine, hair, Marfey's Reagent, LC/MSMS

Enhancing High-Resolution Mass Spectrometry Performance for NPS Analysis With Improved Sensitivity and Characterization

Joe Doktorski and Pierre Negri, PhD, SCIEX

Short Abstract

The technology advancements of the ZenoTOF 7600 system provides the ability to confidently characterize and identify novel psychoactive substances (NPS) present in authentic case samples. at trace levels that were not previously achievable.

Extended Abstract

This presentation will showcase how the benefits of the new technological features introduced on the ZenoTOF 7600 system provide a high degree of sensitivity, selectivity, and confidence for sensitive novel psychoactive substances (NPS) detection and characterization. The addition of a Zeno trap (which improves MS/MS duty cycle) in combination with the use of a hybrid collision cell (which offers an alternative fragmentation capabilities) are leveraged to significantly improve MS/MS sensitivity and provide richer fragmentation for improved structural information, respectively. These technological advancements were investigated for the characterization and identification of NPS, and more specifically novel synthetic opioids (NSO) in a series of authentic case samples. The depth of information extracted from the unique fragmentation capabilities of electron-activated dissociation (EAD) enabled differentiation of structurally related isomeric species that were not previously discernable using convention collision-induced dissociation (CID). In addition, the improved MS/MS sensitivity resulted in confident identification of key drugs and

metabolites at trace levels that were not previously achievable. Overall, the use of the ZenoTOF 7600 system provided a means to characterize and monitor low-levels of ultra-potent NSO in poly- drug intake scenarios. These advancements are shown to support the case of combined opioid drug toxicity leading to death, which offers a clearer picture for help in determining the cause of death.

Learning Objectives:
After having attended this presentation, one will:

a) Learn the instrument features on the ZenoTOF 7600 system that provide qualitative flexibility combined with quantitative power for NPS detection and characterization

b) Understand how the depth of information extracted from EAD-based MS/MS spectra combined with the improved MS/MS sensitivity can be leveraged for characterization of structurally related isomeric species present at low levels in authentic case samples

c) Learn how these new technological advancements on the ZenoTOF 7600 are leveraged to provide more confidence in the quantified amounts of drugs and metabolites detected in authentic case samples which is critical when determining the cause of death following an accidental overdose

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Trace Evidence/Fire Debris & Explosives Abstracts

Understanding the Science and Concerns Behind the Possible Conversion of Helium to Hydrogen Carrier Gas for EI GCMS systems

Kirk Lokits, PhD, GCMS Applications Chemist, Agilent Technologies

Helium has historically, with valid scientific reasons, been the preferred carrier gas for GCMS and the majority of GC analysis. Within the last decade there has been an increase in the difficulties to procure UHP helium in the quantities required for full laboratory operations and or a drastic increase in the overall cost of UHP helium tanks. Due to its chemical and physical characteristics, high resolution chromatographic separations can be achieved with minimal analyte interactions.

GCs with atmospheric detectors often utilize alternative carrier gases such as nitrogen, argon, and hydrogen. However, when the GC is coupled to a mass spectrometer under high vacuum, parameters based on a mean free pathway of ion molecules, vacuum, low background, and high sensitivity come into play. Based on these parameter limitations, of the previously mentioned carrier gases, hydrogen is the practical alternative. Nonetheless, hydrogen does have disadvantages that may cause a GCMS analyst to re-evaluate the urgency to convert to hydrogen carrier based on its reactivity with some analytes, reduced sensitivity, increased peak tailing, and reduced spectral fidelity when compared to helium generated reference spectra.

Ultimately, helium is the preferred carrier gas choice, but if not available, hydrogen may be considered. The purpose of this presentation is to help analysts determine if hydrogen can be used as a carrier gas for their specific analysis. Furthermore, the illustration of best practices, specific MS source configurations, forensic drug data examples, and the acquisition parameters necessary to help determine if the transition of a specific application is or is not compatible for hydrogen carrier gas on a GCMS system, will be discussed.

Automation Possibilities for Fire Debris Analysis using ChemStation Macros

Eugene Zegocki, Monroe County Crime Laboratory

ChemStation macro language provides ample possibilities for automation tasks. Using macros, it is possible to automate screening, pattern recognition, identification and documentation of GC/MS instrument results. The author designed and tested a set of macros for Agilent GC/MS instruments using ChemStation software for Fire Debris analysis.

A set of macros automatically process sequences containing samples for one or several cases. The program screens GC/MS data and identifies ignitable liquids. In certain cases, the program only indicates ignitable liquids and those should be cleared by analyst. If analyst does not agree with the automated identification results, he/she could change suggested results or references manually prior to printout. There are embedded tools for comparison of patterns and peaks, MS library search and searches in folders containing GC/MS data of previously run samples, for example, NCFS samples helping an analyst to confirm or reject suggested result.

There is a predefined directory containing references (previously run ignitable liquids). If an ignitable liquid is identified, the program selects appropriate reference(s), automatically makes extracted ion profiles and/or peak RT and mass spectra comparisons. In the case of low intensity peaks the background could be subtracted, thus making better library hits.

Results are produced in PDF format for final review. The printout includes total ion chromatograms of ASTM test mixture, blanks, items in the case, library searches, extracted ion profiles for identified ignitable liquids and appropriate references and might include all additional manual comparisons.

Data is combined in one PDF file, which is easy to use in a paperless workflow process. Printout could be

automatically paginated. Also, obtained results could be automatically inserted into pdf form notes, if analyst use those.

Determining the Variability in Color in Human Head Hair
Emma Redman and Lawrence Quarino PhD, ABC-GKE, Cedar Crest College

Color has often been used as a parameter in human head hair microscopic comparative analysis [1]. What is not known is the extent of variation of naturally colored hair within an individual and between individuals with the same shade of hair. This study is designed to measure the extent of the variability and to determine if color can be reliably used as a parameter in microscopic hair examination. Fifty head hairs were collected from several Caucasian women displaying the same shade of brown hair. Each hair was mounted on glass slides with DPX mounting media (nD - 1.521) and examined at 200x using an Olympus BX53 polarizing light microscope under Kohler illumination. Color measurements in the RGB system (red, green, blue) was performed using CellSens® software. Color measurements were taken at defined points in the cortex, 100-300mm from the edge of the root. This area was chosen since it was found to show the most consistency in terms of color. Median RGB values for each participant were grouped together and compared using principal component analysis (PCA) and linear discriminant analysis (LDA). Results showed significant data overlap between individuals with little to no discriminate value. Similarly, significant overlap between individuals was observed qualitatively using color charts based on median hair values. In addition, a randomly selected hair was compared to the other forty-nine hairs removed from one participant by calculating a delta E value between RGB values. Only one comparison yielded a delta E value below the threshold of minimal color difference, two comparisons provided values consistent with more subtle but distinct difference, while forty-seven yielded values showing notable differences in color. This implies that the intrapersonal variability of hair color is high. Given the data generated both qualitatively and quantitatively in this study, color may have limited utility in microscopic hair comparisons.

[1]. Robertson J., Brooks E. A Practical Guide to the Forensic Examination of Hair From Crime Scene to Court, 2st Edition, 2021.

Ghost Guns
Pete Diaczuk, PhD, John Jay College

Ghost guns, now classified as Privately Manufactured Firearms, or PMFs, are guns without serial numbers. Not too long ago, these were identified as an issue due to the low rate of traceability (less than one percent) with increased use in crimes. According to the Bureau of Alcohol, Tobacco, and Firearms (BATF), "approximately 45,000 reports of suspected privately made firearms (PMFs) were recovered by law enforcement in criminal investigations — including 692 used in homicides or attempted homicides" --- from January 2016 to December 2021. This presentation will explore the concept of ghost guns in comparison with their legitimate counterparts, including unserialized firearms in general.

Elemental Profiling of Gunshot Residue with Multiple X-ray Fluorescence Spectroscopic Techniques
Samantha A. Gong, BS, Nicole Homburger, BS, and Ling Huang, PhD Hofstra University, Hempstead, NY

Gunshot residue (GSR) is the material from firearm ammunition cartridges that is dispersed into to the surrounding environment during and after the discharge of a firearm. GSR is made up of various organic and inorganic compounds that originate primarily from propellant powder, primer material, and the body of the bullet within the cartridge [1]. In this project, GSR was analyzed using paired scanning electron microscope with energy dispersive X-ray fluorescence spectroscopy (SEM- EDS), portable X-ray fluorescence spectroscopy (p-XRF), and total reflection X-ray fluorescence spectrometry (TXRF). The results of the three analyses were compared and contrasted to evaluate each type of XRF technique for their unique capabilities and weakness.

In crime laboratories, the most important aspect of GSR analysis is to determine if the evidence collected is indeed GSR. The most commonly used instrumentation for the elemental analysis of gunshot residue is SEM-EDS [2]. SEM-EDS is used to analyze a sample of presumed GSR material to determine if a suspect has recently handled a firearm [3]. SEM-EDS searches collected material for particles containing a tri-element composition of lead, barium, and antimony that is characteristic to GSR [3]. SEM has the ability to scan and visualize the microscopic GSR particles and analysts generally look for spherical, flattened, or partially splattered particles to focus on. EDS, a type of X-ray fluorescence (XRF) spectroscopy that is coupled with SEM. After locating a scanning area on SEM, EDS has the ability to determine the elemental composition of these particles and determine if they are consistent with the known makeup of GSR.

SEM-EDS analysis focuses on the materials found on the suspect's hands. There is additional information to be found, though, on the impact site of the projectile. GSR collected on a fabric target from a short-range was found to collect residues from most if not all of the sources of GSR. In a previous study, this collection of GSR was analyzed using TXRF [4]. Samples collected in the same method as the TXRF study could be used for analysis by SEM-EDS to look for the same information as traditional GSR analysis. Additionally, a p-XRF instrument could be used to analyze the same samples and as such was added to the comparison.

Through these analyses, different types of ammunition were found to consistently produce distinct elemental profiles from the GSR. Lead, as the main element composing the bullet, was the primary element of interest. The analyses also extended to copper, barium, antimony, iron, and zinc. Each XRF analysis had unique information to present about the elemental makeup of GSR. For practical forensic uses, the information obtained from these XRF techniques could be used as the basis of a predictive tool to associate ammunition types and the GSR on the targets of close-range shootings. The project, furthermore, shows various pros and cons of each instrument. The findings of the instrumental comparison could then assist GSR analysts in deciding which type of XRF instrument is suitable for their own investigation.

References (1) Dalby, O.; Butler, D.; Birkett, J. W. Analysis of Gunshot Residue and Associated Materials—A Review. Journal of Forensic Sciences 2010, 55 (4), 924–943. https://doi.org/10.1111/j.1556- 4029.2010.01370.x. (2) Guide for Primer Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry, SWGGSR, 2011. https://www.swggsr.org/publications (accessed March 26, 2021). (3) Blakey, L. S.; Sharples, G. P.; Chana, K.; Birkett, J. W. Fate and Behavior of Gunshot Residue-A Review. J. Forensic Sci. 2018, 63 (1), 9–19. https://doi.org/10.1111/1556-4029.13555. (4) Gong, S. A.; Homburger, N.; Huang, L.; Elemental profiling of total gunshot residue using total reflection X-ray fluorescence spectroscopy. J. Forensic Sci. 2022, 67(3), 1198-1207. https://doi.org/10.1111/1556-4029.14988

Optimization in the Identification of Inorganic Ions Found in Home-Made Explosives Using Microcrystalline Tests and Raman Microspectroscopy

Krystal Sears and Lawrence Quarino, PhD, ABC-GKE, Cedar Crest College

Identifying inorganic ions commonly found in homemade explosives provides examiners a strong starting point for the scientific investigation of explosive debris. Common methods of analyzing explosive debris typically require extensive sample preparation, multiple types of instrumentation, and are time consuming and expensive. This presentation is a continuation of a study designed to introduce an efficient and cost-effective method to screen for specific inorganic ions indicative of explosive residue utilizing the Raman microscope. Ions found in firework, fertilizer, and bleach were chosen for examination. Microcrystalline tests were performed on aqueous solutions of inorganic compounds containing the anions and cations of interest using nitron, silver nitrate, and squaric acid. Raman spectra were then generated from microcrystals at 200x using the Raman microscope and corrected for baseline, fluorescence, and smooth. Parameters including type of slide, number of scans, power of laser, scanning

range, and aperture were determined for each ion. Often the parameters for each ion were different. Optimization of parameters resulted in noticeable and distinguishable Raman spectra for each ion tested. Combined with the morphology and habit of the microcrystal generated, the differentiation of seven cations (ammonium, barium, calcium, potassium, silver, sodium, and strontium) and nine anions (acetate, chlorate, chloride, nitrite, nitrate, perchlorate, phosphate, sulfate, and tartrate) was achieved. The generation of microcrystals using the three reagents with subsequent examination by Raman microspectroscopy can normally by achieved in less than ten minutes demonstrating the efficiency of the method making it suitable for application in a crime lab.

Fluorescence in Forensic Fiber Examinations
Michelle Mercer, Trace Evidence/Fire Debris Examiner at the Monroe County Crime Laboratory in Rochester, NY. ASTEE member and current OSAC Trace Materials Subcommittee Fiber Task Group Chair.

The 2021 Collaborative Testing Services, Inc. (CTS) fiber proficiency test had a larger number of labs not reaching the expected answer. It was determined that fluorescence was the primary discriminating property that enabled the majority to correctly exclude a fiber pair. Additional issues were found with the instrumentation that was used among some laboratories, specifically with older fluorescence light microscopes and/or filter cubes with limited ranges.

What began as an informal ASTEE forum discussion became a project for the OSAC Fiber Task Group to investigate further. A meeting with representatives from CTS then led to a survey sent out to the ASTEE membership. The purpose of the survey was to capture the experiences of participating laboratories with the PT, as well as how they use fluorescence in general for fiber examinations. The survey data collected includes: brands of microscopes, types of filter cubes, and mounting medium utilized; discriminating power of specific filter cubes/wavelength range; value of fluorescence microscopy for certain fiber types/colors; and selection of representative samples for testing.

This presentation will give an overview of the 2021 fiber proficiency test, the results of the ASTEE survey, and current recommendations for the use of fluorescence in fiber examinations. Discussion and feedback received will assist the Fiber Task Group to evaluate whether there is a need to write a guidance document specifically on fluorescence.

ATF – National Response Team
John Pijaca, SACFI, IAAI-CFI, ATF, retired

This presentation will touch on the similarities and diversities with transitioning as an ATF Agent within the field of fire investigations to the private sector as an independent contractor.

