



Northeastern Association
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Contents

Scientific Sessions

Forensic Toxicology Abstracts.....	Page 2
Trace Evidence/Fire Debris & Explosives Abstracts.....	Page 6
Criminalistics/Crime Scene & Digital Evidence Abstracts....	Page 13
Drug Chemistry Abstracts.....	Page 21
Forensic Biology/DNA Abstracts.....	Page 32
Poster Session Abstracts.....	Page 39
Author Index.....	Page 47



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Forensic Toxicology Abstracts

LCMSMS Confirmation of Multiple Drug Classes in Oral Fluid

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Oral Fluid Testing for therapeutic, recreational and illicit drugs has existed for a number of years but is rapidly gaining more utility and acceptance. Since salivary glands are well perfused, drugs are quickly transferred into saliva sooner than into urine. This allows for a more accurate estimate of drug administration time. Since oral fluid testing exhibits the parent drug as opposed to metabolites, there is also no question of which drug was taken. Several states are now using oral fluid collections for DUID testing with more states are expected to in the coming years. Other forensic toxicology reference and clinical laboratories are using oral fluid drug testing for medication management purposes, to ensure that patients are taking their medications as prescribed and not misusing, diverting medications or delving into illicit substance use. While law enforcement and rehabilitation facilities are typically equipped to perform observed urine collections with gender specific collectors, many physicians' offices are not. In the clinical arena, a significant portion of the patient population would strongly object to being observed while urinating into a specimen cup. Oral Fluid Drug Testing maintains patient accountability should the patient not be able to give a urine sample and also dramatically minimizes the opportunity for a patient to adulterate a sample. For law enforcement use, sample collection could occur very shortly after traffic stop or accident as opposed to delaying collection for transport to a facility for urine collection or an even longer delay for transport to a medical facility for a blood draw.

This presentation will discuss Champlain Toxicology Labs experience with method development and validation of an oral fluid confirmation method by LCMSMS for over twenty commonly used drugs. This laboratory used Immunalysis Quantisal Oral Fluid collection kits and supported liquid extraction (SLE) columns from Biotage extracted on a positive pressure manifold. Extracts were concentrated to dryness using a TurboVap LV Evaporator and once reconstituted, injected onto a Sciex 4500 QTrap LCMSMS configured with a Shimadzu Nexera HPLC system.

Achieving the Standard for the Analytical Scope and Sensitivity Testing in Impaired Driving using Laminar Flow Tandem Mass Spectrometry

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Introduction: Based on results from the 2017 National Survey on Drug Use and Health, 21.4 million people aged 16 or older operated a vehicle under the influence of alcohol and 12.8 million drove under the influence of illicit drugs in the previous year. Due to the prevalence of driving while impaired, the forensic toxicology scientific community developed ASB Standard 120 "Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing in Impaired Driving Investigations". The scope of the standard provides the minimum requirements for target analytes and analytical sensitivity for the analysis biological fluids such as urine in suspected drugged driving cases. The purpose of this research was to develop one analytical method incorporating laminar flow mass spectrometry to detect and quantify all targeted drugs in ASB Standard 120 at and above the required cut-offs. Case samples of 32 patients were also analyzed to demonstrate the applicability of this method.

Methods: The analytes included different drug classifications, such as opioids, benzodiazepines, amphetamines, and others. The body matrix for this study was human urine. Urine samples were collected and screened using lateral flow immunoassay urine cups (One Step Multi-Drug Urine Test T-Cup). All calibrators, deuterated internal standards and quality control samples were prepared by spiking certified reference standards (Cerilliant, Round Rock, TX, U.S.A.) into human urine. Calibrators were prepared from 50% to 2000% of the minimum cutoff value. Quality Control samples were prepared at 10% of the highest calibrator. The fortified urine samples were hydrolyzed using IMCSzyme® RT and RT Rapid Hydrolysis Buffer (IMCS, Inc. Irmo, SC, USA). The samples were prepared with supported liquid extraction using ISOLUTE SLE+ 1mL columns (Biotage, Charlotte, NC, USA). After evaporation, followed by reconstitution, the samples were analyzed using QSight® LC-MS/MS (PerkinElmer, Waltham, MA, U.S.A). A Phenyl-Hexyl 50x 4.6mm

(2.6 μm) column (Phenomenex, Torrance, CA, USA) was utilized in the LC method, with mobile phase A being 10mM ammonium formate in Millipore water and mobile phase B being 0.1% formic acid in methanol. The total run time was 9 minutes.

Results and Conclusions: All analytes were separated on the chromatograph and detected at and below the recommended cutoffs. All compounds established linear calibration models in the working range, with r^2 values above 0.98. No carryover was observed for any analytes. All analytes eluted the column at 6 minutes. The results of case samples analysis showed that each drug in the method could be identified at or below the required cut-off. Further, analytes not previously detected using lateral flow immunoassay were detected using the developed method. This study demonstrated the QSight® 220 CR was effective in detecting and quantitating all analytes at the required cut-offs defined by “ASB 120: Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing in Impaired Driving Investigation” in one combined method. Fast hydrolysis of patient urine samples using IMCSzyme® RT was accomplished in 15 minutes at ambient temperature followed by SLE sample extraction. The analytical method in this study offers a quick and effective way to extract and quantify analytes of interest in drugged driving cases.

Keywords: ASB 120, sample preparation, laminar flow tandem mass spectrometry

Analysis of Nine Fentanyl Analogs in Urine and Oral Fluid by UHPLC-MS/MS

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The opioid epidemic has become a serious public health problem in the United States. Fentanyl is a synthetic opioid analgesic and has resulted in an increasing number of drug overdoses since 2013. In addition, fentanyl analogs, originally used as analgesics or animal tranquilizers, have emerged in the drug market in the United States. Fentanyl and its analogs, similar to other opioids, work as full μ agonists, binding with μ -receptors in the brain membrane. The synthetic fentanyl and its analogs elicit more potent effects compared to the traditional opioids being abused such as morphine or heroin. With the emergence of fentanyl analogs in the drug market, identifying and differentiating those analogs becomes a challenge due to their structural similarities to fentanyl.

The purpose of this research was to develop a method of identifying and quantifying nine fentanyl analogs in both urine and oral fluid using the PerkinElmer QSight® Triple Quadrupole UHPLCMS/MS. The method was validated based on AAFS Standards Board (ASB) Standard 036, Standard Practices for Method Validation in Forensic Toxicology. The analytes of interest for this study were fentanyl, norfentanyl, acetyl fentanyl, carfentanil, cyclopropyl fentanyl, methoxyacetyl fentanyl, valeryl fentanyl, furanyl fentanyl and 4-anilino-N-phenethylpiperidine (4ANPP). All samples, calibrators, and quality controls were prepared by spiking certified reference standards into donated human urine or human oral fluid. Samples were then prepared using supported-liquid extraction (SLE) with ISOLUTE® SLE+ 1-mL columns followed by evaporation. Samples were then reconstituted with 200 μL methanol. Analyte separation was performed using a C18 column, with all analytes eluting in under six minutes. A 5mM ammonium formate buffer with 0.1% formic acid was the aqueous mobile phase and methanol with 0.1% formic acid was the organic mobile phase for gradient elution.

For urine and oral fluid analysis, the calibration range for all analytes was established from 1 to 70 ng/mL. The r^2 values were greater than 0.988 for all analytes. Bias and precision were evaluated at 3, 25 and 60 ng/mL, and bias and percent coefficient of variation (%CV) for inter- and intra-run precision were within $\pm 20\%$. The limit of detection (LOD) was 0.1 ng/mL for most fentanyl analogs, with a LOD of 0.01 ng/mL for valeryl fentanyl and furanyl fentanyl. No carryover was observed for any analytes in either matrix. Recoveries for all compounds following SLE for both urine and oral fluid were above 50%. Matrix effects were evaluated for both matrices with most analytes within 25% except for valeryl fentanyl in oral fluid at 35%. Aside from cyclopropyl fentanyl, all analytes showed good stability for 72 hours at room

temperature.

This method was found to be suitable for the identification and quantitation of these nine fentanyl analogs in both urine and oral fluid.

What Has Your Lab Been Missing? Accurate Mass Screening for Forensic Toxicology

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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. This is an ideal approach for forensic toxicology screening where unknown compounds in complex samples must be identified from information-rich data sets. The SCIEX X500R QTOF system provides the capability of switching between MS and MS/MS scans instantly, enabling fast acquisition of detailed structural information for easier compound identification. Designed for routine use, the benchtop SCIEX X500R QTOF system could also be used for high-specificity, targeted quantitation as well as for nontargeted screening from single sample sets in a routine testing laboratory environment. Due to its straightforward design and intuitive software workflows, non-targeted data obtained on the X500R can be retrospectively mined for additional analytes missed in initial screens, which is important with the constant emergence of new synthetic drugs. Also, the availability of retrospective analysis on X500R has become increasingly popular in forensic work.

Information-dependent acquisition (IDA), also called data-dependent acquisition (DDA), is a widely-used approach for acquiring MS/MS information for screening purposes. In IDA-MS/MS mode, a survey scan is performed to collect information on precursor ions, followed by multiple, dependent MS/MS scans on several of the most abundant precursor/candidate ions. To efficiently evaluate these complex and data-rich scans, SCIEX OS software platform was developed to automatically choose candidate ions by sorting through the observed intensities of precursor ions. Each MS/MS scans are performed after mass filtration (by Q1) of single precursor ion, resulting in IDA-MS/MS spectra that are free of interfering species aiding in accurate MS/MS library spectral matching.

Herein, we present the SCIEX X500R QTOF system and introduce key concepts of how QTOFs operate. Data will be presented showing how to use QTOF instrumentation for the positive identification of targeted compounds while using the IDA-MS/MS or SWATH workflows. Additionally, we will introduce the concept of non-targeted screening allowing for the identification of new compounds of interest without the reinjection of a sample.



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Trace Evidence/Fire Debris & Explosives Abstracts

“Free Range” Gunshot Primer Residue: A Study on Multiple Transfers of Gunshot Primer Residue

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Gunshot primer residue (GSR) is produced by a firearm when it is discharged. The primer for centerfire cartridges is mainly composed of lead styphnate, barium nitrate and antimony sulfide, The residue from the primer explosion escapes from openings in the gun and can be deposited on a person’s hands and clothing. These particles can be collected and analyzed using automated scanning electron microscopy energy dispersive x-ray. Characteristic gunshot residue primer particles have a molten appearance and are composed of barium, antimony, and lead. There have been several studies that examine the transfer of gunshot primer residue to the interior of police cars from a person who has gunshot residue on their person. There have not been studies conducted that try to determine the number of times gunshot primer residue particles could transfer from one surface to another.

This presentation will detail the results of two studies on the potential transfer of gunshot primer residue. The first study involves the transfer of gunshot primer residue from a gunshot primer residue contaminated area to a clean subject who enters the area and then subsequently transfers the gunshot residue particles to other surfaces outside the contaminated area. One of the analysts of the Trace Evidence section of the laboratory attended firearm familiarization training given by the Firearms and Toolmarks section of the laboratory. This analyst does not handle firearms at all. At the end of the training, the analyst was asked to stub her clothing, her cubicle chair, the driver seat of her car, and any chairs that she might have sat in at her home in the same clothing she had worn to the training.

The second study deals with the potential transfer of gunshot primer residue from a victim’s clothing to a person’s hands that touch the victim. The Firearms and Toolmarks section re-created a shooting victims clothing. An analyst who had no gunshot primer residue on their hands handled the shooting victims clothing. Their hands were then stubbed to analyze for gunshot residue primer.

All of the above stubs were analyzed using scanning electron microscopy energy dispersing x-ray spectroscopy instrumentation using standard laboratory procedures for the analysis of gunshot primer residue.

Determining the Probability of Finding a Random Hair

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Microscopic hair comparison has been under attack for many years by scientists, lawyers, and legal scholars and has caused many forensic laboratories and laboratory systems to discard it. One of the major criticisms is the subjective nature of hair comparison and a lack of statistical evaluation. The presented study hopes to establish a model whereby a likelihood ratio could be calculated to assess the probability of encountering a random hair. In the study, two measurable variables, width and color, were used in the examination of collected hairs. Two hundred hairs were collected from vacuum sweepings taken from several dormitory rooms. Presumptive hairs were tested with aniline blue orange g stain to confirm the presence of keratin then mounted in DPX mounting media (nD - 1.521). The width of each hair was taken at five locations using a Leica DMEP polarized light microscope after the ocular was calibrated with a stage micrometer. Mounted hairs were then assessed in five different locations for RGB color values under an Olympus BX53 polarizing light microscope using CellSens software under standardized illumination. A database was constructed from examined hairs with data entry for each hair based on the highest and lowest width values and highest and lowest color values for red, green, and

blue. In addition, each hair generated a RGB summation value and a RGB integer value. The RGB summation value was simply determined by adding all the red, green, and blue values taken in the hair. The RGB integer was determined by the summed values for red, green, and blue using the following equation: RGB Integer (2562 x Summed Red value) + (256 x Summed Green Value) + Summed Blue Value. Correlation coefficients were generated for pairwise comparisons of each individual color with both RGB summation and integer values. The lowest correlation coefficient was obtained when high and low blue values for each hair were compared to the calculated RGB integer (<0.06). A random match probabilities using the counting method were determined for 15 new collected hairs taken from buildings other than the dormitories by multiplying probabilities generated for width, RGB integer, and blue color value. An upper bound 95% confidence interval was calculated from each random match probability and used to then generate a likelihood ratio. Likelihood ratios ranging from 35 to 541 were calculated for each of the 15 collected hairs. Although likelihood ratios are expectedly small, they still may provide value in particular contexts and with association with other types of evidence. This study may be used as a model for a much larger study which could incorporate greater geographical and perhaps ethnic and racial diversity in obtaining hairs for a database.

