Northeastern Association
of
Forensic Scientists

Proceedings
of the Northeastern Association of Forensic Scientists
November 2019 Annual Meeting

Contents

Northeastern Association of Forensic Scientists

Proceedings of the

November 2019 Annual Meeting

Forensic Toxicology Abstracts

A Comprehensive Approach to Targeted and Untargeted Screening Methodology for Emerging Synthetic Fentanyl Analogues using High Resolution Accurate Mass Spectrometry in Forensic Toxicology

Julie Cichelli, Agilent Technologies, Wilmington, DE

In a rapidly changing environment with new synthetic drugs appearing almost weekly, there is an analytical need to confidently identify these analytes in a timely manner. The use of high-resolution accurate mass technology coupled with a novel sample preparation using Agilent's Captiva EMR-Lipid allows for highly specific, selective and comprehensive screening for the identification of these emerging fentanyl analogues in serum matrix. Captiva EMR -Lipid provides highly selective and efficient lipid/matrix removal without unwanted analyte loss. The novel EMR-Lipid technology removes lipids based on a combination of size exclusion and hydrophobic interaction. Effective lipid removal assures minimal ion suppression of target analytes, which significantly improves method reliability and ruggedness.

This novel sample preparation approach allows for lower limits of detection and quantitation as well as better linearity across the entire dynamic range. Immunoassay-based techniques have historically been the methods of choice for drug screening. Positive presumptive drug screen results are reflexed to more specific, confirmatory testing using gas or liquid chromatography coupled to mass spectrometry. False positives and false negatives with immunoassay techniques are common problems that have substantial down-stream consequences for inaccurate results, laboratory operations, and total costs. Thus, the use of high-resolution accurate mass liquid chromatography mass spectrometry (LC-QTOF-MS) is ideally suited for rapid analysis of emerging drugs without the drawbacks associated with legacy techniques and methodology. Herein, a targeted and untargeted screening workflow by LC/MS/MS using Agilent's 6546 QTOF will be presented for approximately 150 fentanyl analogues.

Developing a Raman Microspectrophotometric Method to Quantitate Carboxyhemoglobin

Haley Melbourn1 , Marianne Staretz, Ph.D1 ; Heather Maldonado, M.S2 ; Thomas Brettell, Ph.D., D-ABC1 1Cedar Crest College, Allentown, PA2 State of Delaware Division of Forensic Science, Department of Safety and Homeland Security, Wilmington, DE

Carboxyhemoglobin (COHb) quantitation is a routine toxicological analysis performed in cases of acute carbon monoxide poisoning. Quick and easy to use methods of COHb quantitation include spectrophotometry and CO-oximetry, although these methods can be inaccurate and imprecise both at low concentrations and in putrefied post-mortem specimens. More reliable gas chromatographic quantitation methods are available as well, but these require extensive sample preparation and therefore require elevated use of consumables and increased analysis time. The goal of this study was to develop a Raman microspectrophotometricCOHb quantitation method and assess its precision and accuracy in estimating the COHb concentration of ten experimental unknown samples.

A Thermo Scientific DXR2 Raman Microscope equipped with a 785 nm laser was used to determine the COHb concentration of the samples analyzed for this study. Stock solutions of 0% and 100% COHb were prepared by bubbling ultra-zero air and carbon monoxide, respectively, through whole human blood (healthy living donor, gender not specified) containing K2EDTA anticoagulant for thirty minutes. A total of eight calibrator sets ranging from 0-100% COHb at 10% increments were prepared via appropriate dilution of the stock solutions. These calibrators were deposited in 20-µL aliquots onto aluminum foil-covered microscope slides and subsequently dried in a fume hood for at least one hour. A representative Raman spectrum of each sample was generated by averaging the spectra collected at ten separate locations within the sample. All spectra were preprocessed in the OMNIC for Dispersive Raman software (version 9.8.372) using a sixthorder polynomial fluorescence correction.

A strong linear correlation (Average R2=0.98) was found to exist between COHb concentration and the ratio of the peak intensities at approximately 1552 cm-1 and 1580 cm-1 . From the eight calibration curves analyzed, the limits of detection and quantitation were found to be 4.4% and 13.2% COHb, respectively. Intra- and inter-day studies of three calibrators (10%, 30%, and 50% COHb) indicated high precision, with the coefficient of variation ranging from 0.85-4.26%. The accuracy of this method was evaluated through the quantitation of ten experimental unknowns, each analyzed in triplicate. Using the proposed Raman microspectrophotometric method, the true concentrations of seven of the unknowns were within the 95% confidence limits of the target COHb concentrations. The unknowns were

subsequently analyzed using a Cary 3500 UV-Visible spectrophotometer, resulting in only six of the unknowns' true concentrations being within the 95% confidence limits of the target COHb concentrations.

This study demonstrates that Raman microspectrophotometry may be a viable alternative to quantitate the COHb concentration of blood samples. Furthermore, this method is comparable with established spectrophotometric methods while eliminating the need for time-consuming sample preparation; limiting the use of consumables and generation of waste.

Using High-Resolution Mass Spectrometry for DUID Screening and Postmortem Analysis
Oscar G. Cabrices1, Pierre Negri1, Adrian Taylor1, Melanie Stauffer2, Dean Fritch2, Nadine Koenig2, Derrick Schollenberger2, Jennifer Gillman2 1AB Sciex, Redwood, CA, USA. 2Health Network Laboratories, Allentown, PA, USA.

Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. The use of high resolution mass spectrometry (MS) in the forensic laboratory enables toxicologists to rapidly obtain complete chemical profiles from biological samples, which subsequently leads to increased confidence in compound identification through accurate mass information at low analyte concentration.

Here, two comprehensive drug screening workflows for the analysis of forensic Driving Under the Influence of Drugs (DUID) and postmortem blood samples are described using SCIEX X500R QTOF System. These comprehensive drug screening workflows enabled reliable compound fragmentation comparison to library spectra for confident drug identification and retrospective analysis to avoid missing potential drugs present in DUID and postmortem samples. These methods are shown to provide accurate mass information for increased confidence and enabled identification of multiple number of the targeted compounds present in authentic forensic DUID and postmortem case samples in comparison to immunoassay-based screening.

Control whole blood samples were spiked with a stock standard solution mixture and extracted for LC/MS screening to determine the retention times. Forensic case postmortem blood samples were extracted by using a protein precipitation procedure followed by centrifugation; supernatant was evaporated and reconstituted in mobile phase for analysis.

Analytes were chromatographically separated at 30°C using a PhenomenexKinetex phenyl-hexyl (50 x 2.1 mm, 2.6 µm) column. Mobile phases were water and methanol with appropriate additives, 1 mL/min flow rate. Mass spectrometric detection was conducted on a X500R QTOF System operated in positive electrospray mode using SWATH® Acquisition. Samples were evaluated against four main confidence criteria: mass error, retention time, isotope ratio difference, and library score for all compounds.

The implementation of a robust method development process for both workflows resulted in high combined scores for all compounds, showing the robustness and reproducibility of the streamlined workflows. Overall, the developed QTOF-MS screening approach enabled the rapid implementation and optimization of the screening workflow for 159 compounds of interest for confident drug identification and retrospective analysis to avoid missing potential drugs present in postmortem samples. Similarly, it enabled the identification of multiple number of target compounds present in authentic forensic DUID case samples that were not detected in the immunoassay-based screening method.

Feasibility of the Analysis of Fentanyl Analogs in Postmortem Blood Using Biocompatible Solid-Phase Microextraction (BIOSPME) followed by Direct Analysis in Real Time Mass Spectrometry (DART-MS) and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)
Gabriella P. Smith, B.S.1 *, Thomas A. Brettell, Ph.D., D-ABC1 , Chandler M. Grant, M.S.F.S.2 , Nadine Koenig, B.S., M.T, TC-NRCC3 , Marianne Staretz, Ph.D., Thomas Pritchett, M.S.1 , and Brittany Laramee4 . 1Forensic Science Program, Cedar Crest College, Allentown, PA 2Forensic Pathology Associates, Allentown, PA 3Health Network

Laboratories, Allentown, PA 4 IonSense, Inc., Saugus, MA

From 2015 to 2017, there was a 65% increase in drug-related overdose deaths in Pennsylvania. In 52% of these cases, fentanyl and fentanyl-related substances were identified in decedents, with heroin being the second most frequently identified substance. Due to the ease at which these illicitly manufactured fentanyl and fentanyl analogs can be synthesized, it is increasingly difficult for laboratories to keep up with the development of methodology that can detect these compounds in post-mortem samples. The fentanyl epidemic has also caused an increase in post-mortem toxicology casework. Therefore, the development of more efficient techniques for screening post-mortem samples for fentanyl and its analogs would greatly benefit toxicology laboratories.

The purpose of this study is to investigate a new in-vivo biocompatible solid-phase microextraction technique, BioSPME, for the extraction of fentanyl and its analogs from postmortem blood samples. BioSPME was developed as an extraction method which could quickly extract drugs from biological fluids without the binding of macromolecules, which was a concern for previous SPME techniques.

In this study, the feasibility for the analysis of fentanyl and six (6) fentanyl analogs in post-mortem blood was investigated utilizing BioSPME methodology. The method being developed uses both direct analysis in real time mass spectrometry (DART-MS) as a screening method followed by liquid chromatography tandem mass spectroscopy (LC-MS/MS) for confirmation. Both C-18 and mixed-mode fibers were used to extract the blood samples. BioSPME fibers were conditioned, washed and directly inserted into postmortem blood. The fibers were subsequently analyzed directly by DART-MS while separate BioSPME fiber extracts were further washed, desorbed into solution, dried down and reconstituted for analysis by LC-MS/MS. Examples of the analysis results of post-mortem blood extracts from overdose victims will be presented to show the potential of the method in forensic toxicology.

Novel Strategies for EtG/EtS Analysis in Human Urine by LC-MS/MS

Ravali Alagandula, Justin Steimling, and France Carroll, Restek, Bellefonte, PA

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are important biomarkers for monitoring alcohol use. Detecting these metabolites has proven beneficial for abstinence enforcement and zero tolerance treatment programs requiring assessment of alcohol consumption. The analysis of EtG and EtS in urine offers many advantages for abstinence monitoring, including a three-day detection window, good stability, and analytical specificity. However, EtG and EtS are both polar compounds, making them difficult to retain via reversedphase chromatography. Both compounds are also very sensitive to matrix interferences and this analysis is further complicated by the inherently variable nature of human urine from person to person and population to population, differing health states, and a myriad of other sources of variation. Typical LC-MS/MS methods for EtG/EtS analysis have several shortfalls: poor retention and resolution of EtG and EtS from matrix components, long run times that limit sample throughput, and short column lifetimes. These common issues are all related to the characteristics of the chromatographic separation, as well as to some sample preparation considerations and mass spectrometer settings. Therefore, it is difficult to have a single analytical solution to be suitable for the numerous urine sample types. This study describes two strategies: 1) A simple and high throughput dilute and shoot LC-MS/MS analysis of EtG and EtS, and 2) A protein precipitation LC-MS/MS method with reduced formic acid concentration in aqueous mobile phase and longer gradient to improve the resolution and sensitivity for lower detection limits and other helpful techniques for mitigating troublesome matrix effects in human urine by the use of a novel column designed for the analysis of EtG/EtS. Both the strategies are tested in our lab by evaluating linearity, selectivity, sensitivity, matrix effects, accuracy and precision and found to be successful for the quantitation of EtG and EtS in human urine using a Restek Raptor EtG/EtS column. Isobaric matrix interferences are easily resolved preventing issues with peak identification and quantitation in both simple and complex urine samples using the two strategies. A combined strategy of optimized instrument setup, sample preparation, and method conditions will provide greater versatility, excellent sensitivity and resolution from matrix interferences for the analysis of EtG and EtS in urine, which is especially important for laboratories that process very high volumes of highly variable samples.

Fentanyl and Fentanyl Analogues: Practical Considerations for a Successful Assay
Jeremy Smith, Biotage LLC, Charlotte, NC

Fentanyl and fentanyl-related analogues have rapidly come to the forefront of national interest following several high-profile overdose cases. Testing of these drugs has increasingly become a priority in many labs due to the opioid epidemic affecting many cities across both the United States and Canada. While whole blood remains a consistent matrix of choice for testing in forensic laboratories, urine is also an option for targeting these fentanyl analogues. Obtaining optimal analytical results often requires sample preparation to remove endogenous interferences and isolate compounds of interest. Extraction protocols may be simple, such as with dual mode extraction (DME+), or they may also require more complex methods involving solid phase extraction (SPE) with mixed-mode cation exchange sorbents.
Several extraction techniques were optimized for commonly tested fentanyl and fentanyl analogues in whole blood and urine samples by LC-MS/MS. Comparisons were made for recovery, matrix effects, and overall robustness of each protocol. Each protocol was successful in isolating the target analytes, yet each offer different benefits for the laboratory when considering assay objectives and cost-saving efforts.

Human whole blood and urine was spiked with a panel of fentanyl analogues, which were then extracted via dual mode extraction (ISOLUTE DME+, Biotage), supported liquid extraction (ISOLUTE SLE+, Biotage), silica-based mixed-mode strong cation exchange (ISOLUTE HCX, Biotage), and a polymer mixed-mode strong cation exchange (EVOLUTE EXPRESS CX, Biotage). Each extraction protocol was performed in accordance with the manufacturer's recommendations. Chromatographic separation was achieved on a Shimadzu Nexera X2 UPLC coupled to a SCIEX 5500 tandem mass spectrometer for analysis. Recoveries, process efficiencies and matrix effects were determined by comparing the area counts of extracted samples (pre-spiked), post-extraction samples (post-spiked), and unextracted samples (neat, no matrix).

100 µL of urine or whole blood was used for each extraction evaluation. Each sample was diluted with 0.1% formic acid (aq) or 1% ammonium hydroxide (aq) before extraction, depending on the extraction technique. The supported liquid extraction (SLE+) protocol examined three distinct elution solvents in dichloromethane, ethyl acetate, and MTBE. Both the ISOLUTE HCX and EVOLUTE EXPRESS CX extractions evaluated the use of two elution solvent mixtures: 78:20:2 dichloromethane/isopropanol/ammonium hydroxide or 78:20:2 ethyl acetate/acetonitrile/ammonium hydroxide.

Following extraction, the samples were evaporated using a SPE DRY 96 and reconstituted in 50 µL of a 50:50 mixture of 0.1% formic acid in water/0.1% formic acid in methanol. Samples were then analyzed via LCMS/MS.
The recoveries using the EVOLUTE EXPRESS CX and ISOLUTE HCX were the highest of the extraction techniques when using the DCM/IPA/NH4OH elution solvent. However, significant signal suppression was noted in the EVOLUTE EXPRESS CX protocol, specifically with whole blood. The ISOLUTE DME+ pass-through extraction was successful for urine samples, but did result in less clean samples due to elevated matrix effects. ISOLUTE SLE+ provided samples with the least matrix effects, although recovery was lower compared to the SPE methods. Each method proved to have a reliable LOQ down to 0.1 ng/mL.

Detection and Quantitation of Cannabidiol and Δ(9)-Tetrahydrocannabinol in Oral Fluid of a Therapeutic-Use Cannabidiol Donor Using UHPLC-Laminar Flow-MS/MS
Gardner, Jenna1 ; Hwang, Hajin1 ; Boyle, Sarah1 ; Hill, Collin2 ; Caldwell, Mikayla,1Kero, Frank2 ; Botch-Jones, Sabra1
1 Biomedical Forensic Sciences, Boston University School of Medicine, Boston, MA, USA 2 Perkin Elmer, Waltham, MA, USA

Background/Introduction: Cannabidiol (CBD) is one of over 80 active cannabinoids found in Cannabis sativa and is the second most abundant cannabinoid derived from the plant following Δ(9)- Tetrahydrocannabinol(THC). As opposed to THC, CBD does not appear to have any psychotropic effects. Legislation regarding the therapeutic or recreational use of CBD varies, however many individuals use CBD products for management of seizures, anxiety, insomnia, and more.

Objectives: During the extraction of CBD from plant material, THC may be co-extracted. Therefore, screening and quantitating potential THC levels in individuals using CBD products is important in instances where the legality of use of THC does not match that of CBD.

Methods: This project evaluated the detection and quantitation of CBD, THC, and two primary metabolites in oral fluid samples of a therapeutic-use cannabidiol donor using Supported Liquid Extraction (SLE) and subsequent testing by PerkinElmer QSight® 220 CR LC-MS/MS (PerkinElmer, Waltham, MA) in positive 86 Northeastern Association of Forensic Scientists Annual Meeting 2019 Lancaster, PA ionization mode using a PerkinElmer® Brownlee C18 2.1x50mm (2.7 μm) column. A method was developed for the detection and quantitation of THC, CBD, 11-hydroxy-Δ(9)-THC, 11-nor-9-carboxy-Δ(9)-THC, Δ(9)- THC-d3, and 11-hydroxy-Δ(9)-THC d3 (Cayman Chemical, Ann Arbor, MI, USA). All samples, calibrators, and quality controls were prepared by spiking certified reference standards into synthetic oral fluid (UTAK, Valencia, CA, USA). Calibrators were prepared at 1, 2.5, 5, 10, 20, 30, and 50 ng/mL, to evaluate the calibration model and to identify the limit of quantitation (LOQ) and limit of detection (LOD) with quality controls analyzed at 3, 15, and 40 ng/mL. Samples were prepared in 5% glacial acetic acid (Acros, New Jersey, USA). SLE was performed using ISOLUTE SLE+ 1mL columns (Biotage AB, Uppsala, Sweden) with elution in hexane:ethylacetate:MBTE (80:10:10), followed by evaporation. All samples were reconstituted in 100μL of 0.1% formic acid (Sigma-Aldrich, St. Louis, MO, USA) in DI water:0.1% formic acid in acetonitrile (70:30). Validation parameters were assessed using ASB Standard 036-Standard Practices for Method Validation in Forensic Toxicology, including carry over, LOD, LOQ, linear dynamic range, internal standards interferences, and calibration model.

Results: All compounds were quantified using linear calibration models. The linear dynamic range was determined to be 1 to 50 ng/mL with a LOQ of 1 ng/mL and a LOD of 0.5 ng/mL. Carryover was assessed by running a double blank following a sample spiked at 50 ng/mL with no analytes observed. Oral fluid samples spiked with only deuterated internal standards were used to determine any potential interferences and none were observed. Total run time including equilibration was eleven minutes. The donor samples were collected at several timepoints during the oral administration of an 8mg dose of CBD. These timepoints included prior to administration, at the time of administration, 30 minutes post administration, 45 minutes post administration, 60 minutes post administration, 90 minutes post administration, and 120 minutes post administration. CBD was quantified within the samples from below LOD to >50 ng/mL, above the highest calibrator. THC was quantified within the samples from below LOD to 0.7 ng/mL. Metabolites were not detected above the LOD.

Conclusion/Discussion: Overall, the use of laminar flow mass spectrometry was effective in detecting various cannabinoids in oral fluid samples following SLE sample extraction.

Method Validation-A view from behind the scenes.

Anisha Paul, Forensic Toxicologist, Vermont Department of Public Safety Forensic Lab, Waterbury, VT

In the early '90's, Congress passed the Prescription Drug User Fee Act (PDUFA) that taxed pharmaceutical manufacturers for each New Drugs Application (NDA) filed with the Food and Drug Administration (FDA). What was thought to be the decline of scientific research and development of novel drugs in 2011, in fact, created an upsurge in drug approvals and the fuel required to create the worldwide problem we have before us. In 2018 alone, 59 new drug therapies were approved by the FDA, which is a phenomenal feat for our healthcare industry; but, what impact does this increase have on clinical and forensic labs across the nation?

Once new drugs are either approved for use or can be obtained illegally, a tedious but quite necessary process begins back at the lab. Scientists have to develop robust methods for qualitative and quantitative analyses of drugs from different kinds of biological matrices; however, prior to practical application of such, forensic chemists are required to determine that said analyses are not only viable but are upheld to the standards set by the accrediting bodies - thus spawning the need for Method development and validation.

There is a plethora of scientific literature that provides the practical scientific data required to develop new methods -

which is tremendously helpful when attempting to reach an end goal. However, as a new validation scientist, I find it relatively daunting that none of the literature shares information on failed extraction attempts, instrument troubleshooting, product selection, etc., which in my opinion, would have been just as helpful! In this presentation, I will discuss my experiences: those that could speak volumes for all new researchers and shed light on the unspoken truth that one will inevitably encounter. Each strenuous, time consuming and, at times defeating mistake I have encountered will serve as a manual for what happens behind the scenes.

Northeastern Association of Forensic Scientists

Proceedings of the

November 2019 Annual Meeting

Trace Evidence/Fire Debris & Explosives Abstracts

Vibrational Spectroscopic Analysis of 3D Printed Polymers Pre- and Post- Manufacturing

Ryan Zdenek, April Bowen, University of New Haven, John Reffner, Ph.D., Maria-Isabel Carnasciali, Ph.D., Brooke Kammrath, Ph.D.

Additive manufacturing, commonly known as 3D printing, is becoming increasingly common in today's society. The ease of use and decreasing cost make this technology accessible to a wide range of individuals. 3D printing has been used to manufacture a range of useful, legal items as well as items that can be used in criminal acts. If a 3D printed item is found at a crime scene, it would be valuable to be able to associate the printed object with an unused spool of polymer. Thus, there is a need for forensic-focused research to evaluate whether an association can be made between 3D printed objects and their raw material (i.e., the polymer filament) as well as the discrimination potential of polymers produced from different batches and different manufacturers. It is the focus of this research to be able to understand the chemical nature of the polymers used in 3D printing, including changes in their crystallinity via the manufacturing process, in addition to methods for their classification and discrimination.

Infrared (IR) and Raman spectroscopy are common instruments used in forensic laboratories and are well known and validated analytical methods for polymer characterization and analysis. In addition to identifying the components of the polymer, such as the main chemical, additives and pigments, vibrational spectroscopy has been used for analyzing polymer crystallinity. Changes in polymer crystallinity can result in peak splitting and changes in peak shape which may result in a shift in the computer-selected peak position.