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Criminalistics/Crime Scene & Digital Evidence Abstracts

Changes in the Bacteroidetes: Firmicutes Ratio of the Thanatomicrobiota in Substance Abuse Disorders
Gulnaz Javan, Alabama State University

The microbiota gut-brain-axis is a bidirectional circuit that links the neural, endocrine, and immunological systems with gut microbial communities. The gut microbiome plays significant roles in human mind and behavior, specifically pain perception, learning capacity, memory, and temperament. Studies have shown that disruptions in the gut microbiota have been associated with substance use disorders. The interplay of gut microbiota in substance abuse disorders has not been elucidated; however, postmortem microbiome profiles may produce promising avenues for future forensic investigations. The goal of the current study was to determine gut microbiome composition in substance abuse disorder cases using transverse colon tissues of 21 overdose criminal cases versus 19 non-overdose-related cases. We hypothesized that postmortem samples of the same cause of death will reveal similar taxonomic relationships. A heatmap was generated of the relative abundances of the 30 most prevalent bacteria per case and their associated substance profile. The results revealed that samples of the same cause of death cluster together, showing a high degree of similarity between samples and a low degree of similarity among samples of different causes of death. Our examination of human transverse colon microflora in decomposing cadavers extends emerging literature on postmortem microbial communities, which will ultimately contribute to advanced knowledge of human putrefaction.

Gun-Shot
Peter Diaczuk, PhD, John Jay College of Criminal Justice

A felon who was carrying a concealed firearm was spotted by law enforcement. The felon turned and fled, and while being pursued on foot, the felon was fired upon. One shot hit the fleeing man, who collapsed about 50 yards later and was pronounced at the scene. Upon being searched, no firearm was recovered from the decedent. Months later, a homeowner who lived along the route taken by the fleeing man found a firearm in his garden. It was turned over to the police immediately thereafter. The question to be answered was whether the recovered firearm could be connected to the felon. Laboratory analysts tested the function and assessed the operability of the firearm, which was then test fired. No shots were fired by the man during this altercation, so traditional firearm examination techniques were not probative. Biological traces were long gone; neither hairs nor fibers were present on the recovered firearm. There was however a subtle imperfection in the grip on one side of the firearm, and a missing piece of plastic on the other. Perhaps this damage was the result of tossing the firearm while on the run, or perhaps it was the result of something else.

Open Fire
Abigail Wilson, Peter Diaczuk, PhD, John Jay College of Criminal Justice

Bullets used in ammunition manufacture can be broadly divided into two categories, full metal jacketed and jacketed hollow points. These can be further divided into numerous subsets within each group, including some specialty groups, such as frangible. This research examines a representative brand of jacketed hollow point bullets used in a common caliber cartridge, the 9mm Luger. A jacketed hollow point bullet is designed to open or expand when it comes into contact with a viscous medium such as water, tissue, or ballistic gelatin. This research used ballistic gelatin as a tissue simulant with a chamois overlay as a substitute for skin. The 9mm Luger caliber jacketed hollow point bullets were fired and subsequently recovered from cotton waste. Cotton waste was used as the recovery medium so any bullet expansion, or lack thereof, would not take place post-gelatin. After recovery from the cotton waste, the bullets were carefully weighed, and their expansion measured. As a positive and negative control, a series of bullets were fired into a full-size block of gelatin to achieve complete expansion and into just cotton waste to resist expansion. There are six petals that compose the tip and ogive of the hollow point bullet used in these tests. The

distance between all of the opposing petals of each bullet were measured, resulting in three measurements per bullet. There were five bullets fired for gelatin thicknesses of 3 inches, 2 inches, 1.5 inches, 1 inch, and 0.5 inches, as well as for the negative and positive controls. The positive control averaged 12.97 mm and the negative control averaged 6.24 mm at maximum width. Bullet expansion through the respective gelatin slices averaged 12.47 mm for 3 inches, 12.23 mm for 2 inches, 11.83 mm for 1.5 inches, 11.84 mm for 1 inch, and 11.49 mm for 0.5 inches. The differences between the average distances of the expanded petals can be explained by the differences in the thickness of the gelatins. As the gelatin slices get thinner, the width of the expanded bullets gets narrower. Velocity data was collected both from the muzzle of the firearm and as the bullets left the varying thicknesses of the chamois-covered ballistic gelatin. The velocity loss decreased as the gel thickness decreased. This research is intended to provide an understanding of how hollow point bullets behave and make it common knowledge to those who are not familiar with wound ballistics.

The Study of Hyoid Bone Fracture Patterns

Grace Stockmal, Roger Sherman, MS, Jennifer Hammers, DO, John Viator, PhD and Pamela Marshall, PhD, Duquesne University

Commonly referred to as the "Hangman's Fracture", the hyoid bone in the neck has been observed to partially or completely break when compressed by a ligature in suicidal hangings and homicidal strangulations. Hyoid bones need to be studied to determine if they fracture differently between these two manners of death. Previous studies have determined differences in neck damage and frequency of thyrohyoid fractures, but scientists need to develop effective comparisons between the manners of death and patterns of fracture within the hyoid bone. It is proposed that homicidal strangulations by ligature will require more force and will result in higher frequencies of fractures to the body than the greater horns of the hyoid than in a suicidal hanging. To test this proposal, hyoid bones were collected from deceased patients, cleaned using an Oxi-Clean solution, and models were created by 3D-printing utilizing FibreTuff polymer. The Torbal FT Odyssey force gauge was used to measure the Newtons required to partially fracture these models and allowed observation of fracture patterns. Ligatures and ballistic head models with inserted 3D model hyoids were used to simulate suicidal hangings and homicidal strangulation methods. Future autopsies with unidentified manners of death involving asphyxiation and abrasive damage to the neck can investigate the hyoid bone for patterns of fracture that correlate to either suicide or homicide. Future cases like Jeffrey Epstein would have further investigation of the hyoid bone to determine a manner of death.

Key words: hyoid bone, fracture, suicidal hanging, homicidal ligature strangulation

The Twig Is Up: Forensic Identification of Endangered Wood Species by DART-HRMS and Multivariate Statistical Analysis to Combat Illegal Logging

Mónica Ventura, Samira Beyramysoltan, PhD, Benedetta Garosi, and Meghan Appley, University at Albany, Edgard Espinoza, PhD, National Fish and Wildlife Forensic Lab and Rabi Musah, PhD, University at Albany

One of the recent trends in forensics is wildlife, with the concern being identification of illegally traded endangered species. While trade in fauna, including elephant parts (such as tusks), rhinoceros horns, and pangolin scales, are well-known examples of wildlife crimes, there are a host of flora that are heavily trafficked, including trees. Illegal trade of endangered wood is common because it is highly prized for making exclusive furniture, cabinetry, musical instruments, and construction materials. This causes environmental and economic damage because it leads to forest degradation and deforestation and deprives countries of billions of dollars in revenue. Illegal logging is one of the most lucrative natural resource crimes and is valued at $52 billion to $157 billion per year, which is a magnet to some of the world's largest organized crime groups. The Convention on International Trade of Endangered Species (CITES) was created to address the conservation of imperiled wildlife by controlling their trade. Regulation status is defined by appendices: CITES Appendix I species are threatened with extinction, and trade of any kind is illegal;

CITES Appendix II species are threatened in the wild and international trade is controlled to aide in their survival; and CITES Appendix III species are regulated by a particular nation. Therefore, depending on the species, trade is either totally or heavily restricted. Moreover, when specimens are intercepted by law enforcement, it is extremely challenging to identify the evidence as either legal or illegal, because many of the species that are illegal to trade have an appearance that is similar to species that are not restricted. A current technique used by law enforcement to differentiate species of wood is direct analysis in real time-high resolution mass spectrometry (DART-HRMS), coupled with multivariate statistical analysis. Here, the added dimension of wood headspace analysis featuring solid phase microextraction (SPME) was used to generate data to complement that acquired using the conventional wood analysis technique. This could facilitate the development of "stand-off" approaches to the differentiation of wood species based on their volatiles profiles. Eight genera, including *Dalbergia*, *Swietenia*, *Pericopsis*, *Araucaria*, *Pterocarpus*, *Cedrela*, *Diospyros*, and *Millettia* were provided by the U.S. Fish & Wildlife Forensic Lab, all of which are listed as CITES Appendices I-III. The headspace volatiles of the wood samples were concentrated on SPME fibers for thirty minutes and analyzed by DART-HRMS. Multivariate statistical analysis processing of the DART-HRMS data revealed intra-genus similarities and inter- genus differences that resulted in the ability to assign genus attribution to the chemical signatures. The classification model that was developed could therefore be used for rapid forensic identification of species based on simple analysis of the headspace of the wood. The results show that this approach can contribute to the enhancement of techniques that enable law enforcement to distinguish between endangered wood at crime scenes.

Fly Curious: A Non-Destructive Approach to Entomotoxicology Through Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) Examination of Insect- Ethanol Suspensions
Amy Osborne and Rabi A. Musah, PhD, University at Albany; Jennifer Y. Rosati, PhD, John Jay College of Criminal Justice

Entomological evidence is well-known in a forensic context as a means by which to estimate the minimum postmortem interval (PMI) in death investigations. This is particularly important in cases where decomposition has advanced to a degree that conventional techniques for PMI estimation cannot be used. However, difficulty in establishing the PMI is not the only challenge associated with remains found in advanced stages of decomposition. When blood, urine, and internal organs no longer remain, toxicological information which may have relevance to the cause of death can be lost or become irretrievable. In these circumstances investigators may turn to less conventional methodologies. One resource that may be utilized is the maggots which have fed on the decomposing remains, which ingest tissue along with any drugs or chemical toxins that are contained therein. To the extent that these chemical compounds and/or their metabolites remain within the flies, their presence can serve as a historical record of the factors that may have led to the cause of death.

The majority of research in forensic entomotoxicology has focused on applying traditional toxicological analyses and drug detection methods to insects. These techniques can involve long, complicated sample preparation that requires the complete destruction of the collected insect evidence. It was previously demonstrated that Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) could serve as a means to extract toxicological information from insects without lengthy sample preparation. It is now further reported that DART-HRMS analysis of the aqueous ethanol solution used to preserve insect specimens collected from remains provides a non-destructive means to screen blowflies for drug or toxin contamination. For this study, *L. sericata* were fed beef liver laced with fentanyl derivatives at physiologically relevant concentrations, and the life stages of the flies following emergence of the maggots from eggs were collected through to the appearance of adult flies from pupal casings. Following standard insect evidence collection procedures, all specimens were stored in 70% aqueous ethanol prior to further analysis. DART- HRMS was then utilized to generate insect metabolome profiles through both the direct investigation of the individual insect specimens as well as through the aqueous ethanol preservative solution. These profiles were subjected to multivariate statistical analyses including kernel discriminant analysis (KDA), discriminant analysis of

principal components (DAPC), and support vector machines (SVM), in order to determine whether the profiles of the liver control (no drug) and the drug-laced liver could be differentiated through pattern recognition techniques. While the results showed strong differences in the metabolome profiles of drug versus control samples for the analysis of the whole specimens, there were also significant differences exhibited in the metabolome profiles obtained solely from the ethanol solutions. These differences are especially pronounced in the metabolome profiles belonging to the ethanol solutions which preserved the pupae and the adults, and pupal casings. These findings provide a new avenue by which to access toxicological information of potential relevance to death investigators while circumventing many of the challenges encountered when using conventional techniques for the toxicological analysis of insects.

Footwear Image Quality Classification: Using Expert Assessments and Image Metrics to Predict Impression Quality

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Shoeprints deposited during the commission of a crime vary in quality as a function of numerous factors, including substrates, media, and the physical activities carried out by perpetrators. This variability impacts the value and quantity of information available in questioned crime scene impressions, and therefore the strength of an examiner's opinion concerning source attribution. To date, limited research has been conducted to quantify footwear image quality. In response, this research aims to develop definitions of footwear image quality as a function of image factors, including totality, noise, contrast, sharpness, and complexity. Modeling is then proposed to combine these factors, and using human assessments as a guide, predict footwear image quality.

The methodology used to achieve this goal is based on a five-pronged approach, of which the first three phases have already been completed. First, a footwear database was created containing nearly 600 images, with variations in media (blood, dust), substrate (vinyl, ceramic, paper), collection method (electrostatic lifter, gelatin lifter, Stat-Lift), and enhancement technique (digital, chemical). Second, automated image processing tools, such as wavelet coefficients and gray-level co-occurrence matrices (GLCM), were employed for feature extraction. Next, online surveys were designed using a R Shiny application that presents impressions to human raters to elicit quality and value assessments. As soon as expert opinions are collected, the fourth step is to relate subjective ratings and image features through an ordinal logistic regression model. Finally, the regression coefficients generated from this model will be evaluated.

During this presentation, pilot study data will be presented, including proposed measures of intra- rater inconsistency and inter-rater reliability, the use of matrix completion for data expansion, and preliminary correlation estimates between human assessments and image factors of sharpness (0.56), noise (0.73), contrast (0.48), and complexity (0.45). Although separately, each correlation coefficient is (at most) modest in magnitude (<0.75), the results are better than anticipated based on how challenging it is to describe footwear image quality using traditional image analysis methods, and given that no single metric can capture all variability. Instead, individual image features demonstrating reasonable correlation coefficient values will be used in combination via regression, which is anticipated to improve overall prediction. Moreover, this preliminary data can be used to inform deep learning methods to explore the use of patent versus latent image features. Once the entire project is completed, it is expected to provide insight into which image features drive human quality and value ratings, thereby ultimately advancing the body of work concerning footwear impression quality.

Analysis of Distortion in BVDA Gel Lift Method of Obtaining Footwear Impressions in Relation to Time of Rest Before Evidence Application

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Purpose: The focus of this project will be to determine whether there is significant distortion among impressions

lifted using Bureau Voor Dactyloscopische Artikelen Gel-lifters, and to establish whether the duration the gelatin sheet is left to rest before application contributes to distortion. This will be done by performing measurements on gel-lifted footwear impressions using various lifting times and comparing these measurements to the test impression of the shoe, with an accepted standard difference of <1mm. This research will help establish the ideal time to allow the gelatin sheet to rest before application, as an effort to reduce significant distortion in lifted impressions. Providing the ideal time of rest before application can enable shoe characteristics to be properly analyzed without having to account for possible deviations due to distortion. This will prevent issues relating to distortion from being raised at court trials, which may result in footwear evidence being deemed inadmissible. Reducing distortion may also help avoid wrongful identification and the dissipation of time and resources due to incorrect interpretations of distorted shoe characteristics.

Background: The gel-lift method targets two-dimensional impressions on porous and non-porous surfaces (1, 2, 3). Gelatin sheets are available in black, white and transparent colors which are selected depending on the print matrix (2, 3, 4, 5). Black gelatin sheets target impressions in soil, dust and developed latent impressions (2, 3, 4, 5). Gelatin sheets are composed of a transparent cover, a gelatin layer and a rubberized linen backing (2, 3, 4, 5). High-quality Impressions of black gelatin sheets are obtained using the BVDA scanning machine, which incorporates oblique lighting and vacuum settings that are adjustable depending on the strength of the impression (2).

Methodology: The total sample size consists of 80 impressions, which include 20 impressions per shoe type, which is further subdivided into four impressions per rest time. The time variables used include 30 seconds, 1 minute, 2 minutes, 5 minutes and 10 minutes. Latent impressions were created on a tile surface, and further developed using white granular fingerprint powder. After allowing the gelatin sheet to rest for the time variable of interest, it would then be applied to the impression using a roller and lifted immediately after application. The subsequent impression would then be placed into the BVDA machine for scanning. Scanning parameters included a 13 x 36 cm image, with a single light source for strong impressions. Measurements of the lifted impression were obtained in Adobe Photoshop using calibration of the built-in scale from the scanned image in conjunction with the ruler tool.