Key Words: Forensic hair examination, RGB, probability, likelihood ratio

Soil Mineral Analysis by Particle Correlated Raman Spectroscopy (PCRS): Method Optimization

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Soil is a valuable and powerful trace evidence that provides linkages and investigative leads. Criticisms of forensic soil analysis (e.g. subjective, labor-intensive, time-consuming) have resulted in a considerable decline in its use in forensic investigations and created countless missed opportunities within the criminal justice community. Consequently, there is a need for a statistically-supported, automated, and objective analytical method for soil analysis. Particle correlated Raman spectroscopy (PCRS), also known as particle driven or morphologically directed Raman spectroscopy (MDRS), is a novel yet reliable analytical technique that can add significant value to the forensic examination of soil evidence. It has proven easy-to-use and nondestructive, and its ability to be automated enables a large amount of data to be collected with minimal time required by the criminalist. Resulting data provides qualitative and quantitative information on a sample. PCRS obtains particle images of soil components (i.e. minerals) and produces microscopic morphological characteristics (e.g. circularity, elongation, brightness) and particle size distribution for the particles present. Particles can then be targeted randomly or based on morphological characteristics for Raman analysis to provide secure mineral identification.

The research presented here focuses on PCRS method optimization for soil mineral analysis using traditional figures of merit and response surface modeling of a multi-level experimental design. Laser wavelength, laser power, magnification, grating, and exposure time were examined for chemical identification of minerals via Raman spectroscopy. Particle size, particle destruction, and detector oversaturation influenced the final parameters to be evaluated for spectral analysis. The optimal parameters were determined based on a complex mineral mixture which reflect the diversity of minerals (based on stability, structure, Raman scattering capability, transparency, and fluorescence) that may be found in a single soil sample. The contrast/illumination method, magnification, and targeted morphological analysis were then evaluated to obtain an optimized method for automated imaging and Raman analysis. The effect of field of view and contrast on particle sensitivity and time of acquisition were important factors considered. At this step, final parameters were set for each soil type based on the individual parameters determined necessary for minerals of that given soil type. Now that the optimal method parameters have been determined, they can be applied to the analysis of a range

of soil samples to generate a robust PCRS dataset for forensic soil analysis.

Simultaneous Optical Photothermal Infrared (O-PTIR) and Raman Microspectroscopy of Automotive Paint

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Paint evidence can provide valuable associative information for crimes involving vehicular accidents and home invasions. The complex chemistry of paint is thoroughly interrogated with vibrational microspectroscopy, with results that are used for its classification and comparison. Traditional IR microscopy is frequently used to identify binders, some pigments and additives, while Raman microspectroscopy affords the additional ability to identify colored pigments thus providing complimentary information. However both traditional IR and Raman microspectroscopy have limitations which prohibit a complete chemical characterization of multi-layered paint samples, especially those with layers thinner than 10 μm .

The mIRage+R, IR and Raman microscope is a new technology that combines optical photothermal IR (O-PTIR) with Raman microspectroscopy to enable the complementary and confirmatory analysis of a sample in a single measurement. O-PTIR uses a visible light probe to measure the photothermal response of the absorption of radiation from a pulsed IR laser focused on the sample. The use of visible light allows for non-contact, reflection mode analysis with submicron spatial resolution that is independent of the IR wavelength. Further, analysis is fast ($\sim 1\text{s}$) and requires little to no sample preparation. The reflection O-PTIR spectra are free of specular and diffuse scatter artifacts and can be directly compared to commercial/custom transmission IR databases. When combined with a Raman spectrometer, the visible light probe also doubles as the Raman excitation laser, thus allowing for IR and Raman spectra to be simultaneously and non-destructively collected from a submicron sized spot in seconds. IR+Raman data collection can be extended to create a line array in under a minute, and map an area of a sample to create a hyperspectral image within a few minutes to a few hours (depending on the size of the area and desired spatial resolution). This presentation will detail the benefits of O-PTIR combined with Raman microspectroscopy for the analysis of automotive paint.

“Who’s Going to Clean up that Garbage?” Cleaning Biological Material Off Plastic Bags for Subsequent Chemical Analysis

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In a previous presentation, the authors discussed the effects on class characteristics of plastic trash bags that were subjected to various conditions. The conditions included the addition of weight inside the bags, weathering and burial in soil. It was noted that, in some instances, the dimensions of the bags changed. Instrumental testing showed that the bags acquired some surface materials during the environmental studies. The chemical composition of the bags themselves, however, did not change. In this study, the effects of decaying biological material on the chemical analysis of the plastic trash bags was investigated. In addition, the study focused on determining the best techniques and reagents to efficiently clean off the biological material from the bags, without altering their original chemical make-up.

Two different types of trash bags were used in this study: thin, 0.5mil bags and thicker, 3mil bags. The bags were filled with an assortment of raw meats from a grocery store. They were then placed into plastic bins. Holes were placed into the tops and bottoms of the bins, and they were placed outdoors for approximately four months.

Initial experiments were conducted using various reagents on clean bags to determine which reagents did or did not leave any residue on the bags. The reagents chosen were: acetone, 10% bleach, Dawn dishwashing solution (in water), ethanol, isopropanol, methanol, deionized water, 3% hydrogen peroxide, Pine Sol cleaner and Oxy-Clean laundry detergent (in water). After washing, the procedure for all reagents included a rinsing in tap water, and a final rinse in deionized water. The reagents that were eliminated based on this first experiment were: acetone, Dawn, isopropanol, deionized water, hydrogen peroxide and Pine Sol.

Cuttings of the contaminated bags were run on a Thermo-Nicolet iS50 Fourier Transform Infrared Spectrometer (FTIR) using Attenuated Total Reflectance (ATR). Additional cuttings of the bags were then cleaned with the remaining reagents listed above by three different techniques. These techniques were: shaking in a conical tube; vortexing in a conical tube; and scrubbing followed by vortexing. The best washing technique was determined to be a combination of scrubbing and then vortexing. Oxy-Clean in water was found to clean the bags well, as was 10% bleach. Both solvents were efficient in cleaning the biological material off the bags without adding additional IR peaks.

Optimization Of Extraction Methodologies For Condom Lubricants And Additives In The Presence Of Biological Fluids

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Over time, criminals have become more aware of the different types of trace evidence that are capable of being identified by forensic analysis. As a result, the frequency of actions taken to prevent the transmission of evidence, specifically biological fluids and subsequent DNA evidence, with the usage of condoms in the commission of sexual assaults, has increased. With the increased use of condoms, comes the increased awareness and probative nature of forensic analysis of the potentially unique chemical profiles residues may leave behind. This includes the identification of lubricant type and of any additives that may be present, such as spermicides, flavoring or topical anesthetics. The two predominate condom lubricants are polydimethylsiloxane (PDMS) and polyethylene glycol (PEG): PEG, a water-soluble lubricant, is soluble in polar solvents, such as methanol, while PDMS, a siliconebased lubricant, is reported to be soluble in non-polar solvents, such as hexane.

A total of thirty condoms representing eight brands, each of a different type, were evaluated by Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR). It has been reported that PDMS is the more prevalent condom lubricant compared to PEG; this trend was reflected in this small subset of products. A direct extraction method was developed to isolate the lubricant from the condoms. Following the direct extraction of the condom lubricants from ten condoms of different brands and types containing PDMS, the extraction capabilities of three solvents: hexane, methanol and methylene chloride, in the presence of blood and saliva separately, were evaluated. Two different biological fluid/lubricant sample types were created: liquid suspensions of lubricant, biological fluid and solvent; and contrived casework samples consisting of a mixture of lubricant and biological fluid dried onto a cotton swab. Hexane was capable of isolating only the PDMS lubricant in the presence of biological fluids. Methanol was used as the direct extraction solvent for condoms marketed as containing the spermicide nonoxynol (N9) and the topical anesthetic benzocaine, separately, as much of the published literature has determined that additives, such as spermicides and topical anesthetics, are often found in combination with PEG, which must be extracted in a polar solvent. The presence of biological fluids in the both sample types prevented the successful isolation of any condom lubricants or additives with the use of methanol. The extraction capabilities of methylene chloride were assessed; in the literature, methylene chloride is often used to eliminate a two-step, or two-solvent, extraction for condom lubricants. The

isolation of PDMS and N9 had mixed results when using methylene chloride as a solvent. PDMS and N9 were successfully isolated and identified in one of the Trojan brand spermicidal condoms; benzocaine could not be isolated from the Durex brand condom lubricant by this extraction method. In the blood and saliva/lubricant contrived casework samples extracted with methylene chloride, the PDMS and PEG in the respective condoms were isolated but N9 was not.

An evaluation of solvent extraction efficiency was made by comparing the ability of each solvent to isolate condom lubricant and additives in the presence of biological fluids. Methylene chloride was found to be the most effective solvent when compared to hexane and methanol for this purpose.

Using the CAMAG Laboratory Method for Detection of Nitramine, Nitro Aromatic, Nitrate Ester, and Peroxide Based Organic Explosives by High Performance Thin-Layer Chromatography

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High Performance Thin-Layer Chromatography (HPTLC) is a rapid, reproducible, and highly sensitive method used to detect various chemical compounds across many fields of forensic analysis. One useful application of HPTLC is the detection of nitramine, nitro aromatic, nitrate ester, and peroxide based organic explosives. New methods of detecting explosives are in high demand due to the increasing number of terrorist attacks devastating society. CAMAG Laboratory has proposed an HPTLC method for identifying cyclotrimethylenetrinitramine (RDX), N-methyl-N,2,4,6-tetranitroaniline (tetryl), trinitrotoluene (TNT), nitroglycerine (NG), pentaerythritol tetranitrate (PETN), hexamethylene triperoxide diamine (HMTD), and triacetone triperoxide (TATP). For the purpose of this work, HPTLC silica gel 60 F254, 20 x 10 cm plates were employed and analyzed using an automatic sampler. Before running the analysis, pictures of the clean plates were taken under 254 nm light and white light using the visualizer. This is done to establish a background, which is later automatically subtracted from the pictures containing spotted samples. Samples of the relevant explosives were spotted on the HPTLC plates, the plates were saturated with a magnesium chloride solution, and chromatography was performed in a sealed chamber using a 9:1:1:2 toluene-chloroform:methyl acetate:heptane mobile phase. After chromatography was complete the plates were photographed under 254 nm light and white light. Since RDX, tetryl, and TNT can be documented prior to derivatization, the TLC Scanner 3 and visionCATS software were used for densitometry, to measure absorbance at 240 nm. The remaining analytes were derivatized as described below. For the purpose of this work, two different derivatization methods were used, one for identification of nitrogen based explosives, and one for identification of peroxide based explosives. To identify nitrogen based explosives, the plates were dipped into 5% potassium hydroxide in ethanol and heated for 2 minutes at 100C. After photographing the plate under 254 nm light and white light, the plates were heated for 10 minutes at 100C, then dipped into Griess reagent, and allowed to dry. After the plates are dry they were photographed under 254 nm light and white light. For the identification of peroxide based explosives the plates were sprayed with the diphenylamine reagent and allowed to dry, then photographs were taken under 254 nm light and white light. After derivatization, densitometry is used to measure absorbance at 480 nm. The goal of this project was to validate the method proposed by CAMAG and expand the number of explosives that are able to be identified using the method set forth by CAMAG Laboratory. In addition to the previously mentioned explosives identified by CAMAG, this method was applied in the identification of Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), nitroguanidine, picric acid, and Ethylene glycol dinitrate (EGDN). In the future, this method could be used to detect single component explosive or explosive mixtures. After viewing this presentation attendees will gain a basic understanding of how to use a High Performance Thin Layer Chromatography system, making them more competent individuals when it comes to laboratory work and the exploration of innovative ways to detect and identify forensic unknowns.

Development and Validation of Systematic Methods for Physical Fit Examinations

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A physical fit is defined as the three-dimensional alignment of items that have been broken, torn, or separated in a manner that is not likely to be replicated. Since a physical fit is the highest degree of association between items in trace evidence analysis, there is an interest in the forensic and legal community to develop standardized methods for the analysis and interpretation of these examinations, and to evaluate error rates.