This research focused on the vibrational spectroscopic analysis of a common polymer used in 3D printing: polylactic acid (PLA). For the polymer, filament spools of different colors within the same brand and also different brands were analyzed. Pre-manufactured samples were analyzed from each spool at increments of 24in for a total of five pre-manufactured measurements per spool. Additionally, for some polymer filament spools, three items were manufactured at three different temperatures for a total of nine 3D printed objects per spool to observe if there were any pre- and post- manufacturing chemical differences.

Infrared and Raman spectral analysis resulted in the identification of the major and minor components (e.g., additives and pigment) of the polymers for classification as well as lot and brand comparisons. It was concluded that different brands and colors of polymers were able to be discriminated based on their vibrational spectra. Within a spool of polymer, there were no meaningful detectable differences indicating that the polymers within a spool are homogeneous. Last, statistical comparisons of the peak positions, splitting and Full Width Half Max (FWHM) values of the pre- and post-manufactured polymers indicated that there were no consistent differences over the range of melting temperatures to indicate a measurable change in crystallinity via the 3D printing process. Overall, vibrational spectroscopy was shown to be a valuable tool for 3D printed polymer identification which can be used to associate a 3D printed object with a specific manufacturer's polymer filament.

Electron Backscatter Diffraction for the Analysis of Common Mineral Polymorphs

Tiffany J Millett The Graduate Center, CUNY. John Jay College of Criminal Justice, EDAX, INC, Shawn Wallace, EDAX, INC.

Mineral identification is a crucial factor in the forensic analysis of soil and sand evidence types. Recently, the field has seen a major decrease in the amount of laboratories analyzing this type of evidence, mainly due to the equipment and training necessary based on traditional methods of analysis. Electron Backscatter Diffraction (EBSD) has been used in the metallurgic and geological sciences since its debut. However, its application has found very few uses in the forensic sciences. This research aims to highlight one of the applications of this technique to the area of forensic mineral analysis, particularly its ability to differentiate between polymorphs, which has often been proven problematic to analysts in mineral identification. Using a scanning electron microscope (SEM) as an electron source, EBSD patterns, or Kikuchi patterns, are produced by the diffracted electrons from a crystalline material and projected onto a phosphor screen. While the EBSD patterns reveal the crystal structure of the sample, the same electron beam can also concurrently allow for the collection of X-rays by the energy dispersive X-ray spectrometer (EDS) to understand the chemical composition, as well as allowing to obtain morphological information of the sample using the SEM. The ability to determine these

three key pieces of data from a sample becomes extremely important especially when analyzing mineral polymorphs, those minerals with the same chemical composition, but different crystal structure.

Conventionally, minerals are analyzed with X-ray diffraction, polarized light microscopy, or infrared spectroscopy. Each of these techniques have their advantages and disadvantages, which will be assessed and compared to the information gained from the coupled EBSD/EDS/SEM technique. Common mineral polymorphs will be used to determine the applicability of this technique to the field.

Evaluation of the Evidentiary Value of Cable Ties

Celeste Lambert B.S., Thomas Brettell, Ph.D., DABC, Lawrence Quarino, Ph.D., D-ABC, Cedar Crest College, Ted Schwartz, M.S., F-ABC, Brandi Clark, B.S., F-ABC, Westchester County Crime Laboratory.

Cable ties are often overlooked as forensic evidence since their characterization has not been extensively studied. However, cable ties may be used in criminal events in a variety of capacities such as binding hands of victims, in the design of homemade explosives, and during strangulations. Cable ties are made by injecting molten nylon plastic into metal molds. Each cable tie mold on the metal plate has a series of numbers and letters. The molten plastic is then ejected from the mold using pins.(1) Due to the nature of the manufacturing process, class and individual characteristics are created.

Class characteristics of the cable ties include general dimensions, color, and the mold number/letter impressions. To document class characteristics for comparison, cable ties were purchased from various national hardware stores. To create a database, a total of 29 bags of cable ties were purchased from Lowe s, Home Depot, and Harbor Freight. From each store, eight inch, 11 inch, and 14/15 inch cable ties were purchased. Ten cable ties were randomly selected from each store and size for examination. Twenty-five areas were selected to be measured or observed from different areas of the cable tie including the clasp, body, and tail. These measurements and observations were made using a calibrated digital caliper and a stereomicroscope. The measurements were averaged over the sampled cable ties and a 95% confidence interval was calculated for each class characteristic.

Pin impressions from each manufacturing mold leave individual characteristic striation patterns on cable ties. When the plastic is ejected from the mold, striations can be imparted onto the malleable plastic. With larger quantity bags of cable ties, there are often multiple cable ties with the same mold number. Thus, cable ties with the same mold number impression can be compared to each other. To study how strongly the striation pattern matches between cable ties of the same mold and between ties of different molds, 12 bags of 100 cable ties were purchased from Nelco Cable Ties (Pembroke, MA). The 12 bags were from four separate lots. The first six bags were from the manufacturer Hua Wei Industrial (Taiwan) and were natural colored. The next six bags were black colored and from the manufacturer Kai Suh Suh Enterprise (Taiwan).

The majority of the bags had mold numbers in numerical order with multiple cable ties with the same mold number. The cable ties with the same mold number were compared to each other and had high amounts of matching striations whereas cable ties of different mold numbers had almost no matching striations. This indicates that matching striations are successfully transferred to cable ties with the same mold numbers. Creating a cable tie database could be an extremely useful tool for forensic investigations. A database containing multiple measurements will yield a better chance for successful identification of brand/manufacturer. A large enough database may even provide an estimate to the likelihood of finding a particular cable tie from a random location, such as a crime scene. The study presented shows the potential evidentiary value of cable ties and should provide a practical reason for their collection by crime scene personnel and their subsequent examination in the laboratory.

Reference:
1. Gorn, M., Hamer, P. The Forensic Examination of Cable Ties. Journal of Forensic Identification 56 (2006):744-755.

Cable Ties, Database, Class and Individual Characteristics

Smokeless Powder Analysis
Claire Page, Penn State University, Jack Hietpas, Wayne Moorehead, Ryan Schonert, Todd Sowers, Penn State University

Small arms propellants (SAP) are readily accessible and cost-effective materials that firearms enthusiasts can acquire for the legitimate assembly of ammunition. Unfortunately, the ease of access to and low cost of these materials is advantageous for their utilization in the construction of improvised explosive devices (IEDs). Typically, the SAP charge is loaded into a metal pipe and sealed with screw-fit end caps. Two recent highprofile domestic terrorist attacks using IEDs (Boston Marathon Bombing and NY/NJ attempted bombings) demonstrate their continued usage. Thus, there is a need to develop robust metrics for the characterization of propellants that are used as explosives as well as for comparisons between exemplar and recovered explosive residues.

The goals of the presented research are to investigate GC/MS additive profiles of SAP and compound-specific stable isotope signatures of SAP for potential brand identification and sample discrimination. 160 one-pound canisters of SAP were purchased from local firearms stores. The samples represent a wide selection of different distributors (n=8) and product brands (n=160). The identified additives were primarily nitroglycerin, ethyl centralite, diphenylamine, and dibutyl phthalate. It was noted that many samples that gave the same GC/MS additive profiles could be differentiated by powder granule morphology.

Nitrogen and carbon isotope signatures were measured using isotope ratio mass spectrometry. The SAP sample isotopic compositions were examined to determine if there were recognizable geographic signatures. SAP manufactured in Canada and Belgium cluster in the most negative δ13C and δ15N space. The samples from Finland tightly cluster with negative δ13C and positive δ15N, thus allowing for discrimination of these powders. Samples from Sweden cluster in the center of the data set. Samples from the United States and Australia tend to show the least negative δ13C compositions and have positive δ15N signatures. This clustering is encouraging but will require a more complete dataset to draw robust conclusions. The results from this study show that there is a need for the fusion of data from different analytical methods for more robust sample differentiation and comparison.

Reference(s): 1. Reardon, M.; MacCrehan, W. Developing a quantitative extraction technique for determining the organic additives in smokeless handgun powder. J Forensic Sci 2001:46(4):802-807.

Interference of Desiccant Packets on Ignitable Liquid Collection and Analysis
Marilyn De Apodaca, Erika Chen, Asst. Dir. Diana Vargas, New York City Police Department Laboratory.

Cotton-tipped swabs with desiccant packets built into the container are designed for DNA collection, but are occasionally used by fire scene processors to collect possible ignitable liquids and residues. However, the desiccant packets are adversely affecting the extraction of ignitable liquid residues from the swabs by adsorbing the ignitable liquid vapors. The swabs themselves displayed substrate contribution that masked the identification of the diesel fuel and gasoline under certain conditions, and the desiccant beads absorbed enough of the compounds to diminish the full recovery of the sample. These swabs and desiccant caps are not recommended for collecting ignitable liquid residues.

The relationship Between Terminal Velocity and Glass Fracture via. 177 caliber steel BBs
Jocenel Julian Beach, Linda Rourke, Peter Diaczuk, John Jay College of Criminal Justice.

Glass is an abundant material in the environment, and can also be an abundant material found at crime scenes. It may often be the only thing between a bullet and its intended target, which is where fractography is necessary. Fractography is the study of fractured materials, such as glass. The majority of bullets will perforate or shatter most categories of glass; however, in some cases, the bullet may not have sufficient energy to shatter the glass at all. Correlating the terminal velocity of the projectile to the degree of glass fracture can provide useful information regarding the muzzle-to-target distance for a particular firearm/ammunition/glass combination.

Mechanical glass fracture from a projectile impact can result in different types of fracture patterns including radial and concentric fractures. The degree of fracture depends on several factors including: type of glass, the thickness of the glass, curvature (if any), distance from the muzzle, and type of projectile. This research focuses on the terminal projectile velocity of .177 steel BBs shot from a pneumatic air rifle. Double strength glass panes were chosen due to its common use in commercial and residential properties. A Doppler radar system was used to measure the projectile velocity from muzzle to target and potentially beyond the target for perforated targets. Other research has been presented on the subject of dynamic impact glass fracture using timekeeping instruments such as traditional chronographs. The use of a Doppler Radar System has the advantage of coupling Doppler processing with pulse radars to provide accurate velocity information with superior precision.

Pilot studies have been conducted to ascertain suitable muzzle-to-target distance ranges, the number of pumps required to pressurize the air rifle propulsion system, and the means of measuring the ricochet distance for projectiles which do not perforate the glass substrate. The experimental methodology was developed from the pilot study results.

The goal of this research is to characterize the relationship between impact velocity and glass fracture pattern. The results of our study can be used to develop future experiments using other types of firearms and ammunition to study glass fracture patterns. Our research will aid in the advancement of glass fracture analysis which can be useful in crime scene reconstruction.

An Overview of The NYPD Police Laboratory
Asst. Director Diana Vargas New York City Police Department Laboratory

The New York City Police Laboratory-Past, Present and Future : With 380 employees, the New York City Police Laboratory is one of the largest forensic laboratories in the United States. This will be an overview of the Police Laboratory, from its history in a shared building at the Police Academy in Manhattan, through the current state and looking toward plans for an exciting future.

An overview of the current structure of the Police Laboratory will be discussed, as well as, the types of services and analysis provided, particularly in the Criminalistics Section. The Forensic Investigations Division consists of the Police Laboratory, the DNA Liaison Unit, the Crime Scene Unit and the Latent Print Section. The Controlled Substance Analysis Section is the largest section of the Police Laboratory, followed by the Criminalistics Section, the Firearms Analysis Section, Quality Assurance and Case Management. The Criminalistics section further breaks down into these units: Trace Unit, Chemistry Unit, Latent Print Development Unit and Questioned Documents Unit.

There are plans in the works for a new expanded laboratory facility with over 1000 expected employees. From its humble beginnings to its expansive future, the Police Laboratory has ensured analysis be done in a manner that ensures the integrity, quality, accuracy and timeliness of all scientific findings, opinions, interpretations and/or conclusions.

Direct Real Time GCMS Analysis of Drugs in Powders, Solids, Liquids, and Trace Level Explosives on Cotton Swabs using Agilent's Quick Probe TM Technology on a 5977B/8890 GCMS System
Kirk E Lokits Agilent Technologies.

During analysis of unknown powders, tablets, and liquids, capillary chromatography coupled to mass selective detectors have become a staple tool for the forensic drug chemist. This analysis usually requires some sample preparation involving acid/base or liquid/liquid extractions. Furthermore, run times can excessively range from 10 to 30 minutes for general sample screenings. In this research, a new technique is demonstrated for fast GCMS analysis in under 2 minutes and requires minimal to no sample preparation prior to analysis. This technique can produce identification of compounds in a variety of sample matrices and is achieved by using the Agilent QuickProbe GCMS spectra and classical EI library searches. The purpose of this research is to demonstrate the ability of the QuickProbe to be successfully incorporated into the current work flow of forensic drug and trace analysis of unknowns.

Allentown Bombing: Overview and Forensic Challenges
Meghan Kane, ATF

In September 2018, a car exploded in Allentown, Pennsylvania, killing two adults and a child. The investigation that followed was in many ways a "typical" post-blast investigation, but it also presented new challenges because of the particular circumstances of the case and the homemade explosive (HME) material that was suspected to have been used in the bombing. While the laboratory was examining the evidence for post-blast residues of those suspected HMEs, the details of the device were exposed in a series of letters mailed by the bomber before the explosion. The challenges of this case, both at the scene and in the laboratory, will be discussed in this presentation.

Black Belt: The Art and Science of Impact Dynamics
Peter Diaczuk John Jay College of Criminal Justice/CUNY, Detective Andrew J. Winter, Centenary University

Earlier this year the Remington Arms Company, known for over two centuries for their firearms and ammunition innovations, unveiled a new ammunition design in its Golden Saber Law Enforcement line called "Black Belt". Currently available in three popular handgun calibers- 9mm Luger, (and 9mm Luger +P), .40 Smith & Wesson, and .45 ACP, it boasts a novel bullet design that is supposed to remain intact and retain its initial weight upon expansion. To accomplish this impressive feat of terminal ballistics, the at Remington Arms have added a band of reinforcing metal to the hollow point bullet's circumference, to what is traditionally only a thin copper or brass jacket enclosing the lead core. Located on the bearing surface just after the ogive, the black belt reinforcement gives the bullet an hourglass cross-sectional profile, resisting case-core separation, the downfall of many other hollow point bullet designs. This presentation examines the microscopy and metallurgy of the new three-part bullet construction and tests its performance in some commonly encountered substrates such as drywall, glass, sheet metal and ballistic gelatin, a tissue.

BLEVE it or not (Boiling Liquid Expanding Vapor Explosion)
Peter Diaczuk John Jay College of Criminal Justice/CUNY

Reaction vessels have a long history of usefulness and success in the commercial and industrial chemical industry. They provide the proper environment to promote a predicted reaction and based on chemical kinetics, the reaction's percent yield can be calculated. Sizes of reaction vessels will vary, as well as their ability to withstand temperature and pressure. A BLEVE, or boiling liquid expanding vapor explosion, can happen when a vessel containing a pressurized liquid reaches temperatures above its boiling point and then succumbs to a mechanical failure. The results of the ruptured vessel can be quite dramatic, including fire if the vessel's contents were flammable, or "merely" the explosion if they were not flammable.

On a much smaller scale, a plastic soda bottle containing the correct ingredients can make quite an interesting combination when sealed before the exothermic reaction begins. Tactfully avoiding the complexity of chemical kinetics, this presentation will take a peek into these homemade chemical reaction vessels and some of their parameters, including the initial thickness of the bottles, temperatures generated during the reaction, unconsumed reactants and the aftermath of the event.

Accreditation, Certification, and Licensing; do we really need 3 programs?
Christopher P. Chany, Texas Department of Public Safety, Austin Crime Laboratory.

Historically, Forensic Science Laboratories were accredited through ASCLD-LAB which then merged with the ANSI National Accreditation Board (ANAB). Since the early 90's, Forensic Scientists have had the opportunity to attain certification through the American Board of Criminalistics (ABC).

As of January 1, 2019, Texas became the first state to require that certain Forensic Scientists who perform analyses on evidentiary materials must be licensed by the Texas Forensic Science Commission (TFSC). The TFSC created the

requirements for this licensing, which includes among other things, a written exam. The TFSC does not consider Forensic Scientist certification through the American Board of Criminalistics as an indicator of competency. Additionally, Texas state law requires that any laboratory, whether in the state of Texas or not, that sends analysts to testify in Texas criminal courts must be accredited. The TFSC is also tasked by Texas state law with the responsibility of accrediting laboratories. While the TFSC does not assess the laboratories, it is responsible for determining which accrediting bodies can perform assessments of the laboratories. The TFSC also has the final vote regarding a laboratory's accreditation.

This presentation will examine differences and similarities between the licensing, accreditations and certifications that govern certain Forensic Scientists and Forensic Science Laboratories using the requirements of ANAB, ABC, and TFSC. It asks the question: does another program increase the reliability of Forensic Science testing?

Disclaimer: the opinions given in this presentation are those of the author and not the Texas Department of Public Safety, the TFSC, ANAB or ABC.

Northeastern Association of Forensic Scientists

Proceedings of the

November 2019 Annual Meeting

Criminalistics/Crime Scene & Digital Evidence Abstracts

Electrifying Gun Shot Residue Revelations Electrifying Gun Shot Residue Revelations
Tim Starn, Ph.D., Sarah Crispin, and Savannah Butala, West Chester University

Choban and Starn recently showed that shooting a firearm generates an electrical discharge from the incomplete redox deflagration reactions of the primer and propellant (1). That study was limited in scope in terms of the ammunition and firearm used. We will discuss current results which expand on the varieties of ammunition and firearms tested. The shape of the electrical discharge waveform, as a function of ammunition and firearm type, needs further explanation.

1. Choban E., Starn T. Electrical Pulse Generated Upon Discharging a Firearm and Its Implication for Gunshot Residue Analysis. J. Forensic Sci., 2019 (doi: 10.1111/1556-4029.14159)

Drug Induced Fit or Homicide … YOU DECIDE
Scott Rubins, New Rochelle High School/Syracuse University

Imagine you are a police officer, you arrive at a house and find a 45 year old male unconscious, naked and in a pool of blood on the floor of his bedroom. The victim has contusions to the body and right hand and deep bilateral knee lacerations. A significant amount of bloodstains were found to have been deposited on the walls, floors and other surfaces in almost every room throughout the residence, some of it over 6 ½ feet high on the walls.

The victim has a long history of involvement with the police as a perpetrator of narcotics related offenses, had been arrested multiple times and had been involuntarily committed to mental health care on numerous occasions. EMS took the victim to the hospital where he remained in a coma for over a month. The victim sent his brother's girlfriend a text message indicating that he was in fear for his safety and identified two individuals as persons who should be investigated if anything happens to him. One of the individuals temporarily resided at the victim's residence, was familiar with the layout of the house and knew there was a hidden house key under a rock in the backyard. The key was missing when the police arrived and was never recovered. There was no forced entry.

Police did NOT treat the incident as an assault and did not analyze the bloodstains or any evidence at the victim's residence. It is unknown if photos were taken. It was treated as an aided case. The Father of the victim questioned the lack of investigation by police and hired a private firm to conduct a post incident investigation to determine if a crime had been committed. Two retired NYPD Homicide Investigators and a retired NYPD Crime Scene Blood Spatter Expert were assigned to investigate the case. Over 350 photos were taken and more than 30 swabs collected to try to identify forign DNA. All the photos and DNA were preserved and packaged into evidence. chain of custody was maintained and the evidence handed over to the private investigation firm for delivery to DA or a private lab if necessary.

How important is solid walk through, a thorough understanding of the crime scene, accurate bloodstain pattern analysis and detailed photo documentation? Crime scene photos alone do not tell the story. See photos from the scene, hear the account of the story and experience the 12 hour post incident investigation at the house. Then you decide.... Did blood end up all over the house as a result of a drug induced fit or was a crime committed?

York John Doe: Using a Forensic Facial Reconstruction to Further a Case
Jennifer (Jenny) Kenyon, Pennsylvania State University, Free-lance Forensic Artist

As the investigation into the York John Doe slowed down, police at the West Manchester Police department turned to Penn State University to help with the forensic facial reconstruction of John Doe. They first turned to Jamie Heilman at the digiFab Lab at the Stuckeman School and School of Visual Arts in the College of Art & Architecture at Penn State for help with scanning and printing the remains. Then, trained forensic artist Jenny Kenyon, also working in the College of Art & Architecture, was brought onboard to create a new facial reconstruction to help move the investigation forward. Starting with the challenges and benefits of scanning the remains, Kenyon will narrate the process of getting a high quality digital scan and an accurate 3D print for the forensic facial reconstruction. She will detail the process of the 3D facial reconstruction, including finding accurate tissue depth datasets, application of the

tissue depth markers, construction of the muscular structures, and finishing of soft tissues. Images of the construction process will be shown. At the police department's request, an additional set of age regressions was also created to connect with those who may have known the individual in years prior to his death. Kenyon will discuss the process of creating the age regression illustrations, explain considerations when creating the regressions, and display the series of age regressions done for the department. Kenyon will also discuss and demonstrate additional digital 3D options for facial reconstructions, and explain other ways that forensic artists can assist in criminal and missing person investigations. After seeing these processes, perhaps you will see how other investigations could be furthered by this unique artistic and scientific collaboration.

Justice Through Science, or Science Through Lawyer/Media?
Dr. Henry Lee, University of New Haven

Since the 2009 National Academy of Sciences (NAS) Report and the 2016 President's Council of Advisors on Science and Technology (PCAST) report, many positive improvements have happened in forensic fields However, many in the press and lawyers took advantage of the reports accusing aspects of the forensic disciplines of issues including lapses in integrity, as well as claims of incompetence in the forensic fields. These accusations also target established forensic science methods/procedures and qualification and training of laboratory scientists. Many lawyers or the media twist the scientific facts in attempts to disgrace the profession and professionals. Also, some of the lawyers and media simply change the science and misrepresent it to courts and the public. These problems might impact the judicial system as well as the testimony of forensic science experts.