Results: This is a preliminary report as there are statistical results that are still needed. Full statistical analyses of these results are pending.

Keywords: forensic science, forensic identification, BVDA gel-lifter, distortion, gelatin lift, impression evidence, time before application, two-dimensional footwear impression

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Estimate of the Random Match Frequency of Acquired Characteristics in a Forensic Footwear Database

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When analyzing footwear impression evidence, one of the goals of an examiner is to determine if an exemplar shoe could be the source of a questioned impression. In order to form an opinion regarding the possible source, examiners evaluate the similarity of class and individualizing characteristics between impressions, typically using individualizing characteristics as the basis to reach the highest degree of source association. Opinions regarding the strength of the association are based upon the quantity and quality of the observed characteristics, but specific descriptions of what is considered sufficient quantity and quality are not well-defined. Instead, these criteria are developed through training and experience. Due to the purported subjective nature of the interpretation of this evidence, the opinions formed regarding footwear evidence can be misunderstood and undervalued. One way to mitigate this criticism is to complement casework with research that includes numerical analyses. This has been successfully accomplished within the discipline of DNA, wherein the term random match probability (RMP) is commonly used to provide an estimate of the chance of randomly selecting a person from a population and observing a predefined DNA profile of interest. This research aims to perform a similar evaluation within a footwear research database comprised of 1,300 outsoles in order to estimate random match frequency (RMF) for randomly acquired characteristics (or RAC-RMF). To accomplish this goal, this study re-analyzes and expands upon previously evaluated/published data, including RAC comparisons performed using a combination of visual comparisons (> 91,000) and mathematical predictions (> 3.2 million). Through visual comparison, 2,182 indistinguishable RAC pairs were previously identified. For the remaining pairs, a mathematical model predicted the conditional probability of indistinguishability based on the degree of geometric similarity between the features on unrelated outsoles, as assessed via overlap in size, shape, and orientation. Re-analysis of the data for the purposes of RAC-RMF indicated that approximately 95% of RAC pairs with positional co-occurrence were distinguishable with a probability of 0.99 based on shape characteristics. Moreover, only 0.2% of non-mated shoe pairs in this database (2,072 out of 844,350 pairwise comparisons) shared at least one indistinguishable RAC pair, with a maximum of four observed on four different outsole pairs. RAC-RMF was estimated for each shoe by determining the number of unrelated outsoles that contained one or more RACs with positional and geometric similarity. The minimum RAC-RMF of 0 out of 1,299 was observed for 32% of the outsoles in the database, with an additional 19% having a RAC-RMF of 1 out of 1,299. Approximately 77% of values were less than 5 out of 1,299, and the maximum RAC-RMF of 49 out of 1,299 was observed for a single outsole. This research demonstrated that, while repetition of RAC features did occur on unrelated outsoles in this database and RAC-RMF values near 1 in 1,000 exist, the majority of RAC pairs were distinguishable from each other and the indistinguishable features were often small RACs that did not possess remarkable attributes.

A Cold Case Homicide Solved After 37 Years

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Key Words: Cold Case Homicide, Case Study, Genetic Genealogy

Learning Objective: By attending this presentation, the attendees will obtain knowledge about the circumstances and the steps taken to solve a homicide that occurred in 1984. The case involved police investigations, forensic science, genetic genealogy, as well as a "death bed" confession.

Impact Statement: This presentation will provide the forensic community actual case information and discussion on the challenges encountered and new technology utilized during the processing of the evidence in this cold case. Interesting case facts will also be discussed, and questions will be addressed.

Abstract: On February 13, 1984, a 64-year-old woman was brutally stabbed multiple times, strangled, and beaten in a small town in Massachusetts. The suspect then remained in her home, ate her food, and took her car out for a joy ride. Several items of evidence were left behind at the scene that contained blood and skin cells. The case was assigned to the author on August 15, 2004, during her work as a Criminalist at the Massachusetts State Police Crime

Laboratory. The assignment included giving the case a second look with fresh eyes and updated technology. Numerous items were examined, and DNA processing was completed. DNA profiles were obtained, and some were entered into CODIS. Years went by without a CODIS hit. Additional DNA work was performed but the case remained unsolved. Fast forward to April 2019 – a Y-STR profile from the crime scene was sent to a private company for comparison to genealogical haplotype databases with the goal of developing a potential surname for the suspect. This provided some leads but did not result in the identification of a suspect. In December 2019, a crime scene sample was sent out for forensic genetic genealogy (FGG). This sample did not contain enough genetic material to produce searchable SNP data. Another sample was sent for FGG in September of 2020 and resulted in the identification of an individual who was eliminated as a potential suspect. In February 2020, police were contacted by a lawyer's office; someone had come forward with information about the case. This information led to the discovery of a "new" crime scene that was processed in June of 2020. New evidence was collected and tested in the Criminalistics Unit by another analyst. In March 2021, the District Attorney's office made a televised announcement of a "death bed" confession that was brought forward and verified by forensic science. A suspect had been identified and his involvement in the crime was confirmed with DNA analysis. Finally, this cold case homicide was brought to a close.

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Drug Chemistry Abstracts

Optimal Extraction for the Identification of Components in Complex Mixtures
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When preparing complex mixtures for the identification of controlled substances at the NYPD Police Laboratory, one sample can potentially be prepared up to four times to identify all components present. This is especially true for mixtures that include heroin, fentanyl, and fentanyl- related compounds. Using current laboratory procedures, some controlled substances can be identified in methanol, while others may require a basic extraction with sodium hydroxide (pH 14) for identification. The extraction process allows for identification criteria to be met for all remaining components of complex mixtures. An optimal extraction for these compounds should be utilized in order to reduce the number of preparations, promote timeliness, and consume less resources. This study aims to determine the ideal solvent and pH for the recovery of all controlled substances in complex mixtures when using Gas Chromatography/Mass Spectrometry (GC/MS) analysis. Based on the literature, a lower extraction pH may be optimal for the recovery of fentanyl and related compounds. Research was conducted using chloroform basic extractions with sodium hydroxide at a lower pH, as well as using ammonium hydroxide as an alternative base. Preliminary results showed that ammonium hydroxide was not optimal for extraction due to its possible assistance of de-acetylation of heroin to 6-monoacetylmorphine. Extractions using sodium hydroxide at a lower pH showed variable results when utilizing mixtures made with standards of fentanyl, quinine, and procaine. The variability may be attributed to the fact that the standards used were not representative of clandestinely manufactured street samples. Future research will be conducted on previously analyzed casework samples to accurately assess if a lower pH yields a more optimal extraction for the identification of these compounds.

Improved Quantitation for 15 Benzodiazepines using LC-MS/MS with a 1.5 mm Internal Diameter Column
Taylor Maslin, William Campbell, Ph.D., The Pennsylvania State University

Benzodiazepines are a well-known class of drugs used to treat a variety of conditions including anxiety, insomnia, and muscle tension. These drugs are often also used to facilitate crimes including sexual assault and robbery due to their ability to sedate and or compromise the memory of the victim. In addition, benzodiazepines are abused by individuals seeking to mediate withdrawal symptoms of other drugs. There are currently numerous variations in the core structure of benzodiazepines which result in various analogues. In this study, a method for the separation and identification of fifteen different benzodiazepines was developed for LC-MS/MS analysis. This study specifically compared the peak area intensities using a column with an internal diameter of 2.1 mm versus a column with an internal diameter of 1.5 mm. At 1.5 mm, the internal volume of the column is roughly half of the internal volume of a 2.1 mm column of the same length. This reduced system volume effectively doubles the concentration of analytes as they elute from the column assuming equivalent mass load. In addition, columns with an internal diameter of 1.5 mm utilize a slower elution flow rate as compared to the 2.1 mm column. The slower flow rate results in a smaller volume of mobile phase being used. As a sample is introduced to the mass spectrometer with a smaller volume of mobile phase there is a higher concentration of ions in the sample introduced due to the decrease in the amount of solvent needing to be stripped away upon exiting the ions source in atmospheric ionization instruments. This means that at the same initial mass load of the sample, the 1.5 mm column will provide increased peak sensitivity when compared to the 2.1 mm column. This study's results demonstrate that there was a twofold or better increase in peak areas for each of the drugs when using the 1.5 mm internal diameter column.

Waters RADIAN^{TM} ASAP Direct Mass Detector – An Alternative Seized Drug Screening Technique
Nicholas Ciccone, M.S., Marisa McNamara, Nassau County Office of the Medical Examiner, Division of Forensic Services

The recent helium shortage has severely affected crime laboratories across the country as most forensic drug chemistry laboratories utilize Gas Chromatography/Mass Spectrometry instrumentation. To combat this, forensic laboratories are exploring alternative techniques to detect and identify controlled substances in drug seized drug evidence. The Nassau County Office of the Medical Examiner, Division of Forensic Services Controlled Substance Section had the opportunity to serve as a beta testing site for a novel chemical and materials testing technique, the RADIAN™ ASAP with LiveID.

The RADIAN™ ASAP is Waters latest development for rapid preliminary detection of controlled substances. Utilizing technology similar to Direct Analysis Real-Time Mass Spectrometry (DART- MS), the RADIAN™ ASAP is a direct analysis system using a robust single quadrupole mass spectrometer combined with an Atmospheric Pressure Solids Analysis Probe (ASAP). The single quadrupole mass detector provides high quality mass spectral data that is delivered in real-time using the LiveID software. This ensures a quick preliminary identification of the unknown sample against a library of known compounds of commonly seen controlled substances analysis without the use of helium.

This presentation will give an overview of the RADIAN ™ ASAP including instrumental operation and sample preparation. Specific performance characteristics evaluated during this beta test period include selectivity, repeatability, interference, and analyte concentration. Recommendations for integration of the RADIAN™ ASAP into a typical drug chemistry laboratory workflow and overall utility for controlled substance analysis will also be included in the presentation.

Qualitative Analysis of Fentanyl Laced Marijuana

Matthew J. Marino, B.S., New Jersey State Police Office of Forensic Sciences

The Connecticut Overdose Response Strategy and the Connecticut Department of Public Health, Office of Emergency Medical Services recently received numerous reports of suspected opioid overdose cases that required naloxone for revival and where no opioid use was acknowledged. 39 such incidents were reported. In each, the patients solely reported the use of marijuana. At one of the overdose scenes, the Plymouth Police Department confiscated a vegetation sample for laboratory testing. The results indicated the presence of both marijuana and fentanyl. Mixtures of marijuana and fentanyl within the illicit drug market could have a significant impact on the quantity of reported overdoses within the Northeast. The New Jersey State Police Drug Monitoring Initiative (DMI) of the Regional Operations and Intelligence Center (ROIC) identified this as a public health concern and established a joint effort between the New Jersey State Police Office of Forensic Sciences (OFS) and the Hazardous Materials Response Unit (HMRU) to aid in the detection of such mixtures. This research project tested the reliability of several screening and confirmatory drug analytical techniques with identifying sample mixtures of fentanyl on vegetation or paper. The expectation was that a laboratory practitioner would not encounter any issues in confirming fentanyl. However, police officers and first responders often have limited techniques that can be used to presumptively identify fentanyl in the field. Solutions of multiple concentrations of fentanyl in acetone and isopropanol were sprayed onto marijuana and paper and tested using an analytical scheme of Modified Duquenois color test (on marijuana only), Marquis color test, BTNX Rapid Response Fentanyl Forensic Test Kits immunoassay and Gas Chromatography/Mass Spectrometry (GC/MS).

The research project confirmed the ineffectiveness of using the Marquis color test on fentanyl laced marijuana samples, as the marijuana control sample resulted in a false positive orange/brown color change. This is significant, as it is the most commonly used narcotic presumptive test by police officers in the field. As expected, the GC/MS was the most effective analytical tool utilized in identifying fentanyl laced samples. The results also verified the effectiveness of BTNX Test Kits on fentanyl laced paper samples and solid fentanyl powder, marijuana mixtures. However, they were not an acceptable presumptive test for the liquid fentanyl laced marijuana samples within this

research project. Further study is necessary to determine whether the BTNX Test Kits would be an effective screening test for higher concentration fentanyl mixtures. This would verify BTNX Test Kits as an efficient screening method for law enforcement to determine the likelihood of fentanyl mixtures at crime scenes.

Trick or Weed – Application of Ambient Mass Spectrometry for the Detection and Quantification of Cannabinoids in Complex Matrices

Benedetta Garosi, Megan I. Chambers, B.S., Rabi A. Musah, Ph.D., State University of New York at Albany

With the increased legalization and decriminalization of marijuana in the U.S., recreational use of Cannabis sativa, as well as the myriad of products derived from Cannabis or prepared with cannabinoids, has increased exponentially. This rise in Cannabis, CBD (cannabidiol)- and THC
(Δ9-tetrahydrocannabinol)-infused products has imposed major challenges related to the analysis of cannabinoid content (i.e., THC/CBD detection and quantification), because highly specialized methods must be developed for each type of complex matrix that is encountered. When applied to complex materials, conventional methods used in current forensic science practices in the U.S generally are resource-intensive, time-consuming, require extensive sample preparation, and involve complex data analysis. To address some of these difficulties, this study focused on the application of direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) for the rapid analysis of CBD and THC in edible and non-edible complex matrix samples for detection, differentiation, and quantification purposes.

Of importance to this approach is the necessity of devising a means by which to differentiate between THC (scheduled) and CBD (unscheduled), and which, when analyzed by mass spectrometry under soft ionization conditions, are indistinguishable because they are isomers with a molecular formula of C21H30¬¬¬O2 and a protonated monoisotopic mass of 315.232. Previous results demonstrated that the presence of the two compounds within a complex matrix such as candies, could be revealed by derivatization using N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). Engagement of the one -OH group in THC and the two -OH groups in CBD with the derivatizing agent results in the differentiation of the two cannabinoids due to the mass [M+H]+ disparities of the protonated adducts formed (m/z 387.272 and 459.312 for THC and CBD, respectively). Thus, derivatization is an important step in the process of differentiating between CBD and THC by DART-HRMS.

While the process proved successful with edible matrices, it remained to be seen whether it could be applied to other types of complex materials such as commercial topicals (i.e., balms). Therefore, CBD-infused balms were treated with MSTFA to reveal the cannabinoid content of these products. The approach was also applied to extracts, the generation of which are essential to the ability to quantify compounds of interest such as THC. The success of this method was demonstrated with gummy candies, chocolates, and marshmallows. In the course of these investigations, it was discovered that the use as internal standards of the deuterated counterparts of analytes of interest was not optimal for the quantification of derivatized cannabinoids by DART-HRMS. Alternative compounds are under investigation (e.g., synthetic cannabinoids) for the development of validated quantification protocols. Development and optimization of these procedures will aid in the investigation of Cannabis sativa evidence in forensic laboratory settings.