To address the need for standard criteria, two systematic methods were developed for duct tape and textiles. Both methods result in a similarity metric referred to as the edge similarity score (ESS), through which the quality of the fit can be associated to a quantitative value. To assess the methods, populations of fractured pairs of tapes and textiles were created to be analyzed by examiners blind to the origin of the samples. The performance rates and distribution of scores were evaluated to determine what factors were the most impactful on the accuracy of the method.

The duct tape dataset consisted of approximately 2300 comparison pairs, made from three grades of tape, two separation methods (scissor cut and hand-torn), and different levels of stretching. Overall, the method produced accuracies of between 84.9-99.8% for the different tape sets, with no false positives reported. It was found that the grade of the tape had a substantial impact on the quality of a fit between edges. Tapes with scores above 80% or higher indicated strong support for a match, while scores below 25% indicated strong support for a non-match.

The textile set consisted of 100 comparison pairs, 10 hand-torn and 10 stabbed pairs generated from each of 5 items of clothing. General and distinctive characteristics were identified for each material. The method resulted in an accuracy of 93% for the hand-torn set and 95% for the stabbed set. The stabbed set did not have any false positives reported, while the hand-torn set had a false positive rate of 2%. A higher misclassification rate was observed in the hand-torn set as a result of the fraying and stretching caused by the tearing, particularly on more deformable fabrics. To evaluate the intraexaminer variation, the original set was relabeled for one of the examiners to blindly re-analyze about six months after the first comparison was completed. The accuracies of the set remained at a high level (96% for hand-torn and 94% for stabbed samples) and the number of overall misclassifications decreased. In addition, the overall average difference in scores between the two examinations was less than 15%, and generally, true matching pairs received similar or higher scores, while true nonmatching pairs received similar or lower scores.

Overall, the results indicated that the ESS was valuable in physical fit examinations. The distribution of scores provided insight into how the composition of the material impacted the features and quality of the fit. The use of the ESS scores can provide examiners with statistical data to support their opinions during physical fit determinations.



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Criminalistics/Crime Scene & Digital Evidence Abstracts

Mass Spec of the Woods: Species-level identification of endangered woods using mass spectral and chemometric techniques

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Illegal logging occurs when timber is harvested, transported, processed, bought or sold in violation of national or sub-national laws. It is estimated that 70% of the timber exports of some countries is illegal, and that this activity nets up to \$100 billion dollars annually. These funds are then used to finance other illegal actions such as drug and weapons trade by organized crime networks, sometimes in collaboration with corrupt government officials. Traditional identification procedures for wood species include DNA profiling, morphological feature characterization and stable isotope analysis. Each of these techniques exhibit challenges including their time-consuming and costly natures, and the requirement for specialized expertise, making them suboptimal for field utilization, such as at border crossings. Therefore, a method is needed that will allow rapid detection and identification of illegally-traded endangered woods. We established how direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) along with thermal desorption coupled with gas chromatography-mass spectrometry (GC-MS) can be used to quickly reveal species-specific chemical fingerprints that, in conjunction with multivariate statistical analysis processing tools, enable species-level identification. We also show how this method can be used for both direct analysis of wood samples as well as with headspace analysis. For this technique, the headspace volatiles of pulverized wood samples were concentrated onto conditioned solid phase microextraction (SPME) fibers for 30 minutes. The fibers were then analyzed using DART-HRMS. The bulk shredded material was also analyzed directly by DART-HRMS. Both sets of results were subjected to multivariate statistical analysis processing, which showed that chemical fingerprints could be produced from the direct analysis of the bulk material as well as the headspace of wood samples. Both techniques were successful in producing models for species-level identification of wood samples. For the identification of the molecules associated with enabling species differentiation, the shredded wood was deposited within a thermal desorption tube, and analyzed using thermal desorption coupled with GC-MS. The molecules associated with diagnostic m/z values that enabled species-level identification by DART-HRMS were detected and identified using this method. Through this technique, the molecules important for species discrimination could be identified. The results indicate proof-of-concept that the mass spectral analysis of wood samples can be used to create a database accessible to Border Patrol Agents that can be used for rapid and cost-effective identification of illegally-traded wood species.

Handwriting Analysis Through Kneser Graph Triangle Decomposition

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Handwriting comparative analysis is often criticized because of the subjective nature of traditional examination. In an attempt to provide objectivity, a study is being conducted where handwriting is being examined with the use of Kneser Graph Triangles. Through the use of Kneser Graph Triangles and their decomposition to analyze handwriting, more specific information can be obtained from each sample and compared both within a source and with multisource data. Gneser Triangles in this study were formed in such a way that each node within a triangle set has a unique color and each edge is unique to its triangle. The characteristics of each handwriting sample that gets marked as a different color node include the beginning of a pen stroke, where that pen stroke ends, any location where the pen line overlaps itself, the lowest point of a word, and the highest point of a word. Triangles are able to provide information on angles, length, area similarity, and congruency. The sample is able to be broken into individual words by a software 'handwriter' created using the statistical program R. By forming Gneser Triangles over these samples, it may be possible to gauge the variation in a single source author and then be able to compare this quantitative value to other samples of unknown sources. Using this information, the study hopes to be able to form a quantitative analysis of handwriting samples and be able to calculate how similar or dissimilar two samples are from one another. One of the

study's main goals is to be able to form these triangles and multiple samples from multiple different sources and be able to group, identify, and accurately determine what samples came from what source. Once this is accomplished, a similarity statistic may be generated to verify inclusions or exclusions.

RUVIS: Is this useful examination tool a DNA destroyer?

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In laboratories where items may be examined for latent prints and swabbed for DNA analysis simultaneously, a study should be conducted to determine the potential threat of degradation of DNA after the exposure to certain latent print visualization techniques. One specific visualization technique used by latent print examiners that poses a potential threat of degradation to the DNA is the Reflective Ultra Violet Imaging System, or RUVIS. RUVIS is an optical system consisting of an illumination source and a viewer, with the illumination source operating in the short wavelength UV region of the electromagnetic spectrum. The reflectance of the ultraviolet radiation off of the item allows for the latent print to become visible and/or enhanced after superglue fuming when viewed with the proper optical device. The potential risks of using RUVIS prior to swabbing for DNA must be explored since ultraviolet radiation, especially short wavelength UV, is detrimental to DNA. Short wavelength UV is often used to decontaminate instruments, equipment, and laboratory hoods with the intent of eliminating any residual DNA after DNA analysis techniques have been performed. The study performed at the Nassau County Medical Examiner's Office assesses the degradation of the sample after exposure to the RUVIS for varying lengths of time using blood, saliva and mock touch DNA. This study assisted in determining a proper workflow for items with both latent print and DNA analysis as well as what precautionary steps may be necessary for using the RUVIS technique when processing items for latent prints that may also be tested for DNA.

Complete Automation and Digitization of a Forensic Laboratory Workflow: Crime Scene to Court

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With the enactment of the 2019 Criminal Justice Reforms specific to bail and discovery procedures, forensic laboratories are faced with significant increases in case submissions and the number item requests per assignment. Provisions within NYS CPL 245.20 for automatic discovery have set unprecedented target dates for forensic laboratories; with as little as 20 days for “in custody” defendants and for “not in custody” defendants, as soon as practical but no later than and within 35 days of arraignment. As a result, increases in the time required to accession, analyze, and report on case evidence has increased and in a significant number of instances, have exceeded the time requirements of bail and discovery reform. Fragmented information management systems (IMS) used to accession evidence collected by police agencies into independent laboratory information management systems (LIMS) require duplicate entries that are prone to transcriptional errors. In situations where case circumstances are necessary to establish the probative nature of evidence, perform comparative analysis, and applicable database submission, lengthy periods of time delaying testing are common due to the unavailability of case detectives due to revolving shifts. Lengthy delays cannot be afforded, especially in unsolved violent offenses requiring investigatory leads to identify possible perpetrators. To provide customers with timely service, conventional methods of data entry and sharing must be restructured to meet the demands of current legislature.

The Nassau County Medical Examiner's office Division of Forensic Services has implemented a fully digital LIMS

which incorporates evidence intake, analysis, review, dissemination of reports, and complete discovery production in one fluid workflow. Using a combination of software provided by Porter Lee Corporation (BEAST), Mideo Systems (CaseWorks-CW), and the Nassau County Department of Information Technology (NCIT), the laboratory has customized an evidence workflow which minimizes the bottlenecks associated with a paper or partial electronic system. Facilitated by secure database connections between the laboratory and the Nassau County Police Department (NCPD), the BEAST evidence prelog system (.NET Prelog) allows for the review of submitted evidence by the assigned detective and prosecutor. Analysis requests can be modified by the submitting agencies prior to submission to the lab for review and acceptance. Within .NET prelog, environments exist to (1) provide case commentary in the form of evidence questionnaires which are designed to determine database eligibility across all disciplines, (2) provide the names and metadata of victims/subjects (DOB, Arrest No., NYSID, etc.) and allows the customer to associate specific items to specific subjects, (3) enable the modification of the initial NCPD analysis request by the assigned Assistant DA preventing expedited requests of items left of the submission inventory, (4) provides complete electronic submission to the laboratory for review and acceptance. During the review process, .NET Prelog allows the laboratory the ability to accept/reject and/or add additional items from the submission request and provide commentary to the customer. Approval of the submission request by the laboratory launches an auto-notification by means of email with instruction for submission of the evidence. The submission form can be scanned by the lab populating all the case related information and section specific assignments into BEAST, creating an electronic case file. If required, a 24/7 unstaffed locker system can be used by including the submission form with items placed into locked custody storage. Once accessioned, multi-disciplinary evidence is processed from evidence documentation to instrument data within the LIMS without the need to transcribe the item information captured during case accessioning. Data is processed in batch format utilizing import/export scripts and automated worksheets between BEAST and CW systems, providing for electronic review and approval of all data. As batches are approved electronically, the data generated by instrumentation is auto-populated into specifically customized compartments within LIMS providing all supportive administrative and technical data required to render opinions and publish scientific reports. When discovery is required, customers can access a discovery portal designed by NCIT which hosts all scientific, quality control (QC), and personnel data for all cases and witnesses. The requests are made electronically, launching a BEAST discovery service which prepares all materials associated with the case file, while supporting QC and personnel data can be obtained within the portal environment. This fully automated LIMS system integrates all aspects of forensic investigations and provides all agencies involved in a criminal case with the ability to streamline their workflow.

Microscopic and Elemental Analysis of Anthropological and Modern Buried Hair Compared to Soil Composition: A Case Study of a Male Child and Adult Female from the Arch Street Project, PA

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Several hundred human remains were unearthed during a 2016-2017 construction project at 218 Arch Street, site of the former First Baptist Church of Philadelphia (FBCP). Local archaeologists launched a salvage archaeological project to recover and relocate these remains in what became known as the Arch Street Project. The large quantity of hair recovered on the skulls of two of the remains (a male child G-9 and adult female G-33) provides a unique opportunity to conduct a case study of anthropological hair from the FBCP cemetery, analyzing its morphological features and intrinsic chemical composition compared to the surrounding soil after years underground. The value of buried anthropological hair as well as the effect of different sample preparation procedures on the hair samples (i.e. hair washing procedures to remove exogenous contamination without damaging the hair) is investigated through visualization with light microscopy and chemically using inductively coupled plasma – optical emission spectroscopy (ICP-OES) multi-elemental analyses. Simulated burials of modern hair provide insight as to the degradative effects of the environment through various burial intervals (from one week to one year across different soil types), the efficacy of the different hair washing procedures, and the prevalence of postmortem hair morphologies across samples. ICP-

OES multi-elemental analysis focused on the parts per million (ppm) level of a select 14 elements including major and trace elements in hair and soil (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, and Zn) as well as heavy metal toxins (As, Cd, and Pb) found at levels below the detection limits (<2 ppm) of the method.(1-5) Statistical analysis (t-tests and ANOVA) of the simulated burial remains yielded significant results across experimental conditions for some, but not all, the elements studied. Washed versus unwashed hair showed significant ($P<0.05$, two-tailed t-test) differences for Zn in unburied controls and Ca in buried hair samples as well as significant ($P<0.01$, two-tailed t-test) differences in Na, K, Cu, and Zn levels between the burial durations compared to unburied controls. Results indicate that burial has a significant effect on mineral content of hair and that surrounding soil should be investigated along with hair samples in these cases.

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Overview of the Evofinder Ballistic Identification System

Jake Kurth, Leeds.

This presentation will be an overview of the forensically validated Evofinder Ballistic Identification system, which is helping advance the science of firearms examination. The Evofinder has highly unique capabilities of 3d scanning both bullets and cartridge cases in addition to providing the statistical context of matches. With the dramatic improvements in 3D virtual comparison microscopy and algorithmic comparisons, the Firearms analysis discipline is going through quite possibly one of the largest technological advancements since the advent of the comparison microscope system in the early 1900s.