As a result of these attacks, will the forensics field of the future be dominated by work performed by forensic technicians without interpretation or expert opinion, or with forensic scientists being relegated to providing reports to attorneys and judges, rather than testifying? Will future forensic scientists simply be technicians producing analytical reports for lawyers, judges and journalists who will then decide what the results really mean in both criminal and civil courts, and the courts of public opinion?

If that is where the profession is going, it will be mandatory to educate the media, press, judges, prosecutors and defense attorneys in the fields of forensic science because they are becoming the voices of and the gatekeepers of forensic evidence. The public can be easily misled by false, twisted and misleading statements from purported scientific or legal experts, or others claiming to know the truth and to speak the truth of forensic science.

Case examples will be presented to illustrate how forensic reports and testimony was misinterpreted or deliberately misrepresented. Suggestions will be present on how to work together in handling those situations, and how to avoid being a victim of your cases.

Video Comparison Cases
Keith A Mancini, Westchester County Forensic Laboratory

A description will be provided of what the OSAC Video/ Imaging Technology and Analysis subcommittee is and what standards they are working on; what the Scientific Working Group on Digital Evidence (SWGDE) is and what standards they are working on. As well, a description of how both groups are moving their own documents forward independently, while also working together to not duplicate efforts.

Video/Imaging OSAC and SWGDE Update
Keith A Mancini, Westchester County Forensic Laboratory

Several cases of comparisons of clothing from video to known items of clothing will be presented. The presentation will include the effects of lighting and Infrared Radiation (IR) on the comparison examination.

Evaluation of the SERATEC PMB Test for Menstrual Blood

Amy Brodeur, Boston University School of Medicine

Body fluid identification is an important aspect of forensic work. Blood is one of the most commonly found body fluids at a crime scene, and while visually distinguishable from other biological fluids, an accurate method is needed to differentiate between peripheral blood and menstrual blood. One difference between peripheral blood and menstrual blood is the relative concentration of D-dimer present in each fluid. Elevated levels of D-dimer, a fibrin degradation product, are produced during menstruation, thrombosis formation, and as part of the postmortem breakdown process.

SERATEC PMB Test is a relatively new lateral flow immunochromatographic assay that targets human hemoglobin and D-dimer simultaneously in order to distinguish peripheral blood and menstrual blood at the same time; both components are detected in menstrual blood while only human hemoglobin is detectable in most other blood samples. To evaluate the SERATEC PMB Test, menstrual blood samples were selfcollected from anonymous donors on up to three consecutive days of menses. Peripheral blood samples from living donors as well postmortem blood samples were also collected. All samples were tested in triplicate, at a minimum.

All of the menstrual bloodstain samples (n = 12) reacted positively for the presence of hemoglobin. Nine menstrual bloodstain samples also reacted positively for D-dimer in each of the triplicate samples. All of the postmortem blood samples (n = 20) reacted positively for the presence of both hemoglobin and D-dimer. All of the antemortem peripheral bloodstain samples (n = 10) reacted positively for the presence of hemoglobin, while none reacted positively for the presence of D-dimer.

In addition, the SERATEC PMB Test was evaluated against two other commercial tests for menstrual blood, the DIMERTEST Latex Assay and the LGC ParaDNA Body Fluid ID Test, using a subset of menstrual blood samples. DIMERTEST Latex is a rapid agglutination assay which uses a solution of specially coated latex beads to detect D-dimer. ParaDNA Body Fluid ID Test is a rapid, one-step, mRNA profiling method that screens for multiple body fluids simultaneously and gives automated results in approximately 90 minutes. The SERATEC PMB Test outperformed the other methods, in both sensitivity and accuracy, for the detection of menstrual blood.

Overall, SERATEC PMB Test is an inexpensive screening test for menstrual blood, with a short run time and minimal training required. All postmortem samples showed positive results in D-dimer detection, suggesting that SERATEC PMB Test could also be a suitable method for distinguishing postmortem blood from antemortem peripheral blood.

Nondestructive Identification and Analysis of Biological Stains Using Vibrational Spectroscopy for Forensic Purposes

Ewelina Mistek, University at Albany, SUNY

The identification and characterization of biological traces found at a crime scene is critical. The ideal method for forensic investigation should be nondestructive, rapid, and universal for a wider range of samples. Vibrational spectroscopy is a nondestructive technique which examines the (bio)chemical composition of specimens resulting in a unique spectrum of an investigated sample. Our laboratory applies different spectroscopic techniques, Raman and attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy, in combination with chemometrics to analyze biological stains for forensic purposes. The automatic approach enabled the identification of various body fluids. Differentiation between human and animal blood was achieved with possible identification of specific species. Moreover, human phenotype profiling has been studied, and vibrational spectroscopy with statistical analysis was able to determine human biological sex, race, and age form several biological fluids. This aspect is extremely important in order to narrow down the search for a suspect and when DNA analysis is not successful or no matching profile is found in the DNA database. Vibrational spectroscopy with chemometrics shows great potential for forensic examination of biological traces in a nondestructive manner. Portable instruments offer entire examination to be performed in the field immediately after a crime scene is discovered.

Development of a Method to Estimate Measurement Uncertainty in the Creation of Test Panels for GSR Distance Determination

Amy Brodeur, Boston University School of Medicine

All quantitative measurements have a degree of measurement uncertainty. While the term uncertainty can be essentially defined as doubt, measurement uncertainty in this sense instead inspires assurance in a quantitative value to a certain degree of confidence.

Gunshot residue (GSR) patterns of distribution are used to establish a range of possible distances that the muzzle of the firearm was from the target in order to piece together a particular series of events. Because different distances can have considerably different residue patterns, it is important that a method for creating the test panels minimize uncertainty in order to be considered reliable and reproducible. When establishing a protocol for determining the measurement uncertainty in the creation of test panels, the two most important factors are the measuring device and a repeatability study.

In this experiment, a total of 238 measurements was taken by eight members of the Boston Police Department Crime Laboratory on eleven different days over the course of a month. The measurements were divided into eight baseline distances to which the firing device had been set : 3", 6", 9", 12", 18", 24", 36", and 42". The data was analyzed as a whole, as well as split into two groups: analysts who are proficient and authorized to perform GSR distance determination testing (Group A), and analysts with no GSR distance determination training or experience (Group B). At a confidence interval of 95.45%, the reported uncertainty was found to be 0.082 inches for the total group, 0.045 inches for the group trained in performing GSR distance determination, and 0.043 inches for the group with no experience in distance determination testing. F-test statistical analysis of the standard deviations of each distance, along with a comparison of the uncertainties, indicates no significant difference between the abilities of the two groups. Thus, calculating a new uncertainty of measurement will not be required when current GSR distance determination analysts leave or new analysts are hired and trained, given that all other variables remain constant.

The outlined method and experiment for determining measurement uncertainty was successful in that it met the four main requirements set forward by the American National Standards Institute (ANSI) National Accreditation Board (ANAB): (a) include the specific measuring device or instrument used for a reported test result in the estimation of measurement uncertainty for that test method; (b) include the process of rounding the expanded uncertainty; (c) require the coverage probability of the expanded uncertainty to be a minimum of 95.45%; and (d) specify a schedule to review and/or recalculate the measurement uncertainty.

Chemometric Processing of DART-HRMS Derived Chemical Signatures of Carrion Insects to Establish Insect Species Identity to Facilitate Postmortem Interval Determination

Amy Osborne, Justine E. Giffin, and Rabi A. Musah, Ph.D., University at Albany, and Jennifer Y. Rosati, Ph.D. University at Albany

An important aspect of death investigation is the estimation of time since death, also known as the postmortem interval (PMI). This can be difficult to assess for remains that have entered advances stages of decay for which traditional methods of PMI determination such as liver temperature are no longer applicable. In such cases, carrion insects found on and near the body can assist forensic investigators in accomplishing this task, as there is a well-established correlation between insect colonization of the remains and stage of decomposition. Since the timeline associated with insect progression through life stages is thoroughly documented, knowledge of the species of collected entomological evidence can be used to estimate PMI. Therefore, accurate species identification is critical. However, this process is challenging because different insect species are often morphologically similar at a given life stage. Thus, species identification often requires the time-consuming exercise of rearing eggs or larvae to maturity so that the species can be identified by examination of the gross physical features of the adult.

It is demonstrated here that chemometric processing of direct analysis in real time- high resolution mass spectrometric (DART-HRMS) data acquired from the analysis of insects can be used to rapidly accomplish species identification.

Eighteen species of forensically relevant insects belonging to the Diptera and Coleoptera Orders were studied, such as Muscidae sp., Creophilus maxillosus, and Nicrophorus orbicollis. DART-HRMS analysis was performed on ethanol suspensions in order to mimic the form of samples collected in the field. However, as many insect repositories preserve adult specimens in dried form, the optimal conditions for insect rehydration prior to mass spectral analysis were also determined. With regard to sample preparation, while it was determined that species-specific DART-HRMS chemical fingerprints could be acquired using dried, non-hydrated samples, consistent results were obtained when dried samples were suspended in aqueous ethanol for at least 24 hours. There was no additional advantage conferred from suspending samples for more than 24 hours, nor was there an advantage to rehydrating the insects with steam prior to placing them in suspension. The results showed that the ethanol suspensions of each species exhibited a unique chemical fingerprint, which was consistent for members of the same species but different between species. Kernel Discriminant Analysis (KDA) was applied to the data and showed that clear species distinction is possible not only for insects in different families, but also for insect species within the same genus.

Ruffling a Few Feathers: Forensic Identification of Illegally Traded Endangered Macaw Species Using Direct Analysis in Real Time - Mass Spectrometry

Dr. Rabi Musah, Ph.D., Meghan Appley, M.S., and Samira Beyramysoltan, Ph.D., University at Albany – State University of New York

It is estimated that the illegal wildlife trade is a multibillion-dollar/year industry that profits from the sale of plants as well as animals as pets or for other purposes including the creation of medicinal products. Macaws (giant new world parrots --Family: Psittacidae), which include several different species, are one type of animal that are traded both for their use as pets but also for jewelry and ritual practices. The high level of trade in both macaw's and macaw-derived products has resulted in a steady decline in the wild macaw population worldwide. To combat illegal trade, the Convention on International Trade in Endangered Species (CITES) lists the Scarlet Macaws as Appendix I, which represents the highest threat and indicates the greatest endangerment. With this classification, no trade is allowed. The Red-and-Green Macaws are listed as CITES Appendix II, indicating that while trade is allowed, it is closely regulated. The tracking of trade in these species hinges on the ability to distinguish between them, which is extremely challenging even for highly trained ornithologists, because of the visual similarities of the birds and in particular, their feathers. Therefore, a technique is needed that would enable the rapid and straightforward differentiation of macaw species, specifically the Scarlet, and the Red-and-Green Macaws, which are visually very similar. We proposed the idea of analyzing the feathers of these two species by direct analysis in real time - high-resolution mass spectrometry (DART-HRMS), and subjecting the results to multivariate statistical analysis for classification that would be based in species specific differences in chemical profiles. The individual barbs of several feathers from several individuals from both species were analyzed directly in the DART gas stream. The spectra that were produced were then subjected to multivariate statistical analysis. To identify potential outliers, the logarithmically transformed spectra were explored by principal component analysis (PCA) and Hoetlling's Tsquared distribution. To select primary discriminative features, the binned MS data matrix of the species was subjected to an elastic net feature selection method. The results from PCA-discriminant analysis (PCA-DA) and partial least square-discriminant analysis (PLS-DA) methods were fused to create a robust classifier for reliable discrimination between the two species. The performance analysis of the method showed 96% accuracy by ten-fold venetian blind cross validation and a 90% accuracy by external validation determination. The variables that were important for discrimination were ranked in importance through the projection scores derived from PLS-DA and PCA-DA. The results demonstrate a powerful proof-of-concept for the development of a technique that enables the rapid forensic identification of macaw species.

Forensic Pseudoscience?

David San Pietro and Brooke Kammrath, Lee College of Criminal Justice and Forensic Sciences, University of New Haven

Over the last decade, forensic science has received considerable negative attention and criticism. Several forensic specialties, including but not limited to hair and fiber trace evidence, arson, forensic odontology, bloodspatter, and DNA mixture analysis, have been branded as pseudoscience or junk science. The implication behind these claims are

that there is no scientific premise or validity behind these forensic methods. The criticisms are not isolated incidents, and have been steadily reported across media platforms - from newspaper and magazine articles to television shows and podcasts. Obviously forensic science is not free from error, and when incidents of wrongdoing or flawed information is discovered, it should be dealt with honestly and with full transparency. However, the authors of this presentation vehemently disagree with this pseudoscience branding of forensic science, and propose that the forensic science community needs to have an open discussion about these criticisms with the goal of creating action items to help rectify this false perception.

First, we need to understand the root causes for these attacks on forensic science. Are they merely stimulated by the exoneration of wrongfully convicted individuals and the unethical & illegal actions of "bad" forensic scientists, or is there something else initiating questions about the reliability of forensic science? It is only through an understanding of the source of the criticisms that we will be able to develop solutions.

Next we ask, how should forensic science practitioners and educators, address these critics? Do we merely write them off as unaware and unknowing of the true nature of forensic science? Should we cease doing these analyses, like the FBI did in response to the criticisms of bullet lead? Are there additional measures the forensic science community can take, such as practitioner certification? Is there specific research that forensic science educators and researchers should be undertaking? Will the inclusion of statistics, such as likelihood ratios or Bayesian analyses, be the cure-all some suggest it to be? A 95% confidence still indicates that 5% of the time the wrong answer will be given.

This directed discussion with potential audience participation will focus on first understanding these claims of pseudoscience, and then potentially developing ways forensic scientists can address and/or combat them. Whether you are a forensic science practitioner, educator or student, we hope this presentation will stimulate your thinking about the bigger picture of forensic science in modern society.

This discussion will be fostered by the use of "Poll Everywhere", a web-based audience response system. The audience will be able to respond in real time to the questions posed during this presentation via the web or via SMS texting on their phones. The authors request that attendees download the Poll Everywhere free app onto their mobile devices prior to attending this presentation in order to facilitate discussion. This is not mandatory because attendees will be able to respond via texting.

Northeastern Association of Forensic Scientists

Proceedings of the

November 2019 Annual Meeting

Drug Chemistry Abstracts

Validation of Opiate/Opioid Method using LC-Q-TOF Technology

Brynn Vitrano-Stocker and Daniel Harrington, Suffolk County Crime Laboratory

We are presenting a method development and validation for separation and identification method for 12 common compounds found in illicit drug submissions using an Agilent 1260 Infinity HPLC and Agilent 6545 Quadrupole time of flight mass detector. The purpose of this method is to separate and identify opiates and opioids Tramadol, heroin, noscapine, fentanyl, acetyl fentanyl, furanylfentanyl, carfentanil and butyrylfentanyl, precursor 4-ANPP, cocaine and diluents and adulterants caffeine and quinine. This is the first method developed on this instrument at Suffolk County Crime Lab.

Reference standards were used to create an in-house database and library, and were compared to the database and library provided by Agilent Technologies. QTOF was operated in positive mode utilizing both Data Dependent and Data Independent Acquisition (DDA and DIA) modes. Using the HPLC to separate the 12 compounds and the QTOF in all 3 manners (TOF, Targeted QTOF and Auto Q-TOF) to identify the compounds based on retention time, molecular ion, and fragmentation pattern.

Hundreds of injections of a standard mix were done to determine retention time parameters and also verify accurate mass. A total of 42 case samples were prepared from discarded case extracts (identification redacted). Compounds were extracted from case sample submissions of powders and hard substances. Samples in Methanol were diluted in LCMS grade methanol. Samples in chloroform were dried down and then diluted in LCMS grade methanol, approximately 1mL in 200mL for both types.

An Investigation of Extraction, Thermal, and Instrument Conditions on Cannabidiol to delta9-Tetrahydrocannabinol Cycloiosomerization

Mark Filandro, Joseph Cusick, Ph.D., Drug Enforcement Administration, Northeast Laboratory

Delta-9-tetrahydrocannabinol (Δ9-THC), and artifacts thereof, can be observed in samples of pure cannabidiol (CBD) that have been subjected to acid/base extraction schemes as well as upon introduction to a gas chromatograph inlet. Cycloisomerization of CBD to Δ9-THC is a known transformation in chemical literature that can most easily be affected by heating CBD in an acidic solution.

To test the effects of acid and base extractions, a CBD standard (purity of 98.18% and checked by LC-MS to ensure no presence of Δ9-THC) was subjected to various conditions (i.e. both extraction types, heating, and multiple injection port temperatures). All CBD extraction and heating conditions resulted in the observation of slight to significant creation of Δ9-THC and other cannabinoids at the lowest injection port temperature of 150 ºC. At a 200 ºC injection port temperature, most conditions did not lead to the observation of Δ9-THC. At a 280 ºC injection port temperature, the only conditions that led to the observation of Δ9-THC involved acid extractions and heating before the injection, as expected.

Through the course of this investigation, it was determined that at lower GC injection port temperatures, Δ9-THC and other cannabinoids were created from a CBD standard. As the injection port temperature increased, the artifacts disappeared for all but a few conditions. This issue can be mitigated by setting GC inlet temperature to approximately 280 ºC. Heating samples in acidic media for extractions will result in the observation of Δ9-THC, as well as the possibility of other cannabinoids, which can be of significant consequence under the current legal threshold of 0.3% Δ9- THC content.

HPTLC Analysis of Cannabinoids in Cannabis sativa L.

Yifan (Lisa) Liu, B.S., Thomas Brettell, Ph.D., D-ABC, Marianne Staretz, Ph.D., Cedar Crest College, Matthew Wood, Ph.D., F-ABC, Ocean County Sheriff's Department

Marijuana casework continues to burden crime laboratories since medical marijuana has become available and recreational marijuana has been legalized in many states. Thin-layer chromatography (TLC) has historically been used

as a routine test method for marijuana analysis. It is critical to have an optimized mobile phase system when analyzing cannabis products using TLC. A variety of mobile phases have been used for this purpose yet the literature review of different mobile phase systems is either out of date or some of the mobile phase systems that are currently used in forensic laboratories have not been reported in the literature. In addition, even though traditional TLC is an easy-toperform and useful tool for screening seized drugs in forensic laboratories, it often results in poor resolution due to systematic errors rising from hand-spotting, temperature/humidity control, and measurement of the retardation factor (Rf). High-performance thin-layer chromatography (HPTLC) on the other hand can be used to eliminate systematic errors and increase resolution. This study evaluates 10 different mobile phase systems from the literature and laboratories in an attempt to find the most optimized mobile phase for the analysis of cannabinoids in Cannabis sativa L. using HPTLC. All systems were run in triplicate. Retardation factors (Rf) were recorded and the resolution was calculated for 11 different cannabinoid certified reference standards. A CAMAG HPTLC instrument setup, which included an Automatic TLC Sampler 4, an Automatic Development Chamber 2, a TLC visualizer and a TLC Scanner 3, was used for all analyses. Samples were visualized on the plate under white light, and using wavelengths of 254 nm and 366 nm . A CAMAG Chromatogram Immersion Device III was used to dip the developed plate into 0.5% Fast Blue B salt solution for five seconds. VisionCATS CAMAG HPTLC SOFTWARE (version 2.5) was used to control, document, and analyze all results from experiments. HPTLC Silica gel 60 F254 20 x 10 cm plates were used for nine systems and a RP-18 WF254 10 x 10 cm plate was used for one additional system. Two systems, xylene-hexane-diethylamine (25:10:1) and 6% diethylamine in toluene, gave the best results in separating the three major cannabinoids: Δ9-trans-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD), and cannabinol (CBN). Both systems were then repeated seven more times to meet the SWGDRUG validation requirements. The results of the analysis of various cannabis products from casework will be presented. Using the proper mobile phase system with HPTLC is a superior method when compared to traditional TLC systems for qualitative identification of the common cannabinoids in cannabis products. This method has the potential to provide better resolution and to generate reports for more convenient documentation for peer review of casework in crime labs.

Investigating the Optimal Gas Chromatographic Parameters of Thirty Fentanyl Analogues

Delilah DeWilde, M.S.F.S.., NMS Labs, Thomas Brettell, Ph.D., D-ABC, and Thomas Pritchett, M.S., Cedar Crest College, Matthew Wood, Ph.D., D-ABC, Ocean County Sheriff's Department

Fentanyl abuse has become an epidemic in the United States and is a major source of opioid related deaths due to overdose. Data collected from crime laboratories during the period of 2016-2017 as reported in the 2019 National Forensic Laboratory Information System (NFLIS) Report shows an overall nationwide increase in the abuse of fentanyl analogues well over 100%. The sheer number of different fentanyl analogues and their close structural similarity makes traditional identification methods difficult. Gas chromatography-mass spectrometry (GC-MS) remains the gold standard for analysis of seized drugs, including fentanyl and its analogues. The chromatographic conditions are thus extremely important for the separation and identification of this class of drugs.

This presentation will discuss the significant findings from the development of a confirmatory method using GC-MS for the analysis of 30 fentanyl analogues. Each GC parameter was analyzed independent of each other to create a robust method for the optimum resolution of multiple fentanyl analogues. Multiple inlet temperatures, injection volumes, split ratios, and temperature programmed methods were compared. Six GC stationary phases were investigated. Two columns with phases ranging from slightly non-polar to completely non-polar were investigated for their utility for fentanyl analogue analysis. One was comprised of a 5% diphenyl: 95% dimethyl polysiloxane stationary phase and the other had a 100% dimethyl polysiloxane stationary phase. Four other columns were included in this project to determine if any could offer contrasting data as a useful confirmation column. The stationary phases utilized in these columns were comprised of a mid-polarity stationary phase (50% diphenyl: 50% dimethyl polysiloxane), a low to mid-polarity stationary phase with some phenyl, methyl, and cyano functional groups, a mid-polarity phase column with a 100% trifluoropropyl methyl polysiloxane phase, and a chemically treated column with a 5% diphenyl: 95% dimethyl polysiloxane phase.

The generation of data in this project was used to produce linear retention indices, which are comparable to those found in literature. The best conditions for analyzing fentanyl analogues will be reported and hopefully will be an asset for practicing forensic scientists.