Understanding the Science and Concerns Behind the *Possible* Conversion of Helium to Hydrogen Carrier Gas for EI GCMS systems

Kirk Lokits, Ph.D., Agilent Technologies

Helium has historically, with valid scientific reasons, been the preferred carrier gas for GCMS and the majority of GC analysis. Within the last decade there has been an increase in the difficulties to procure UHP helium in the quantities required for full laboratory operations and or a drastic increase in the overall cost of UHP helium tanks. Due to its

chemical and physical characteristics, high resolution chromatographic separations can be achieved with minimal analyte interactions.

GCs with atmospheric detectors often utilize alternative carrier gases such as nitrogen, argon, and hydrogen. However, when the GC is coupled to a mass spectrometer under high vacuum, parameters based on a mean free pathway of ion molecules, vacuum, low background, and high sensitivity come into play. Based on these parameter limitations, of the previously mentioned carrier gases, hydrogen is the practical alternative. Nonetheless, hydrogen does have disadvantages that may cause a GCMS analyst to re-evaluate the urgency to convert to hydrogen carrier based on its reactivity with some analytes, reduced sensitivity, increased peak tailing, and reduced spectral fidelity when compared to helium generated reference spectra.

Ultimately, helium is the preferred carrier gas choice, but if not available, hydrogen may be considered. The purpose of this presentation is to help analysts determine if hydrogen can be used as a carrier gas for their specific analysis. Furthermore, the illustration of best practices, specific MS source configurations, forensic drug data examples, and the acquisition parameters necessary to help determine if the transition of a specific application is or is not compatible for hydrogen carrier gas on a GCMS system, will be discussed.

Development of an Analytical Platform for the Rapid Testing of Drug Paraphernalia Residue
Meghan G. Appley, Ph.D., Elizabeth L. Robinson, Edward Sisco, National Institute of Standards and Technology

To better initiate public health and public safety responses related to drug overdoses requires the ability to capture real-time tracking of drug trends to identify new substances and poly-drug mixtures as they hit the streets. This type of information is typically gathered using spectroscopic or immunoassay-based techniques which present several limitations. To capture the drug landscape more completely, begin monitoring the presence of cutting agents and adulterants, and minimize the need to handle bulk drug powders, the use of ambient ionization mass spectrometry techniques, specifically direct analysis in real-time – mass spectrometry (DART-MS), for analysis of trace residue from drug paraphernalia was proposed. To evaluate the feasibility of this approach, wipes of trace residue samples from over 500 pieces of used drug paraphernalia were collected from multiple syringe service programs throughout the state of Maryland. The wipes were mailed to the laboratory for analysis by DART-MS where the resulting spectra were analyzed for the presence of drugs and other compounds of interest using the NIST/NIJ Data Interpretation Tool (DIT) and NIST DART-MS Forensics Database. The entire process produced near complete chemical profiles of the drug residue within minutes with little sample preparation and no bulk material handling. As valuable and efficient as DART-MS was, additional testing with more targeted mass spectrometric techniques (DART-MS/MS and liquid chromatography-MS/MS) was needed to provide supplementary information for a small subset of samples. When new substances or substances not well differentiated by the screening approach of DART-MS (e.g., isomers) the targeted approaches of DART-MS/MS and LC-MS/MS were used to provide confirmation of the presence or absence of a compound identified. By using the combination of different techniques, DART-MS can rapidly identify possible compounds so that public health and law enforcement officials can quickly inform the public, while confirmatory testing can provide specific information that is necessary for the tracking of new compounds and drug trends. This presentation will discuss the successful development of this analytical platform created to provide near-real time results to help map the drug landscape within the state of Maryland. In addition, the limitations of the techniques, data obtained from the ongoing pilot study, and the future goals of this project will also be presented.

Separation of Geometric Isomers (±)-11-nor-9-Carboxy-Δ9-THC and (±)-11-nor-9-Carboxy-Δ8-THC by LC-MS-MS
Paola Roldán-Arroyo, William Campbell, Ph.D., The Pennsylvania State University

The separation and identification of the geometric isomers, (±)-11-nor-9-CarboxyΔ^9 -THC and (±)- 11-nor-9-Carboxy-Δ^8 -THC are important in clinical and forensic drug testing due to the variation in legal status of the delta 9 and delta 8 variants of THC in multiple states. The compounds in question are metabolites of THC and it is the metabolites that are analyzed in drug screens and other testing. Many laboratories cannot distinguish these isomers since both compounds have the same molecular weight and have similar chromatographic behavior. Baseline separation is essential for LC/MS/MS analysis. Analysis will depend on both retention characteristics and MS/MS transitions, since transitions for both compounds are the same. In this study, a method for separation of these metabolites was developed using LC/MS/MS. Furthermore, this method was developed for the inclusion of a larger panel of drugs if so desired. Additionally, we evaluated the Halo® C18 column technology comparing a 2.1 mm ID column with a 1.5 mm ID column.
Baseline resolution greater than 5% was achieved using both columns. We wanted to explore the advantage of using the 1.5 mm column. The smaller column geometry provides elution of equivalent mass load of the desired drug but into a smaller volume giving a higher average concentration across the eluted peak. This results in at least a two-fold improvement in sensitivity. Further, using a lower flow rate provides enhanced ionization from the atmospheric pressure ion source in our system. We will provide quantitative data for these compounds and hope to transfer this method to analysis of biological fluids.

Things WE'ED Like to Know – How to Differentiate Hemp and Marijuana Varieties of Cannabis sativa with a Combined Ambient Mass Spectrometric and Chemometric Approach

Megan I. Chambers, Samira Beyramysoltan, Ph.D., Benedetta Garosi, and Rabi A. Musah, Ph.D., State University of New York at Albany

Hemp and marijuana are two major varieties of Cannabis sativa, both of which contain Δ9- tetrahydrocannabinol (THC), the primary psychoactive component of the plant. However, these two varieties differ in the amount of this active ingredient that is present. Federal law currently stipulates that C. sativa that contains greater than 0.3% THC is the drug-type (marijuana), while plant material that contains less than or equal to 0.3% THC is the fiber-type (hemp). The differentiation of hemp and marijuana has become a challenging aspect of analyzing Cannabis evidence in a forensic laboratory setting because of the increased workload that arises from the need to analyze and quantify the THC content of all C. sativa samples to enable proper designation of seized materials. This project aimed to develop a combined direct analysis in real time – high- resolution mass spectrometry (DART-HRMS) and chemometric approach for the rapid differentiation of hemp and marijuana plant materials.

Commercial hemp products from multiple vendors were purchased. Marijuana plant material was obtained from two DEA-registered suppliers. Furthermore, DART-HRMS data from analysis of recreational marijuana strains was provided by an industrial collaborator. All plant materials were analyzed by DART-HRMS with no sample pretreatment in both positive- and negative-ion modes at a range of orifice 1 voltages, including +/- 20, 30, 60, and 90 V. The application of preliminary statistical analysis methods to the chemical profiles revealed the potential for differentiating hemp and marijuana by DART-HRMS. These results prompted the use of advanced multivariate data analysis (e.g., principal component analysis (PCA), Random Forest) for the optimized differentiation of the two C. sativa varieties with a high level of certainty. In addition to developing the model and performing external validations (i.e., test samples), several m/z values were identified as diagnostic for distinguishing between the hemp and marijuana materials in the model. The identities of m/z values that were determined to be important for the optimal differentiation of hemp and marijuana are currently under investigation by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and DART-HRMS. Using these methods, several markers appear to correspond to cannabinoids and terpenes known to exist in Cannabis. Fragments of these major molecules are also formed during analysis under ambient conditions. This combined mass spectrometric and chemometric approach would significantly aid in the investigation of C. sativa plant materials in a forensic setting, prior to launching confirmatory

analysis testing.

Distinction of cathinone isomers and fentanyl isomers based on statistical comparison of mass spectra

Andrew Sacha, Forensic Science Program, School of Criminal Justice, Michigan State University, Victoria L. McGuffin, Ruth Waddell Smith, Ph.D., Department of Chemistry, Michigan State University

In this presentation, a method to statistically compare electron-ionization mass spectra will be discussed, with particular emphasis on the distinction of structurally similar synthetic cathinones and fentanyl analogs. The method uses the unequal variance t-test to compare the intensity of corresponding ions in the two spectra. The null hypothesis (H0) states that the difference in intensity is equal to zero, whereas the alternative hypothesis (Ha) states that the difference in intensity is not equal to zero. If H0 is accepted for all corresponding ions, the two spectra are statistically indistinguishable at the specified confidence level. In contrast, if H0 is not accepted for at least one ion, the two spectra are statistically distinct. In these cases, the number and m/z value of the ions responsible for discrimination are determined.

Previous work in our group demonstrated the ability to distinguish spectra of positional isomers of ethylmethcathinone and fluoromethamphetamine at the 99.9% and 99% confidence levels, respectively. In the current work, the robustness of the comparison method is evaluated, specifically addressing the effects of inherent instrument variation and compound concentration. A set of synthetic cathinone structural isomers (dibutylone, eutylone, propylone, and pentylone) and a set of fentanyl positional isomers (isovaleryl, valeryl, and pivaloyl fentanyl) were analyzed by GC- MS multiple times over a 12-month period. The resulting mass spectra were statistically compared to evaluate association of corresponding compounds and discrimination from other compounds in the set.

At the 99.9% confidence level, spectra of corresponding cathinones were associated across the collection period. Each cathinone was readily distinguished from the other three cathinones, with 9 – 33 ions responsible for discrimination. As concentration was decreased, discrimination was maintained albeit with fewer ions (2 – 10 ions) responsible for discrimination. Similar trends were observed for the fentanyl analogs, with discrimination among the three compounds achieved at the 99.9% confidence level, with 3 – 6 ions responsible for discrimination. However, as concentration was decreased to 0.1 mg/mL, discrimination of isovaleryl fentanyl and valeryl fentanyl was not possible at 99.9% confidence level but was achieved at lower confidence levels.

This presentation will discuss the statistical comparisons in more detail and will highlight ions that are consistent and, therefore, reliable for discrimination of the synthetic cathinones and fentanyl analogs.

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Update

Tiffany A. Ribadeneyra, M.S., ABC-CC, Nassau County Office of the Medical Examiner, Division of Forensic Services, Sandra E. Rodriguez-Cruz, Ph.D., ABC-DA, DEA Special Testing & Research Laboratory

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) was formed in 1997 in a joint effort between the U.S. Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). Historically, SWGDRUG recommended minimum standards for the forensic examination of seized drugs and sought their international acceptance. Considering the formation of the Organization of Scientific Area Committees (OSAC), SWGDRUG continues to work as part of the international community to improve the quality of the forensic examination of seized drugs. In addition, the extensively utilized resources provided on the SWGDRUG website will continue to be updated and available including free spectra libraries and monographs.

This presentation will provide attendees with an update on SWGDRUG activities during the year 2020 and currently in 2022. Upcoming publications will include revisions to Part IVA of the SWGDRUG Recommendations: Quality Assurance/General Practices and Supplemental Document SD-5: Reporting Examples. Recent activities include revising Parts IVB of the SWGDRUG Recommendations: Quality Assurance/Validation of Analytical Methods, revising Supplemental Document SD-2: Validation of Analytical Methods and the formation of an outreach and training subcommittee. Lastly, the current state of SWGDRUG as well as future initiatives will be reviewed.

Introducing the "Database of Psychoactive Plants" aka "DoPP": A Graphical User-friendly Application for the Rapid Forensic Identification of Psychoactive Plant Materials

Rabi A. Musah, Ph.D., Samira Beyramysoltan, Ph.D.; Megan I. Chambers; Amy M. Osborne; Mónica I. Ventura, State University of New York at Albany

The abuse of "legal high" psychoactive plants is a world-wide public health concern that exposes users to dangerous health consequences and even death. A major challenge for law enforcement in regulating widespread abuse of these plants is the paucity of methods by which to identify them. In general, identification of psychoactive plants is limited to only a few species using methods such as color tests, visual examination, chemical/biochemical methods, and DNA. While DNA analysis is the gold standard, the genomes for most of the relevant psychoactive plants have not been mapped, and therefore, this approach cannot be used. By and large, the other tests are presumptive, and definitive identification is laborious and time-consuming, rendering cases involving these substances un-prosecuted. Development of accurate, fast, efficient and cost-effective techniques for forensic identification of psychoactive plant materials is crucial. Direct Analysis in Real Time- High Resolution Mass Spectrometry (DART-HRMS) was investigated as an approach for building a species-specific chemical signature database that could be mathematically interrogated to reveal differentiation between and identification of psychoactive plants species. The rapid acquisition of DART-HRMS mass spectra (i.e. a few seconds per analysis) and the ability to analyze the materials in their native form without pre-treatment steps, enabled the generation of the vast number of spectral replicates required for database construction. A machine learning based workflow was implemented in Python for the generation of a discrimination model and identification of plant unknowns. An interactive graphical user interface named "database of psychoactive plants" or "DoPP" was also developed to simplify the workflow for identification of plant materials for end users.

To create the database, 57 psychoactive species including plants such as Mitragyna speciosa (aka Kratom), and Salvia divinorum (aka diviner's sage) representing a range of sample types including flowers, stems, seeds, leaves, roots, extracts and brews, were analyzed by DART-HRMS in multiple replicates. A DART-SVP ion source coupled with a JEOL AccuTOF high-resolution time-of-flight mass spectrometer (JEOL USA) operating in positive ion mode was used to collect soft-ionization mass spectra in the m/z 40-800 range. The spectra were corrected for background and mass shifts and aligned along common m/z values for further multivariate analysis. A three-level hierarchical classification tree was designed (i.e., family, genus and species) based on the taxonomic relationships between the plant species, to reduce the 57 multi-class problem into several simplified multi-class problems. Within each node of the tree, the supervised classifiers support vector machine (SVM), Random forest (RR), and k-nearest neighbor (KNN) were used to train the discrimination models, and their outputs were then fused for sample prediction. Performance analysis using 5-fold cross validation revealed the hierarchical classifier to have 95% prediction accuracy. Therefore, the workflow enabled prediction of plant species identity from the raw DART mass spectra of unknowns, despite the complexity of their matrices and the absence of sample pretreatment. The developed screening tool can be readily utilized by crime labs and forensic scientists and does not require sample preparation steps or knowledge of botany.

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Forensic Biology/DNA Abstracts

Rapid identification of semen using STK Sperm Tracker Spray

Taylor Zekri, Michael A. Marciano, Jessica Haresign, Elise McInnis, Syracuse University

The identification and confirmation of semen stains on items of evidence can be a critical step toward corroborating stories and obtaining a profile suitable for comparison. An alternative light source (ALS) is one of the most common methods to screen for the presence of body fluids, which then leads to presumptive or confirmatory testing. Although most semen stains will be identified on clothing it is not uncommon to identify semen stains on other items at a scene, for example, items or surfaces in a bathroom. These larger items or surfaces may not be easily transported to a lab or may be time-consuming to screen at the scene. This study examines the use of STK Sperm Tracker™ Spray, used in conjunction with ALS, for the identification of semen stains. The STK Sperm Tracker™ is specific to seminal acid phosphatase and uses reagents that cause the stain to fluoresce more brightly under ALS making detection faster and more efficient. The aim of this study is to (1) compare the accuracy and precision of semen identification with STK Sperm Tracker™ Spray and ALS compared to the ALS in isolation, (2) determine the sensitivity of the spray, and (3) test the specificity of the spray. Bathroom surfaces and structures were stained with dilutions of semen, blood, urine, and mixtures of these fluids.