Discrimination of menstrual and peripheral blood traces using attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy and chemometrics for forensic purposes

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Body fluid traces can provide highly valuable clues in forensic investigations. In particular, bloodstains are commonly found and the discrimination of menstrual and peripheral blood is a crucial step for casework involving rape and sexual assault. Most of the current protocols require the detection of characteristic menstrual blood components using sophisticated procedures that need to be performed in a laboratory. The present study uses attenuated total reflection Fourier transform-infrared (ATR FT-IR) spectroscopy as a nondestructive technique for discriminating menstrual and peripheral blood traces. This method incorporates statistical analysis and was evaluated by internal and external validation testing. A partial least squares discriminant analysis (PLSDA) classification model was created for differentiating the two types of blood in a binary manner. Excellent separation between menstrual and peripheral blood donors was achieved during internal validation. External validation resulted in 100% accuracy for predicting a sample as peripheral or menstrual blood. This study demonstrates that ATR FT-IR spectroscopy combined with chemometrics is a reliable approach for rapid and nondestructive discrimination of menstrual and peripheral bloodstains. It offers a significant advantage to forensic science due to the availability of portable instruments and the

potential of bloodstain analysis at a crime scene.

Detect More Evidence: Extend Your Search Beyond the Visible

Rebecca Nick, Scientific Sales Specialist.

Various types of evidence are often overlooked at a crime scene because they are not visible to the naked eye. When using an alternate light source with the proper corresponding filter, it is still possible to miss items of evidence due to background interference or improper angle of illumination. The purpose of this workshop will be to introduce attendees to various methods utilized in detecting evidence beyond the visible range. An introduction to light theory and techniques utilizing wavelengths of light beyond the visible range on notoriously difficult surfaces will be discussed. This will include ultra-violet and infrared examination to detect the presence of biological fluids, gunshot residue, and fingerprints on difficult backgrounds. Novel oblique lighting techniques will be explored to detect evidence on raised surfaces and footwear impressions. Bandpass filtering techniques will also be explored to aid in the detection of evidence at crime scenes. Attendees will be introduced to nonvisible lighting techniques and digital capture of fingerprints developed using various treatment methods including cyanoacrylate fuming and IR fluorescent fingerprint powders. A new technique for developing fingerprints on fired cartridge casings and other difficult surfaces will also be introduced in this workshop.

Flour Power: A Fresh Approach to Insect Detection in Stored-Product Forensic Entomology Assays Using Direct Analysis in Real Time-High Resolution Mass Spectrometry and Chemometrics

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In the United States, the processing of agricultural products is federally regulated with health and safety guidelines set by the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA). The failure of food producers and manufacturers to meet the limits of insect and pest infestation set by these regulations can result in litigation leading to fines and other legal consequences. It is therefore essential that both regulating bodies and food producers are able to accurately test for, and detect the scale of, insect infestation in agricultural products in a timely manner. Unfortunately, conventional techniques in stored-product forensic entomology for determining insect presence generally require significant specialized expertise, are time-consuming and resource-intensive, and exhibit variable sensitivity and selectivity in detecting the type and severity of insect infestation. This speaks to the continued need for simple, rapid, and reliable techniques for the assessment of the presence of food pests in order to establish liability in court cases regarding food health and safety violations.

It is hypothesized here that many of the challenges associated with conventional methods of insect pest detection could be addressed using a chemical approach. Following Locard's Exchange Principle, it is anticipated that insect presence in milled grain would result in the transfer of species-specific insect biomarkers to their surrounding environment (i.e. the milled grain). Further, the levels of these chemical markers in the milled grain should correlate to the insect population present. Direct analysis in real time high-resolution mass spectrometry (DART-HRMS) has unique capabilities which make it ideal for accomplishing this type of rapid analysis. In a proof-of-principle study, all-purpose flour was deliberately infested with the common agricultural pest *Tribolium castaneum* (the red flour beetle). These insects were introduced into five separate batches of flour, each in replicates of five, while an equal number of samples of non-infested flour were used as controls. The samples were then analyzed over the course of several months by DART-HRMS in positive-ion mode. The collected spectra were used to generate chemical profiles for the control and infested flours, which were then evaluated using several methods of statistical analysis including ANOVA-simultaneous component analysis (ASCA). This analysis revealed masses that enabled the differentiation of infested and non-infested flour. One of these masses was m/z 137, which GC-MS analysis confirmed to be 2-ethyl-1,4-

benzoquinone (EBQ), a molecule known to be produced by red flour beetles. These results indicate the potential for DART-HRMS to be used as a forensic tool by regulating bodies to rapidly screen milled flour to determine not only the presence of insect pests, but also their amounts.

Richard Saferstein: Life, legacy and contributions to forensic science

Tiffany Roy, ForensicAid, LLC.

Dr. Richard Saferstein retired after serving 21 years as the chief forensic scientist of the New Jersey State Police Laboratory, one of the largest crime laboratories in the United States. Dr. Saferstein held degrees from the City College of New York and earned his doctorate degree in chemistry in 1970 from the City University of New York. From 1972 to 1991, he taught an introductory forensic science course in the criminal justice programs at the College of New Jersey and Ocean County College. These teaching experiences played an influential role in Dr. Saferstein's authorship in 1977 of the widely used introductory textbook *Criminalistics: An Introduction to Forensic Science*, currently in its 13th edition and the widely used professional reference books *Forensic Science Handbook*, Volumes I, II and III currently in its 3rd edition. Saferstein's basic philosophy in writing *Criminalistics* is to make forensic science understandable and meaningful to the nonscience reader, while giving the reader an appreciation for the scientific principles that underlie the subject. This talk will discuss the trials and tribulations Saferstein encountered in his personal and professional over the course of his career, as well as his contributions to the field of forensic science. Finally, the legacy of Dr. Saferstein will be discussed and how his contributions have been and will be maintained in the future.

Analyzing the Scent of Death Utilizing Solid Phase Microextraction

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By-products of cadaveric decomposition, such as volatile organic compounds (VOCs), provide scientists with vital forensic information that could improve search and rescue operations in police investigations. Human decomposition is a very complex process and has not been well studied at the chemical level. Despite the fact that there has been an increased interest in researching VOCs related to decomposition, there continues to be a lack of an in-depth cadaveric VOC profile. Additionally, the inherent impact of environmental factors on the decay process and insect activity have not been fully investigated or well understood. While insect succession patterns have been thoroughly studied for hundreds of years, understanding why there is a sequential arrival pattern of different insect species that are attracted to decomposing cadavers remains unanswered. In this study, the VOC temporal profile of pig (*Porcus*) carcasses as human proxies are used to determine if there is a correlation between the presence of specific chemical compounds or mixtures and insect succession.

Stage 1 of the study involved collecting, identifying, and quantifying VOCs emitted from decaying swine carcasses. A solventless headspace collection via solid phase microextraction (SPME) fibers and Gas Chromatography-Mass Spectrometry (GC-MS) was used analysis. The project focused on the examination of released compounds in a controlled, indoor environment as well as in an outdoor setting. The pig carcasses were monitored at varying time intervals ranging from hours to days. The results were studied to determine whether or not insect activity conditions had any impact on the formation and distribution of the VOCs from the cadaver during the decomposition process. Stage 2 entails preliminary studies on the relationship between released cadaveric VOCs and insect succession using fly traps. These traps contain a variety of released chemical compounds during decomposition placed for outdoor field testing in 48-hour increments in hopes of determining ecological correlation. After the allotted time period, the fly traps are collected in order to identify any collected insects. These results will hopefully aid in correlating insect succession and human decomposition to impact and improve cadaver detection techniques commonly used in

forensic applications.

Evaluating the Accuracy of Bloodstain Pattern Analysis Using Hemodynamic Factors

Paul M. Yount, University of Providence.

This comparative survey explores the relationship between the discipline of bloodstain pattern analysis (BPA) and hemodynamic blood properties, such as viscosity and hematocrit. In BPA, forensic researchers examine the phase change of blood when in contact with air and surfaces, but little forensic literature investigates physiological blood alterations that could affect BPA measurements. Red blood cell count, or hematocrit, in females (37-48% of blood volume) is slightly lower than males (45-52% of blood volume) from menstrual red blood cell loss, etc. Strong evidence suggests that erythrocytes influence blood viscosity because of their high concentration (4-6 x 10⁶ RBC/mm³ or 40-45% of blood volume in healthy individuals). Blood that is physiologically altered by a disorder/disease or alcohol intake will accommodate by changing hematocrit and viscosity levels. With this knowledge, it is hypothesized that traditional BPA measurements can be inaccurate when blood is physiologically altered by a disease/disorder. Intravenous blood samples were drawn from nine volunteers (all women, including eight with blood alterations and one healthy control) into collection tubes containing ethylenediaminetetraacetic acid. Each sample was tested for viscosity using a CannonFenske viscometer and hematocrit levels using a ZipCombo centrifuge. Each sample was used to make several bloodstains at varying degrees of impact (10°, 30°, 60°, and 90°). ANOVA ($\alpha = 0.05$) and Tukey HSD statistics were used to compare experimental and expected angles of impact within the nine participants. Hemodynamic factors among diabetic and hypothyroid participants were found to significantly influence BPA measurements while blood alcohol concentration did not influence traditional BPA methodologies. This survey demonstrates a connection between hemodynamic properties and angle of impact measurements in BPA. Additionally, this research discusses how bloodstain reconstruction can be miscalculated in unique situations. By examining blood among several individuals, this research creates a predictive framework for analyzing bloodstains created by physiologically altered blood. Future research is recommended to better understand hemodynamic properties in BPA and fulfill the recommendations made by the 2009 National Academy of Sciences (NAS) Report.



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Drug Chemistry Abstracts

High Performance Thin-Layer Chromatography (HPTLC) Separation of Benzodiazepines

Katlyn Beidler, Cedar Crest College

Gas chromatography-mass spectrometry (GC-MS) is the gold standard for identification of seized drugs and is commonly used for the detection and quantification of benzodiazepines. The use of gas chromatography introduces problems with benzodiazepines analysis. Since they are thermally unstable, some benzodiazepines decompose due to the temperature of the injector port. Other problems such as co-elution, and lack of differentiation of some positional isomers and diastereomers also exist. Thin-layer chromatography (TLC) has also been used for the separation and identification of benzodiazepines. TLC can be very helpful in the screening of seized drugs for the commonly used benzodiazepines. TLC solvent systems for the separation of some benzodiazepines have been reported in the literature, but TLC produces poor resolution, is hard to reproduce, and is difficult to document for peer review. High performance thin-layer chromatography (HPTLC) provides increased sensitivity and selectivity over traditional TLC. Automation of HPTLC allows for faster analysis of up to 15 samples at a time, quantitation, and high reproducibility. In this study, 51 benzodiazepine drugs were analyzed for their separation and identification on four different mobile systems using HPTLC. Retardation factors (R_F) were measured using the different mobile phase systems. 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 using light at a wavelength of 254 nm. VisionCATS CAMAG HPTLC SOFTWARE (version 2.5) was used to control, document, and analyze all results from experiments. HPTLC Silica gel 60 F 254 20 x 10 cm plates were used for the four mobile phase systems. The information presented will provide forensic scientists a good option to be used in an identification protocol consistent with SWGDRUG recommendations. Further work will concentrate on optimizing the separation of closely retained benzodiazepines.

Determination of the Ultraviolet Absorption Properties of Twenty Synthetic Cathinones

Jane Berger, Cedar Crest College.

Presently, cathinones are the second largest group of novel psychoactive substances (NPSs) and there are at least 130 different synthetic cathinones that have been identified. With the large number of different NPSs, it has been an arduous task to accumulate analytical data and methods to aid in the detection and identification of these dangerous drugs. The continuous effort to accumulate analytical data is only exasperated by the slow development of reference standards which can cause delays in case reporting. For the forensic drug chemist and forensic toxicologist it is very important to have known analytical data, including spectral databases and standards, available for the purpose of identification of these drugs. The most widely used instrumental method for analyzing seized drugs, including synthetic cathinones, is gas chromatography-mass spectrometry (GS-MS). Although other techniques such as liquid chromatography, infrared and Raman spectroscopies are now used in crime laboratories, ultraviolet spectroscopy is not as commonly used. The most likely reason for this is that even though a UV spectrum can detect certain functional groups that act as chromophores it does not provide unambiguous identification of a drug. Many of the NPSs have not had their ultraviolet spectroscopic properties reported. Synthetic cathinones are one class of NPSs that such information is lacking. Properties such as maximum absorbance (λ_{max}), molar absorptivity (ϵ) and specific absorbance (ρ) are valuable for characterizing drugs. Specific absorbance (ρ) is defined as the maximum absorbance of a 1% solution over a 1-cm path length as measured by ultraviolet-visible spectroscopy (UV-Vis). The determination of a reliable ρ is a useful alternative to quantitatively verify the concentration of analytical standard solutions.