Identification of a new class of thermolabile psychoactive compounds, 4-substituted 2-(4-X2,5-dimethoxyphenyl)-N-[(2-hydroxyphenyl)methyl]ethanamine (25X-NBOH, X= Cl, Br, or I) by gas chromatography-mass spectrometry using chemical derivatization by heptafluorobutyric anhydride (HFBA)

Thomas Brettell, Ph.D., D-ABC, Cedar Crest College, Benny J. Lum, M.S.F.A., Broward Sheriff's Office Crime Laboratory, Joseph J. Brophy Ph.D., D. Brynn Hibbert, Ph.D., University of New South Wales Sydney

4-Substituted 2-(4-X-2,5-dimethoxyphenyl)-N-[(2-hydroxyphenyl)methyl]ethanamine (25X-NBOH, X= Cl, Br, or I) are new psychoactive substances (NPS). N-2-methoxybenzyl substituted 2C compounds, "NBOMe", were initially designed in 2003 as research chemicals for use as potent agonists of the 5-hydroxytryptamine receptor subtype 2A but became notorious as highly dangerous recreational drugs a decade later. 25I-NBOMe, the most widely encountered 25X-NBOMe, is known on the street as "NBomb", "Smiles", "Dots", "legal acid", and "N-Boom". It is sold impregnated in paper squares, similar to lysergic acid diethylamide (LSD), which can include images and logos from popular culture. Although originally marketed as a "legal high" 25I-NBOMe and its analogs are now proscribed as prohibited substances in most jurisdictions. Recently the N-[(2-hydroxyphenyl) methyl] derivatives, 25X-NBOH, have come to the attention of law enforcement agencies.

Identification of these NBOH analogs by gas chromatography-mass spectrometry is difficult because they are found in very low concentrations in the blotting paper in which they are sold, and because of the thermolabile nature of NBOH. They break down to the known NPS 2,5-dimethoxy-4-Xphenethylamine (2CX) thereby giving no indication of the initial compound present. A method to identify 25X-NBOH compounds is described using heptafluorobutyric anhydride (HFBA) derivatization and subsequent gas chromatographic-mass spectrometric analysis. The major peak is the bis-derivative where both the amine and phenol are derivatized, with a minor peak of the monoderivative in which only the amine is derivatized. There is a small amount of degradation of 25XNBOH to the 2C-X amine which is then derivatized to the amide in-situ. Reaction with HFBA is an efficient and simple derivatization that works well with NBOH and NBOMe analogs and is recommended for routine forensic analysis.

Automation Possibilities for Drug Analysis using ChemStation Macros

Eugene Zegocki, Monroe County Crime Laboratory

ChemStation macro language provides multiple possibilities for automation tasks. Using macros, it is possible to automate screening, identification and printing GC/MS instrument results. The author designed and tested a set of macros for Agilent GC/MS instruments using ChemStation software for Drug analysis. These macros could automate identification of controlled substances and documentation and printing process. That will save time, which is particularly important for cases with numerous items.

A set of macros automatically process sequences containing samples for one or several cases. The program screens GC/MS data, and identifies controlled substances or any other defined compounds from the list made by analyst. If the analyst does not agree with the automated identification results, he/she could change suggested results or references manually prior to printout. There are options for changing integration threshold for each item and manually checking of any integrated peak prior final printout.

There is a predefined directory containing references (previously run controlled substances). If a substance from the list is identified, the program selects the appropriate reference and makes a comparison of mass spectra and retention times. In the case of low intensity peaks the background is automatically subtracted, thus making better library hits.

Results are produced in PDF format for final review. The printout includes annotated total ion chromatograms for all items in the case, blank samples, RT and MS comparisons with references and library searches for each item.

Data is combined in one PDF file, which is easy to use in a paperless workflow process. Also, results could be automatically inserted into pdf form notes, if the analyst is using those.

Simple and Cost-Effective Synthesis of an Alprazolam Analogue

Emily Langlois, Koby Kizzire, Ph.D., University of New Haven

Benzodiazepines have the potential to become another facet of the drug crisis with a large number of prescriptions being written and frequent reports of recreational use. Alprazolam is one of the most overprescribed and abused benzodiazepines. It is listed as a schedule IV substance in the Controlled Substances Act, meaning that the possession of alprazolam requires a legitimate prescription. To skirt the legalities of the Controlled Substances Act, some clandestine laboratories synthesize analogues, or compounds with the same pharmacophore but varying certain moieties and atoms. Although the Federal Analogue Act was enacted to deter the synthesis of analogues, the act specifically applies to analogues of schedule I and schedule II substances. Since alprazolam is a schedule IV substance, alprazolam analogues are not included as potential targets of enforcement. This allows clandestine laboratories to make substitutions on the compound's structure that cause similar psychoactive effects with a lesser chance of legal ramifications. As a likely result of this issue, these compounds can pose analytical challenges in forensic laboratories as they may not be present is spectral libraries or be readily available for purchase as standards for comparison.

A synthesis for alprazolam achieved via a small number of steps and using cost-effective reagents and techniques is currently available online. As a simple method for synthesis, the publication has potential for abuse by clandestine laboratories, especially if it might be adapted to synthesize alprazolam analogues. The goal of this study was to demonstrate the application of this synthesis in a potential clandestine laboratory setting to generate a non-controlled analogue of alprazolam.

The synthesis of a deschloro alprazolam analogue (1-methyl-6-phenyl-5,6-dihydro-4H- [1,2,4]triazolo[4,3-a][1,4]benzodiazepine) was attempted following the procedure included in the published synthesis with an alternate precursor, but initial attempts to replicate multiple synthesis steps were unsuccessful. However, the published procedure did provide a framework of possible intermediates for completion. To monitor the progress and outcome of each reaction, gas chromatography-mass spectrometry, infrared spectroscopy, and thin layer chromatography were used. All intermediates have been successfully synthesized and completion of the final product is anticipated.

Upon completion of the synthesis, this study will demonstrate the utility of a synthetic method intended to produce alprazolam in the manufacture of a non-controlled analogue and its feasibility in a clandestine laboratory. The data provided from this work can be used to aid future identification of this analogue, should the need arise. This data may also be used to determine a potential route of production, if intermediates and/or reagents are identified in seized materials. Parallel comparisons of the intermediate data for the analogue could provide clues should the same method be used to clandestinely produce alprazolam or other alprazolam analogues.

Classification of Synthetic Cannabinoids using GC/FTIR in Vapor Phase

Lewis Smith, Cape May Country Forensic Laboratory

Since the advent of commercial Fourier Transform Infrared Spectrometry in the late 1960's, much progress has been made in both instrument sensitivity and data retrieval. The multiplex nature of interferometry combined with MCT cryrogenic detectors has vastly improved the signal-to-noise ratio of many older microscopic and reflective (ATR) sampling techniques. Modern fast scanning FTIR spectrometers have also made hyphenated techniques such as GC/IR/MS possible.

The most widely used confirmatory method of choice for most forensic labs is Mass Spectrometry. There are many compounds encountered in drug analysis which yield poor fragmentation patterns upon electron-impact. In addition, positional, chiral, and optical isomers cannot be unequivocally identified from their mass spectra alone. GC/FTIR using flow cell technology yields infrared vapor phase spectra that provides supplemental data with solutions to solve these problems.

The most complex and vast group of Forensically encountered drugs are the Synthetic Cannabinoids. Spectral comparisons between solid phase (condensed) and vapor phase will be made illustrating the importance of physical state and molecular association. Vapor phase allows for rotational isomers to exist creating unique additional bands

for identification. Unlike solid-deposition GC/FTIR, these spectra are completely free from the effects of polymorphism, intermolecular hydrogen bonding, and water.

A classification system will be taught that will organize all derivatives into their proper sub-categories. Properties of vapor phase make it possible to resolve every carbonyl band present within the Synthetic Cannabinoid framework. The Bridge Frequency Table is based on the use of this unique carbonyl cluster found within a 200 wavenumber interval. Data from this table can quickly identify the proper sub-category from unknown Synthetic Cannabinoid spectra and offers the following advantages:

1. Quickly gain insight to the proper Synthetic Cannabinoid Category and molecular structure
2. Gain information without the use of reference materials - especially if none are available
3. An aid to mass spectral interpretation from molecular pieces revealed in table.
4. Knowledge of the correct category will narrow the search for proper standards or data

Spice Up Your Life – An Ambient Mass Spectral Technique for the Rapid Detection and Quantification of Psychoactive Compounds in Pepper Plant and Magic Mint-derived Complex Matrices

Megan I. Chambers, Amy M. Osborne, Justine E. Giffen, Rabi A. Musah, Ph.D., University at Albany – SUNY

Piper methysticum and Salvia divinorum, commonly referred to as Kava and Salvia respectively, are psychoactive plants designated by the UNODC as "plants of concern." The non-characteristic appearance of these plants and their derived products makes it difficult for law enforcement and forensic laboratories to distinguish them from innocuous plant-based materials such as spices or herbs. This investigation focused on developing a rapid screening method to enable forensic identification of such products, as well as quantification of their psychoactive biomarkers. Direct analysis in real time-high resolution mass spectrometry (DART-HRMS) was chosen as the instrumental approach because it is an ambient ionization technique that enables direct analysis of samples, rapid screening and identification of compounds, and semi-automated quantification.

The six major kavalactones including psychoactive yangonin (in Kava) and salvinorin A (in Salvia) were rapidly detected in various products using DART-HRMS, thus confirming that the plant material was derived from the respective plants. Standard curves were developed and validated to quantify the yangonin and salvinorin A content in various complex matrix plant products. The yangonin levels in 16 of the 18 Kava products were successfully quantified, as was the salvinorin A content in 8 Salvia products. Kava tinctures had yangonin concentrations of 1.03 to 4.59 mg/mL, while the solid samples exhibited a range of 2.71 to 8.99 mg/g. Salvia leaves had an average salvinorin A concentration of 1.54 mg/g, while the enhanced leaf extracts had concentrations ranging from 13.5 to 53.2 mg/g. The Salvia results were generally consistent with previously reported concentrations. In summary, a DARTHRMS protocol that enables rapid identification of plant-based legal-high substances, as well as efficient quantification of their psychoactive components within complex plant matrices, was successfully developed.

Pharm to Table: Mass Spectral Analysis of Synthetically-Derived Psychoactive Materials

Meghan G. Appley, Rabi A. Musah, Ph.D., University at Albany – SUNY

The United States is currently in the midst of an opioid epidemic. Individuals are turning to these often unscheduled drugs because of the belief that their legal status makes them safer alternatives to outlawed substances. Although these drugs are not scheduled, they are dangerous not only to the individuals abusing them, but also to law enforcement personnel and laboratory analysts who are exposed to them as an occupational hazard. Therefore, there is a need for methods that will allow for the rapid detection and identification of these drugs before dangerous exposures occur. We proposed that this could be accomplished through the use of headspace analysis coupled with mass spectrometry. The headspace volatiles of several classes of synthetically-derived psychoactive compounds (cathinones, tryptamines and opioids) were concentrated onto conditioned solid phase microextraction fibers by exposing the fiber to the volatiles for 30 mins. The fibers were analyzed by direct analysis in real time - high-resolution mass spectrometry (DART-HRMS). This process was repeated in replicates of ten for each compound. The DART mass spectra were then subjected to multivariate statistical analysis (kernel discriminant analysis). The headspace results were compared

to the spectra acquired from direct analysis of the powders, both under soft ionization and collision induced dissociation (CID) conditions, as well as to those obtained by gas chromatography - mass spectrometry (GC-MS). The spectra acquired from headspace analysis exhibited consistent similarities between replicates and differences between compounds, that were visually apparent. The data were first classified using KDA by type of synthetically-derived psychoactive compound (i.e. cathinones, tryptamines and opioids). This analysis showed that classes could be separated and classified correctly with a leave-one-out cross-validation (LOOCV) of 90%. Each class was then subjected to statistical analysis separately, to determine if individual compounds could be classified correctly. The results for this step showed that individual compounds could be classified with a LOOCV of 90% or greater using KDA. In comparison with other techniques, it was determined that headspace analysis could provide similar if not more accurate prediction results than direct analysis. The observations illustrate that chemometric processing of headspace chemical profiles determined by DART-HRMS, can enable identification of psychoactive synthetics, and could provide a safer alternative to direct sample analysis by decreasing exposures to dangerous compounds. Parts of this project were supported by the National Institute of Justice through Award Nos. 2017-R2-CX-0020 and 2018-R2-CX-0012.

Northeastern Association of Forensic Scientists

Proceedings of the

November 2019 Annual Meeting

Forensic Biology/DNA Abstracts

Determination of the Optimal Method for the Detection of Vaginal Fluid

Melissa Rogers, B.S., Master of Science in Forensic Science Program, Cedar Crest College; Lawrence Quarino, Ph.D., Amrite Lal-Paterson, M.S. and Janine Kishbaugh, M.S., Cedar Crest College

With the advent of DNA and the potential to be corrupted by bias, many people, including forensic scientists, have forgotten the importance of applying context to the evidence of a particular case. DNA may be present at a crime scene, but without context, that DNA evidence may be interpreted incorrectly. However, identifying the body fluid from which that DNA came can provide the information of how that DNA came to be there. Forensic Science literature presents a number of tests to confirm the presence of various bodily fluids; however, such a test for vaginal fluid is still needed.

A confirmatory test for vaginal fluid will provide critical context for forensic casework, especially in sexual assault cases where no semen is present. In this study, different methods were examined to detect and confirm the presence of vaginal fluid on "used" condoms donated by women of different ages and then tested over periods of time. Following Institutional Review Board (IRB) approval, multiple samples were obtained from over twenty volunteers. After extensive validation, a tandem method is proposed that provided robust results over all demographic groups tested.

The best method utilizes the Periodic Acid-Schiff (PAS) reagent to stain glycogen present in vaginal epithelial cells in a histochemical reaction (1). The reagent proved to be an improvement over Lugol's iodine which was more commonly used in forensic labs (2). Of twenty vaginal fluid samples tested on women ranging in age from 24-82, all produced positive results with no false positives observed with corresponding saliva and urine dried stains. These visual results were bolstered by using CellSens software with an Olympus Polarized Light Microscope to photograph the stained slides and measure the RGB values of each photo.

Not only do these results bring to light more effective detection methods for vaginal fluid, but also provides the foundation for a potential confirmatory test that could be used in serology laboratories. Utilizing and validating these methods is vital for aiding in sexual assault investigations. The detection of vaginal fluid in cases could provide the context needed to solve these heinous crimes.

References: 1. Jones EL. The Identification of Semen and Other Body Fluids. In: Saferstein R, editor. Forensic Science Handbook: Volume II. Upper Saddle River, 2005;360-68. 2. Rothwell TJ, Harvey KJ. The Limitations of the Lugol's Iodine Staining Technique for the Identification of Vaginal Epithelial Cells. J Forens Sci Soc 1978 Jan;18:181-184.

Comparison of DNA Profiles Recovered from Cotton-Tipped versus Nylon-Flocked Swabs from Post-coital Cervicovaginal Samples

Kathleen M. Maguire, B.S., and Janine M. Kishbaugh, M.S., Cedar Crest College; Jillian Conte, Ph.D., Keystone College; Lawrence Quarino, Ph.D., Cedar Crest College

Cotton-tipped swabs are routinely used as a method of collecting biological material by medical personnel and forensic investigators. Standard practices include collecting vaginal samples from a female victim as part of a sexual assault kit. Swabs used to collect biological material should be able to maximize the cellular material collected and then elute intact cells for DNA extraction and profiling. A swab with higher extraction and recovery efficiency can lead to more DNA available for genotyping. The differences in swab morphology between cotton-tipped swabs (wound swabs) and the hydrophilic open fiber structure of flocked swabs may account for the higher rates of sample elution from flocked swabs (1). This study aims to compare male profiles obtained from vaginal samples taken with cotton-tipped swabs and nylon-flocked swabs.

Following Institutional Review Board (IRB) approval, sets of four vaginal swabs were collected from sexually active, heterosexual participant couples. The couples varied in age and birth control methods. Swabs were collected over three distinct post-coital time intervals, with the cotton and the nylon-flocked swabs being inserted concurrently following the NIJ recommendations for sample collection (2). Control swabs of the vaginal cavity were taken after a sexual abstinence period of at least seven days and collected immediately prior to intercourse. Vaginal swabs were

collected after 72, 120 and 168 hours post-coitus with ejaculation. Each collection was based on a separate act of intercourse. Male and female participant buccal swabs were collected to obtain reference profiles. Reference swabs were extracted with 5% Chelex®. The vaginal swabs were extracted using QIAamp® DNA Investigator Kit. Both reference and vaginal swab extracts were amplified using the PowerPlex® Y23 System.

For one 72-hour sampling period, the profile obtained from the cotton swab showed 3/22 (13.6%) alleles while the profile obtained from the nylon swab showed 12/22 (54.5%) alleles. A second set of participants completed the 72-hour sampling period and full profiles (22/22 alleles) were obtained from both the cotton and the nylon swabs. Two sets of participants completed the 120-hour sampling period, with the cotton profile returning 2/22 (9.0%) alleles and the nylon profile having 13/22 (59.0%) alleles present in one set. The second set of 120-hour swabs had 22/22 alleles obtained from the cotton swab, and 14/22 (63.6%) alleles obtained from the nylon. The 168-hour sampling period was completed by one participating couple, with 0/22 alleles obtained from the cotton swab and 2/22 (9.0%) alleles obtained from the nylon. Overall, profiles obtained from nylon swabs were more complete in 3/5 (60%) sample sets, profiles obtained from cotton swabs were more complete in 1/5 (20%) sample sets and 1/5 sample sets (20%) returned full profiles.

These results provide data that could be used to improve the collection methods used in sexual assault evidence collection. The improvement of the swabs used in post-coital vaginal sampling could have positive downstream implications when attempting to obtain a DNA profile.

References 1. Benschop C, Wiebosch D, Kloosterman A, Sijen T. Post-coital vaginal sampling with nylon flocked swabs improves DNA typing. J Forensic Sci Int:Gen 2010;4:115-121. 2. National Institute of Justice. National best practices for sexual assault kits: a multidisciplinary approach. 2017. NIJ 250384;15-19

Human Identity from Mosquito Midgut using GlobalFiler™ Express

Mollie Comella Undergraduate student, Forensic Science Program, Joann Butkus, Scott E. Lindner and Reena Roy, The Pennsylvania State University

There are currently three main forms of the Anopheles stephensi mosquito: typical, intermediate, and mysorensis. Despite the intermediate and mysorensis forms having poor vector statues, the typical form is found in many urban areas and is known to spread various diseases such as Malaria. While these insects are generally considered as pests and carriers of disease, the female mosquitoes can be useful in forensic cases as they feed on human blood. Nucleated white blood cells found in the blood stored in the midgut of the mosquitoes can be useful in identifying the donor on which the female mosquito has fed. In the context of a forensic case, the ingested blood could help identify a suspect or link a victim to a crime scene.

Generation of short tandem repeat (STR) DNA profiles by direct amplification is useful for identification of the donor of the blood that was ingested by the mosquito. Commercially available direct amplification kits allow for detection of the donor of a body fluid and bypass the labor intensive and timeconsuming steps needed for extraction and quantification of DNA.

Previous research in this laboratory using another direct amplification kit indicates that DNA stored within the white blood cells of the mosquito gut starts to degrade after 8 hours post-feed and the DNA is completely degraded 72 hours post-feed. Based on those results, in this current study, mosquitoes were fed on warm blood meals and euthanized at 0 hours, 4 hours and 8 hours post-feed. Several mosquitoes were euthanized at each time interval having fed on a single donor. Mixture of blood, using two donors, was also used in this study, and these mosquitoes were euthanized at the same time intervals.

This project utilized the GlobalFiler™ Express PCR Amplification Kit to perform direct amplification and generation of STR profiles. To assist in the direct amplification process, COPAN microFLOQ® Direct Collection Devices were used for puncturing the midgut of each mosquito. These swabs contain a nylon-fiber tip with a lysis agent that helps to break open the nucleated white blood cells. Once a minute quantity of blood meal was collected on the tips, the tips were cut and placed into the PCR amplification tubes where they remained immersed in the direct amplification

reagents during the thermal cycling process. Blood sample from each donor was used as reference to compare the results of profiles generated from the midguts of the mosquitoes.

This research indicated that DNA profiles obtained from the mosquitoes using GlobalFiler™ Express amplification kit were consistent between and within each donor. In addition, both donors could be identified in the mixture profiles. Full profiles for all sample types were obtained for 0 hours, 4 hours, and 8 hours post-feed. Additionally, it is hypothesized that variability between mosquitoes both in their feeding and digestion patterns affected the quality of the profiles obtained. In conclusion, DNA contained in the human blood meal ingested by mosquitoes can be useful in determining the donors' identity using the GlobalFiler™ Express PCR Amplification Kit. This is a robust method to establish the presence of one or more individuals at a location-of-interest within a specific time window.