Items were screened with the Vilber ALS at 365nm and Rofin Polilight Flare 2 ALS at 365nm to compare efficiency. Preliminary testing indicates that the STK Sperm Tracker™ Spray enhances the brightness of the semen stains, thus making it easier and faster to identify potentially probative evidence. Downstream analysis will be conducted on stains observed with STK Spray to determine whether the product interferes with the ability to obtain DNA profiles.

Validation and Implementation of Three Next Generation Sequencing Systems

Rachel Oefelein, DNA Labs International

Next Generation Sequencing (NGS) technology has been available for quite some time; however, the forensic science community, up until this point, has been slow to adapt. In 2021, going in to 2022, the tides have turned. Over 6 major government laboratories and multiple private laboratories have begun to adopt NGS workflows. This presentation will go over the validation and implementation process of three ForenSeq NGS systems; whole genome mtDNA, Signature Prep Primer Set B, and Kintelligence for forensic genetic genealogy. Challenges and lessons learned regarding validation, automation, protocol drafting, report templates, scope of accreditation changes, and training will be highlighted. Finally, a successful case using NGS technology will be highlighted.

Using Genetic Genealogy to Overcome a Non-Paternity Event and Solve an Unknown Parentage Case

Tobi Kirschmann, Nora Cheek, Don Carola, and Kevin Sullivan, DNA Investigations LLC

Genetic Genealogy (GG) is a human identification technique that places a person's sequenced DNA file into a genealogy database for comparison amongst relatives and combines that information with traditional genealogy research methods. This technique can solve family mysteries and deliver suspect leads for law enforcement. A common obstacle in GG cases is the non-paternity event (NPE), arising when genetic data and genealogical records conflict. Although problematic, an NPE can be overcome with thorough genealogical analysis and target DNA testing. Using an example of an unknown paternity case, the identification and resolution of an NPE is described, and the steps taken for target DNA testing are reviewed. When the target DNA matches the starting DNA in the predicted way, the NPE is confirmed, and the true genetic lineage is identified.

Extraction of Challenging Forensic Samples Using the MicroGEM DNA Extraction Kit

Lauren Chwatt, Pace University, Falyn Vega, John Jay College of Criminal Justice, James Prinston & Andrew Schweighardt, NYC Office of Chief Medical Examiner

DNA extraction is an essential but sometimes tedious process in forensic investigation that may require a significant investment of time and resources. Proteinase K has been an industry standard for DNA extraction for several decades due to its reliability of protein denaturation when performing an extraction. Some of the drawbacks of proteinase K are that its use requires multiple ionic detergents and washing steps, while only being active above 65 ℃. Here, we analyze the potential of a new enzyme being used in DNA extraction known as forensicGEM by the manufacturer MicroGEM. This novel enzyme is temperature-dependent, which enables it to be compatible with mesophilic enzymes. The forensicGEM protocol offers complete DNA extraction in about 20 minutes in a single tube, thus limiting contamination, loss of sample, and working time -- ultimately increasing efficiency. One of the main attractions of forensicGEM is its ability to extract DNA from highly degraded samples, potentially leading to more complete STR profiles in samples where a profile may have previously been poor or unattainable by conventional extraction procedures. To assess the efficiency and potential uses of forensicGEM, we collected highly degraded tissue and bone samples and extracted DNA using the MicroGEM kit, altering different parameters such as incubation times, enzyme amount, bone preparation method, and post-extraction purification. We then compared the results of samples extracted with MicroGEM to the results of the same samples extracted with a standard organic extraction to assess whether this new technology could be utilized routinely on highly degraded samples.

Half of the degraded samples extracted with MicroGEM had detectable DNA. The highest success rate was observed for bone samples. One tissue sample in particular yielded higher average peak heights when extracted with MicroGEM. No statistically significant pattern was apparent with respect to identifying superior MicroGEM optimization parameters. Success with bone profiling was notable given that there was much less sample input for MicroGEM (10 mg) compared to the organic extraction (2 g). An ancillary finding of this study is that the bone reparation method of scraping yielded higher DNA quantities and better quality profiles compared to samples treated with the standard method of milling. Since the initial results were promising, this new technology was utilized on remains from the 9/11 World Trade Center attacks from which no detectable DNA had been previously extracted. Ultimately, MicroGEM was able to yield a 22-locus and a 15-locus profile on two of these highly degraded samples.

Future work will focus on further investigation of the bone scraping method for universal application, and continued optimization of experimental parameters in the MicroGEM extraction protocol.

New Standards and Best Practice Recommendations for Forensic DNA Testing
Charlotte Word Ph.D., Consultant

Since its inception in 2014, the Organization of Scientific Area Committees for Forensic Science (OSAC) has facilitated the drafting of many new Standards and Best Practice Recommendations for forensic science service providers in multiple disciplines. Many of these documents are now published and available for implementation having been further developed by various Standards Developing Organizations (SDOs), such as the American Academy of Forensic Sciences Academy Standards Board (ASB), and are also available on the OSAC Registry. Currently thirteen Standards and Best Practice Recommendations for forensic DNA testing laboratories have been published and/or listed on the OSAC Registry, and many others are in various stages of the drafting or development process. These documents cover various aspects of analyst training as well as DNA laboratory testing processes, including DNA mixture interpretation, probabilistic genotyping software and serological testing method validation and protocol development. Other documents address contamination prevention, monitoring and mitigation including the use of elimination databases as well as the reporting of profiles impacted by contamination or failed controls. This presentation will provide a brief summary of the thirteen Standards and Best Practice Recommendations available for implementation in forensic DNA testing laboratories along with a preview of other documents currently in progress. Information regarding how individuals can get involved and participate in the standard development process and the resources available to assist with implementation by laboratories will also be presented.

Examination of Saliva for Determination of a Confirmatory Test
Sierra Soletsky, Claire Glynn Ph.D., University of New Haven

Saliva can be very important in criminal cases for confirming activities claimed took place or victims or suspects were involved in the crime. The way saliva is identified in forensic cases is through the use of three tests: SALIgAE®, RSID™ Saliva, and Phadebas® Amylase Test.

These tests work to identify salivary a-amylase which is an enzyme that is present in saliva. However, there is no general consensus across laboratories on if these are all presumptive or if any can be used as confirmatory. This is due to amylase also being present in small quantities in other bodily fluids like vaginal fluid, semen, and breast milk. It is also present in plants and animals. These items can cause false positives in the tests along with even household items like laundry detergent causing false positives in some studies. These false positives lead to these tests not being selective enough to be considered confirmatory by all labs, yet still some labs believe RSID™ saliva is selective enough to be considered confirmatory. This study examined the selectivity and the sensitivity of these three tests to see if RSID™ saliva should be considered confirmatory by all labs, or if there were too many false positives possible that RSID™ saliva and the other two tests interact with, to display that all should be solely presumptive. This study used citrus fruits and laundry detergents which are known to have forms of amylase in them. This study confirmed that citrus fruits is a false positive that interacted with RSID™ saliva and laundry detergents are false positives that interacted with Phadebas® Amylase Test. This proves that Phadebas® Amylase Test and RSID™ saliva are solely presumptive tests since false positives were easily obtained by these tests. SALIgAE® was the only test to not react positively to the laundry detergent or the citrus fruits showing it had higher selectivity than the other two tests, but further research must be done on possible false positives and the sensitivity to these false positives before it can be fully considered confirmatory by all labs.

DNA Mixture via Transplantation
Jennifer Thayer, New Jersey State Police Office of Forensic Sciences

Y-STR DNA profiles obtained in a sexual assault case were originally described as 2-person mixtures; however after a suspect reference was submitted it was theorized that he was, in fact, a chimera. This presentation describes the DNA results for the case, the subsequent retesting of reference samples, the potential causes of a chimera, and the final reporting for the case.

Applying complex trait and statistical genetics concepts to forensically relevant phenotypes
Frank Wendt Ph.D., Brendan Newton, University of Toronto, Andrea Quintero Reis, American University of Antigua College of Medicine

DNA can be used in a forensic science context to make inferences about the source of biological material collected from an event. Short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) also may be aggregated into "profiles" that may predict eye color, hair color, skin color, hair balding patterns, etc. Collectively, these forensically relevant phenotypes have garnered considerable attention for generation of investigative leads in criminal casework.

Commercially available kits to predict such phenotypes rely heavily on whether the unknown profile matches some reference profile(s). As all humans are a mosaic of genetic information blended from ancestral populations, the appropriate consideration of admixture is not appropriately addressed in these predictive algorithms. This talk will describe a series of investigations into (i) the population genetic features of DNA phenotyping SNPs across diverse global populations, (ii) the relationship between these loci and potentially probative features of the human phenome, (iii) overcoming statistical challenges to pattern-matching algorithms in forensic DNA phenotyping, and (iv) an example use of statistical genetics investigation of various definitions of suicidal thoughts and behaviors. Collectively, this talk is designed to shed light on the power of complex trait genetics, statistical genetics, and genetic epidemiology to rapidly advance the forensic science field with rigorous and statistically powerful computational investigation of traits routinely investigated in the legal system. Furthermore, this talk will emphasize one major weakness of the forensic DNA community related to the inclusion and appropriate adjustment for measures of population stratification in genetic studies.

Application of K-means clustering to Images of Immunochromatographic Test Strips for Saliva Detection
Katelyn Rivera, Lasell University, Frank Wendt Ph.D., University of Toronto

The identification of body fluids on evidentiary items is an essential presumptive step in forensic casework. Lateral flow immunochromatographic tests are regularly used as a confirmatory test for the detection of human saliva, seminal fluid, and/or blood and determines whether or not these tested items will be submitted for DNA extraction. The positive and negative results of these test strips rely on visual recognition of a colored band, making the results highly subjective from analyst to analyst. We hypothesized that machine learning analysis of image data can accurately discriminate between negative and positive detection of saliva from immunochromatographic assays. As a proof of concept, we used a total of 10 images of saliva test strips from Old, et al. Each image showed a control line indicating a reliable test result and a test line resulting from various volumes of saliva: 0.5nl, 0nl, 5nl, 10nl, 25nl, 50nl, 250nl, 500nl, 1250nl, and 2500nl. Image colorization was performed with custom K-means clustering algorithms written in R studio. Control lines from each test strip were analyzed to determine the most appropriate K (i.e., the number of clusters of data points in the images) using three methods: (i) within sum of squares (WSS), (ii) average silhouette coefficients ("silhouette"), and (iii) gap statistics. We decomposed the test lines into red, green, and blue color spectra and projected these values onto the two- dimensional feature space of the spectra from control lines. Pixels were assigned to a reference cluster (e.g., "Background, "Test Line," and "Unclustered") based on their relative position to the centroid of the reference clusters. Any data point within the 95% confidence interval of the reference cluster centroid position was considered a member. Control line analyses support K=2 as the most appropriate number of clusters in the data (mean WSS=7.99±1.32; mean silhouette coefficient=0.856±0.010; mean gap statistic=0.780±0.030). At K=2, image colorization was able to detect 500nl of saliva with approximately 6.7% of data points assigned to the test line. Some immunochromatographic card images supported K=3 with some of the clustering algorithms; however, across all cards, K=3 was less well supported than K=2 by all model fit metrics (K=3 mean WSS=3.59± 0.483; mean silhouette coefficient=0.798±0.020; mean gap statistic=0.766±0.047). Our work has established one workflow to assess images of RSID Saliva cards for the presence of positive test lines. Based on these findings, future work includes collection camera setting parameterization, cross-card compatibility, and cross-substrate portability. We are actively collecting high-quality immunochromatographic card images and assessing cross-tissue portability of the algorithm.

Utilizing Y-SNPs for samples containing low amounts of nuclear DNA

Elise Anderson, Arwin Ralf, Manfred Kayser, Charla Marshall, Kimberly Sturk- Andreaggi,
AFMES-AFDIL

Y-chromosomal single nucleotide polymorphisms (Y-SNPs) can provide paternal lineage identification and paternal ancestry prediction in cases with samples of limited nuclear DNA, such as aged skeletal remains in missing persons cases. Additionally, due to reduced amplicon size, Y-SNPs can be recovered from poor quality samples when typical Y-chromosomal short tandem repeat (STR) testing fails to yield a useful profile. The Ion AmpliSeq HID Y-SNP Research Panel was designed for Ion sequencing to detect 884 Y-SNPs and allow the interference of 640 Y-haplogroups. The assay includes ~600 amplification targets multiplexed into a single primer pool, with amplicons averaging 120 bps (65 to 250 bps). To assess this kit for the application to forensic-type samples, the assay was tested on blood serum samples from diverse U.S. populations. Serum contains minimal quantities of high-quality nuclear DNA, which limit Y-STR recovery. Furthermore, this study evaluated the ability to generate Illumina data with the AmpliSeq HID Y-SNP panel. The Y-SNP targets were amplified with Qiagen Multiplex Disclaimer: The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, or the Armed Forces Medical Examiner System.

Post-Conviction Testing: A Case Study
Devora Goldberg, New York City Office of Chief Medical Examiner, Jonathan S. Kui, Office of the Hudson County Prosecutor

The New York City Office of Chief Medical Examiner's Department of Forensic Biology is an accredited laboratory with approximately 170 analysts who process over 20,000 cases per year. In addition to homicides, assaults, robberies, firearms and property crimes under active investigation, and cold cases and missing persons identifications, the laboratory also tests evidence requested in post-conviction contexts, where testing may yield results that would support exoneration. The laboratory tests a wide variety of evidence types in post-conviction inquiries, as the use of modern techniques may represent one of the last remaining options in uncovering elusive answers in these enduring cases. Here we present a post-conviction case study on a violent 1996 kidnapping and sexual assault by three perpetrators. Three suspects were convicted at trial in 1997, though the DNA testing at the time had only shown possible associations to two of the three suspects. A 2015 request for post- conviction testing sought to explore remaining 1996 evidence to assess any contribution/exclusion of the third suspect. The results of two years of testing efforts from 2017-2019 demonstrates the process and challenges involved in post-conviction testing at OCME.

SupreMEtric: A commercialization effort for a body fluid identification test for forensic laboratories
Alexis Weber, SupreMEtric LLC, Igor K. Lednev, Ph.D., University at Albany, SUNY

The ability to identify body fluid traces at crime scenes, while preserving any DNA present, is critically important in forensic science. Currently in forensic science laboratories, the identification can be difficult and many of the current techniques are specific to one body fluid. Additionally, typical biochemical methods are destructive – preventing any further analysis. When there is a problem within the scientific field, research laboratories are the main group to solve this problem. After conducting research in the laboratory, the next step in the process is to commercialize the research. Commercialization is bringing a product to market and selling it for financial gain. Within the Lendev Laboratory, in order to develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, the specificity of Raman spectroscopy was combined with the analytical power of statistical modeling. All six forensically relevant body fluids (blood, semen, saliva, sweat, urine, and vaginal fluid) were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids. This development of this product has occurred over several years to prepare it for sale, with the culmination of this being the creation of the start-up company SupreMEtric LLC. SupreMEtric's mission is to streamline the forensic analysis of biological stains by creating a universal nondestructive method for the identification of all main body fluids. This presentation covers the process from research to commercialization process of this technology.