Using the specific absorbance of a drug via spectrophotometry is a very easy and reliable method to verify the purity of a drug standard solution, including synthetic cathinone standards. The UV spectral properties, λ_{max} , ϵ , and ρ of 20 synthetic cathinones were determined in methanol using three different UV-Vis spectrophotometers. A Beckman Coulter DU-800 UV-visible spectrophotometer, Mettler Toledo LabX UV-visible spectrophotometer, and a Cary

3500 UV-visible spectrophotometer were used to create calibration curves based on dilutions sets prepared using Cerilliant and Cayman Chemical certified reference standards. All spectral data acquired were processed using Microsoft® Excel for MAC (version 16.30) to determine molar absorptivity, standard deviation and CV%. Information presented can potentially be helpful to forensic chemists in an identification scheme for the analysis of synthetic cathinones in seized drugs. The specific absorbance () data for the 20 synthetic cathinones in methanol can be used to verify concentrations of standard reference samples to be used for quantitative analysis in seized drug analysis and forensic toxicology.

The Efficacy of Multi-Spectral Imaging in Detecting Product Fraud and Adulteration

Brady P Carter, PhD, Neutec Group.

Counterfeiting and economically motivated adulteration of products is an increasing threat to consumers and pose severe challenges to the manufacturing industry. They pose not only a financial risk, but a safety risk as well. The challenge for forensics teams searching for evidence of maleficence is that the perpetrators are becoming more sophisticated, making it increasingly difficult to identify instances of fraud. These teams need superior detection techniques. Once candidate technology is multi-spectral imaging, which consists of a high-resolution camera that takes a series of images at wavelength bands ranging from ultraviolet to near infrared light. Because a unique pixel is taken at each wavelength band, multivariate analysis can be utilized to identify differences on a pixel by pixel basis across the wavelength range. The objective of this study was to utilize multi-spectral imaging to identify counterfeit pharmaceutical products. Three different tablet types consisting of both genuine and counterfeit samples were analyzed using multi-spectral imaging. A color image of the products did not reveal any differences, nor were differences detectable by the naked eye. However, while distinguishable differences did not exist in the visual band, differences could be observed in the near infrared region. Authentic and counterfeit samples were then compared using normalized canonical discriminant analysis (nCDA) to identify the best way to differentiate between the samples. Once trained by nCDA, the system was able to provide a spectral fingerprint to correctly identify genuine product from counterfeit in blind samples. Multi-spectral imaging was concluded to be an effective tool for forensics teams to identify counterfeit products in a fast, non-destructive and versatile way.

Who's Ready for Halloween? – Detecting Cannabinoids in Candies, Chocolates, and Other Edible Products by DART-HRMS

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As cited in the National Institute of Justice (NIJ) 2019 Report to Congress: Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices, some of the greatest challenges to emerge in recent years are consequences of the “legalization and decriminalization of marijuana.” The report indicated that the issues require the “implementation of new testing strategies,” and that “testing methods must be developed to test THC (tetrahydrocannabinol),” in a variety of plant-based substances, edibles, and extracts. Current protocols, among which there is little uniformity, can be cumbersome and often require extensive sample preparation and complex data processing. This study focuses on the unique capabilities of direct analysis in real time-high-resolution mass spectrometry (DART-HRMS) for the rapid detection of cannabinoids in complex edible matrices. This ambient, high-resolution technique permits direct analysis of samples in their native forms, and as well as rapid interrogation of samples following their chemical alteration through exposure to derivatizing agents. A wide variety of samples were investigated, including commercial cannabidiol (CBD) products, edible certified reference materials (CRMs) infused with cannabinoids, and edible samples prepared in-house such as candies, chocolates, and other food products. DART-HRMS analyses rapidly identified the presence of cannabinoids in the products without sample pretreatment. While several cannabinoids were detected, including cannabigerol (CBG) and cannabinal (CBN), each DART-HRMS-

derived spectrum had a peak at m/z 315.232, which is consistent with protonated THC and CBD. Because these two molecules share the same molecular formula of $C_{21}H_{30}O_2$, and therefore the same protonated mass, they are indistinguishable by DART-HRMS under soft ionization conditions without sample pretreatment. Furthermore, they remain indistinguishable even under in-source collision-induced dissociation (CID) conditions that induce fragmentation of the molecules. As such, an alternative approach must be taken to identify and differentiate these compounds. It was found that THC and CBD are readily distinguishable under both soft ionization and CID conditions when the derivatizing agent N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was applied. Engagement by the derivatizing agent of the single $-OH$ group in THC and the two $-OH$ groups in CBD results in the two compounds being converted to molecules with protonated $[M+H]^+$ masses of 387.272 and 459.312, respectively. Therefore, when analyzed by DART-HRMS after derivatization, chemical standards, commercial products, and edibles yield chemical signatures that provide additional cannabinoid-related information. In summary, the data demonstrate that DART-HRMS can detect cannabinoids in complex matrices and provide a method to triage edible marijuana evidence in crime laboratories, while circumventing problems often encountered using conventional methods.

Evaluation of presumptive color tests for anticoagulant adulterant's identification

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Introduction/Background: Warfarin and superwarfarins are anticoagulant agents widely used in commercial rodenticides and in the treatment of bleeding disorders. The major compounds of the class include brodifacoum, bromadiolone and difenacoum, all of which have a history of involvement in many types of forensic Northeastern Association of Forensic Scientists 46th Annual Conference 36 casework such as suicides, homicides, and accidental and deliberate poisonings. Recently, these substances have emerged as toxic adulterants in synthetic cannabinoid, cocaine and marijuana casework, and have led to hundreds of hospitalizations and at least five deaths in the US. Presumptive color tests are an important step in the analytical process, leading the analyst to the next step in the identification of unknown material.

Objective: The purpose of this work was to evaluate the response of the target chemicals to the commonly used chemical color test reagents. The anticoagulant agents evaluated in this work included: brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, diphacinone, flocoumafen, pindone and warfarin.

Method: All target chemicals were evaluated as analytical standards, and diphacinone, bromadiolone, brodifacoum and warfarin were also evaluated as commercial products. 10 commercial products were selected randomly, from those, 7 contained diphacinone as the active ingredient, 1 contained bromadiolone, 1 contained brodifacoum and 1 contained warfarin. The color test reagents selected for this assay were: Cobalt Thiocyanate, Dille-Koppany, Duquenois-Levine, Mecke's, Marquis, Froehde and Mandelin's. Small amounts of the commercial products (approximately 1-5 mg) and 2 drops (approximately 0.1 mL) of analytical standards (1 mg/mL) were added to the wells of a plastic spot plate, and each reagent was then added. Color changes were recorded immediately after.

Results/Discussion: The 10 different target chemicals reacted with the majority of the color test reagents, except for DilleKoppany and Duquenois-Levine, which did not produce any color changes for any of the target analytes or commercial products. Of the commercial products tested, products containing diphacinone, bromadiolone, and brodifacoum all yield some color reactions with the various tests. However, when the color reactions in the commercial products were compared to those of the standards, the results were not very consistent. The reason for that could be the potential interference from the dyes present in the formulation of commercial products, as well as the difficulties of dissolution of these solid products when mixed with the liquid color test reagents. Also, the very

low concentration of the active ingredients in the commercial products may make the reaction with the color test reagents even more difficult to detect.

Conclusion: The detection of anticoagulants in forensic casework is challenging due to the small amounts present in samples and their chemical properties. The most common chemical color tests were not effective as presumptive tests for commercial products. There is a need for development of more suitable tests for identification of this class of compounds.

Key-words: Anticoagulant adulterants, presumptive color test

Microscopy and Field Color Test Analyses of Commercial CBD Plant Products

Sarah Snider Leonhauser, Arcadia University.

Cannabis is a large genus of a variety of plants that contain multiple distinct compounds.(1) Of the three main species of cannabis, *Cannabis sativa* is the primary producer of the two most popular plant types- marijuana and hemp.(2) While both marijuana and hemp are derived from the same species, the single quality that distinguishes them legally is the concentration of delta 9-tetrahydrocannabinol Northeastern Association of Forensic Scientists 46th Annual Conference 37 (Δ 9-THC) present within the plant. The Agriculture Improvement Act of 2018, which is commonly referred to as the 2018 Farm Bill, removed hemp and other derivatives of cannabis that have less than 0.3% Δ 9-THC by dry weight from the definition of marijuana in the Controlled Substances Act (3,4). In other words, hemp that contains less than 0.3% Δ 9-THC is legal, while marijuana that contains any amount more than 0.3% Δ 9-THC is not. This differentiation by law of hemp and marijuana removes all Drug Enforcement Administration (DEA) oversight on hemp and hemp products and makes these products legal federally (5). This now requires forensic laboratories to determine the concentration of Δ 9-THC to determine whether or not a sample is marijuana or hemp.

Microscopic examinations along with color tests are common analyses that are performed on suspected cannabis plant material in forensic laboratories. To simulate this initial analysis of suspected cannabis, a microscopic examination followed by two field color test kits were performed on 27 different CBD plant products, sold as smokable hemp, purchased from a variety of stores and online sites. The microscopic examination was performed to search for cystolithic hairs, which are a main identifying feature of cannabis products. The first field test kit contained the Duquenois-Levine reagent, which indicates whether a substance contains cannabis by reacting with THC. The second field test kit contained the 4-aminophenol reagent, which claims to indicate the concentration of THC relative to CBD by turning one of two distinct colors.

After microscopic examinations under 40x, 100x, and 400x, it was determined that all 27 CBD plant products showed a positive microscopic examination for cystolithic hairs (6,7). All 27 CBD plant products had a positive test result with the NIK Test E field test kit, indicating the presence of cannabis in all of the products. The CBD Quick Test containing the 4-aminophenol reagent did not produce any color despite a positive NIK Test E, so these tests were therefore considered inconclusive, with the possibility that they were defective. This work reveals a potential issue for forensic labs that are only using these two analyses to identify suspected cannabis evidence. If no further distinguishing quantitative tests are performed, legal CBD products yield the same results as illegal marijuana products.

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Rapid In-Field Screening for Fentanyl Analogs Using Coiled Microextraction and Portable GC/MS

Tom Mancuso, PerkinElmer Inc.

In recent years there has been an increase in fatalities related to the use and consumption of opioids, a class of synthetically manufactured pain-relieving drugs similar to naturally derived opiates like morphine, opium, codeine, etc. The Torion T-9 (Coiled MicroExtraction) CME-GC/MS method successfully collected, analyzed and identified the compounds of interest in relevant forensics scenarios involving adulterated heroin and synthesized fentanyl analogs. The Torion T-9 GC-TMS features a low thermal mass capillary gas chromatograph (GC) with high speed temperature programming (>2 °C/sec) and a miniature toroidal ion trap mass spectrometer (TMS) with a nominal unit mass resolution over a mass range of 45 to 500 Daltons. The system is totally self-contained and has an on-board helium GC carrier gas supply cartridge (2500 psig, 90 cc). The Torion T-9 GC-TMS features electronic pressure control (EPC) of the helium GC carrier gas. New additions to the Torion T-9 include expanded on-board library search capabilities, calibration and performance validation routines, addition of the NIOSH database, quantitative results, and the addition of two new sampling accessories.

From Patrol Car to Lab Bench: Implementing the Thermo Scientific™ TruNarc™ Analyzer into a Seized Drug Laboratory Workflow

Stephanie Minero, M.S., F-ABC, Nassau County Office of the Medical Examiner, Division of Forensic Services.

For many municipalities, it is common that law enforcement personnel utilize commercially prepared chemical color test kits to presumptively identify narcotics in the field during an investigation. It is well documented that its limitations include false positives/negatives due to the specificity of the colorimetric reaction and limited discriminating power. Additional factors such as sample matrix, kit storage conditions, training, sample introduction, and cross-contamination may also negatively affect the accuracy of the result.

Law enforcement agencies have begun to utilize the Thermo Scientific™ TruNarc™ handheld Raman spectrometer as an alternative to traditional color test kits. Classified as a SWGDRUG and ASTM Category A technique due its increased level of selectivity, the technology of the forensic laboratory can now be used in field environments. The device has the ability to rapidly identify drugs of abuse, precursors, and common diluents with little or no sample preparation required. An accompanying Type H test kit allows for the analysis of many fluorescent compounds and some low concentration analytes. The utility of the device is purported to result in cost-saving for agencies who no longer need to purchase and dispose of color test kits and reagents, in addition to the generation of reviewable data for judiciary proceedings. Most importantly, it reduces the exposure of hazardous substances as scans can be performed through Raman-inactive drug packaging such as glass, plastic, and wax paper. The Nassau County Office of the Medical Examiner, Division of Forensic Services recently purchased several units to implement into its seized drug laboratory workflow. The goal of this new technology is to increase the efficiency of laboratory analysis in routine cases without sacrificing the quality of the work product. Integration of this device as a screening technique has shown promising results to reduce case turnaround time in both the analytical and technical review phases.