Key words: GlobalFiler™ Express, mosquitoes, direct amplification

Single Cell Analysis Using the DEPArray™ NxT System: Implications for Forensic DNA Mixture Deconvolution

Davis Watkins, Forensic Science and National Security Institute, Syracuse University; Olivia D'Angelo and Michael A. Marciano, Syracuse University

DNA profiling has become the gold standard in forensic identification however it has significant challenges, namely, the interpretation of samples consisting of multiple donors. Mixed DNA samples have become ubiquitous in routine casework and pose a significant interpretational challenge due to a variety of issues including the potential number of contributors, the presence of low level contributors, instrumentation noise and high levels of allele sharing between individuals. Many software solutions are available to aid in the analysis of these samples such as PACE for contributor number estimation and STRmix™ for deconvolution. However, few solutions exist that attempt to deconvolute the mixture prior to DNA extraction. Selectively recovering and analyzing single cells will avoid the complexity involved with DNA mixture interpretation, whereby the profile of a single cell will be of a single donor. Historically, the success of single cell analyses in forensic science has been inconsistent. Single cell analyses have become more tangible with the development of new technologies, such as recently developed "global" human DNA amplification kits and single cell recovery platforms such as the DEPArray™ NxT. We propose the use of a workflow that takes advantage of this new technology to re-examine the validity and robustness of single cell analysis in a forensic setting. This study examines the ability to successfully obtain interpretable DNA profiles from single epithelial and white blood cells, recovered using the DEPArray™ NxT using standard forensic amplification methods (Promega PowerPlex® Fusion 6c amplification kit with varied cycles from 29-31). As expected, average peak heights increased as cycles number increased, with 82RFU, 137RFU and 181RFU for 29, 30 and 31 cycles respectively. We observed a relatively consistent percentage of locus and allele dropout across the different PCR cycles, with allele dropout at 38.1%, 34.3% and 39.6% for 29, 30 and 31 cycles, respectively; and locus dropout at 31.7% 30.0% and 30.4% for 29, 30 and 31 cycles, respectively. The results demonstrate that single cell analysis using the DEPArray™ pipeline can be effective using the recommended PCR cycle number and with increased cycles. Furthermore, this study highlights the importance and use-case for single cell DNA profiling in forensic investigations.

Comparisons of Allele Sizing Between Genotyping Software

Ashley Ruddy, Forensic Science and Law Program, Department of Forensic Sciences, Duquesne University; Dr. Jillian Conte, Ph.D., Department of Forensic Biology, Keystone College; Lindsey Domonoski, Department of Forensic Sciences, Pennsylvania State University Dr. Jillian Conte, Ph.D.(1), Ashley Ruddy B.S.(2), Lindsey Domonoski(3) Forensic Biology Program, Keystone College, La Plume PA, 18440, USA (1)Department of Forensic Biology, (2)Department of Forensic Sciences, Duquesne University, (3)Department of Forensic Sciences, Pennsylvania State University

There are multiple genotyping software programs available for use and analysis of electrophoretic data. There is a gap in determining if these programs are equally accurate or if they have differences between kits, in injection time, and analysis method. This study fills the gap in determining these differences using a database of samples, PROVEDIt, via Laboratory for Forensic Technology Department and Integration (LFTDI), by focusing on allele sizing(1).

Genotyping software can be freely available for download or available for purchase. This study uses GeneMapper by Thermo Fisher Scientific and GeneMarker HID by SoftGenetics, which are both commercially available, in addition to OSIRIS, which is freely available for download. GeneMapper and GeneMarker HID both use the Local Southern Method for sizing alleles, while OSIRIS analyzed multiplex STR profiles using internal lane standard (ILS) and allelic ladder. The local southern method used fragment length and mobility relationship to determine size. The timing of the ILS and ladder peaks was compared to the sample peaks for sizing in the OSIRIS method. Sample genotypes were verified between software, followed by construction of single-source profiles with two different injection times and STR chemistries. Statistics were calculated to determine differences between the software. It was found that allele sizing is dependent on software, injection time, and STR typing kit.

Keywords: ILS, STR, allele sizing

References: (1)Alfonse, L.E., Garrett, A.D., Lun, D.S., Duffy, K.R. & Grgicak, C.M. A large-scale dataset of single and mixed-source short tandem repeat profiles to inform human identification strategies: PROVEDIt. Forensic Sci. Int. Genetics 32, 62-70.

Deadmen DO Tell Tales!

David San Pietro & Claire Glynn, Lee College of Criminal Justice & Forensic Sciences, University of New Haven

In 1717 the Whydah Gally, the main ship of the pirate "Black Sam" Bellamy, went down in a nor'easter off the coast of Cape Cod. Of the 145 man crew, only two survived, with Bellamy going down with his ship. At the time 4.5 - 5 tons of varied treasure was lost along with him as well. In 1984, the wreckage was found. Over the last 35 years explorers have recovered pieces of ship and treasure, with a very small amount of possible human remains recovered in 2018. This presentation will discuss the subsequent DNA analysis that was conducted in 2018 to attempt to ascertain whether the remains were those of Black Sam. The methods employed, as well as the results obtained, will be discussed. The use of Y-STR analysis and it's application for historical kinship purposes will also be examined. A brief discussion regarding haplogroup determination will also be provided.

Qualitative Assessment of visible nuclei hair roots

Maria Lawas, Counterterrorism and Forensic Science Research Unit, Visiting Scientist Program, FBI Laboratory Division; Linda M. Otterstatter, Trace Evidence Unit, FBI Laboratory Division; and Joseph Donfack, Counterterrorism and Forensic Science Research Unit, FBI Laboratory Division

DNA Recovery from Semen and Saliva Immunochromatographic Test Cassettes

Jillian Conte, Keystone College; Ashley Ruddy, Duquesne University; Lindsey Domonoski, Penn State University

Immunochromatographic tests strips are being used increasingly to quickly and presumptively identify bodily fluids of forensic interest such as blood, semen, and saliva. The forensic sample travels across a membrane and interacts with embedded antibodies to indicate the presence of a bodily fluid antigen of interest, and after documentation, the testing cassette gets discarded. Commonly, forensic samples are of limited quantities. In the practice of conserving limited samples, it would be ideal to be able to recover the genetic material deposited on these testing membranes. The goal of this research was to determine if genetic material can be recovered from immunochromatographic test strips following use. The SERATEC® PSA and Amylase Tests had semen and saliva samples, respectively, deposited and analyzed. The testing membrane was then removed from the cassette and three different DNA extraction methods performed. The extractions included: organic, QIAamp DNA Mini kit, and New England Bio's Monarch Genomic DNA Purification kit. Equivalent volumes of sample that were not analyzed by the immunochromatographic test strips underwent DNA extraction. The samples were quantified and percent recovery from the strips determined. Percent recovery ranges from less than 10% to over 80%. This wide variation in recovery is likely due to the stochastic effects present in the experimental design. Regardless, this study has determined that DNA can be recovered from immunochromatographic testing strips. By using the evidence that remains on these testing cassettes, evidence loss is decreased. On going work on this project includes serial dilutions of semen and saliva for detection and recovery and a timed study to determine if DNA can be recovered after storage of the cassettes

following testing.

Six months to 30 days in 24 hours: A Laboratory's Journey to Meet Statutory Requirements Relative to Sexual Assault Evidence Collection Kit Processing
Lynn Schneeweis, Stefany Harman, Darina Griffin and Kristen Sullivan, MA State Police Crime Laboratory

A national dialogue emerged several years ago as a result of the discovery of thousands of Sexual Assault Evidence Collection Kits (SAECK) sitting untested in law enforcement agencies' evidence storage facilities. This prompted demand for legislative reform by advocacy groups, scientific agencies and law enforcement nationwide. The subsequent reaction of many jurisdictions has been one of action; identify the scope of the problem in their respective jurisdiction and implement measures to reform current practices with the goal of ensuring such a situation never repeats itself. In April 2018, the Commonwealth of Massachusetts passed an Act Relative to Criminal Justice Reform (CJR) containing many initiatives designed to overhaul the Commonwealth's Criminal Justice System. Included in the reforms were sections that addressed topics directly applicable to forensic analysis providers within the Commonwealth. Specifically, one section directed the Commonwealth to identify and test previously unsubmitted SAECK while another outlined requirements for testing current and future kits. An Act Relative to Criminal Justice Reform, Chapter 69, Section 214, mandated law enforcement agencies to submit to the laboratory all previously unsubmitted investigatory kits to the Crime Laboratory. To accomplish this, each agency had to complete an inventory of sexual assault evidence collection kits within their custody. Additionally, the section further prescribed that all identified previously unsubmitted investigatory SAECK must be tested within 180 days of submission. Chapter 41 section 97B ½ addressed the processing of current and future kits by virtue of the following mandates: all investigatory kits would be submitted to the crime laboratory and tested within 30 days. Additionally, a SAECK tracking system would be implemented statewide between June 30, 2019 and December 1, 2019. While well intentioned, the CJR Act posed significant challenges to address before the mandates could be effectively met. Massachusetts has several hundred law enforcement agencies including local, county, state, and academic institutions. By statute, all of these agencies were now required to complete the inventory, report on any SAECK within their custody that fit the criteria for testing, and submit all identified kits to the laboratory. However, the statute did not specify the logistics or ultimate responsible party for ensuring this task was completed. Additionally, given that the number of potential kits subject to the new legislation was widely unknown, determining an appropriate facility to store what could be a significant number of kits posed another potential obstacle. The 30-day turn-around time mandate for testing was progressive and likely intended to provide both expeditious justice for potential defendants as well as forensic answers for survivors. However, at the enactment of this bill, the MA State Police Crime Laboratory (MSPCL) lacked adequate personnel, space, and equipment necessary to meet this mandate. Further compounding this, the statute was effective immediately, meaning no transition period was contemplated to achieve compliance. Criminal justice reform is not unique to Massachusetts; many other jurisdictions have or may soon have mandates related to SAECK processing. This presentation will discuss the challenges associated with the reforms enacted within Massachusetts and the steps MSPCL has taken towards meeting these mandates. Specifically, the initial process for determining what kits fit the criteria of "previously unsubmitted" as well as the coordination of the inventory, collection and testing of said kits will be detailed. Additionally, the challenge to meet a 30-day turn-around time including identification of resources needed and the data to support requests for these resources as well as optimization of current workflow will be discussed. Lessons learned from the perspective of MSPCL will be shared as well as suggestions for other agencies facing similar scenarios will be presented.

An Overview of the National Institute of Justice's (NIJ) Forensic Science Programs
Luther Schaeffer, Office of Investigative and Forensic Sciences, National Institute of Justice

The National Institute of Justice (NIJ) is the research, development, and evaluation agency of the U.S. Department of Justice and is dedicated to improving knowledge and understanding of crime and justice issues through science. NIJ provides objective and independent knowledge and tools to reduce crime and promote justice. Within the NIJ, the Office of Investigative and Forensic Sciences (OIFS) develops programs to support publicly-funded forensic laboratories, medical examiner and coroner offices, and forensic science research. This presentation will introduce attendees to some of the opportunities that NIJ OIFS has offered.

Wet-vacuum based collection versus wet-swabbing of diluted blood from multiple substrates
Jessica McLamb, FBI Laboratory/ORISE; Lara Adams and Mark F. Kavlick, FBI Laboratory

The recovery of DNA from evidentiary items is fundamental for forensic investigations. Swabbing, cutting, and taping are commonly used for DNA collection; however, these conventional methods can be limited by the physical characteristics of the item being sampled, e.g., porous, irregular and/or rough substrates. Therefore, alternative techniques are sought to maximize DNA recovery from such challenging items of interest. The purpose of this study was to determine the efficacy of a wet-vacuum based collection system, the M-Vac®, as a possible DNA collection method for use at crime scenes by collecting diluted blood spotted on 22 substrates of varying porosity. Total DNA yields obtained from the wet-vacuum were compared to those from a standard operating protocol (SOP) which uses a wet cotton swab.

A volume of 1.44 mL of blood diluted 1:100 (14.4 µL of whole blood) was spotted onto most substrates in ~100 cm^2 areas and, once dried, was collected using the wet-vacuum or a wet cotton swab, in triplicate. Swabs were extracted according to a DNA casework SOP and wet-vacuum samples were extracted according to the manufacturer's recommendations. All samples were quantified for nuclear DNA (nDNA) via Quantifiler® HP and for mitochondrial DNA (mtDNA) via an in-house mtDNA qPCR assay.

The wet-vacuum recovered more nDNA on 18 porous substrates compared to the SOP, eight (8) of which were significantly greater. The method also yielded on average 12 times more DNA than swabbing. However, both methods yielded comparable amounts of total DNA on two (2) porous and two (2) non-porous substrates. In no instance did swabbing significantly recover more DNA. Ten (10) selected substrates, which were previously swabbed, were subsequently subjected to wet-vacuum collection for additional DNA recovery. For nine (9) of these substrates, wet-vacuuming recovered additional DNA which was, at minimum, equivalent to the initial swabbing, and maximally 46-fold more. Lastly, the wet-vacuum protocol was compared to a modified wet-vacuum protocol which utilized the SOP extraction buffer as a replacement buffer, but was otherwise identical. Those experiments resulted in comparable nDNA yields for both protocols indicating that the SOP extraction buffer could successfully serve as a substitute without sacrificing nDNA yield. All mtDNA quantification outcomes were generally similar to the nDNA quantification outcomes described above.

This study has demonstrated that wet-vacuuming may serve as an alternative collection method to swabbing on difficult porous substrates and could even be used in follow up to swabbing to potentially recover additional DNA. However, swabbing may remain the preferred collection method on substrates with visible stains since DNA quantities may be abundant. In addition, when stains are not visible, swabbing may be most appropriate on flat, smooth, or non-porous substrates for reasons of convenience, simplicity, and lower costs relative to the wet-vacuum method. Future studies are expected to evaluate alternative body fluids, alternative DNA concentration filters, and STR quality of the recovered DNA.

Integrating validated a posteriori probabilities on the number of contributors into forensic interpretation pipelines for full DNA profile interpretation
Catherine M. Grgicak, Department of Chemistry & Center of Computational & Integrative Biology, Rutgers University, Camden; Slim Karkar, Lauren E. Alfonse, Ken R. Duffy and Desmond S. Lun

Forensic DNA signal is notoriously challenging to interpret and requires the implementation of computational tools that support forensic DNA interpretation. While data from high-copy, low-contributor samples result in electropherogram signal that is readily interpreted by probabilistic methods, electropherogram signal from forensic stains can consist of low-copy, high-contributor-number samples that are often obfuscated by allele sharing, allele drop-out, stutter and noise peaks, all of which greatly complicate interpretation. Since forensic DNA profiles are too complicated to quantitatively assess by manual methods, continuous probabilistic frameworks have been developed to draw inferences on the Number of Contributors (NOC) and the Likelihood Ratio (LR) of the electropherogram given the prosecution and defense's hypotheses. In this work we present the validation of a new version of the NOCIt inference platform that determines an A Posteriori Probability (APP) distribution of the number of contributors given an electropherogram. NOCIt is a continuous inference system that incorporates models of peak height (including

degradation and differential degradation), forward and reverse stutter, noise and allelic drop-out while taking into account allele frequencies in a reference population. We established the algorithm's performance by conducting tests on samples that were representative of types often encountered in practice. In total, NOCIt's performance was tested using 815 degraded, UV-damaged, inhibited, differentially degraded, or uncompromised DNA mixture samples containing up 5- contributors. We found that the model makes accurate, repeatable and reliable inferences about the NOCs and significantly outperformed methods that rely on signal filtering. By leveraging recent theoretical results of Slooten and Caliebe (FSI:G, 2018) that, under suitable assumptions, establishes the NOC can be treated as a nuisance variable, we further demonstrate that when NOCIt's APP is used in conjunction with a downstream likelihood ratio (LR) inference system that employs the same probabilistic model, a full evaluation across multiple contributor numbers is rendered. This work, therefore, illustrates the power of modern probabilistic systems to report holistic and interpretable weights-of-evidence to the trier-of-fact without assigning a specified number of contributors or filtering signal.

"Who you going to call? Okay not Ghostbusters but still a pretty cool team of scientists"
Danielle J. Brownell, Promega Corporation

As forensic laboratories strive to meet demands to process increasingly higher number of samples, establishing automated workflows can improve efficiency to meet that demand. The Maxwell® FSC and Maxwell® RSC 48 instruments are compact, personal DNA purification systems that process up to 16 and 48 samples, respectively. Both systems use the proven DNA IQ™ technology for fast and efficient purification of highquality DNA from casework samples. Additional workflow efficiency can be achieved by integrating the Maxwell® systems with the Maxprep™ Liquid Handler. In addition to pre-extraction sample processing, Maxprep™ will also provide post-extraction methods for DNA quantification setup, normalization, STR amplification setup, and CE setup. These intuitive and easy-to-use pre-loaded methods are being developed and tested by Promega, and will be supported by Promega. Additionally, the Promega portal software maintains sample traceability from the first DNA extraction step to the final CE setup step. Together, these systems provide a modular automated workflow that can expand with and adapt to throughput needs while maintaining flexibility in number of samples to process and reduce human error through increased sample tracking and automated sample preparation.

Northeastern Association of Forensic Scientists

Proceedings of the

November 2019 Annual Meeting

Poster Session Abstracts

Analysis of d and l-Amphetamines using reversed-phase LC-MS/MS
Ravali Alagandula, Restek Corporation; Frances Carroll, Restek Corporation

Amphetamine (AMP) and methamphetamine (MAMP) are psychostimulant drugs and occur as two enantiomers, dextrorotary and levorotary due to their chiral center. The dextro-methamphetamine (disomer) form is highly abused and typically found in illicit preparations. However, detection of abuse is complicated because consumption of some over-the-counter and prescription medications may yield positive results if the analytical method cannot distinguish between the enantiomers. Chiral separation of d- and l-methamphetamine and their metabolites d- and l-amphetamine can help determine whether the source was licit or illicit, but chiral columns can be expensive, may necessitate a dedicated instrument, and are not as broadly useful as ubiquitous C18 columns. In order to provide labs with a high-throughput assay that effectively separates d- and l- amphetamine and methamphetamine enantiomers in urine without the use of a costly and specialized chiral column, a LC-MS/MS method was developed using a standard reversed-phase Raptor C18 column. The method employs a simple pre-column derivatization followed by dilution and results in a selective, specific analysis of d- and l-amphetamine and methamphetamine enantiomers that is free from sample matrix interferences. The LC-MS/MS method was developed using a Raptor C18 2.7 µm, 100 mm × 2.1 mm column with water and methanol mobile phases modified with 0.1% formic acid. The LC gradient resulted in a total analysis time of 7.0 minutes cross a linear range of 50-5000 ng/mL, including column equilibration time with good baseline resolution of the target compounds, allowing easy peak identification and quantitation. Carryover was not observed. Validation across this range demonstrated good linearity with r2 values of 0.998 or greater and acceptable method accuracy and precision. An enantioselective method for d- and l-methamphetamine and amphetamine determination in urine was successfully developed. To accomplish AMPs chiral resolution, pre-column derivatization with CDR (Marfey's reagent) was performed with minimal (dilute and shoot) sample preparation. Although CDR utilization in the present method increased the sample preparation time by approximately 2 h, conventional reversed-phase conditions on a C18 column were utilized and the chromatographic separation time was limited to 7 min. Additionally, the use of deuterated internal standards for each enantiomer allowed the method to maintain accurate and reliable quantitative results and able to accurately identify/ quantify licit vs. illicit methamphetamine.

Comparison of STR Allelic Recovery Post-UV Damage Utilizing the PreCR® Repair Method: Singleplex versus Multiplex PCR Amplification
Morgan Barrett; Dr. David San Pietro, University of New Haven

Deoxyribonucleic acid (DNA) is subject to many environmental factors that cause various types of deleterious damage to the molecule, including oxidative, hydrolytic, and ultraviolet (UV) light-induced. This is a major issue in forensic DNA profiling as forensic samples are often found in a damaged or degraded state. The damage inflicted on biological samples results in a degraded DNA template to be used in polymerase chain reaction (PCR) amplification and ultimately causes the loss of short tandem repeat (STR) peak signals in forensic DNA profiling. The PreCR® Repair Mix by New England BioLabs is composed of enzymes used in the cellular mechanisms of DNA repair. Studies have shown the ability of this mix to repair most types of DNA damage prior to PCR amplification of the core STR loci4. However, this repair process does not always result in complete STR profile recovery. Standard STR analysis kits are designed as multiplex PCR amplification reactions, consisting of forward and reverse primers for multiple STR loci. The purpose of this study was to determine whether or not singleplex amplification reactions post-UV damage repaired with PreCR® would result in greater STR allele recovery with the idea that singleplex reactions would allow for less competition between individual STR loci primers and primer binding sites, allowing for more efficient amplification.

As larger STR markers tend to be more susceptible to environmental damages, as observed by the 'ski slope effect',

this study focused on three STR loci of differing lengths, all with simple, tetrameric repeats for the singleplex amplifications; TH01, D18S51, and CSF1PO. Primer sequences for these loci were obtained from STRbase and all forward primers were developed to include a 6-FAM fluorescent tag. Multiplex and singleplex amplifications were performed in triplicate under three different conditions; no damage, no repair (NDNR), damage, no repair (DNR), and damage, repair (DR). Additionally, two damaging events were performed as the consistency of UV damage to a particular sequence is unknown.

Many observations were made with the data obtained in this study. These include the interpretations of any differences in peak height percent recovery post-PreCR® repair within each locus between multiplex and singleplex amplifications, any consistencies or inconsistencies between the two damage events, any allele size shift, alterations in peak height ratios, or introduction or disappearance of nonspecific peaks, and differences in peak height percent recoveries post-repair between shorter and longer loci and homozygous and heterozygous loci. Further studies that focus on more STR loci, both heterozygous and homozygous, as well as a larger sample size and more damage events are warranted.

A Microspectral Analysis of Synthetic Wig Fibers
Jaclyn Beshlian; Dr. Thomas Kubic, John Jay College

In order to determine whether differentiation between 20 visually similar light brown synthetic wig fibers was possible the use of a microspectrophotometer (MSP) was employed. Visually similar fibers were chosen to display the power of the instrument over the human eye. An MSP is an instrument that has the ability to aid in the analysis of certain microscopical samples. With this particular instrument, one is able to collect the absorption and fluorescence spectra of microscopical colored samples without destroying the piece of evidence itself. Prior to any analysis with the MSP, the material of the fibers were verified through FT-IR ATR, as well as their optical properties. Using a lab-assembled MSP the absorbance measurements were obtained perpendicular and on a 45-degree angle to the polarizer. After the absorbance measurements were complete the fibers were then exposed to fluorescent light. The fluorescent lighting used contained a series of UV, blue, and green excitation filter combinations. No single fiber displayed the exact same spectra to another, allowing differentiation. When combining the information obtained through the absorbance measurements and the three excitation filter combinations, differentiation of all 20 visually similar light brown synthetic wig fibers was multiplied.