Trace DNA Detection Using Diamond Dye: A Recovery Technique to Yield More DNA
Leah Davis, Heather Coyle Ph.D., University of New Haven

The current approach to the recovery of trace DNA on larger pieces of evidence is blind swabbing in an area where there may be a source of DNA. When using this technique, the chance of missing the area that does contain trace DNA increases which could lead to retaining a partial DNA profile or no profile in general. This study aspired to find a new approach to trace DNA recovery techniques to yield a higher quantity of trace DNA from larger items of evidence. It took the path of visualizing trace DNA on items of evidence with potential DNA so analysts can swab a

more localized area rather than through the general swabbing technique currently used for touch DNA recovery. Specifically, the use of Diamond Nucleic Acid Dye in a spraying mechanism was used to distribute the Diamond Dye solution (Young & Linacre, 2020).

The first part consisted of the solution being tested on porous and non-porous surfaces to investigate the appearance of the dye on different materials when being excited by a mini crimescope at different light wavelengths (Kanokwongnuwut, Kirkbride, & Linacre, 2018) . These tests helped to access the amount of dye that needed to be distributed from the spraying mechanism to ensure the retention of DNA was possible. Images were captured to display the effects of the dye on the different materials. The next part of the experiment involved Diamond Dye being sprayed onto brand new and laundered brassieres that had touch DNA placed by donors on the porous cup area and the non-porous clasp area to replicate potential evidence from an assault. The stained brassieres were visually analyzed using a Dino-Lite magnifier to locate areas that fluoresced, meaning that touch DNA is present, and images were captured to display the effects on the mock evidence. The final part of the study consisted of double swabbing of laundered brassieres using the general blind and localized Diamond Dye swabbing techniques and analyzing the swabs for the quantity of DNA present. Donors placed trace DNA on the cup and clasp areas of laundered brassieres that were used for either swabbing technique. General blind swabs of randomly selected brassieres were collected. The other brassieres were first sprayed with the Diamond Dye solution and placed under the mini crimescope at multiple wavelengths in order to excite the dye for locating the trace DNA present and swab fluorescing areas of the brassieres. The swabs are currently being put through DNA extraction and quantification using the Qiagen QIAmp DNA Investigator Kit and the Quantifiler Trio DNA Quantification Kit in order to quantify the amount of DNA was recovered from each area of the brassiere. The quantified data can then be input into charts in order to compare recovery quantities between the two techniques in both porous and non-porous materials of mock evidence items and determine if there's a statistical difference in the quantity of DNA being yielded between the two techniques.

Forensic Investigative Genetic Genealogy (FIGG): A Cautionary Tale and a Call to Action
Claire Glynn Ph.D., University of New Haven

The use of Forensic Investigative Genetic Genealogy (FIGG) has rapidly increased across the United States since early 2018. It is estimated that at least 500 cases within the US alone have been resolved using FIGG in just a few short years. However, the number of cases solved is not the same metric, nor is it as informative, as the clearance rate of FIGG cases. As there is no required reporting of the use of FIGG in criminal investigations to any governing body, there exist many questions regarding the number of cases FIGG was not successful in resolving, and why. While several law enforcement agencies have established their own in-house FIGG investigation units, it remains that the majority of cases are outsourced to private FIGG providers and/or vendor laboratories. Some FIGG providers/vendors are cognizant of forensic standards and adhere to robust quality assurance and quality control practices. It is critical however that all FIGG providers and vendor labs do the same. This presentation will highlight some of the risk factors that can compromise the integrity of a case and will offer some recommendations to safeguard the use of FIGG in the future.

The Impact of Manually Degraded SNP Microarray Data on GEDmatch Top Genetic Matches for Forensic Genetic Genealogy Purposes
Justin Rivera, Claire Glynn Ph.D., University of New Haven

Forensic Genetic Genealogy (FGG) has recently become a valuable tool in the forensic science community and is having a great impact on the resolution of unresolved cases, including homicides, sexual assaults, and Unidentified Human Remains (UHRs) cases. In forensic investigations, following traditional Forensic DNA (STR) analysis and CODIS upload (within the United States), and failure to produce a candidate match in CODIS, FGG could produce investigative leads to identify an unknown individual. FGG employs SNP sequence data uploaded to genetic genealogy databases (i.e., FamilyTreeDNA® and GEDmatch PRO®) to identify genetic relatives (i.e., genetic matches) of the unknown individual. Family tree(s) are then constructed using the genetic matches to reach a possible candidate identity of the unknown individual. SNP sequencing (i.e., SNP microarray) typically requires high-quality/high-quantity DNA samples. Degraded DNA samples however are regularly encountered in forensic investigations.

Therefore, a critical analysis of the impact of degraded DNA/SNP data is necessary to investigate the downstream effects this may have on the subsequent FGG analysis within the genetic genealogy databases. Addressing this potential issue, this study investigates how manually degraded SNP DNA data files affect the top ten genetic matches generated in GEDmatch. Following informed consent, three volunteers provided their own downloaded raw DNA SNP microarray data. Once received by the principal investigator, the data files were anonymized and subjected to a randomized manual deletion protocol using Microsoft Excel. This process is composed of increasing increments of deletion percentages from the overall SNP data profile with a total of nine modified files for each donor (minus 5%,10%, 15%, 20%,25%, 30%,- 50 deletion), each file was uploaded to GEDmatch as "Research Files", and a list of the top ten genetic matches based on shared DNA (total shared cM value) was produced. Each modified file was examined using autosomal One-to-Many matching, autosomal One-to-One Q-Matching, and Segment Searching, to investigate how values and top matches were altered with increased deletion of data.

Currently, this specific protocol and analysis is being completed for SNP profiles generated through whole genome sequencing (WGS), which has been valuable in FGG. The results highlight various changes among top matches, including, but not limited to; matches that decrease/increase in total shared cM value, decrease/increase in quality scores of matching segments on a one-to-one basis, and changes to percentage confidence in predicted relationships. Additionally, the ranking of each donor's top ten genetic matches became altered with increasing deleted percentages, with some moving up in rank, some moving down in rank, and some lost completely (from the top ten list) when compared to the original full DNA SNP data file. Practically, these findings highlight potential issues for match assessment as typically the top ten genetic matches are the most valuable starting point in an FGG investigation. As FGG use grows, it is important to understand how to assess the information coming from a subject's matches, particularly when dealing with degraded DNA samples. Overall, this research emphasizes the need for further empirical research to assess the impact of degraded DNA samples in FGG investigations.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2022 Annual Meeting

Poster Session Abstracts

P1. Getting Confident Answers to Serious Questions via "Classical & Searchable EI Spectra" in Under a Minute using Agilent's QuickProbeTM Technology on an existing GCMS System

Kirk Lokits, Agilent Technologies

Routine analysis of unknown powders, tablets, and liquids by forensic drug chemist, has routinely utilized capillary chromatography with mass selective detectors (MSD). However, this usually requires some sample preparation and or acid/base extraction and includes runtimes routinely from 10 to 30 minutes for general sample screenings. QuickProbe analysis can produce "classical EI" spectral identification of compounds in a variety of sample matrices with minimal to no sample prep. The purpose of the research is to demonstrate the ability of QuickProbe™ to be successfully incorporated into the current forensic workflow on existing GCMS systems. This work illustrates how the technique can be configured on existing 5977B/7890B or 8890 GCs in 3 different configurations, while maintaining the current conventional capillary GCMS capabilities. QuickProbe is controlled by Agilent MassHunter software with access to acquisition control, qualitative analysis, unknowns' deconvolution, and reporting templates.

P2. Increased Accuracy and Precision in the Detection and Identification of Human Semen Stains on Clothing Fabrics using the STK ™ Sperm Tracker STK Lab

Jessica Haresing, Syracuse University; Elise McInnis, Taylor Zekri, Michael Marciano, Forensic and National Security Institute at Syracuse University

The detection and identification of seminal fluid is many times a critical step in forensic analyses, where these stains can be present on items collected at a crime scene or from a sexual assault evidence collection kit. The alternate light source, or ALS, is the first analytical tool that is used to screen evidence for the presence of body fluids, primarily used for the identification of semen. However, this is not specific, as other types of body fluids and chemical reagents can fluoresce under ALS, making the search and identification of semen stains potentially difficult and time-consuming. Additionally, diluted semen or semen mixed with other bodily fluids can go unnoticed even with the use of an ALS. STK Sperm Tracker - STK Lab paper has been developed allowing for a more sensitive and specific means of quickly screening evidence for the presence of semen on porous items such as clothing fabrics. This product has chemical reagents impregnated onto one side of the paper that reacts with human seminal acid phosphatase, producing a bright fluorescence when viewed under a 365nm UV light. This study investigates the sensitivity of the STK Sperm Tracker - STK Lab paper on clothing made from a diverse array of fabric types, such as cotton, polyester, and wool. The speed and sensitivity of identifying semen stains using the STK Lab paper was compared to an ALS, (Arrowhead Forensics 455nm), and the resulting evaluation of semen positive STK Lab paper will be compared using the two different 365nm ALS, the Vilber VL- 6.L and Rofin Polilight Flare + 2 UV. Preliminary studies have shown that semen stains fluoresce brighter with the STK Lab with ALS versus the 455nm ALS by itself. Also, it is evident that Vilber VL-6.L (365nm) increases the fluorescence of the semen stains with STK Lab compared to the Rofin Polilight Flare + 2 UV (365nm). Further studies will analyze how accurate and how rapid the STK Lab is when identifying semen stains on clothing fabrics with varying thicknesses, compositions, and colors.

P3. Increasing the Precision and Accuracy of the Detection of Semen Stains on Household and Vehicle Fabrics Using STK Sperm Tracker Lab

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Initial efforts in searching for the presence of semen involve the use of an alternative light source (ALS). There are many types of fabrics on which semen stains may be deposited, some types may be more difficult to detect stains using this method. This study uses STK® Sperm Tracker Lab, a new product to be used along with ALS as a presumptive test for human semen. STK Lab is a paper impregnated with reagents that react with seminal acid phosphatase, making it highly sensitive and specific to semen. The reaction results in greater fluorescence of the semen stain under ALS, including for diluted semen stains and stains mixed with other body fluids. Greater fluorescence allows for greater efficiency when searching for the presence of semen and greater sensitivity. This study aimed to compare the accuracy and precision of semen identification using STK Lab and ALS compared to ALS alone. To achieve this, various household and vehicle fabrics were stained with semen dilutions, blood, and a mixture of semen and blood to simulate evidence in a real scenario. Fluorescence with the ALS was compared on items using Arrowhead 455 nm and Vilber VL-6.L 365 nm before using the STK Lab. Following the use of STK Lab, fluorescence was compared on items using Vilber VL-6.L 365 nm and Polilight® Flare + 2 365 nm ALS. Preliminary testing has found the STK Lab increases the ability to identify the stain including showing a more precise size and shape of the stain and increasing the ease of identifying mixed stains of semen and blood.

P4. What Came First, the Crime or The Egg? Analyzing Necrophagous Insect Eggs by DART-HRMS for Species Identification

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Forensic entomology focuses on the use of necrophagous insects to inform investigators of the time elapsed since death, or post-mortem interval (PMI). Blowflies are often used to determine the PMI since they are typically the earliest arrivers and due to the well-known correlation of their colonization of human remains to the various stages of decomposition. It is crucial that the insect species are accurately identified to correctly utilize their lifecycle for this prediction. This is challenging and laborious because species identification of juvenile fly life stages is difficult due to the fact that multiple species have similar appearances. For this reason, it is common practice to identify species by rearing the juvenile life stages to adulthood in order to base the assessment on the gross morphological feature characteristics of the adult specimens. This is time consuming, requires specialty laboratory resources and entomological expertise.

Described here is the development of a method for determination of the species identity of blow fly eggs based on their chemical fingerprints attained by direct analysis in real time-high resolution mass spectrometry (DART-HRMS). Eggs from various species within the blowfly Calliphoridae family, such as Calliphora vicina, Lucillia illustris, and Cyonoma cadaverina were collected and suspended in aqueous ethanol. The suspensions were analyzed via DART-HRMS, producing unique chemical profiles. The chemometric processing of the ethanol suspensions revealed interspecies differences and intraspecies similarities amongst the samples. The application of Kernal Discriminant Analysis (KDA) to the DART-HRMS data enabled species identification with an accuracy of 99.18%. Subsequent research aims to create a database of DART-HRMS chemical profiles for blowfly species eggs that can be utilized by law enforcement for species identification of entomological evidence, thereby increasing the evidentiary value of this underutilized insect life stage.

P5. Manifestation of TASER drive stun burn marks on fabric

Hannah Rufo, University of Toronto, Mississauga; Eugene Liscio, Wanying Cao, Yu Ran Zhou, Corrin Doucette, University of Toronto, Mississauga

The TASER® is a type of conducted energy weapon (CEW) used with increasing frequency by law enforcement to subdue subjects in circumstances where compliance is necessary. When operated in the drive stun method of deployment, the electrodes at the head of this CEW are intended to make direct contact with a surface, generating

heat and light which may result in burn marks as a by-product of the electrical discharge that occurs. This research aims to tackle a crucial gap in CEW research that fails to address the appearance of burn marks on fabrics. A drive stun duration (DSD) of 1, 3, and 5 seconds was used with three TASER models (X26P, X2, & TASER 7) on three fabrics (white 100% cotton, 100% polyester, 35:65 cotton-polyester blend) with an underlying backing of pork hock. Using a Keyence VHX-6000 confocal microscope, high magnification images were taken to observe any qualitative changes to the fabric. On polyester fabric, with increasing DSD, darker brown discolouration occurred. Additionally, on polyester fabric, the spatial orientation of the burn marks corresponded with that of the electrodes at the muzzle of each TASER model. These features enabled the correct identification of the TASER model and DSD on polyester fabric in the blind tests performed. Evidence of burn marks on cotton and blend fabrics were both limited and inconsistent such that no features were sufficiently unique to link them to any TASER model or DSD.

P6. Development of a spectroscopic screening tool to determine optimal sampling sites for DNA recovery from human skeletal remains

Kathleen Smith, University of New Haven; Cody Silverman, University at Albany, SUNY.

Forensic experts estimate the number of unidentified dead in the United States to be between 40,000 and 60,000. Numerous challenges exist with forensic genetic testing of human skeletal remains due to diagenesis patterns in bone microstructure, DNA degradation, and the presence of PCR inhibitors. Diagenesis is the microscopic breakdown of the bone matrix, which consists primarily of mineralized calcium hydroxyapatite and collagen. The process of diagenesis occurs in a heterogeneous, non- uniform manner along the diaphysis of a long bone, and determining the region with the most intact bone microstructure is not possible with the naked eye. Therefore, taking cuttings from the diaphysis for DNA testing is a blind process, and decades of research and casework have demonstrated that differences in DNA recovery do exist between cuttings along the shaft of the same long bone. An additional consideration is that forensic genetic testing of bones is a time-consuming and labor- intensive process. Development of an effective screening method to determine the optimal sampling site(s) on the diaphysis could reduce time, labor, costs, and the degree of destructive sampling to obtain a DNA profile. This approach could help maximize DNA recovery and improve success rates in unidentified human remains (UHR) investigations.