This presentation will discuss how the initial instrument was validated for use in casework, effective ways to train staff, and how to implement the device into a seized drug scheme of analysis to best mitigate false positives/negatives. Interesting cases, alternative sample preparations, discussions with technical support, and key differences between

field and laboratory techniques will also be discussed.

Development of Color Test and Sweat Patch Extraction Protocols for Synthetic Cathinones

Nicholas Negri, The Pennsylvania State University, Department of Forensic Science.

With the rise in synthetic cathinone usage, a presumptive testing scheme that can detect synthetic cathinone analogs with a high degree of specificity is needed. Synthetic cathinones are an amphetamine-type stimulant that have many names, flakka, bath salts, and, simply, cathinone, among others. Cathinones are psychoactive compounds that cause paranoia, hallucinations, panic attacks, restlessness, and increased movement, among others. Cathinones are classified as novel psychoactive substances (NPS) and designated as designer drugs. NPS are illicit drugs that mimic the effects of established drugs. Illicit drugs that can be synthesized to specifically evade law enforcement are known as designer drugs, and, generally, rely on functionalizing the base chemical structure. Presumptive tests remain important for unknown powder identification, both in the field and in laboratories.

Presumptive tests, like color tests, should give immediate and clear responses when testing samples to facilitate downstream analytical testing. This research modifies the synthetic cathinones themselves, creating a chromophore directly from the cathinone. The reaction scheme tests multiple functional groups on the cathinone structure: the benzene ring, the amine group, and the carbonyl bond. The sequential testing procedure first nitrates the benzene ring then utilizing gold supported titanium dioxide (Au/TiO₂) catalyst, convert the nitrobenzene to an azobenzene, an orange-red liquid. The amine saturation test procedure will be from a pre-existing forensic color test that can differentiate between 1o, 2o and 3o amines. The last test will be to modify the carbonyl bond to form a new, colored compound. The second phase of this research is to develop a synthetic cathinone extraction protocol from sweat patch matrices that is liquid chromatography mass spectrometer (LCMS) compatible. Sweat patches are a non-invasive technique for the detection of illicit drug use; sweat containing these drugs are absorbed into the sweat patch while it is on the skin for up to 10 days. The best solvent for eluting cathinones will be determined and ran on the optimized LCMS method for separation of cathinones. This extraction and identification procedure could then be implemented in industry.

Tour of a Cannabis Extract Manufacturing Facility in Maine

Jennifer A. Paris, BS, F-ABC*, New Hampshire State Police Forensic Laboratory.

After attending this presentation the audience members will have a better understanding of the operation of a small state licensed and inspected cannabis extract manufacturing facility.

The State of Maine enacted a medical marijuana law after a citizen led ballot initiative passed in November 1999. Since that point in time multiple amendments have been made to the statute following feedback from patients, caregivers, medical providers, and a specially developed task force. Although a 2016 law was passed to allow for the recreational use of marijuana and a 2017 law was passed concerning the taxation and other regulations surrounding recreational marijuana, the commercial sale of the plant and products made from it has not yet begun.

Today the State of Maine permits both growing and processing of marijuana by individuals holding a medical card (“patients”). In addition, patients and caregivers are allowed to use a third-party manufacturing facility to convert their harvested marijuana or hemp into concentrated forms. These facilities are registered, inspected, and licensed by the State of Maine and are expected to adhere to strict procedures regarding site safety and security, the quantity and type of plants on site, the quantity of harvested product on site, the use of “inherently hazardous substances”, and the disposal of waste materials, among other standards.

This presentation will briefly cover the regulations in place for Maine's program and then focus on the specifics with respect to a small registered manufacturing facility. Chemists from the New Hampshire State Police Forensic Laboratory were given the opportunity to tour one such facility in the summer of 2019 while extractions were in progress. This cannabis extract manufacturing facility employs Super Critical Carbon Dioxide Processing of harvested marijuana and / or flowering hemp grown by patients or their caregivers to produce highly purified concentrates devoid of terpenes. In addition the facility was equipped with a closed loop extraction system to produce butane honey oil, shatter, and "diamonds". Discussions regarding purity, concentration, cannabinoid profiles, and all aspects of the operation, including the evolution of the facility's practices over time, took place with the owner/operator of the facility. Attendees will be shown the outdoor grow area, extraction and purification processes, preparation of food products, and product packaging material at the visited facility.

Given the increased number of states where cannabis products are legal for medical or recreational use it is important for those involved in the analysis of seized drugs to have an understanding of the process used to concentrate cannabinoids from harvested marijuana and flowering hemp, and how products must be diluted, prepared, and packaged in order to comply with the relevant laws and regulations. Finally, the information presented may be helpful for attendees who are assisting to build a local or state jurisdiction's medical or recreational marijuana program.

Cannabis, Manufacturing, Extract

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) 2020 Update

Tiffany Ribadeneyra, Nassau County Office of the Medical Examiner - Division of Forensic Services.

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

This presentation will provide attendees with an update on SWGDRUG activities during the year 2019 and currently in 2020. Recent publications include version 8.0 of the SWGDRUG Recommendations with revisions to Part III B related to Methods of Analysis/Analytical Scheme for Identification of Drugs as well as a new supplemental document (SD-7) exemplifying the Construction of an Analytical Scheme. Recent activities include revising Parts IVA and B of the SWGDRUG Recommendations, SD-2: Validation of Analytical Methods and SD-5: Reporting Examples. Lastly, the SWGDRUG spectral libraries and monographs remain an extensively utilized resource within the forensic community and current status as well as future plans will be reviewed.

Gas Chromatography-Mass Spectrometry of Eight Aminoindanes

Amber Rose, Cedar Crest College.

Aminoindanes are one of the new types of psychoactive designer drugs that have emerged. The base structure, 2-aminoindane, can be chemically altered to produce a variety of aminoindane analogues. Minimal analytical data has been reported on these compounds, and limited standards are available for forensic science laboratories, which presents a challenge to analysts who lack the necessary information needed for identification and differentiation of aminoindanes. The main goal of this study was to help strengthen the analytical profiles of 2-aminoindane and seven analogues to assist forensic science practitioners. The aminoindanes investigated in this study were 2-aminoindane (2-

AI), N-methyl-2-aminoindane (N-methyl-2AI), rasagiline, 5-methoxy-2-aminoindane (MEAI), 5-methoxy-6-methyl-2-aminoindane (MMAI), 5,6-methylenedioxy-2-aminoindane (MDAI), 4,5-methylenedioxy-2-aminoindane (4,5-MDAI), and 5-iodo-2-aminoindane (5-IAI), and all were analyzed as free bases. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the eight aminoindanes using two different column stationary phases, Rtx-1Sil MS and Rtx-5Sil MS, with the same configuration (30 m x 25 mm x 0.25 µm). Split injection (25:1) was used, and retention times and retention indices were determined for the aminoindanes on both columns. The separation of a mixture of the aminoindane analogues was optimized on both columns as well. The mass spectral fragmentation patterns were interpreted for each compound and will be discussed in the presentation. Based on retention data, it was observed that all but two analogues, 4,5-MDAI and MDAI, could be separated within the mixture. Further studies will include gathering retention data on additional stationary phases and using derivatization reagents to better distinguish between these structural isomers on a single column.

QuEChERS Sample Preparation for Extraction of delta-9-Tetrahydrocannabinol (THC) from Edible Matrices

Sarah Roseman, Nassau County Medical Examiner- Division of Forensic Services.

Alleged marijuana is commonly submitted to forensic laboratories for identification. While extraction of delta-9-THC (THC) from marijuana in the vegetative form is a fairly simple and quick procedure, its extraction from other matrices (i.e. cookies, brownies, and candies) poses a challenge for forensic analysis. These samples, often referred to as “edibles”, are particularly difficult as most of them do not allow for the common procedure of a single solvent dilution prior to GC/MS analysis. The traditional methods of sample preparation for THC edibles have many limitations, including lengthy sample preparation processes, the potential for instrument contamination or carryover, and the need for more frequent instrument maintenance. After analyzing the different preparation methods for these types of samples, it was determined that the use of QuEChERS extraction tubes might be the best course of action to limit these potential problems.

The QuEChERS extraction is a sample preparation method that can be performed on many food substrates believed to contain controlled substances such as THC. Originally intended to isolate pesticides from produce, the QuEChERS sample preparation is also useful for extracting THC from edible matrices. This two-step process extracts the analytes of interest into an organic solvent through the use of aqueous salts, and subsequently “cleans up” the extract using dispersive solid phase extraction (dSPE). In the first step, the analyte of interest is extracted from the sample through the use of an organic solvent and an aqueous blend of salts. The salts enhance the efficiency of extraction and allow the organic solvent to separate from the water in the sample. During the second step, a sample of the organic solvent extract from step 1 is cleaned up using dSPE. The dSPE step is particularly important for analysis of controlled substances in edible matrices, as it removes many organic acids, fats, lipids, and sugars that can interfere with instrumental analysis. While this technique was found to be suitable for both hard candies and brownies, it was also found that the use of a hexanes extraction was suitable for hard candy analysis.

This presentation will discuss the results of the evaluation of several different sample preparation techniques used to extract THC from hard candies and brownies in the laboratory. Each sample preparation technique was evaluated based on several parameters, including carryover, interference (matrix effects), and the ability to cleanly extract and detect THC from the matrix of interest. Each preparation technique was assessed for their ability to successfully extract THC from both a hard candy matrix and a chocolate brownie matrix.

FTIR and FT-Raman for Safe Identification of Illicit Drugs

Ron Rubinovitz², Shea A Schleman¹, Manuel Gomez¹, Suja Sukumaran², Rui Chen²; ¹ Albuquerque Police

Department,² Thermo Fisher Scientific.

The growing opioid epidemic, driven mainly by illicitly manufactured fentanyl and the ever-increasing number of new synthetic opiates, challenges authorities to identify these emerging chemical entities and to understand their pharmacology and toxicology. In addition, the high potency of fentanyl and fentanyl analogues poses a potentially life-threatening safety risk to law enforcement, first responders, and forensic lab personnel. It is therefore imperative that mitigating the risk of accidental exposure is an integral part of the overall analytical method development.

Vibrational spectroscopy, including both FTIR and Raman, has long been used as viable analytical tools for the detection and identification of illicit drugs. In this presentation, we will discuss the use of high throughput mid-infrared fiber optic ATR (FO-ATR) FTIR and FT-Raman for the safe identification of illicit drugs including fentanyl and fentanyl analogues.

Leveraging the inherent advantages of ATR, including suitability for strongly absorbing material, minimal sample preparation and maintaining sample fidelity, FO-ATR-FTIR enables remote analysis of hazardous materials in a glove box. On the other hand, FT-Raman enables sampling through glass vials, polymer blister packs, and plastic evidence bags. Hence, greatly mitigating the risk of accidental exposure to high potency drugs through ingestion, inhalation, and skin absorption. Real-life examples of identifying illicit drugs using FO-ATR-FTIR and FT-Raman will be presented. Details of Fentanyl Raman library containing 18 fentanyl associated compounds, including several pairs of isomeric fentanyl analogues, will also be discussed.

Trip or Treat: Data Fusion and Multivariate Statistical Analysis Treatment of DART-HRMS Collision-induced Dissociation Data to Enable the Rapid Detection and Identification of Tryptamines

Mónica Ventura, University at Albany-SUNY.

Forensic crime laboratories across the United States continue to grapple with the challenges imposed by the unrelenting rise in the circulation of new psychoactive substances (NPS), a term that refers to recreationally used unscheduled products that elicit a psychoactive response and have a high potential for addiction. There are several classes of NPS including opioids, cannabinoids, cathinones, and tryptamines. Opioids have rightfully received significant attention because of the high toxicity of some of the compounds that fall under this category, such as fentanyl and its derivatives. However, the focus on opioids has masked the significant problems associated with the emergence of other NPS subsets. Tryptamines serve as a case in point. This is a class of psychoactive monoamines that generally contain an indole ring. Many are structural derivatives of serotonin or DMT. Because of the structural similarity to serotonin, tryptamines usually bind to the 5-HT receptors, causing mild euphoria, visual and auditory distortions, and hallucinations. These substances can have devastating impacts on human health and can cause hypertension, rhabdomyolysis, tachycardia, mydriasis, and catalepsy.