An Exploration of Protein and DNA components in Fingerprint Components
Ashley Borrego, John Jay College

The main focus of this project was to investigate the protein and DNA components in both sebaceous and eccrine fingerprints. This study investigated the relative content of DNA and proteins in eccrine fingerprints to sebaceous fingerprints. All volunteers were instructed to wash and dry their hands prior to depositing parallel thumbprints. Twenty volunteers were instructed to touch their face to produce sebaceous prints, and 5 volunteers were instructed to wear gloves over a heat source to produce sweaty or eccrine prints. Microscopy was used to score the cellular debris of the right fingerprint on a scale of 1-4 based on density of cellular debris. The score was then compared to the DNA yield and proteins detected in the left fingerprint(s). The results of the study illustrated that sebaceous samples had an average DNA yield of 1101.4 ± 1344.0 pg and eccrine samples had an average DNA yield of 131.7 ± 219.5 pg. The difference in DNA yield between the two sample types was significant ($p=0.023$). The sebaceous samples ($n=20$) had an average count of 26 ± 22 proteins, and the eccrine samples ($n=5$) had an average count of 39 ± 20 proteins. The difference in the number of proteins detected was not significant ($p=0.153$). The sebaceous samples had better STR quality resulting in 75% full profiles compared to the 20% full DNA profiles of eccrine samples. Linear regression

results indicate a lack of correlation between cellular debris scores and DNA yields in sebaceous samples (R=0). A strong correlation between the cellular debris and DNA yield in eccrine samples (R=0.80) needs to be confirmed with more samples. Linear regression results show a moderate correlation between the cellular debris and number of proteins detected for sebaceous and eccrine samples (R=0.39, 0.55, respectively). The results of this study provide additional information about donor variability/shedder status, and the content of DNA and proteins in fingerprint samples.

Statistical Association and Discrimination of Fired Cartridge Residue using PCA and HCA
Becca Boyea; Dr. Ruth Waddell Smith, Michigan State University

Fired cartridge residue analysis typically involves the identification of characteristic inorganic components that originate from the primer. However, as primer formulations progress to eliminate toxic inorganic components, there has been increasing interest in analyzing the organic components of the propellant, which is typically a smokeless powder. The overall objective in this work was to investigate the potential to associate fired cartridge residue to the original, unburned smokeless powder based on the organic composition.

Unburned smokeless powders were removed from 18 ammunition types from different manufacturers and of different calibers. The powders were analyzed in replicate by gas chromatography-mass spectrometry (GC-MS) to generate organic compound profiles. To investigate similarities and differences among the unburned powders, the chemical profiles were subjected to principal components analysis (PCA) and hierarchical cluster analysis (HCA). From PCA, the unburned powders were separated into five groups, primarily based on differences in abundances of dibutyl phthalate and ethyl centralite. HCA of the unburned powders resulted in clusters consistent with the groups identified by PCA. Thus, association and discrimination of unburned powders was achieved based on differences in organic compound profiles and abundances of major compounds.

Four of the ammunition types were also fired using the appropriate firearm and the cartridges were collected. The cartridge residue was extracted with methylene chloride and then analyzed by GC-MS to generate organic compound profiles for the fired cartridge residue. The fired cartridge residue profiles were first compared to the profile of the corresponding unburned powder. When ethyl centralite and dibutyl phthalate were present in highest abundance in the unburned powder, these compounds remained highest in the fired cartridge residues although, as expected, overall abundance was substantially lower in the fired cartridge residues.

Association of the fired residue to the corresponding unburned powder was then investigated, again using PCA and HCA. Due to lower abundances of compounds, the fired cartridge extracts were not closely associated with the corresponding unburned powders by PCA. Greater success was possible using HCA, although the extent of success was dependent on the original composition of the powders. Powders that contained ethyl centralite or dibutyl phthalate as the most abundant compound in the unburned powder resulted in fired cartridge residue with similar composition, resulting in successful association using HCA. In contrast, in powders for which the most abundant compound was the explosive compound, association of the fired cartridge residue to the unburned powder was limited and in some cases, not possible. This presentation will discuss the application of the statistical methods for association and discrimination of smokeless powders, discussing the success and the limitations of each method.

Evaluation of Sample Preparation Techniques for the Detection and Quantitation of Benzodiazepines in Human Urine and Whole Blood Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS)
Mikayla Caldwell; Cassandra Swart, Massachusetts State Police Crime Laboratory; Michael Moretto, M.S., Boston

University; Andrew Ziegler, M.S., Boston University; Jenna Gardner, B.A., Boston University; Nichole Bynum, M.S., RTI; Megan Grabenauer, PhD, RTI; Katherine Moore-Bollinger, M.S., RTI; Sabra Botch-Jones, M.S., M.A., Boston University

Benzodiazepines are a class of drugs generally prescribed for treatment of anxiety, depression, and insomnia. Based on the U.S. Drug Enforcement Administration's National Forensic Laboratory Information System 2018 Mid-Year Report, three benzodiazepines (alprazolam, clonazepam, and diazepam) reside in the top 25 most frequently identified drugs. Thus, toxicological analysis and detection of benzodiazepines remains forensically relevant.

The purpose of this research was to evaluate three sample preparation methods: liquid-liquid extraction (LLE), solid phase extraction (SPE), and supported liquid extraction (SLE) for the reliable and accurate identification of 6 benzodiazepines in human urine and whole blood, including: alprazolam, alpha-hydroxyalprazolam, clonazepam, etizolam, diazepam, and 7-aminoclonazepam. Further, analytical methods were validated using all three of these techniques. A six-point calibration curve and three quality control samples each in triplicate were extracted using SLE with ISOLUTE cartridges (SLE, Biotage, Uppsala, Sweden), SPE using Clean Screen XCEL I cartridges (SPE, United Chemical Technologies, Inc., Bristol, PA, USA), and LLE. Samples were analyzed on a high performance liquid chromatograph (Shimadzu, Kyoto, Japan) with a 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometer (SCIEX, Waltham, MA, USA) in positive ionization mode. The method was validated in accordance with proposed AAFS Standards Board Standard 036, First Edition 2018 for quantitative analysis by evaluating calibration model, precision, bias, limit of detection, limit of quantitation, carryover, interferences, analyte recovery, and ionization suppression and enhancement.

With this developed method, analysis time totaled 9 minutes. A linear dynamic range of 10 - 1000 ng/mL was used for all analytes. Recovery of all analytes for blood and urine was similar between SLE, SPE, and LLE, ranging from 26.13% (SPE, blood) to 143.9% (LLE, urine). The limit of detection for urine and blood utilizing LLE (0.5 - 1ng/ml) was lower than those for SLE (0.5 - 1 and 1 - 5 ng/mL, respectively) and SPE (1 ng/mL and 1 - 2ng/mL, respectively). All compounds were stable up to 72 hrs after being processed using SPE and LLE, while compounds processed with the SLE method had varying stability from 24 hrs (clonazepam, etizolam and diazepam), to 48 hrs (alprazolam), and 72 hrs (alpha-hydroxyalprazolam). Ionization suppression and enhancement was evaluated, and although some samples had >25% suppression/enhancement, it did not negatively impact LOD/LOQ. Additionally, the validation met proposed ASB 036 guidelines for precision (<20%), bias (<20%), LOQ, carryover, and interference.

The development and validation of these sample preparation methods demonstrates reliable and reproducible results to identify and quantify six commonly encountered benzodiazepines in human urine and whole blood. The use of LLE proved to be efficient, however sample preparation was laborious and costly due to the associated chemical waste of the solvent. The SLE cartridges used were approximately twice as expensive as SPE, but was the fastest method to prepare samples, whereas SPE consumed a large amount of solvent and took the most amount of time to prepare samples. Given the data above, each of the sample preparation methods were demonstrated to provide valid and accurate results, with varying advantages and limitations.

Acknowledgements/Disclaimer: This work was supported by the National Institute of Justice under Award No.: 2016-DN-BX-0192. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

The Formation and Examination of Bloody Friction Ridge Patterns on Common Textile Materials using Different Enhancement Techniques

Kaitlyn Chetney, Pennsylvania State University; Ralph R. Ristenbatt III, MS, Pennsylvania State University

The presence of fingerprints at a crime scene has proven to be useful during an investigation due to the great variability of ridge features. Finding and analyzing fingerprints may provide criminalists with information allowing an individual to be linked to an incident. It is common to find fingerprints, along with other friction ridge patterns, at crime scenes on a multitude of surfaces. For example, textiles of various types and complexity are often present at crime scenes. If a textile contains differentiating pieces of evidence, such as fingerprints composed of blood, it could be instrumental in the resolution of the investigation. However, bloody fingerprints on textiles may often be difficult to observe or locate, causing them to remain undetected or be deemed unsuitable for comparison. The enhancement of bloody fingerprints on fabrics could be crucial in increasing their probative value in an investigation.

There are several techniques that may be used to enhance fingerprints generated by bloodcontaminated friction ridge skin. Criminalists must first characterize the fabric containing the pattern. Composition, structure, color, and textile porosity must be considered before selecting an appropriate chemical or dye. Three different classes of compounds that can be used to enhance fingerprints made primarily of blood are protein dyes, heme-reactive compounds, and amino-reactive compounds.

A series of five bloody fingerprints created with a blood-covered finger were deposited onto a variety of textiles. This was accomplished by depositing 20 L of defibrinated ovine blood onto the fingertip of the index finger (palmar surface of the distal phalange) and redistributing the blood with a Teflon spatula, followed by placement of the fingertip onto the substrate. Four subsequent fingerprints were deposited onto the substrate without applying additional blood. The fingerprints were deposited onto both washed and unwashed fabric swatches of different colors with different backing materials.

The fabric swatches were allowed to dry for a specific amount of time (1 hour, 5 hours, 10 hours, or 24 hours) and then fixed and/or enhanced using a variety of different dyes and chemicals (Amido black, Coomassie blue, Leucocrystal Violet, DFO, Hungarian Red, or Acid Yellow 7). After enhancement, a Nikon D810 DSLR camera was used to record the results of each trial. Different optical techniques were employed in an attempt to provide further enhancement.

The enhancement of patterns of friction ridge skin composed of blood on different fabrics may be important in forensic investigations. If better understood, criminalists may be able to decide which technique would provide optimal results for the enhancement patterns of blood on fabric.

DNA Sequence Analysis in Forensic Science: A case for motif-specific stutter modeling

Olivia Ann D'Angelo, Syracuse University; Brian Young, Luigi Armogida; Tom Fairs, Jonathan Adelman; Michael A Marciano

Forensic DNA analysis is among the most well recognized and well-developed forensic disciplines. However, the field's use of DNA markers known as short tandem repeats (STRs) both offer a useful tool in discriminating individuals and make analyses challenging. The PCR amplification of STRs produce a non-biological artifact known as stutter that can significantly complicate the analysis of DNA mixtures. In the simplest form, this results in the formation of a child sequence that is one repeat unit shorter or longer than the parent sequence. The formation and amplification of these stutter products can occur at rates as high as 15-20% of the parent allele. The challenge inherent in this process is differentiating stutter fragments from true alleles, particularly in the presence of a minor contributor. Traditionally, DNA profiles are obtained through the use of capillary electrophoresis, where amplified DNA fragments are separated by size, not sequence, and the identification of stutter is performed on a locus-specific or locus-longest uninterrupted stretch-specific (LUS) level [1]. The use of CE-based fragment data, and not sequence-based data, has limited the community's understanding of the precise behavior of stutter. The use of massively parallel

sequencing (MPS) data provides an opportunity to better characterize stutter, thus permitting a more accurate means of detecting this phenomenon using not only size-based information but also allele and motif-specific characteristics. This study uses DNA sequence data to characterize stutter through a locus, allele and motif specific manner. Analysis and characterization of stutter sequences was performed using data generated from 550 samples amplified using the PowerSeq 46GY library preparation kit and sequenced on the Illumina MiSeq FGx. Within each locus, the rate at which a particular motif or allele stuttered was modeled to determine if different elements of a sequence showed distinct stutter rates. The models indicate that the stutter from one motif or allele can be distinguished from another motif or allele based on their unique stutter rates. Motif- and allele-specific stutter models provide the most comprehensive analysis of sequence stutter rates and provide the ability to more accurately differentiate stutter sequences from true allele stutter. This information provides a higher confidence in the resulting conclusions.

[1] S.B. Vilsen, T. Tvedebrink, P.S. Eriksen, C. Bøsting, C. Hussing, H.S. Mogensen, N. Morling, Stutter analysis of complex STR MPS data, Forensic Sci. Int. Genet. 35 (2018) 107–112. doi:10.1016/j.fsigen. 2018.04.003.

Forgery Examining various human body fluid concentrations using Fourier Transform Infrared Spectroscopy

Nicole DiRado, Alfred State SUNY College of Technology; Coral Smith, West Virginia University

Current serological techniques used to identify human body fluids are destructive and involve many consumables. This study sought to examine various concentrations of human body fluids using a Fourier Transform Infrared Spectrometer with an Attenuated Total Reflectance head (FTIR-ATR) because the technique is fast, it is non-destructive, and it does not require consumables. Five concentrations of human blood, semen and saliva were allowed to dry on three different intermediary materials; a white, cotton t-shirt, a red, cotton t-shirt, and a white, fluid-impermeable laboratory coat. The resultant stains were scanned on a FTIR-ATR, and their spectra were analyzed for unique lipid, protein and nucleic acid peaks. The spectra of each body fluid were compared to determine whether the body fluids could be differentiated by their spectra. The spectra of each concentration of body fluid were compared to determine the limit of detection of this method. This study sought to determine whether the FTIR-ATR could be used as a precise method to identify body fluids. The results of this study demonstrated that the FTIR-ATR is not a suitable technique for human body fluid identification on cotton. Because the medium is so porous, the spectrum of the body fluid samples was indistinguishable from the spectrum of the cotton, even at the highest body fluid concentration. The FTIR-ATR was a suitable technique for human body fluid identification on the fluid-impermeable laboratory coat, as the spectra of the body fluids were distinguishable from one another. These results suggest that this technique may be useful when examining a human body fluid stain on a non-porous medium, such as a raincoat or a plastic bag, but not when examining a human body fluid stain on a porous medium, such as a cotton sheet.

Inorganic Gun Shot Residue (GSR) Analysis using Laser-Induced Breakdown Spectroscopy (LIBS) on Nasal Swipes

Margaret Duffy, Alvernia University; Nick Laraia, NMS labs; Marianne Staretz, Cedar Crest College; Rosemarie C. Chinni, Alvernia Unversity

Gunshot residue (GSR) is the product after any firearm is fired; this ultimately leaves behind trace amounts of residue that can be collected and processed for further analysis. Any time lapsed between the initial firing of a firearm and evidence collected creates a window of opportunity for loss of evidence. GSR evidence can be lost in a short period time due to washing, sweating, or transfer loss. GSR is also inhaled via the nasal cavity of any person naturally, upon firing any firearm. Evidence found in the nasal cavity would normally be overlooked and therefore, theoretically, should be present for longer a window of time.

This study presents analyzing nasal swabs using laser-induced breakdown spectroscopy (LIBS). In LIBS, a high powered laser is focused on the sample; the focused laser pulse heats, ablates, atomizes and ionizes the sample and results in the formation of a plasma. The light from the plasma is spectrally resolved and detected. Elements contained within the sample are identified by their unique spectral signatures. LIBS data can provide both qualitative and quantitative results.

Testing for GSR using LIBS has great benefits, which include no sample preparation, rapid sample analysis, and multi-element detection. A study was performed to determine the best nasal swab material. That material was then used on a 12 shooter study and included 25 control samples. The shooter data was collected at three different points, (1) prior to shooting, (2) after one shot expelled from the firearm, and (3) after ten shots were expelled from the firearm. The elements targeted were barium (Ba), lead (Pb), and antimony (Sb); these are the three primary inorganic elements found in the composition of gunshot residue primer. Sb was not detected and this is most likely due to its low concentration found in the original primer. Pb and Ba were detected in 92% and 100% of the time in both the 1 shot and 10 shot samples, respectively. The common presence of Ba in the nasal swabbings of persons who fired a weapon could provide as a useful beacon in distinguishing a shooter of a firearm versus a non-shooter.

A quantitative study was then performed to determine the detection capabilities of Pb and Ba on the nasal swabs. Synthetic nasal mucosa was purchased, applied on the nasal swab, and allowed to dry. Known amounts of Pb and Ba were placed on the nasal swabs. Calibration curves were created for Pb and Ba. Their detection limits and sensitivities were determined. This study shows LIBS application for successfully determining GSR on nasal swabs and provides information on the detection capabilities of using LIBS for Pb and Ba detection on the nasal swabs.

Investigating the use of DNA Methylation Analysis for Donor Age Estimation
Rachel C Graziano, University of New Haven

Within the forensic science community, there is a challenge that scientists have long sought to address that could prove useful in various cases. This challenge is the ability to predict the chronological age of the donor of a biological sample. DNA methylation within a person's genetic sequence is a type of epigenetic change that occurs throughout a person's life, due to various factors. The methylation change refers to the addition of a methyl group to cytosine molecules at cytosine-guanine dinucleotides (CpG sites). Methylation of these sites is correlated to gene expression of the gene's in which the cites lie in. The aim of this research was to determine if there are CpG sites that could be targeted, using quantitative real-time polymerase chain reaction (qPCR), that have methylation patterns that can be used to predict the chronological age of the donor. FHL2 was chosen as the target gene as it was reported to be associated with age prediction, and the housekeeping gene for control comparison was EF1a.

After obtaining Institutional Review Board (IRB) approval, buccal swabs were collected from fourteen donors, aged 18-85 using sterile cotton swabs, with written informed consent. The samples were stored at -20°C until extraction. The QIAmp DNA Investigator Extraction Kit (Qiagen®) was utilized to extract DNA from three swabs for each donor. Next, the DNA was quantified for each sample using the Qubit 3.0 Fluorometer (ThermoFisher Scientific) using the double stranded (ds) DNA High Sensitivity (HS) assay. Following this, the EpiTect Bisulfite Kit (Qiagen®) was utilized to bisulfite convert the DNA samples. MethPrimer 2.0 online software was used to design the MethyLight specific primers and Taqman™ probes necessary. The bisulfite converted samples were amplified using the EpiTect Methylight PCR + Rox Vial Kit on the Applied Biosystems™ 7500 Real Time-PCR Instrument, using protocols provided by the manufacturer.

The samples were run in technical duplicates for both genes, for both the unmethylated and methylated primer/probe

combinations. Only five samples produced results in the duplicates, and only Sample 2A obtained Ct values for both the methylated and unmethylated regions in duplicates. However the Ct values obtained within each duplicate varied considerably, and therefore showed inconsistency in the measurements. Due to the vast amount of undetected results, and the inter sample variation, it was determined that this methodology is not suitable for methylation analysis. It is suggested that the bisulfite conversion damages the samples to such an extent that reproducible results are not obtainable. Future research should continue to look into other methods, including pyrosequencing, and Sanger sequencing. This research highlights that DNA Methylation analysis using qPCR based methods is not the optimal approach for assessing donor age estimation and other methods of analysis should be explored.

Determination of the Efficacy of Two Processing Methods for Molar Teeth as a Source of DNA in Missing Persons and Unidentified Human Remains (UHR) Investigations

Lily Josephs, University of New Haven; Ira Titunik DDS; Timothy Palmbach JD; Angie Ambers PhD

The goal of this presentation is to provide attendees with an empirical evaluation of two different processing methods for molar teeth as a source of DNA in missing persons and unidentified human remains (UHR) casework. This presentation will impact the forensic science community by demonstrating that the specific approach used to prepare a molar tooth for DNA extraction can impact DNA recovery and downstream STR typing results.

Bones and teeth are a valuable source of DNA in forensic, anthropological, and archaeological investigations. There are many scenarios in which bones/teeth may be the only viable sample type for DNA testing, including fires, explosions/bombings, natural disasters, war conflicts, airline crashes, homicides, and mass graves. Typically, compact weight-bearing long bones (such as the femur or tibia) and molar teeth are the preferred sample types for forensic genetic testing of skeletonized or highly decomposed remains. There currently is not a standardized protocol for processing molar teeth for DNA extraction. Some forensic laboratories pulverize the entire tooth, while others horizontally transect the tooth and drill into the inner cavity of the crown and roots to collect tooth powder. Pulverization of the entire tooth is a less time- and labor-intensive approach, and it requires less manual manipulation of the sample (an important consideration in contamination prevention). However, pulverization is a destructive process that completely consumes the sample, eliminating the possibility of re-testing and/or additional analyses that may be informative (e.g., amino acid racemization, isotopic analysis). In contrast, horizontal sectioning preserves the structural integrity of the tooth (which may be important for museum or other precious specimens), although it generates less powder for DNA extraction. In highly degraded remains, the latter approach may be prohibitive to recovery of sufficient DNA to make an identification or association.

Understanding the structural, molecular, and biochemical composition of teeth is an important consideration for forensic genetic testing. A molar tooth can be divided into two physical regions: the crown (the portion that visibly extends above the gumline) and roots (which are embedded within the alveolar bone of the maxilla and mandible). Additionally, teeth consist of four molecularly differentiated layers: enamel, cementum, dentin, and pulp. Enamel (the hardest substance in the human body) and its adjacent layer (cementum) provide a strong physical barrier which protects endogenous DNA from environmental insult. However, fractures or caries in enamel expose the inner layers of the tooth (and endogenous DNA) to damage, as well as to exogenous contamination. Dentin, which lines the inner pulp cavity, is targeted for DNA testing because it is rich in hydroxyapatite. Hydroxyapatite directly interacts (binds) with the backbone of the DNA molecule, purportedly protecting DNA from degradation. Often the pulp (soft tissue) within the crown of the tooth has decomposed by the time skeletal remains are recovered, leaving the rigid microstructure of the tooth as the only potential source of DNA. A principal rationale for using targeted drilling to process molar teeth lies in the protection afforded to the DNA molecule via direct interaction and binding to hydroxyapatite in the dentin layer.