Non-destructive Raman spectroscopy could serve as a reliable screening tool to obtain information about bone microstructure and stage of diagenesis which, according to previous research, often correlates to the quantity and quality of endogenous DNA within that region of bone. In the first phase of this research, Raman spectroscopy was evaluated for its effects on known quantities of human DNA extracted from buccal swabs. This step was implemented to determine if exposure to the Raman laser would damage endogenous DNA, which would preclude the use of spectroscopy in genetic casework involving human skeletal remains. Additionally, a fresh non-human (mammal) bone was scanned to serve as a reference for high quality (non-degraded) bone microstructure. In the second phase of this research, Raman spectroscopy was used to scan various pre-marked sections of the diaphysis of long bones from three sets of human skeletal remains with varying post-mortem intervals (9 months, 5 years, 50 years). Compositional analysis of each scanned section provided information about degree of diagenesis within the bone microstructure. The scanned regions of each long bone diaphysis were subsequently sectioned with an autopsy saw (Mopec), pulverized into fine powder using liquid nitrogen and a SPEX SamplePrep 6770 Freezer/Mill, and the associated bone powder fractions were then extracted for DNA. DNA extraction from buccal swabs and bone powder were performed using the QIAamp™ DNA Investigator Kit (Promega) and a modified organic extraction method, respectively. Total DNA recovery and a Degradation Index (DI) were determined using the Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio™ 5 Real-time PCR System (Thermo Fisher Scientific). Data on both DNA quantity and quality were compared to the Raman spectroscopy data to evaluate the correlation between bone diagenesis and DNA recovery.

P7. Investigation and Quantitation Using Ultraviolet-Visible Spectrophotometry of the Products of the 4-Aminophenol Reaction with Cannabinoids

Juliet Pearsall, Cedar Crest College; Marianne Staretz, Ph.D., Cedar Crest College; Matthew Wood, Ph.D., ABC-GKE, Ocean County Sheriff's Department; Jeanne Berk, Ph.D., Cedar Crest College

Delta-9 tetrahydrocannabinol (THC) and cannabidiol (CBD) are two major cannabinoids often derived from the plant Cannabis sativa L. Uses of cannabinoids range from recreational drug use to medical uses. A new test being utilized by law enforcement and forensic science analysts is the "Swiss test" or the 4-aminophenol (4-AP) test which is a presumptive color test used to determine if a sample potentially contains THC or CBD which is important in distinguishing a legal hemp sample from a marijuana sample. In the current study, a visible spectrophotometric analysis of the products of various cannabinoids with 4-aminophenol was performed. The wavelength maxima for the products of the 4-AP reaction with delta-9 THC, CBD, cannabinol (CBN), delta-8 THC were 650 nm, 525 nm, 685 nm, and 650 nm, respectively. The kinetics of the reaction was studied at a THC concentration of 159.0 μM and the time required for maximum formation of product was observed to be 15 minutes. Most of the other cannabinoids had a similar kinetic profile. The formation of products was found to be linear with increasing concentration of starting cannabinoid for all cannabinoids. Standard curves were generated using the absorbance of the 4-AP reaction products at the respective maximal wavelengths using a concentration range of 1.590 μM to 159.0 μM for CBD, 1.590 μM to 119.2 μM for both delta-8 and delta-9 THC, and 1.611 μM to 80.54 μM for CBN. The ability to quantify THC in the presence of CBD using visible spectrophotometric analysis of the 4-AP product was also investigated. Delta-9 THC and CBD were combined at the following ratios (THC: CBD) 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, and 1:10. THC was effectively quantitated at each ratio using the maximum absorbance of the THC/4-AP product with biases ranging between -7% and 22%.

Keywords: Cannabinoids, 4-Aminophenol, Ultraviolet-Visible Spectrophotometry

P8. The Importance of a Comprehensive Raman Spectral Library for the Identification of Minerals in Soil

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Raman spectroscopy is a valuable tool for elucidating the chemical structure and more of an unknown sample. A comprehensive collection, or library, is required for the proper identification of any material. Searchable spectral libraries have demonstrated value for the identification of a plethora of forensic samples, such as drugs, organic pigments and polymers. Mineral analysis is another opportunity where a comprehensive searchable Raman spectral library could aid in the identification of samples for both geological and forensic purposes. However, while there have been several collections of mineral spectra created, there remains to be one comprehensive searchable Raman spectral library. Programs like KnowItAll currently only have a few hundred profiles, and other databases like RRUFF do not have a capability to compare an unknown sample to the library, thus a comprehensive database is required. Another important consideration is the challenge associated with the natural variations within a mineral variety which can cause spectral differences. This research aims to create a comprehensive spectral library of minerals that addresses these issues and also is evaluated for its ability to accurately identify samples from a known set of 60 common soil minerals.

P9. Analysis of Kratom using High Performance Thin Layer Chromatography Coupled with Surface Enhanced Raman Spectroscopy

Ana Monogan, Cedar Crest College; Marianne Staretz,, Cedar Crest College

Kratom is an herbal substance, derived from the leaves of the tree Mitragyna speciosa, which can produce opioid-like effects and is currently legal in the United States. Given the increasing use of Kratom products, there is a growing need for methods that can be used in the analysis of these products. Thin layer chromatography (TLC) has often been applied to the screening of substances of abuse with high performance TLC (HPTLC) offering improved resolution, reproducibility, and automation over traditional TLC methods. Raman spectroscopy has also been used to aid in the identification of substances of abuse. Surface-enhanced Raman spectroscopy enhances the Raman scattering of molecules on particular surfaces leading to greater sensitivity. The current research investigates the use of HPTLC in combination with surface enhanced Raman spectroscopy for the analysis of Kratom samples. Kratom components were separated by HPTLC, sprayed with a gold colloid solution, and then Raman spectra were collected and compared to standards. The HPTLC separation was performed using a 95:5 acetone:methanol solvent system. The gold colloid solution was synthesized by adding 48.5mg gold (II) chloride hydrate ($HAuCl_4$) in 100mL of ultrapure water, heating to 85°C, and adding 10mL of a 1% trisodium citrate solution. The reaction was stirred until the solution turned from spring yellow to wine-red. The reaction was then removed from heat and placed in an ice water bath. Standard Raman spectra were created by running standard 1 mg/mL solutions of the five main components of Kratom (mitragynine, 7- hydroxymitragynine, speciociliatine, speciogynine, and paynanthenine) on the same type of HPTLC plate used for separation. This combination of HPTLC and SERS shows promise in the identification of Kratom products.

P10. The Effect of Degradation on IrisPlex SNPs

Maria Gruber, University of New Haven; Dr. Heather Coyle, University of New Haven

The information from short tandem repeats (STRs), the current standard forensic DNA typing technology, does not always result in identification. In these cases, there are other informative markers in DNA that can be used to help generate investigative leads, one of which is single nucleotide polymorphisms (SNPs). One of the uses of SNPs is for phenotype determination. Among the phenotypic characteristics that can be predicted is eye color. A commonly used tool for eye color prediction is the free, online statistical prediction model IrisPlex, which includes 6 SNPs known to control eye color.

While SNPs can be useful, they, like all DNA, are susceptible to degradation. Research on the effect of degradation on SNPs has already been done, but much of it uses massively parallel sequencing (MPS), which is not currently feasible for most crime labs. A stronger consensus is needed regarding the value of SNPs with instrumentation and techniques currently available to crime labs, such as real- time PCR. The ultimate goal is to test IrisPlex SNPs on teeth samples and ancient human remains from the Gobi Desert.

Because SNPs are such small fragments, they will be highly resistant to the effects of degradation. Buccal swabs from 3 donors were degraded using two methods: UV exposure and burial in soil. For the UV exposure samples, swabs were put in a UV crosslinker with an energy level of 120 $\mu J/cm^2$ for 1 min, 5 mins, 10 mins, 15 mins, 20 mins, and 30 mins. For the soil burial samples, swabs were buried 2 inches deep in individual containers of potting soil. Temperature was recorded and a controlled amount of water was added each week. The soil pH was kept within 1 unit of the original soil pH. Samples were buried for 7 days, 14 days, 30 days, 60 days, and 90 days. The DNA was extracted and quantified, and the degradation index (DI) was assessed. So far, the UV exposure samples follow the expected pattern of decreasing DNA quantity and increasing DI with longer exposure times. The DI roughly doubled with every 5 min increase between 5-20 mins. The soil samples show sharply decreasing DNA quantity with increasing time, compared to untreated control swabs.

The current sample extracts are being used with custom SNP assays to generate allele data for all 6 IrisPlex SNPs. For the UV exposed samples, the allele calls were consistent throughout the 0-30 min exposure times for the OCA2 and LOC105370627 SNPs. The SNP assays were not hindered by the degradation caused by UV exposure for these time intervals. The additional SNP assays are in progress.

The resulting SNP data will be input into IrisPlex to generate predictive eye color estimates, which will be assessed for accuracy and compared within each degradation method and between similar degradation indices. Ultimately, the goal is to develop a predictive strategy at the quantitation step for success in SNP assays for extreme samples such as those recovered from human remains.

P11. Forensic Analysis of 3D Printed Polymers Using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

Jenna Covey, University of New Haven; Dr. Brooke Kammrath, University of New Haven; Dr. Brian Musselman, Ionsense, Inc.; Dr. Maria-Isabel Carnasciali, University of New Haven

Additive manufacturing, or 3D printing, is a burgeoning industry with examples of its products existing in all aspects of everyday life including automotive and other mechanical parts, house and bridge construction, medical and emergency equipment, food and pharmaceuticals, and items of aesthetic value (e.g., jewelry, clothing, and shoes). As 3D printer technologies continue to evolve with concerted improvements in quality and decreased costs, its ease of access for providing parts for nefarious endeavors has been exploited. A variety of 3D printed parts have been used in criminal activities, including firearm components, knuckle dusters, pipe bomb components and ATM skimmers. In order to assess the evidentiary significance of 3D printed materials, the nature and variability of polymer materials used in their assembly must be investigated and understood.

The goal of this research was to evaluate the ability of Direct Analysis in Real Time-Mass Spectrometry (DART®-MS) for the source identification and discrimination of polymers used in the manufacturing of 3D printed objects. DART®-MS is a rapid, non-contact and non- destructive ambient ionization technique which enables near instantaneous determination of sample composition when paired with a mass spectrometer. DART®-MS is a proven technique for the identification of a range of samples of forensic interest, such as explosives and drugs. There is also concerted interest in evaluating its utility for the identification and discrimination of other materials, including ink, paint and polymer fibers. DART®-MS has been demonstrated to differentiate polymers from different manufacturers due to its ability to detect a diversity of its complex components. A variety of chemical additives (e.g., dyes, pigments, UV absorbers and plasticizers) may be added to a polymer product in order to produce a certain chemical or physical property of the final material. Although it is known that differences in chemical components of polymers exists between manufacturers, analytical techniques which may be uses for their discrimination need to be evaluated. In particular, this research focused on understanding the advantages and limitations of DART®-MS as it applies to the brand classification and source identification of 3D printed objects made using polylactic acid (PLA). PLA filament spools of different colors within the same brand and also different brands were analyzed using DART®-MS. Intra-sample variability was assessed for pre-manufactured samples through analysis at several locations along the filament. To evaluate brand classification and source identification, seven different colors of PLA filament from three different manufacturers were analyzed by DART®-MS. Last, the ability to associate a 3D printed part to an unused spool of polymer was assessed through a comparison of the DART-MS analysis of a PLA polymer filament and its post-3D printed part. It was ultimately concluded that DART-MS is a rapid and reliable tool for the forensic analysis of 3D printed PLA polymers which provides chemical information that can be used for its classification and discrimination.

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P12. Evaluation of successive DNA extractions from different types of swabs

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DNA evidence has been incredibly useful to law enforcement for the identification and conviction of criminals as well as the innocence of others. Many cases rest on the ability of the DNA evidence to clearly and decisively determine to whom the DNA belongs to. Samples at crime scenes can be collected in different ways, and the use of swabs to collect biological samples for DNA analysis is a current practice. For decades, the cotton swab has been an important device for collecting biological samples since they are inexpensive and can be applied on several items and surfaces. Alternative swab types, such as nylon-flocked swabs, have been developed and applied for DNA sampling. Comparisons of the recovery efficiencies of different swab materials have shown diverging results depending on the sample and on the surface or item. But independently of the type of swab chosen for sample collection, crime labs face a challenge in regards to swab storage or swab discard after DNA extraction. In many crimes, only one or a couple of swabs are collected due to the lack of biological material available for collection at the crime scene. Once a sample is collected, the DNA is extracted and it can often yield low DNA amounts, which may not be enough for use in different DNA tests done at the crime lab. In order to increase the DNA yield of a swab and subsequently increase the probability of there being enough DNA for all the necessary tests, the swab should go through the extraction process multiple times. The goal of this study was to investigate the success rate of obtaining good DNA yields from previously extracted swabs. Buccal cells were collected from volunteers using cotton and flocked swabs. To mimic case-type samples, two volunteers drank from the same type of coffee cup and the cups were swabbed with different types of swabs. Genomic DNA was extracted from samples using only the QIAamp® DNA Investigator Kit (Qiagen, CA), or using this kit in combination with the Investigator Lyse&Spin Basket Kit (Qiagen, CA). Each swab went through the process of extraction four different times. DNA samples were amplified using the GlobalFilerTM PCR Amplification Kit (ThermoFisher Scientific, MA) and the amplification products were separated on a SeqStudio Genetic Analyzer (ThermoFisher Scientific, MA). According to the results, both types of swabs were able to produce complete DNA profiles for buccal swab samples up to the third extraction, and for some even up to the fourth extraction, independently of the type of extraction method used. As for case-type samples, both flocked and cotton swabs generated complete DNA profiles up to the second extraction, and most up to the third extraction. Our results showed that it is possible to obtain complete profiles from multiple extractions of the same swab, and that this technique is incredibly advantageous for forensic scientists since it increases the amount of DNA evidence obtained from swabs and that is available for multiple genetic tests.

Keywords: Cotton swab, Flocked swab, DNA extraction, DNA profiling

P13. The Detection of Backspatter Bloodstains on Horizontal Surfaces at Different Heights

Nicole Millis, University of New Haven; Dr. Peter Valentin, University of New Haven

Bloodstain pattern analysis (BPA) is the interpretation of the shape, size, distribution, location, and appearance of bloodstains. BPA can provide information on the location of blood sources at the time of events at a scene, which can assist in crime scene reconstruction. There is an inverse relationship between the energy in a bloodletting event and the size of the droplets produced during that event. The blood is broken into many sub-millimeter droplets in

high- energy events, such as gunshot wounds. While the diameter of the droplets can indicate the force used in the event, the location can provide information about the position of the person when they were shot.
Two types of spatter stains that can be produced from a bullet striking a person are forward and back spatter. Backspatter travels in the opposite direction of the bullet and originates from the entrance wound. Forward spatter, which travels in the same direction as the bullet, only occurs when there is an exit wound, so it is not always produced.