While several are scheduled compounds, there are numerous sites within their scaffolds to which structural modifications can be introduced, resulting in the continued emergence of novel variants that retain their psychoactivity. These “legal highs” enjoy increasing use without the risk of criminal prosecution by law enforcement agencies because of their non-scheduled status. There are several bottlenecks to the scheduling of these compounds, including: (1) the challenge of rapidly detecting and structurally characterizing emerging variants; and (2) development of protocols for their routine detection and identification. In this regard, one obstacle encountered by crime labs is that closely related structures 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 of tryptamines as a class of molecules. We demonstrate in this study that these issues can be circumvented through chemometric analysis of Direct Analysis in Real-Time High-Resolution Mass Spectrometry (DART-HRMS)-derived Collisioninduced Dissociation (CID) data. Fifty tryptamines were subjected to DART-HRMS under CID conditions at 60 V and 90 V. Generation of “neutral loss” spectra, which were created by subtracting each of the fragment masses from that of the protonated precursor, enabled the extraction of the fragment identity information that is essential to interpretation of the spectra. This resulted in virtual spectra that featured peaks representing the masses of the neutral losses that had resulted in the original CID spectra. A PLS-DA model of the data generated from fusion of the 60 V and 90 V neutral loss spectra revealed ten compound classes that could be used to screen and classify unknowns. 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.



Northeastern Association of Forensic Scientists
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Forensic Biology/DNA Abstracts

Development of qPCR Based LDH Isozyme Detection Assay for Blood Samples

¹Alaina Albino, ¹Robert Powers, PhD, ¹David San Pietro, PhD., University of New Haven.

After attending this presentation, attendees will gain a better understanding on the detection of lactate dehydrogenase (LDH) isozyme mRNA in blood samples utilizing qPCR. This information may help forensic scientists use LDH isozyme mRNA levels as a potential diagnostic tool in the attempt to distinguish specific tissue stress or damage.

Lactate dehydrogenase (LDH) is a ubiquitously expressed enzyme family, comprised of five isozymes, which are differentially associated with various organs. LDH-A is associated with the lungs, LDH-B with the heart and blood, and LDH-C with the male testes and spermatozoa. LDH levels in the blood can be reflective of erythrocyte damage and varying levels of LDH expression can occur as the result of damage to various tissues. The presence of increased levels of specific LDH isozymes has been utilized as an indicator of specific tissue damage. Increased levels of LDH-B may indicate tissue damage to the heart. The main interest was to develop a qPCR based LDH isozyme detection assay, to determine baseline expression levels within humans and examine impacts of age and gender on LDH expression. Since LDH is commonly expressed in the body, it was expected that a mRNA sequence could be isolated, and reverse transcribed to detect LDH isozyme expression levels via qPCR utilizing custom-made LDH primers.

In this study, LDH-B was detected in human blood samples by using the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. Blood was collected from 22-27 and 35+ year-olds. RNA was extracted using the RNeasy Mini Kit and quantified using the Qubit® 3 Fluorometer according to the manufacturer's protocols. Then, qRT-PCR analysis was performed on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System utilizing custom-made LDH-B primers by ThermoFisher. Primers were used for the housekeeping gene, EF1A, and SYBR green was used as the basis for the dye detection reaction. This process was repeated at 1 week, 4 weeks, and 5 weeks post collection.

Initial testing demonstrated LDH-B was detected in human blood samples. Samples tagged with the LDH primers crossed the CT threshold later, indicating a decline of cDNA content over time. However, the samples tagged with the EF1A primers, the CT threshold values indicates more cDNA from week 1 to week 4, but less cDNA from week 4 to week 5. This indicates an initial increased stability of EF1A over time when compared to LDH-B. The data also demonstrates that over time, RNA content from certain blood samples were too low to detect and therefore, not continued to the reverse transcriptase step. Initial testing did not reveal a statistical distinction between age or gender for this sample set.

In conclusion, this ongoing study provides information that a mRNA sequence can be isolated, and reverse transcribed to detect LDH-B in human blood samples. Differentiating LDH isozyme mRNA expression levels in humans could be used as a medical diagnostic tool for tissue differentiation. Further research will be completed with larger sample sizes.

Investigating the use of the ANDE 6C Rapid DNA Platform for use with Environmentally Degraded Body Fluids

Erin Dimino, Claire Glynn, PhD, University of New Haven.

ANDE Rapid DNA systems were originally designed to profile buccal cell samples at booking stations with the ability to produce DNA profiles within two hours. With the creation of the I-Chip, the scope of the system has since been expanded to include a variety of sample types e.g. bone, tissue, and other forensically relevant body fluids. The ANDE system has been used to make identifications after mass disasters, such as the California Wildfires, to get identifications to family members quickly. While there are several peer-reviewed published articles on the use of various Rapid DNA platforms with buccal samples, and a smaller amount on various tissues and bone samples, there is very little published literature on their use with other forensically relevant body fluids such as those collected after sexual assaults (e.g. semen, saliva, etc.), and also the impact of environmental insults on those body fluids. The aim of this research was

to determine if the ANDE 6C Rapid DNA system with a FlexPlex assay could be used to produce partial or full DNA profiles from environmentally degraded body fluids, such as semen.

Following IRB approval and written informed consent, semen samples were collected from a healthy male volunteer. 100µL of semen was deposited on glass microscope slides in duplicate. Individual samples were created for each environment and each time interval. Over the course of six months, the samples were environmentally degraded through different temperatures (room temperature, 4°C, and 30°C). Four time intervals were used; 48 hours, 1 month, 2 months, and 6 months. At each time interval, the samples were collected with sterile ANDE swabs, inserted into I-Chips, and run on the ANDE 6C Rapid DNA system. Samples were also collected in tandem at each time interval and each environment for full traditional STR profiling. The traditional DNA testing used the QIAamp® DNA Investigator kit, QuantiFiler™ Trio DNA Quantification kit, and the GlobalFiler™ PCR Amplification kit. The Electropherograms produced using the ANDE 6C Rapid DNA System were directly compared to those produced using traditional STR profiling.

The results of this study show there to be little impact of time and environment (of those tested) on the ability of the ANDE 6C Rapid DNA System to produce full concordant profiles from semen samples. Some partial profiles (26 out of 27 loci) were obtained, however it is not thought that time or environment impacted this. This research will be extended to include longer time periods. The results of this study highlight the successful use of the ANDE 6C Rapid DNA System with semen samples exposed to different environments and time. This shows the potential of this system for implementation into operational crime labs to assist with sexual assault investigations.

The results gathered from this research will impact the forensic science community by shortening the amount of time it takes to obtain a DNA profile to just two hours. The ANDE 6C Rapid DNA instrument has paved a new path in forensic DNA analysis and continues to do so with its capability of use on environmentally degraded body fluids.

Assessing the Publics Opinion on the Use of Forensic Genetic Genealogy in Criminal Investigations

Claire L. Glynn, Rachel Graziano, Jessica Flynn, Glenn McGee, University of New Haven, Department of Forensic Science.

Forensic Genetic Genealogy (FGG) has emerged as a novel investigative tool and has gained much attention in the last two years since the Golden State Killer was identified using this technique. While this was not the first case solved using this technique, the high profile nature of the case certainly brought this method a lot of media attention, leading to a rapid increase in its interest and use in cold case investigations. FGG broadens the field of forensic DNA analysis and combines novel scientific/genetic methods with traditional genealogical methods. FGG differs from traditional Forensic DNA profiling in both the type of DNA technology used and the DNA databases employed. As this is a relatively new, and a much more encompassing technique used as an investigative tool in criminal investigations, questions have arisen regarding its use, ethics, and privacy issues. The aim of this survey is to assess the public's opinions on the use of FGG in criminal investigations. A thirtytwo question survey was created using the Qualtrics XM survey platform. The questions collected demographics of the respondents, followed by questions designed to assess their opinions on the use of FGG in criminal investigations. The survey received ~1,400 responses. The survey questions addressed the use of both public and private genetic genealogy databases. Private genetic genealogy databases refer to Direct-To-Consumer (DTC) DNA testing companies such as AncestryDNA, 23andMe, MyHeritage, Family Tree DNA, etc., from which consumers purchase kits and submit biological samples to. The raw DNA data is available from these companies for users to download and uploaded to public genetic genealogy databases. Public genetic genealogy databases refer to public online databases such as GEDmatch to which users can voluntarily upload their raw DNA data to search for genetic relatives. The results of the survey show there is a willingness among the public to allow law enforcement to access public and private genetic genealogy databases, however mostly only for major crimes. The results also show the opt-in/opt-out function for law enforcement access

in both private and public genetic genealogy databases is favorable as it allows the user to control their own data. Interestingly, 57% of respondents believe a search warrant should be required. FGG has benefited hundreds of criminal investigations in recent years and may soon become routine practice for investigating major crimes as more crime laboratories begin utilizing FGG in their case work investigations. As the database sizes grow, so does the power of Forensic Genetic Genealogy.

Use of the methylation status of the MyoD Family Inhibitor (MDFI) for saliva identification

Kimberly Hane and K. Joy Karnas, P.h.D, Cedar Crest College.

Collecting and accurately identifying bodily fluids at a crime scene is a vital aspect of forensic science. Some of the most common fluids, including saliva, semen, urine, and blood, all have well-studied presumptive tests that analysts use for identification; however, these presumptive tests can be less than definitive as they often use proteins that can be found in multiple fluids. For instance, saliva is typically identified by the detection of α -amylase, an enzyme found not only in saliva, but vaginal fluid, urine, breast milk, and fecal samples as well. Previous research in our lab has found that the differential methylation status of specific genes in various bodily fluids can be identified by qPCR high resolution melt analysis and used to discriminate crime scene stains origins. Our previous report describes an analysis method for the identification of human semen. This study expands the analysis to include saliva, discriminating it from other fluids such as peripheral blood, vaginal fluid, menstrual blood, and urine using the promoter region of MyoD Family Inhibitor (MDFI). DNA was extracted using the QIAamp DNA Investigator Kit followed by a bisulfite conversion, chemically altering any unmethylated cytosines to uracil residues. This converted DNA, when amplified during qPCR, results in an amplicon that has a higher A/T content in saliva than the same amplified region from other tissues, allowing us to distinguish saliva based on the melt temperature. In addition to expanding the options for fluid identification, advantages for this methodology include the small amount of sample required for the analysis and its compatibility with STR DNA identification of the individual. Utilizing a common instrument found in forensics labs also makes this method more accessible to labs when compared to other proposed fluid identification systems relying on the methylation status of genomic DNA.

Optimizing the Isolation and Analysis of Exogenous Trace DNA from Fingernail Evidence

Mary Nagle, Boston University.

Fingernail evidence is often collected in criminal cases of violent and/or sexual assault. In acts of aggression and self-defense, foreign deoxyribonucleic acid (DNA) can be transferred from the perpetrator to beneath the surface of the fingernail of the victim. It is possible to recover this foreign, exogenous DNA from the victim's fingernails and potentially identify the perpetrator via DNA analysis. When attempting to recover this DNA from fingernail clippings, a couple of problems can occur. Oftentimes, not enough exogenous DNA gets trapped underneath fingernails, so there is not usually much DNA to work with for recovery. The other major problem is the presence of endogenous DNA from the fingernail donor. Not only is there donor DNA in the fingernail itself, but the donor's own DNA can build up underneath their nails simply by rubbing their face or combing their fingers through their hair. This means that there can be more donor DNA present that can mask the presence of the foreign DNA and cloud the results.

In an attempt to improve the recovery of foreign DNA and produce a reportable, informative profile, a time course study was developed. Typically, when using forensicGEM as an extraction method, the samples would incubate for 15 minutes before going through protease inactivation. For this study, the extraction period was broken up into four 5-minute periods of incubating, for a total of 20 minutes, before inactivating the protease. This was done to pinpoint the time period at which more foreign DNA is being extracted from the surface of the nail before endogenous DNA is extracted in excess and clouds or even hides the presence of foreign DNA altogether. Female fingernail clippings were spiked with neat male saliva to observe the ratio of male to female DNA during quantitation and on the

electropherograms.

The quantitation results depicted a strong presence of male DNA through the entirety of the time course, and female DNA did not appear to be extracted in greater levels until the 15 minutes of incubation. The resulting profiles exhibited the male saliva profile as the major contributor for most of the samples, especially at the 5- and 10-minute markers. In 50% of the profiles, a minor female contributor could be identified as the nail donor. One sample produced a single, male profile for each time point with no indication of a female donor present in the extract; another sample produced a profile with a male major contributor with only 3 to 6 loci having additional detectable alleles of a minor contributor at each time point. These alleles could not be conclusively attributed to the female nail donor, but she could not be excluded.

These preliminary results indicate that a shortening of the forensicGEM extraction period could be beneficial for improving the recovery ratio of exogenous to endogenous DNA from fingernail evidence.

Missing and Unrecovered Aircraft: The Overlooked Missing Persons Crisis

Stephen L. Richey, BA, RRT Kolibri Forensics.

In the states from which NEAFS members are drawn, there are no fewer than 130 cases where military or civilian aircraft were lost and at least one of the crew or passengers remain unaccounted for. Some of these cases date back to World War II and some are as recent as the first decade of the 21st century. There are several additional cases where the original recovery efforts, occurring decades ago, likely were incomplete and human remains are potentially still on site. The matter of bringing closure to these cases is a daunting task but is not an insurmountable problem. The presenter, a forensic anthropology doctoral student, will discuss his nonprofit's efforts to research and plan search and recovery efforts in many of these cases. The presentation will bring to light many cold cases that attendees may be unaware of in their own jurisdictions.