This research specifically focused on comparing the effectiveness of two common mechanical processing strategies - complete pulverization vs. targeted drilling - for 1) burned molar teeth and 2) teeth that have been subjected to burial in acidic soil. These conditions reflect two of the most common forensic casework situations in which skeletonized remains are recovered. Effectiveness of each tooth processing method was determined via comparison of total DNA recovery (ng), average signal per locus (peak heights, in RFUs), and total number of alleles detected (i.e., completeness of STR profiles). Additionally, special considerations for targeted drilling techniques (and potential associated complications) will be discussed.

High-Throughput Analysis of Biofluids with Direct Analysis in Real Time Mass Spectrometry (DART-MS)
Paul Liang, IonSense, Inc.; Brittany Laramee, Frederick Li, Brian Musselman, IonSense, Inc.

In forensic toxicology, biological specimens such as blood or urine are collected to confirm presence of toxins, such as drugs of abuse. However, biological specimens often affect detection sensitivity of target analytes. In order to improve sensitivity, conventional analytical techniques such as mass spectrometry are coupled to chromatography to separate target analytes from matrices prior to analysis. Chromatography based mass spectrometry is very sensitive but is often lengthy and limited in high through-put capability.

In contrast, Direct Analysis in Real Time - Mass Spectrometry (DART-MS) is a rapid analytical technique with lower sensitivity due to lack of separation. In order to improve sensitivity, DART-MS can be coupled with solid phase microextraction (SPME) for sample cleanup to reduce matrix effects. The SPME workflow requires solvents for conditioning, washing, extracting, and eluting, all of which can be facilitated with liquid handling robots for higher throughput. Liquid handling robots can also be utilized to deposit the eluents onto wire mesh substrates for transmission mode DART-MS. The SPME workflow can be performed in 60 minutes, and 96 samples currently can be analyzed by DARTMS in as little as 20 minutes.

For preliminary testing with SPME-DART-MS, bovine blood was fortified with fentanyl and analyzed directly with DART-MS. Samples were analyzed with and without SPME extraction for comparison. The direct analysis workflow resulted in fentanyl detection limits as low as 5 parts per million (ppm or μg mL-1), and SPME workflow resulted in fentanyl detection limits as low as 100 parts per billion (ppb or ng mL-1). The results show that SPME-DART-MS reduces matrix suppression and improves detection sensitivity by 50-fold.

An Investigation into the Use of Amino Acid Ratios to Distinguish Between the Hairs of Similar Individuals
Allison Macri, University of New Haven; Alyssa L. M. Marsico, Ph.D.; Robert H. Powers, Ph.D., University of New Haven

Currently, forensic hair analysis is accomplished by microscopic hair comparison (MHC) and DNA analysis. However, MHC is very subjective and is not supported with statistical analysis, while DNA analysis can be problematic if there is not enough undamaged nuclear DNA to sample from or if the nucleated cells within a hair follicular tag are not present. In this study, amino acid analysis was investigated as a new method to analyze hair proteins. Human hair is made up of several proteins, such as keratin, and these proteins can have slight genetic variations in the form of genetically variant peptides (GVPs). These GVPs contribute to individuals' genetic variation because they can alter amino acid sequences. This means the amount of amino acids can vary in individuals' hair based on their genetics.

Hair samples were obtained on various days over one month from two consenting individuals. To account for intra-variability, hair samples from one individual were collected from four areas of the head (left, right, top, and back). Hair samples were first washed with deionized water and methanol to remove any surface contaminants and/or hair

products. Then, the hair samples were cut into smaller pieces to increase surface area for protein digestion. Next, the hair proteins were broken down into their amino acids via acid hydrolysis with hydrochloric acid. After protein digestion, L-norvaline, an amino acid not synthesized by humans, was added to the hair samples as an internal standard. The amino acids were then derivatized with N,O-Bis(trimethylsilyl)trifluoroacetamide, and the derivatized hair samples were diluted with ethyl acetate. Gas chromatography-mass spectrometry was used to identify the amino acids present in the hair samples.

Eight derivatized amino acids were detected and positively identified from the hair samples. These amino acids were confirmed by comparing them to the standards run as well as conducting library searches. The amino acids were then quantified relative to the internal standard, and the values were used to construct twenty-eight amino acid ratios. Once this method was confirmed to be successful in detecting and quantifying amino acids, the intra-variability of these ratios from hair samples taken from different areas of the head was investigated. It was determined that the amino acid profile of hair proteins does not vary among different areas of the head. This was confirmed by conducting one-way ANOVA tests. Furthermore, the reproducibility of this method was also investigated. Hair samples that were collected on different days were prepared and analyzed multiple times over the course of one month. The resulting amino acid ratios were averaged, and the standard error of the mean was used to represent the error associated with reproducibility. Results indicate that the amino acid profile in hair does not vary greatly over one month. This data signifies that amino acid ratios are consistent and are a promising supplemental technique for hair analysis when MHC cannot distinguish between individuals or DNA analysis is not feasible. Current research is being conducted into a comparison of morphologically similar hair samples from two demographically similar individuals.

Pigment Analysis Using Laser Induced Breakdown Spectroscopy (LIBS) and Digital Image Processing
Amber Malloy; Megan Olsson, Alvernia University; Shijun Tang; Rosemarie Chinni, Alvernia Unversity

The purpose of this project is to show how laser-induced breakdown spectroscopy (LIBS) and digital imaging processing can be used for indirect dating, authentication, and identification of oil paint pigments. Pigments are the organic or inorganic substances that give paint their colors. The samples consisted of ten oil paint pigments from traditional and modern palettes. Since synthetic paints (modern) were not available prior to the 19 century, the presence of a synthetic paint could allow for determination of whether the artwork was conserved in the past or it if was a forgery. The oil paint pigments were analyzed in pure form and binary mixture form using both LIBS and digital imaging processing.

In LIBS, a laser pulse is focused on a surface. This heats, ablates, atomizes, and ionizes the surface material and results in the formation of a plasma. The light emitted by the plasma is collected, spectrally resolved, and detected. Elements contained within the samples can be identified by their unique spectral signatures. LIBS provides qualitative and quantitative results and has many applications from environmental to defense including metal sorting, explosive and fissile material detection, lead in paint determination, heavy metal contamination in soil, identification of cultural heritage objects, etc. In LIBS, the data was taken on the pure and binary mixtures using ten shot accumulation analysis. The data was analyzed to determine what spectral signatures could be used to uniquely determine one pigment from another.

Image processing technology and algorithms were used to analyze the pure and mixture pigments in single and multilayer samples. Image histograms were created from the digital images. The peak location of the image histogram was used to determine if there were differences among the pure and binary mixture pigments and whether there were unique peak locations in the histograms of the pure pigments or mixture pigments.

Recovery of Human DNA from the Coat of Domestic Animals: Application of DNA Transfer to Animal Cruelty Investigations

Elaina Marcotte, University of New Haven; Angie Ambers; Virginia Maxwell, Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven

Companion animals, such as dogs and cats, are an important part of human lives. It is estimated that approximately 84.6 million (68%) of American households contain at least one domesticated pet. Current investigative efforts in response to animal cruelty and/or neglect cases involve veterinary intervention and treatment of surviving animals, as well as necropsy of deceased animals to determine cause of death and severity of injuries. Animals who perish as a result of abuse and/or neglect are often dumped or discarded in separate or remote locations, away from the site of the original crimes perpetrated upon them. Identifying and collecting evidence that would support a direct association between discarded abused animals and their human perpetrators is challenging, but is necessary to physically link offenders to victims and to provide support for indictment and/or prosecution proceedings. Recent advances in forensic DNA technology, particularly in reference to "increased sensitivity" methods, may enable detection of human DNA from the coats of abused animals. When human hands contact or grip a surface or object, epithelial cells are shed onto the surface or object. This phenomenon, known as "primary DNA transfer," is believed to occur more readily when the surface contacted is rough in nature, and as the duration of contact and the amount of pressure or friction increases (as would be expected in violent or forceful contact that occurs in animal abuse cases). The cuticle (outer covering) of animal hair consists of overlapping scales. Presumably, these overlapping scales provide sufficient irregular surface features to dislodge and trap epithelial cells during human contact. The aim of this study was to determine if human DNA transferred to an animal's coat can be recovered in sufficient quantities for STR genotyping. Small sections of hair were cut from the coats of pet volunteers and subsequently stroked between the fingers according to a standardized laboratory protocol. Hair samples then were added to microcentrifuge tubes, and DNA extractions were performed. In order to assess the most robust extraction approach to maximize human DNA recovery, two different extraction methods were investigated: a solid-phase technique that incorporates the use of silica columns (Qiagen QIAamp® DNA Investigator Kit) and a modified organic extraction method designed for low quantity samples. DNA was quantified using the Quantifiler® Human DNA Quantification Kit. Human DNA was recovered from 98% of all animal hair samples tested (n=48), with the organic extraction approach yielding higher quantities of DNA. Recommended input DNA amounts for most commercially-available STR kits range from 0.5ng to 1ng, with 1ng of genetic material being the optimal amount for DNA profiling. This recommended range of input DNA was recovered from 94% of the samples tested in this study. Results demonstrate proof-of-concept for the use of "touch DNA" from human offenders in animal cruelty investigations.

Compact Mass Spectrometry for the Detection of Smokeless Powder Additives

Connor McCausland, West Chester University; Dr. Monica Joshi, West Chester University of Pennsylvania Chemistry Department

Smokeless powder additives such as diphenylamine, ethyl centralite, and dinitrotoluenes are of forensic interest in the detection of low explosives and organic gunshot shot residues. Gas Chromatography (GC) or Liquid Chromatography (LC) coupled to Mass Spectrometry (MS) are the traditional techniques of choice for the analysis of these compounds. In this poster, we demonstrate the use of compact mass spectrometry for the sensitive detection of various smokeless powder additives and their related degradation compounds such as the nitrodiphenylamines. Standalone mass spectrometry significantly reduces the overall analysis times while retaining the ability to analyze complex mixtures. We demonstrate the selectivity and sensitivity of the affordable and versatile compact mass spectrometer by using a selected ion monitoring (SIM) mode with an Atmospheric Pressure Chemical Ionization (APCI) method. The instrument also allows for various sample introduction formats such as liquid injection, solid-phase microextraction (SPME), and a direct atmospheric solids analysis probe (ASAP). With these sample introduction techniques, we can

fully take advantage of the technique and demonstrate fast, sensitive, and reliable analysis of smokeless powders as liquid extracts and solid residues on surfaces.

References: 1. Lennert, Emily, and Candice Bridge. "Analysis and Classification of Smokeless Powders by GC-MS and DART-TOFMS." Forensic Science International, vol. 292, Nov. 2018, pp. 11 -22. 2. Taudte, Regina V, et al. "Detection of Gunshot Residues Using Mass Spectrometry." BioMed Research International, vol. 2014, 22 May 2014, pp. 1 -16. 3. Advion Inc., Screening of explosive residues without sample preparation using a direct sample Analysis probe on the expression CMS, Application Note: www.advion.com/resource-center/

Sex and race determination based on attenuated total reflection Fourier transforminfrared (ATR FT-IR) spectroscopy of a bloodstain

Ewelina Mistek, University of Albany; Lenka Halámková; Igor K. Lednev

Human phenotype profiling is one of the most important analyses during a forensic investigation. It can play a crucial role in the early stages of investigation. All current methods of phenotype profiling based on the analysis of body fluid traces are destructive to the sample and, most importantly, need to be performed in a lab and not at a crime scene. In this study, attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy was evaluated as a potentially nondestructive, rapid, and in situ method for discriminating sex and race based on the analysis of human bloodstains. It is known from the literature that the biochemical composition of blood changes with donor sex and race. ATR FT-IR spectra were acquired from dry bloodstains, and an advanced statistics methodology was utilized to enhance the differentiation capability of the method. Specifically, partial least squares discriminant analysis (PLSDA) was employed to create models for differentiating Caucasian (CA), African American (AA), and Hispanic (HI) donors according to their sex and race. This approach was evaluated by subject-wise leave-oneout cross-validation (LOOCV), resulting in over 90% correct classification. In addition, the main models were validated externally with four hold-out samples, which were not used for the training data set. The validation with hold-out samples resulted in 100% accuracy for both sex and race predictions at the donor level. Overall, this proof-of-concept study demonstrated the great potential of ATR FT-IR spectroscopy and chemometrics for phenotype profiling for forensic purposes based on dry bloodstains. Translating this technology to a portable instrument would provide an excellent opportunity for conducting human phenotype profiling immediately after the crime scene is discovered.

Investigating DNA Methylation Analysis for the Individualization of Monozygotic Twins

Malorie Nitz, University of New Haven; Rachel C. Graziano, Claire L. Glynn, PhD., University of New Haven

A known issue that can arise in forensic investigations is the differentiation between monozygotic (identical) twin DNA. Monozygotic twins share the exact same DNA sequence, therefore identifying them with traditional STR profiling is not possible. Epigenetics, and more specifically, DNA methylation analysis, is a novel field in genetic and forensic science research. DNA methylation is a process of the addition of a methyl group to a cytosine base of a DNA molecule, occurring at cytosineguanine dinucleotides (CpG sites). DNA methylation patterns change over a person's lifetime and can be unique for each individual, and therefore can theoretically be used to individualize monozygotic twins. It is crucial to investigate the ideal methodology and candidate CpG sites that provides individualization in order to implement this novel method into forensic science laboratories. The aim of this research was to investigate, using real-time quantitative-PCR (q-PCR), the DNA methylation patterns of the KIFC3 and EF1α gene markers, reported to be associated with differentiation of monozygotic twins.

Following ethical approval from the Institutional Review Board (IRB), buccal cell swabs were collected from four monozygotic twin pair volunteers with written informed consent. DNA extractions were performed using the QIAamp DNA Investigator Kit (Qiagen®) according to the manufacturers protocol. Following extraction, the

samples were quantified using the Qubit 3.0 Fluorometer (ThermoFisher Scientific) using the double stranded (ds) DNA High Sensitivity (HS) assay. The EpiTect Bisulfite Kit (Qiagen®) was utilized to perform bisulfite conversion on the DNA extracts in order to prepare the samples for quantitative-PCR, following the manufacturers protocol. The Epitect MethylLight PCR +ROX Vial Kit (Qiagen®) was used for quantitative methylation analysis with methylation-specific primers and probes, targeting KIFC3, and EF1α as the housekeeping gene. MethyLight specific primers and Taqman™ probes for the KIFC3 and EF1α markers were designed using the MethPrimer 2.0 online software. The prepared DNA samples were quantified using the Applied Biosystems™ 7500 Real Time-PCR Instrument according to the Epitect MethylLight PCR +ROX Vial Kit (Qiagen®) protocol.

Several samples for the KIFC3 gene marker were undetected, and the gene marker EF1α was not detected in any sample. Both twins in twin pairs 3 and 4 obtained Ct values for the methylated and unmethylated KIFC3 markers; however, these values yielded statistically insignificant results. The gene marker EF1α, while reported to be endogenous in human samples, was not detected in any sample after bisulfite conversion and the KIFC3 gene marker did not produce statistically significant results. Conclusively, this study shows qPCR based methods using the Epitect MethyLight PCR +ROX Vial Kit (Qiagen®) is not an optimal method for DNA methylation analysis in these circumstances. It is suggested that the bisulfite conversion step is deleterious to the samples therefore impacting the ability to obtain usable data. Other methods of DNA methylation analysis, such as pyrosequencing, should be researched to address this current challenge.

The Use of Laser Induced Breakdown Spectroscopy (LIBS) for the Identification of Bullet Holes

Noah Scarpelli, University of New Haven; Virginia Maxwell D.Phil., Brooke Kammrath Ph.D., University of New Haven

Firearms are used in the majority of murder cases and a large percentage of other violent crimes. For many of the investigations related to these events, a shooting reconstruction is completed using trajectory determination. Without first identifying what is and is not a bullet hole at the scene, the reconstruction will not be as forthcoming. In cases where the hole is not obviously from the passage of a bullet, such as if there is an intermediary target, it could be overlooked and left out of the reconstruction. To date, bullet hole identification has been primarily accomplished through chemical tests, such as the sodium rhodizonate and griess tests. While these tests are efficient in identifying individual chemical elements and functional groups commonly attributed to gunshot residue (GSR) and other components present in the bullet wipe (e.g. copper and nickel from a jacketed bullet), they have significant drawbacks. Some of these include the amount of time required to mix and apply the chemicals, the narrow identification of the chemical components of the suspected bullet hole, and the application of chemicals directly to the item of evidence. With the growing backlog of forensic laboratories, a rapid method for bullet hole identification would be of value for the forensic community. This technique must increase throughput and the level of confirmation while also decreasing the destruction of the evidence in question.

In this study, laser induced breakdown spectroscopy (LIBS) was evaluated as an elemental analysis method for the identification of bullet wipe. Five common clothing fabrics (t-shirt jersey, sweatshirt jersey, nylon, denim, and fleece) were chosen. These fabrics were shot with two calibers of full copper jacketed ammunition (.22 and .380) at three angles (10°, 45°, and 90°). Five replicates of each sample were analyzed using LIBS from each of the cardinal directions. The resulting bullet holes were analyzed, and the LIBS spectra were examined for the elements lead (Pb), barium (Ba), antimony (Sb), copper (Cu), and nickel (Ni).

Results indicate that this methodology is promising for the identification of these elements using the major non-interfering emission lines for the five elements, which resulted in the analysis of a total of eighteen spectral peaks. Pb peaks were completely absent in the control spectra of the fabrics but were present more than 91.33% of the time in

the experimental samples. The Ba peaks were absent from four of the five the controls (it was detected in nylon) and were present in all the experimental samples. Sb had one peak without spectral interferences, which proved useful for the identification of this element in 96.00% of the samples shot with the .380. However, Sb was not detected on the bullet holes produced with the .22. Cu was able to be identified in more than 98.33% and absent in the controls. Ni was able to be identified in 38.50% of the samples compared to all of the controls, excluding nylon, analyzed at one peak on the spectra and was present in 98.50% of the samples compared to absence in the controls at the other peak examined. There were no observable trends in elements detected based on the bullet's incident angle.

Based upon results obtained in this preliminary study, it was concluded that LIBS has the potential to be a useful method for the identification of bullet holes in fabrics. Future research will include examination of additional calibers and types of ammunition, alternative target materials, determining the capability of LIBS for analyzing bullet holes through bunched or layered materials, and the use of LIBS in conjunction with commonly used colorimetric chemical testing.

A Preliminary Assessment of Prostate Specific Antigen in Transfers Under Various Conditons

Keryne Skead, University of New Haven; Dr. David San Pietro, Henry C. Lee College of Criminal Justice and Forensic Science University of New Haven

Prostate specific antigen (PSA, also known as p30), is a protein used as an effective marker for the identification of seminal fluid lacking spermatozoa cells. The ABAcard® p30 test, manufactured by Abacus Diagnostics®, is extremely sensitive and can be used to detect the presence of the prostate specific antigen protein in levels as low as 4 ng/mL.1 Most current research surrounding the stability of PSA over time looks at it within the female reproductive tract, however very little examines the detection of PSA outside of the human body where other environmental factors can influence its detection.2 The aim of this study was to investigate the persistence of prostate specific antigen detection in transfer stains under various conditions.

The conditions examined in this study were dry versus wet transfer receiving substrates, pH, drying time, and differing semen dilution concentrations. For all variables manipulated, the ABAcard® p30 test was used for detection. The reaction environment for PSA was manipulated with differing pH levels, starting from a pH of 7, with detection of the protein still possible up to a pH of 12. The length of time each sample was allowed to dry prior to testing for PSA did not appear to affect detection, however the rate of appearance of the test band on the ABAcard was found to be directly dependent on the sample's dilution concentration with lower concentrations yielding longer development times on the test cards. Fifteen microliters of semen were allowed to dry on a nitrile glove for varying time periods before being transferred to a cotton swatch (receiving substrate) with the use of constant pressure upon application. It was found that the transfer stains were typically nondetectable by the ABAcard® p30 test, under the dry receiving substrate conditions. When the cotton receiving swatch was treated with a K-Y jelly™ lubricant, chosen for its water-based formula, the test was positive regardless of the length of time waited prior to transfer. For the wet and dry receiving substrate conditions, as well as the dilution series, Adobe® Photoshop® LAB mode was utilized to examine test band intensity on the ABAcard p30 cards from photographs taken under controlled conditions to see if we could use that method to more accurately assess differences between samples versus development time. Wet conditions utilized K-Y jelly™ lubricant in an attempt to replicate possible moist environments the protein could be transferred to. It was found that the wet conditions yielded more color intense test bands than the dry conditions. Additionally, the wet conditions seemed to stabilize the detection of PSA at differing dilution concentrations, causing the test to behave more consistently. The differences observed between the wet and dry conditions of test band intensity were found to be statistically significant through t-testing ($p<0.0054$). For the dilution series it was found that lower concentrations yielded fainter test bands on the ABAcard® p30 tests. Based on results obtained, prostate specific antigen is detectable as well as durable under these different environmental conditions. Further research examining

longer time periods under certain conditions, as well as increasing the overall number of replicates, would help to more accurately assess any possible statistically significant differences in the findings.

An Analytical Approach for the Detection of Gunshot Residue on Animal Hides to Estimate Shooting Distance

Alyssa N Smale, Pennsylvania State University; Ralph R. Ristenbatt III, MS, Pennsylvania State University; Jason W. Brooks, VMD, PhD, Pennsylvania State University

An important area of forensic science, particularly the reconstruction of shooting incidents, includes estimating the distance between the muzzle of the firearm and the target after a shooting has occurred. This can provide valuable information regarding the relative positions of the shooter and the target to each other. Animals are occasionally shot in reported self-defense and the estimation of muzzle-totarget distance may assist in the reconstruction of an incident with a primary aim of differentiating self-defense from animal abuse. Often, a key piece of physical evidence in estimating shooting distance is the presence and distribution of gunshot residue (GSR). On animal fur, the detection and visualization of GSR is challenging due to hair and its variety of colors, particularly darker colors. The ability to detect and visualize a GSR pattern around a gunshot wound may provide an estimate of shooting distance. The application of several techniques to assist the detection and visualization of GSR on animal hides has produced valuable information regarding the methodology and efficacy of this approach.