The sub-millimeter size of these droplets cannot travel far and, generally, can only be seen when there is a surface near the injury for the droplet to land on. But what if the shooting occurs in a location with no surface close enough to the wound for the blood to deposit?

This research examines whether it is possible to locate these characteristic bloodstains land on the floor by being visualized with blood-detecting reagents. If so, can the blood be visualized while preserving its characteristic size and shape so it can be recognized as back or forward spatter stains and not the other types of bloodstains that might be seen on the floor at the scene of a shooting? If the research yields useful results, we hypothesize that the visualization of these characteristic spatter stains could be used to indicate the position of a shooting victim in a scene.

P14. Rapid quantification of 65 drugs in biological fluids by QSight UHPLC/MS/MS
Jacob Jalali, Perkin Elmer; Cole Strattman, Marc Elie, Perkin Elmer

The main objectives of this work were to develop a rapid LC/MS/MS method for the separation and detection of 65 drugs in urine and blood, and to evaluate the selectivity, linearity, and sensitivity of the QSight® 420 LC/MS/MS system. In this work different categories of drugs such as opiates, benzodiazepines, barbiturates, THCs, amphetamines, antidepressants, stimulants, and their metabolites were determined. Analysis of drugs has always been a challenge due to the number of target analytes, matrix effects and heavy ion suppression. Achieving proper detection limit, quantitation limit, recovery, accuracy, precision, stability of analytes and being able to hit the regulation's LOQs in matrix, are some of the analytical challenges in the analysis of drugs in biological fluids. In this study, blank urine and blood were spiked with all the drugs and deuterated internal standards. Urine samples were then diluted 25-fold with LCMS grade water and blood samples went through a simple protein precipitation using 1/10 Acetonitrile then transferred to HPLC vial for LC/MS/MS analysis. A mix of 20 deuterated internal standards were used across the run for correction of any matrix effect. PerkinElmer SPP Biphenyl HPLC column (50x2.1mm, 2.6um) was used for separation of the drugs. Ionization on Mass spectrometer is achieved with Electrospray ionization. Standards used for this analysis were from Cerilliant. Mobile phases used for this method were 0.1% formic acid & 5mM ammonium acetate in water for A and Acetonitrile for B channel.

P15. Automated Sperm Identification Using MetaSystems Metafer Imaging System
Itunu Alao, Boston University; Boston University; MetaSystems; Caitlyn Taveira; Amy N. Brodeur

Many crime laboratories across the country face a backlog of sexual assault cases awaiting to be processed. Microscopic visualization of sperm cells is a time consuming but important part of sexual assault evidence examination. This study evaluated an automated scanning, imaging and analysis system for its ability to recognize spermatozoa. The Metafer system (MetaSystems Medford, MA) includes a compound microscope with a motorized stage, high resolution digital camera and a software platform that uses specialized algorithms (classifiers) to recognize and group objects of interest.

Slides were prepared using dilutions of human semen and various combinations of buccal cells, yeast, bacteria, mold spores and soil particles. Contaminants were included to mimic difficult casework samples and challenge the limits of the software. Two microscopic staining techniques (Christmas Tree stain and Hematoxylin and Eosin) were applied to each sample type prior to examination. Up to 8 slides were scanned and analyzed at a time. All objects identified by the software as a possible sperm candidate appeared as thumbnail images that included a quantitative value correlating to the relative strength of the classification. A subset of the images was reviewed by the researcher to assess accuracy of the classifier. The slides were subsequently examined using traditional microscopy while blinded to the contents of each sample to compare hands-on time for the user with each technique as well as the occurrence, if any, of false negative results.

Results showed that an artificial intelligence-driven forensic sperm cell detection microscope can significantly reduce the time it takes to find sperm cells and estimate sperm cell quantity compared to a lengthier and more tedious manual search. This method also allows an accurate quantification of the number of sperm cells present in a sample, which can inform downstream DNA testing. Additionally, the Metafer system was successful in sperm cell identification amid different cell types and contaminants which often causes difficulties during a manual search.

P16. Enzymatic Assay Development for SAM-dependent Phenylethanolamine N-Methyl transferase activity in Human S9 cytosol fraction

Mackenzie Pavlik, Department of Forensic Science, University of New Haven; Abby Veeser, Department of Forensic Science, University of New Haven; Robert H. Powers, Ph.D. Department of Forensic Science, University of New Haven

The N-methylation of amphetamine (AMP) yielding methamphetamine (MAMP), is chemically analogous to the N-methylation of norephedrine, a biosynthetic step in the formation of ephedrine, catalyzed by phenylethyanolamine N-methyltransferase (PMT) with S-adenosyl methionine (SAM) as the methyl donor. This enzyme was shown to be functional with a number of neurotransmitters and analogue substrates (e.g. AMP) by Axelrod in 1962. However, the methylation of AMP to MAMP is not generally thought to play a significant role in the metabolism of AMP, for which the primary metabolic pathways involve either ring or C1 hydroxylation, or oxidative deamination, yielding 4-hydroxyamphetamine, norephedrine, or phenylacetone, respectively.

Methamphetamine (MAMP) is relatively rapidly metabolized in the body via N-demethylation, yielding amphetamine (AMP). This pathway is well recognized as the basis for the appearance of AMP in biological fluid and hair samples of individuals using MAMP as a recreational drug, and AMP is an expected finding in such cases. In contrast, there is no expectation that individuals either abusing, or receiving AMP for therapeutic purposes (e.g. as treatment for ADHD) will generate any significant levels of MAMP, and the appearance of that species in drug abuse monitoring samples is routinely presumed to reflect MAMP abuse, often with significant medical or legal consequences. We have hypothesized however, that some individuals receiving therapeutic AMP (e.g. Adderall) may generate low levels of MAMP, as a function of an equilibrium between formation from AMP via PMT, and the reverse demethylation reaction. We expect the equilibrium between AMP and MAMP is determined by both the steady-state concentration of AMP, and product inhibition of PMT by MAMP.

As an initial step in exploring the relationship between the N-methyl and -demethylation reactions, we have developed a method for the n-methylation of AMP analogs in human S9 based on Axelrod's 1962 study on Serotonin. We chose to utilize norephedrine, the C1-hydroxy analogue of amphetamine, as the basis our experiments.

Enzyme assays were completed in 1mL aliquots with a 200mM Trizma buffer at pH 7.6 and consisted of 200L S9 (Sigma), 15L 20mg/mL norephedrine, and 350L 55mM s-adenosylmethionine (SAM; Sigma). Enzyme incubations were performed at 37°C and were stopped at 0 and 60 minutes via the addition of 300 L 1M pH 9.0 Trizma buffer and 100 L 0.5g/mL KF. The solution was then extracted 3 x with 0.5mL EtOAc. The mixture was centrifuged, and the supernatant organic phases were combined and evaporated to ~ 100 L under N2 and analyzed by GC/MS for ephedrine. Our result indicated that N- methyl transferase from human S9, normally functional in neurotransmitter synthetic pathways, is capable of generating MAMP from AMP in this incubation system.

Axelrod, J. (1962). The Enzymatic N-Methylation of Serotonin and Other Amines. Journal of Pharmacology and Experimental Therapeutics, 138(1), 28–33.

P17. Differentiating Human and Canine Saliva through the Genetic Expression of AMY1 and AMY2

Nancy Lay, Cedar Crest College; Lawrence Quarino, K. Joy Karnas, Cedar Crest College

Body fluid identification provides crucial context to crime scene reconstruction. Saliva is often found in criminal investigations and its presence can be used to corroborate witness or victim statements. As a biological fluid, saliva is also likely to contain DNA that can be analyzed to potentially generate a DNA profile. Non-human biological material is forensically relevant due to canine bite-related cases, the transfer of pet or wildlife biological material, and crimes involving animals. At least in some cases, bitemark analysis may not be able to reliably differentiate human from canine bitemarks using morphological analysis. Most methods for saliva identification rely on the detection of amylase, an enzyme involved in digestion. There are two forms of amylase that exist: salivary amylase and pancreatic amylase. Salivary amylase is expressed in human saliva, but this enzyme is not observed in canine saliva. However, both humans and canines express pancreatic amylase. AMY1 and AMY2 are the genes that code for salivary amylase and pancreatic amylase, respectively. No DNA-based method currently exists to differentiate canine saliva from human saliva, despite the potential of encountering non-human biological fluids in a forensic setting. A DNA-based method would offer advantages over current protein- based saliva tests due to the fact that DNA is more stable than proteins, which is critical when it comes to the degradation of biological material at a crime scene. False negatives may occur because of the inability to detect a degraded enzyme, and not due to the actual absence of saliva itself. A DNA-based method also offers the potential for multiplex PCR. In this preliminary study, the sequences of each amylase gene were aligned between the two different species in order to identify regions of interest to target when designing primers. Primers were designed using information obtained from literature searches, as well as from the data generated by the sequence alignments of AMY1 and AMY2 for canines and humans. Differences in the amplicon sizes of the PCR products generated from these designed primer sets were used to distinguish saliva between the two species of interest.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2022 Annual Meeting

Educators' Forum Abstracts

ForensicXR: Creating a Virtual Lab

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Recreating a crime scene can be very challenging as every single detail can be important in understanding what took place. Hence investigators take many pictures and make very detailed notes. However, no matter how detailed the recreation, it can never replace the actual experience of being in a real scene. However, it is still important to come as close as possible to recreating that experience in a safe environment. For investigators and jurors, this allows them to explore the crime scene without worrying about accidentally altering it. For students and educators, the repeatability and lessons that can be learned are vital for providing a consistent and complete education. However, in many cases, it is difficult to recreate a scene in such a manner, oftentimes due to a lack of space, time, or human resources, even more if the organization intends to create several mock crime scenes. To solve these issues, our team has developed an extended reality application - ForensicXR. ForensicXR is an educational platform that supports crime scene processing in virtual or augmented reality environments. Both options provide a consistent and repeatable view of a recreated crime scene. The scene is either a 3d-scanned scene or a scene recreated using accurate measurements from a 3D scan.

These scenes can either be viewed in virtual reality, which entirely replaces the surroundings with the recreated scene, or in augmented reality, which overlays virtual objects and interactions over the existing surroundings. The application mirrors many of the actions that take place in a real investigation: taking pictures of the scene, lifting fingerprints, collecting and labeling evidence, and communicating with other departments and labs via a virtual tablet interface. Each scene contains a briefing and debriefing, which is stored on a database alongside the scene, allowing the application to scale and accommodate a variety of different scenarios. The system is a work in progress as we are still developing the tools required for scene processing, the database structure required to support each scene, and the educational components required to support efficient and successful learning for future investigators. In the future, we plan to perform a series of usability studies to help develop an effective and successful extended reality application. These usability studies will cover, among other things, how to best train people in using the application, and how well different types of scenes (car crash, arson, theft, murder, etc) can be represented in VR and AR formats.

Visual Communication in Undergraduate Forensic Science Courses

Taylor Hopkins, Duquesne University, Kelly Martin, PhD, Rochester Institute of Technology

Educators are tasked with creating a curriculum that uses effective strategies to promote student engagement and learning to prepare them for the workforce and beyond. The use of visual communication in higher education is crucial to ensure instructors are conveying information that students can effectively and efficiently comprehend by offering a variety of learning formats.

Education in STEM disciplines, including forensic science, often follow lecture-style classes and laboratory courses that may use slideshows and procedural handouts to relay information. However, visual communication goes beyond including images in a lecture or handout. The goal of this multi- part project was to discover innovative ways that visual communication was being utilized in pedagogical decisions by undergraduate educators. For this portion of the project, instructors from the forensic science discipline were solicited via email to share visual teaching practices and assignments used in their courses. Data was collected through two different methods: by virtual interview or completion of a survey form, with participants choosing their preferred method of data collection.

Both formats asked instructors to describe their teaching practice or assignment, the motivation behind its development and implementation, and how they felt it has been beneficial to their students. The data presented common themes across the responses of the various educators that provided insight on how visual communication can be implemented at the undergraduate level. Ultimately, this project will contribute to a multi-disciplinary guidebook for university educators to showcase unique visual techniques to encourage continued innovation in higher education courses.

Scientific Criminal Investigation Education for Law Enforcement Officers
Dennis Hilliard, RI State Crime Laboratory, University of Rhode Island

The Rhode Island State Crime Laboratory in conjunction with the University of Rhode Island's Feinstein College's Office of Strategic Initiatives, has been diligent in providing training opportunities to the Forensic Science, Law Enforcement and Public Safety Communities. The training
includes: crime scene processing, blood spatter interpretation, trace evidence collection, latent print processing, firearms examination, and crime scene reconstruction. It is important to the criminal justice community that opportunities for professional development in these areas are continuously being offered.

The primary training opportunity for Law Enforcement Officers is a program that started nearly seventy years ago within the University's Department of Chemistry through the efforts of Harold C. Harrison, Ph.D. a professor of Chemistry and the director of the Laboratories for Scientific Criminal Investigations, which eventually became the RI State Crime Laboratory.

The presentation will explore the genesis of this course offered by the State Crime Laboratory at the University of Rhode Island; its development over the course of the past seventy years; and how it is presented currently as a credited undergraduate two semester course. It will discuss how the elements of the current course were designed to meet the requirements for applying for and qualifying for the basic Crime Scene Investigator Certification (CCSI) offered by the International Association for Identification (TheIAI.org).

Being Learner-Centered: Moving Toward Equity in the Classroom
Yadilette Rivera-Colon, Ph.D., Bay Path University

Diversity, equity, inclusion and belonging (DEIB) work is essential in all fields. In education, making physical and remote classes accessible to all students leads to greater success in achieving learning outcomes. Moving from awareness to action is a difficult but necessary step. Steps toward action may not only be challenging, but also confusing. In this session, participants will learn about the use of equity audits to reveal areas of inequity and how to plan for improvements as a next step in the DEIB road. We will discuss how to apply equity audits in education and how these findings can lead to programmatic equity, quality equity and ultimately move us closer to learner-centered practices.

Northeastern Association of Forensic Scientists
Proceedings of the
October 2022 Annual Meeting

Author Index

The presenting author index can provide a quick reference to find when and in what section presenting authors were scheduled to present at the 2022 Annual Meeting. The reference table below assists you in finding the section in which the presentation was given. Letters correspond to the scientific discipline/section in which the presentation was made while the number corresponds to the page number of the abstract in this booklet.

A Forensic Toxicology
B Trace Evidence/Fire Debris & Explosives
C Criminalistics/Crime Scene & Digital Evidence
D Drug Chemistry
E Forensic Biology/DNA
F Poster Session
G Educators' Forum

Author Last Name

Northeastern Association of Forensic Scientists
Proceedings of the
October 2022 Annual Meeting