Cotton T-shirts in a variety of colors, white cotton twill fabric, and sections of light-colored sheep hide were shot using a 9 mm handgun with Speer Gold Dot LE Duty 124gr +P GDHP ammunition. Substrates were attached to a corrugated cardboard backing and shot from a range of distances.

The proposed analytical approach uses a series of enhancement techniques and chemical tests in order to detect gunshot residue. Samples are initially subjected to documentation under ambient lighting conditions with the assistance of low magnification examination using a stereomicroscope when necessary. Subsequent visual enhancement methods include infrared (IR) photography and examination with an alternate light source (ALS) using a variety of visible bandwidths, with best results from the 455 nm bandpass filter. Finally, an adaptation of the reverse modified Griess test followed by sodium rhodizonate testing are performed, both of which produce chromophoric products in the presence of specific GSR components.

Following optimization of techniques using T-shirts and twill fabric, the methods were further improved for use with animal hides. On light-colored samples, GSR can often be observed using the unaided eye. The use of IR photography shows that GSR particles may absorb light, while ALS may cause GSR particles to fluoresce. The reverse modified Griess test with an added alkaline hydrolysis step produces an orange dye in the presence of nitrite residues from burned and unburned propellant. The sodium rhodizonate test generates a scarlet product upon reaction with ionic lead. The combination of these results can provide important information about the distribution of the gunshot residue components. Comparison of a GSR pattern from a shot fired from an unknown distance to patterns from shots fired at known distances may provide an approximate muzzle-to-target distance.

A Comparison of Extraction Techniques for Fentanyl Analogues in Whole Blood

Jeremy P Smith, Biotage, LLC; Jillian Neifeld, Stephanie Marin, Mohamed Youssef, Mario Merida, Elena Gairloch, Biotage, LLC

A string of recent notable drug overdoses has been attributed to the presence of fentanyl and fentanylrelated analogues. Testing of these drugs has rapidly become a priority in many labs due to the opioid epidemic affecting

many cities across both the United States and Canada. Whole blood remains a consistent matrix of choice for testing in forensic laboratories, although it contains many endogenous interferences. Obtaining optimal analytical results often requires sample preparation to remove these interferences and isolate compounds of interest. Extraction protocols may be simple, such as with supported liquid extraction (SLE+), or they may also require more complex methods involving solid phase extraction (SPE) with mixed-mode cation exchange sorbents.

Three extraction techniques were optimized for commonly tested fentanyl and fentanyl analogues in whole blood and urine samples by LC-MS/MS. Comparisons were made for recovery, matrix effects, and overall robustness of each protocol.

Human whole blood was spiked with a panel of fentanyl analogues, which were then extracted via supported liquid extraction (ISOLUTE SLE+, Biotage), silica-based mixed-mode strong cation exchange (ISOLUTE HCX, Biotage), and a polymer mixed-mode strong cation exchange (EVOLUTE EXPRESS CX, Biotage). Each extraction protocol was performed in accordance with the manufacturer's recommendations. Chromatographic separation was achieved on a Shimadzu Nexera X2 UPLC coupled to a SCIEX 5500 tandem mass spectrometer for analysis. Recoveries, process efficiencies and matrix effects were determined by comparing the area counts of extracted samples (pre-spiked), post-extraction samples (post-spiked), and unextracted samples (neat, no matrix).

100 µL of urine or whole blood was used for each extraction evaluation. Each sample was diluted with 0.1% formic acid (aq) or 1% ammonium hydroxide (aq) before extraction, depending on the extraction technique. The supported liquid extraction (SLE+) protocol examined three distinct elution solvents in dichloromethane, ethyl acetate, and MTBE. Both the ISOLUTE HCX and EVOLUTE EXPRESS CX extractions evaluated the use of two elution solvent mixtures: 78:20:2 dichloromethane/isopropanol/ammoniumhydroxide or 78:20:2 ethylacetate/acetonitrile/ammonium hydroxide.

Following extraction, the samples were evaporated using a SPE DRY 96 and reconstituted in 50 µL of a 50:50 mixture of 0.1% formic acid in water/0.1% formic acid in methanol. Samples were then analyzed via LC-MS/MS.

The recoveries using the EVOLUTE EXPRESS CX and ISOLUTE HCX were the highest of the extraction techniques when using the DCM/IPA/NH4OH elution solvent. However, significant signal suppression was noted in the EVOLUTE EXPRESS CX protocol, specifically with whole blood. ISOLUTE SLE+ provided samples with the least matrix effects, although recovery was lower compared to the SPE methods. Each method proved to have a reliable LOQ down 0.1 ng/mL.

Each extraction technique displayed their own merits. It is essential to consider the analytes of interest, limits of quantitation, desired extract cleanliness, compound recoveries, matrix effects, and process time when determining the optimal method to implement. For ISOLUTE SLE+, MTBE or DCM will provide the cleanest extracts. For ISOLUTE HCX and EVOLUTE EXPRESS CX extractions, the DCM/IPA/NH4OH yielded high recovery, but elevated matrix effects.

Assessment of Cell Suitability of Touch DNA Samples for Forensic DNA Profiling

Jessica Surratt, University of New Haven; Dr. Claire Glynn, University of New Haven

Touch DNA, also known as trace or transfer DNA, is typically deposited on surfaces by a person touching an item and leaving behind cellular material. It has been hypothesized that the DNA comes from nucleated cells from the hands or other parts of the body that have been touched, cell-free DNA from residual body fluids, and cell-free DNA from cells that have undergone apoptosis.1 In forensic investigations, touch DNA is commonly collected from

surfaces using the double swab method, or by collection using adhesive tape (e.g. mini-taping). As these cells are microscopic, the investigator is blindly sampling areas where it is suspected to have been touched. These sampled areas may contain little to no DNA, which is not realized until DNA extraction and quantitation has been performed. Therefore, a screening method to visualize the cellular material present, would serve to assess if the samples contain sufficient cellular material for further processing. Nucleic acid binding dyes have recently arisen as a novel tool for this purpose. Nucleic acid binding dyes will bind to double-stranded DNA and can be visualized using fluorescence microscopy. There are several commercially available dyes, and this research investigates five different nucleic acid binding dyes, on touch DNA samples collected using both the double swab and mini-taping methods.

Following ethical approval from the Institutional Review Board (IRB), with informed written consent, touch DNA samples were deposited on different surfaces. A range of surfaces that are common in forensic casework were tested, including white cotton, black cotton, blue denim, polyester, glass, ceramic tile, and wood. The methods used to collect the samples from the surfaces were mini-taping and the double swab method. Eight different types/brands of swabs were investigated including cotton, polyester, and foam swabs. The dyes used were GelGreen Nucleic Acid Gel Stain (Biotium), EvaGreen dye (Biotium), DiamondTM Nucleic Acid Dye (Promega), and RedSafe TM (Intron). Following collection, the swabs/tapes were dyed and visualized using the Dino-Lite fluorescent microscope with an excitation wavelength of 480 nm and an emission wavelength of 510 nm. Images were captured using the DinoXcope software (version 1.24). Samples treated with each dye were STR profiled to assess interference with profile generation.

The results of this research showed that all touch DNA samples collected from each surface using both collection methods were visualized using each dye. The mini-taping collection method provided more clarity in the images collected compared to the swabbing method, thereby allowing more optimal assessment of the cellular material present. The foam-tipped swabs were the preferred material of all the swab types as they produced the least background fluorescence. The Diamond™ Nucleic Acid Dye was shown to provide the strongest fluorescence compared to the other dyes, it is a cost effective method, easy to use, and is non-mutagenic. All samples STR profiled produced full profiles. This research highlights the potential of this novel screening tool to assess the suitability of touch DNA samples collected from evidence items and crime scenes prior to submission to the DNA profiling laboratory. This screening method will allow investigators to sample more areas for screening, with the purpose of submitting only the samples known to contain sufficient cellular material, thereby recovering more probative information.

Reference: 1. Burrill, J., Daniel, B., Frascione, N. A review of "Touch DNA" deposits: Variability factors and an exploration of cellular composition. Forensic Sci Int: Genet 2019:39:8-18.

Kratom- Extraction and Quantitation Methods of Mitragynine in Urine Samples

Danai Taruvinga, Syracuse University; Ei Thanda Tun; Ulrich Englich

Plant material from the species Mitrogyna Speciosa – commonly known as Kratom - is widely consumed in the subtropical regions of Southeast Asia for its psychoactive effects. The herbal plant uniquely produces both stimulant and analgesic, opioid-like effects depending on the consumed dose. It has gained popularity in the U.S. as both a recreational drug, and as a means to cope with withdrawal symptoms from opioid addiction. While Kratom is not currently a scheduled drug in the United States, the federal government lists it as a "drug of concern" and has insisted that it poses a risk to individuals who abuse it. Mitragynine presents itself as one of two main active ingredients and thus the focus of our study.

Our research is concerned with the comparison of different protocols for the extraction and quantitation of the active ingredient mitragynine in urine samples. Currently, we are developing improved methods for the detection and

quantitation and will discuss results from solid phase extractions, compare results from HPLC-UV experiments with HPLC-MS, and report on detection and quantitation limits for both in the range of 10 to 1000 ppm.

Bleach Decontamination in the Forensic Laboratory and at the Crime Scene: Investigating the Efficacy of DNA Damage in Native versus Naked Templates

Alyssa Tuccinardi, University of New Haven; Dr. Angie Ambers, Henry C. Lee College of Criminal Justice and Forensic Sciences, Forensic Science Department, University of New Haven

In forensic casework, there are three major factors which significantly impact successful recovery of a DNA profile from evidence, including low-quality (degraded) DNA, low quantity DNA, and the presence of endogenous or environmental inhibitors. DNA damage/degradation is inherent in an evidentiary sample when it arrives in the laboratory. The degree and spectrum of DNA damage present depends on the environment to which it was exposed and the length of exposure time. In the natural environment, ultraviolet light, acidity, heat, and humidity all contribute to various forms of damage in the molecular structure of DNA. In addition to environmental insult, chemicals can be used to damage DNA.

Household/commercial bleach (6% NaOCl, sodium hypochlorite) degrades DNA through oxidative damage, production of chlorinated base products, and cleavage of DNA strands (breaking it into smaller and smaller fragments). The presence of these lesions significantly impacts the ability to generate a full genetic profile from forensic evidence. In fact, knowledge of the damaging effect of bleach on DNA is the basis for its use in forensic laboratories to clean workbenches and prevent cross-contamination of samples between cases. Additionally, bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence. A previous study demonstrated that bleach has a decreased effect on native DNA that is still encompassed within a body fluid (compared to naked DNA that has already been extracted) [1]. This research project expanded on the previous study, with an increased sample size and expanded data set. Numerous variables were tested, including dried blood, wet (uncoagulated) blood, native DNA, naked DNA, and varying concentrations of bleach. DNA in whole human blood (native conformation) and extracted (naked) DNA were immersed in two different concentrations of bleach for a 1-hour exposure period. Solid-phase DNA extraction and human-DNA-specific quantification revealed that sufficient quantities of DNA were recovered for STR typing, for both native and naked DNA templates and after exposure to both bleach concentrations (with higher DNA recovery from native samples vs. naked templates).

The ultimate goal of this research was to investigate differences in the efficacy of bleach in generating damage to native and naked DNA templates. Results indicate that current decontamination methods using bleach in the laboratory may not be as effective as perceived (at least for DNA complexed with other materials). Additionally, it is often assumed that if a criminal has cleaned a crime scene with bleach, any underlying DNA evidence has been destroyed (which might prevent crime scene technicians from swabbing the area and submitting samples to laboratories for DNA analysis). Hence, this research will impact the forensic science community by demonstrating that amplifiable DNA often can still be recovered from human blood that has been exposed to bleach, especially if the DNA is still encompassed in its native tissue upon initial exposure (i.e., still protected within the body fluid). Decontamination of laboratory workbenches may actually be partially due to physical removal of DNA from a surface ("wiping away") as opposed to chemical destruction or damage.

An Evaluation of Laser-Induced Breakdown Spectroscopy (LIBS) for Comparative Bullet Lead Analysis

Lauren Vallee, University of New Haven; Chuck Sisson, MS; Dr. Peter R. De Forest, D.Crim.; Peter Valentin, MS; Brooke W. Kammrath, Ph.D.

Bullets are a frequently encountered component of physical evidence recovered during autopsies and at the scenes of shooting crimes. Evidential significance of bullets is typically evaluated based on the comparison of striations and other surface markings to those produced by a specific gun barrel. However, there are many situations in which physical comparison is not a feasible method for evaluation due to the severe fragmentation or deformation of a bullet, or a specific firearm is not recovered. For these, analytical methods which determine and compare the trace elements present in the lead component of bullets can be valuable.

Early in the 21st century, a considerable controversy arose concerning the evidentiary significance obtained with comparative bullet lead analysis. In 2003, the NRC of the NAS convened a committee to evaluate comparative bullet lead analysis. Although it was determined that the analytical chemical approach had a firm scientific background, cited examples of poor testimony were criticized and questions arose about the significance of associations by comparative bullet lead. Unfortunately, instead of investing in additional research, the FBI announced in 2005 that they were no longer conducting comparative bullet lead analysis in their labs. Ultimately, the FBI deemed that the costs, resources, and claimed relative probative value of bullet lead evidence made it unfavorable for forensic investigations. Some scientists criticized this decision for more than a decade, with many concluding that the FBI ultimately `threw the baby out with the bathwater' because it effectively eliminated the ability for comparative bullet lead analysis to be used in all forensic casework despite its proven scientific merits and utility for resolving specific forensic questions. There is a need to revisit comparative bullet lead analysis, especially with the recent availability of new instrumental methods of elemental analysis which will enable more forensic laboratories to perform this type of scientific investigation.

In this research, laser-induced breakdown spectroscopy (LIBS) was evaluated for its ability to perform comparative bullet lead analysis. This three-part study first explored the detection capabilities of LIBS, determined an appropriate element menu, and outlined the optimal parameters for LIBS such as: laser pulse energy, spot size, pattern size, gate delay, and repetition rate using three NIST lead standards. Part two applied the optimal parameters gathered in part one to a twelve-bullet study with two aims. First, the micro- and macro-homogeneity of individual bullets was analyzed. Second, different methods of quantification were examined for the differentiation of lead samples from bullets of different caliber and manufacturer. Quantification strategies that were tested included analyzing element ratios, calibration curve development using known standards, and the use of chemometric/multivariate statistics to appropriately differentiate the experimental samples. In part three, bullet lead from two boxes of ammunition were analyzed using LIBS to explore the elemental composition of bullet lead within a single box of bullets. This fit-for-purpose study sought to evaluate whether that LIBS can be used to perform comparative bullet lead analysis and potentially initiate its revival as part of a more readily available forensic laboratory procedure.

Trippin' on Tryptamines: The Use of Chemometric Processing of DART-HRMS Data for Identification of This Subset of Psychoactive Substances

Monica I Ventura, University of Albany; Rabi A. Musah, Ph.D., University at Albany-SUNY

Tryptamines are a class of psychoactive monoamines that generally contain an indole ring. Many are structural derivatives of serotonin and are subject to abuse because of their mind-altering effects. While several are scheduled compounds, there are numerous sites within its scaffold to which structural modifications can be introduced, resulting in the generation of novel variants that retain their psychoactivity. These emerging new psychoactive substances (NPSs) are "legal highs" which, because of their non-scheduled status, enjoy increasing use without the risk of criminal prosecution by law enforcement agencies. There are several bottlenecks to the scheduling of these compounds, including: (1) the challenge of rapidly detecting and structurally characterizing emerging compounds; and (2) developing protocols for their routine detection and identification. In this regard, one obstacle encountered by crime labs is that closely related structural variants can often exhibit nearly identical electron ionization (EI) mass spectral fragmentation patterns, making it challenging to utilize this conventional approach for their definitive identification.

Another is that some tryptamines fragment so extensively that their EI fragmentation patterns are rendered useless or minimally informative for identification purposes. Because of these limitations, a technique is needed that would allow for the rapid detection and accurate identification of tryptamines as a class of molecules. We demonstrate in this study that many of the aforementioned issues can be circumvented through chemometric analysis of Direct Analysis in Real-Time High-Resolution Mass Spectrometry (DART-HRMS)-derived in source Collision-induced Dissociation (CID) data of tryptamines. To demonstrate proof-of-concept, 42 tryptamines were subjected to direct analysis by DART-HRMS under CID conditions (i.e. 90 V) without the need for any sample preparation steps. Unlike their corresponding EI fragmentation patterns, the resulting spectra furnished a significant number of prominent fragment peaks, while retaining the peak corresponding to the protonated precursor molecule. While the mass spectra obtained were too similar to enable isomers to be distinguished based on visual examination, the generation of neutral loss spectra circumvented this issue. These were created by subtracting each of the fragment masses from that of the protonated precursor. This resulted in a virtual spectrum that featured peaks that represented the high-resolution masses of the neutral losses that had resulted in the CID spectra originally observed. Linear discriminant analysis (LDA) of these neutral loss spectra revealed that the tryptamines could be subdivided into the five classes: methoxy-, acetoxy-, hydroxyl- , no side chain-, and "other" hydroxyl-containing groups, and were clustered accordingly in the LDA plot. Internal validation using the leave-one-out cross validation algorithm indicated that the model exhibited a prediction accuracy of 84.05%. The results show that this approach can be used as a screening tool for the rapid structural identification of emerging tryptamines that fall into the NPS category.

Triggernometry

Andrew Winter, Middlesex County Prosecutor's Office (NJ); Pete Diaczuk, Pennsylvania State University; Kaity March, Centenary University

The Las Vegas Mandalay Bay hotel shooting in October 2017 brought significant attention to the bump fire device utilized by the shooter. The bump fire system was designed to circumvent the federal regulations of the National Firearms Act by not altering the internal mechanism of the semi-automatic firearm. The question being addressed in this research is whether or not a sound recording can be used to distinguish an actual full-automatic firearm and a semi-automatic firearm equipped with the bump fire attachment. This question arose in the Mandalay Bay mass shooting incident. The three firing mechanisms are compared: unaltered semi-automatic, semi-automatic with the bump fire attachment installed, and a full automatic rifle. High speed photography, a digital auto software program, and a shot timer was used to gather data on the three platforms. Barrel temperature was also recorded to compare the semi-automatic rifle with the bump fire attachment to a full-automatic rifle.

Detection of Saliva on Combustible and Electronic Cigarettes and Subsequent DNA Quantity Analysis

Kangning Zhang, Boston University, Robin W Cotton, Season Seferyn, Amy Brodeur; Boston University

Saliva can be detected on items including cigarette butts, glassware, clothing, human skin and condoms, and the identification of saliva on these types of evidence may be important to provide linkages or investigative leads in forensic cases. Sometimes when the presence of saliva is indicated, the item will be sent for deoxyribonucleic acid (DNA) analysis and may be used for identification of individuals involved in a crime. The detection of saliva mostly depends on the activity and the presence of amylase. The SERATEC® Amylase Test (SERATEC GmbH, Goettingen, Germany) is a lateral flow immunochromatographic test that targets the presence of human α-Amylase using two monoclonal anti-human-α-Amylase antibodies. This study investigates the effectiveness of using the SERATEC® Amylase Test to detect amylase on cigarette butts and vaping devices. In addition, the possible correlation between the SERATEC® Amylase Test result and the amount of DNA extracted from cigarette butt samples is evaluated.

Results indicated that the cigarettes and vaping devices tested had no inhibitory effect on the SERATEC® Amylase Test. The SERATEC® Amylase test was able to detect amylase from various brands of cigarettes, marijuana cigarettes, JUULpods™ (JUUL Labs™ Inc., San Francisco, CA) and an additional vaping device. Negative amylase test results (22 of 114 samples) may be attributable to personal smoking habits and the texture of the cigarette butt wrap paper or vaping device. DNA quantification results indicated that the majority of cellular material was retained on the wrap paper even after submersion in the SERATEC® Amylase Test buffer. It is recommended that the wrap paper from the cigarette filter and the remaining extract from preliminary testing be combined prior to DNA extraction in order to maximize total DNA recovered from a cigarette sample. The correlation between the SERATEC® Amylase Test result and the quantity of DNA extracted from the same source was not linear. The presence of saliva and DNA concentration are controlled by different factors, thus using the detection of saliva to predict the recoverability of DNA on cigarettes may be valuable in some situations, but is not precise.

Experimental study: The effects of excessive female DNA in sexual assault samples amplified using PowerPlex® Y23 System

Danielle Jardel, Thomas Walsh, Joanne B. Sgueglia, Laura McComsey, Emily Davis; NMS Labs

NMS Labs recently conducted a validation of the PowerPlex® Y23 System using the Applied Biosystems 3500 platform. As part of the Mixture Study, samples were prepared to mimic those that would typically be encountered in casework, specifically when processing intimate sexual assault type samples. Extract from a male buccal swab with varying quantities of DNA from the Sensitivity Study (4 ng to 16 pg) were spiked with excessive amounts of female DNA (4 µg - 7.6 µg total input). Additionally, as part of the Contamination Study, five vaginal swab extracts, containing excessive quantities of female DNA (0.6 µg - 14.1 µg total input), were amplified to assess cross reactivity artifacts.

The results indicated that samples amplified with an excessive amount of female DNA (≥7.6 µg total input) can either overwhelm the reaction entirely, essentially inhibiting the amplification of the male DNA or produce artifacts. In almost every sample containing a mixture of male and excess female DNA, the expected average peak height of the male was significantly less than the peak height of the male when run as a single source. For samples where the male input was ≥1 ng total input, the male component was lost entirely.

There have been papers published regarding the artifacts that are commonly observed in samples typed with Promega's PowerPlex® Y23 System, including those observed with excessive amounts of female DNA. Many of these artifacts are published in the kit's manual and can be attributed to the input of excessive amounts of female DNA, typically encountered in intimate sexual assault type samples. Some of these artifacts appear as actual alleles and fall within bins, making interpretation of profiles difficult if analysts are not aware of the female artifacts. This could potentially lead to false inclusions or exclusions.

Northeastern Association of Forensic Scientists
Proceedings of the
November 2019 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
November 2019 Annual Meeting