All in the Family: Problems with relatedness and Probabilistic Genotyping

Tiffany Roy, ForensicAid, LLC.

Complications related to the interpretation of mixed DNA profiles has given rise to new technologies aimed at aiding forensic DNA analysts. Interpretation of DNA mixtures is compounded when relatives are expected to be part of the DNA mixture on an evidence sample. One of the most widely used tools developed for addressing DNA mixture interpretation, ESR's STRmix program, has shown utility on a wide range of mixed samples. Extensive validation has revealed the program can indicate when relatives may be contained within an evidence DNA mixture. When close relatives are suspected in a mixed sample, the program has demonstrated that it can falsely include non-contributor relatives and inflate the LR for a person of interest. This talk will discuss instances where this has been observed in validation as well as casework. This talk will describe the steps a lab should take to understand how to detect when this may be occurring at each STRmix user lab, including validation, review of peerreviewed published literature, and methods for detecting and analyzing samples containing close order relatives.

Manual Assessment of Complex Mixtures

Joanne B. Sgueglia, Laura McComsey, Emily Davis and Justin Shaffer, NMS Labs.

A complex mixture validation was performed for manual DNA interpretations. A series of 3-, 4- and 5-person mixtures were amplified using the Promega® PowerPlex® Fusion 6C System run on an Applied Biosystems™ 3500 Genetic Analyzer using GeneMapper™ID-X Software v1.5. A range of DNA target inputs were evaluated for both

individual components and total target DNA. Allelic information was culled to investigate allele recovery and drop out for unshared (single donor) and shared (allelic overlap) components. Peak height information was determined for all components, including an assessment of the single source components to compare expected and observed mixture weights. Degradation effects were studied using a 3-person mixture at a 10:5:1 ratio, with and without a degraded component for the highest donor (10 parts). Additional analyses were conducted from this Mixture Study to compare average peak heights of various component target DNA to the expected peak height ranges established from the Sensitivity Study.

Low template (≤ 100 pg) and/or degraded DNA resulted in deviations from the expected trends. Interpretation of such low template DNA is subject to stochastic effects and drop out that will result in uncertain data that cannot be interpreted accurately and reliably without the use of probabilistic genotyping tools. Such samples will contain partial profiles with missing data that would be difficult to create a set of potential genotypes at the various loci prior to comparisons with knowns. Such data may not be acceptable to use for either exclusions or inclusions and may be considered unsuitable for interpretation.

Determination of Shedding Propensity Based on STR Results

Genevieve Trapani, Xiao Chen, Tebah Browne, Niti Dalal, Mechthild Prinz, John Jay College of Criminal Justice.

Trace DNA evidence is often discovered at various crime scenes due to either active or passive transfer. Based on previous studies, passive transfer depends on the individual's shedding propensity, or probability of depositing a detectable amount of DNA through touch. Determining the shedding propensity of a person of interest can aid in trace DNA interpretation in forensic casework. The primary purpose of this project was to determine the shedding propensities of individuals in a general community using STR profiles acquired from palmar skin samples. Three collections (spaced one week apart) of skin tape lifts using D-Squame adhesive disks were acquired from unwashed and washed fingers for 28 individuals. Each donor then provided a buccal swab as a reference sample. All samples were extracted with QIAamp® DNA investigator kit (Qiagen) and tested using GlobalFiler™ PCR amplification kit (Thermo Fisher Scientific); STR profiles were analyzed using GeneMarker® HID (SoftGenetics). A consistency check was performed to determine whether shedding propensity is a fluctuating property.

As expected, STR results for unwashed hands showed a higher percentage of DNA mixtures. Accordingly, shedding propensity was determined based on STR profile quality and number of the expected alleles present for washed hands only. Out of the 28 volunteers, three (one female and two male) individuals were consistently low shedders (10.7%), 20 (10 female and 10 male) individuals were intermediate shedders (71.4%), and five (two female and three male) individuals were high shedders (17.9%). No gender trend was seen for shedding propensity. Handedness did not seem to have an effect on right versus left hand STR profile quality and/or mixture detection. Consistency over all three collections was mixed, but 52.7% of the sample sets showed all high and 10.7% all low quality profiles. Approximately 14% of the 112 sample sets showed inconsistent profile qualities over all three collections. Since STR profiles can be affected by various factors, such as PCR inhibition and pipetting efficiency, the determination of shedding propensity from this data alone may be inaccurate. Future research will compare the STR results with quantification data to develop a more precise method of shedding propensity determination.

Analysis of DNA methylation markers for tissue identification in individuals with different clinical phenotypes

Rebecca Weeden¹, Johnisa Walcott¹, Haley Ecker¹, Juliette M. Gorson¹, Deborah S.B.S. Silva¹; ¹ Hofstra University.

Identifying a particular tissue is highly important for an investigation, and the type of analysis is critical since the

destructive nature of a test may be important when only a small amount of sample is available. Many of the common techniques used are considered as presumptive tests, such as identifying heme in blood, acid phosphatase in semen, and amylase in saliva. To address problems with sensitivity and stability of these tests, new methods involving the detection of specific mRNA have been developed. However, while RNA and protein markers may be used for tissue identification, DNA presents the ideal source since it provides quantitative results and is more stable than RNA. In addition, as DNA is already routinely extracted in forensic laboratories, no additional sample processing is needed. This is important since in many cases the sample available for analysis is limited. Many studies have demonstrated that some regions in the DNA show different methylation patterns according to the type of tissue being analyzed. These regions are known as tissue-specific differentially methylated regions, and they provide the base for a tissue identification assay. DNA methylation is the most wellcharacterized epigenetic modification and is usually associated with transcriptional repression. The addition of a methyl group usually occurs in the cytosine of CpG dinucleotides. Even though different studies have demonstrated that various CpGs sites present tissue-related methylation levels, these genotype-phenotype associations have not been analyzed in individuals with different clinical phenotypes. Because underlying diseases and different clinical conditions can alter DNA methylation levels, it is important to investigate if the clinical phenotype of an individual can alter methylation patterns and if it is necessary to incorporate changes to data interpretation when using this technique for tissue identification in criminal investigations. In this study, DNA methylation markers for tissue identification were first identified by reviewing previously published data. Then, the Gene Expression Omnibus (GEO) data repository was used to analyze gene expression profiles from different studies. After gathering and reviewing all data, it was possible to understand how DNA methylation patterns are affected by different clinical conditions and if this should be taken into consideration when using DNA methylation for tissue identification.



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Exploring the limits of modern DNA technology: Forensic genetic investigation of two adult male skeletons recovered from the La Belle shipwreck

Angie Ambers, Ph.D., University of New Haven.

In 1995, the historical (17th century) shipwreck of La Belle was discovered off the coast of Texas. One partial human skeleton was recovered from alongside cargo in the rear portion of the ship; a second (complete) skeleton was found atop coiled anchor rope in the bow. In late 2015, comprehensive forensic genetic testing began on multiple samplings from each set of remains. For the partial skeleton recovered from the ship's rear cargo area, results were obtained for 26/27 Y-STR's using traditional CE; with MPS technology, results were obtained for 18/24 Y-STRs, 56/56 ancestry-informative SNPs (aiSNPs), 22/22 phenotype-informative SNPs (piSNPs), 22/27 autosomal STRs, 4/7 X-STRs, and 94/94 identity-informative SNPs (iiSNPs). For the complete skeleton of the second individual, results were obtained for 7/17 Y-STRs using traditional CE; with MPS technology, results were obtained for 5/24 Y-STRs, 49/56 aiSNPs, 18/22 piSNPs, 15/27 autosomal STRs, 1/7 X-STRs, and 66/94 iiSNPs. Biogeographic ancestry for each set of skeletal remains was predicted using the ancestry feature and metapopulation tool of the Y-STR Haplotype Reference Database (YHRD), Haplogroup Predictor, and the Forensic Research/Reference on Genetics knowledge base (FROG-kb). Phenotype prediction was performed using piSNP data and the HIrisPlex eye color and hair color DNA phenotyping webtool. Mitochondrial DNA (mtDNA) whole genome sequencing also was performed successfully. This study highlights the sensitivity of current forensic DNA laboratory methods in recovering the molecular signatures of historical and archaeological human remains. Using advanced sequencing technology provided by MiSeq™ FGx (Verogen) and Ion S5™ (Thermo Fisher Scientific) instrumentation, degraded skeletal remains can be characterized using a panel of diverse and highly informative markers, producing data which can be useful in both forensics and genealogical investigations.

Rapid DNA technology for investigation of exhumed human remains: Resolution of an alleged case of mistaken cemetery burial using the ANDE® 6C platform

Angie Ambers, Ph.D., Timothy Palmbach, J.D., University of New Haven.

In October 2019, two adult female decedents were processed by the same funeral home in Connecticut. According to traditional Jewish funeral practices, the bodies of both women were prepared for burial according to tahara (ritual purification, no embalming) by a hevra kaddisha (sacred burial society), wrapped completely head-to-toe in a tachrichim (simple white shroud), and interred in a simple wooden casket (aron). Decedent #1 was emaciated at the time of death due to a long illness; Decedent #2 was reportedly obese. Pallbearers at the funeral of Decedent #1 remarked that her casket seemed exceedingly heavy based on her low body weight at the time of death. A few months later, family members of Decedent #1 visited her gravesite, noticed that the date-of-birth on her headstone was incorrect, and suspected that there may have been an accidental "burial switch" at the cemetery. Consequently, the families of both women convened and petitioned the funeral home to exhume Decedent #1 to investigate the matter. In June 2020, human remains were exhumed for DNA testing to confirm the identity of the woman interred in Decedent #1's burial plot. A small section from the diaphysis of the exhumed decedent's right femur was collected for DNA testing, along with family reference samples (buccal swabs) from two of Decedent #1's biological daughters and the sole biological daughter of Decedent #2. Traditional forensic DNA casework approaches for human skeletal remains are time-consuming and labor-intensive, often taking weeks-to-months to obtain genetic testing results. Rapid DNA technology offers the capability to provide a more timely resolution (< 2 hours processing time) and closure to families on such sensitive issues. Bone samples collected during the exhumation and buccal swab (family) exemplars were processed on the ANDE® 6C Rapid DNA System (ANDE Corporation, Waltham, Massachusetts) using the FlexPlex® 27 assay (which incorporates the 20 core CODIS loci mandated by the FBI for casework) and the patented I-Chip™ and A-Chip™ (for bone samples and reference swabs, respectively). Kinship analysis was performed using the Claimed Relationships module of ANDE FAIRSTM software (ANDE Corporation).

Handwriting Analysis Through Kneser Graph Triangle Decomposition

A. Arabio, A. Quiricury, L. Quarino, J. Taylor, J. Hammer, D. Ommen, Cedar Crest College, CSAFE

Handwriting comparative analysis is often criticized because of the subjective nature of traditional examination. In an attempt to provide objectivity, a study is being conducted where handwriting is being examined with the use of Kneser Graph Triangles. Through the use of Kneser Graph Triangles and their decomposition to analyze handwriting, more specific information can be obtained from each sample and compared both within a source and with multisource data. Gneser Triangles in this study were formed in such a way that each node within a triangle set has a unique color and each edge is unique to its triangle. The characteristics of each handwriting sample that gets marked as a different color node include the beginning of a pen stroke, where that pen stroke ends, any location where the pen line overlaps itself, the lowest point of a word, and the highest point of a word. Triangles are able to provide information on angles, length, area similarity, and congruency. The sample is able to be broken into individual words by a software 'handwriter' created using the statistical program R. By forming Gneser Triangles over these samples, it may be possible to gauge the variation in a single source author and then be able to compare this quantitative value to other samples of unknown sources. Using this information, the study hopes to be able to form a quantitative analysis of handwriting samples and be able to calculate how similar or dissimilar two samples are from one another. One of the study's main goals is to be able to form these triangles and multiple samples from multiple different sources and be able to group, identify, and accurately determine what samples came from what source. Once this is accomplished, a similarity statistic may be generated to verify inclusions or exclusions.

Bladder Wash, a (not-so) Alternative Specimen for Postmortem Forensic Toxicology

Kylie Candela B.S., Luke N. Rodda, Ph.D., Amy P. Hart M.D. and Karen S. Scott Ph.D., Arcadia University.

Urine is considered the ideal sample in postmortem forensic toxicology to provide historical evidence of drug use. However, there are many cases in which the deceased's bladder is voided prior to their autopsy or dehydration occurs postmortem. In these cases, it is possible to wash the bladder with distilled water or saline and thus collect the bladder wash and any available residual urine for drug screening and confirmation. The San Francisco Office of the Chief Medical Examiner (OCME) has made the collection of bladder washes at autopsy an option when urine is not available. While bladder washes are not conventional, this study aims to determine its use in postmortem forensic toxicology. Data from analysis of bladder wash (BW) samples collected at the OCME were analyzed to assess the efficiency of this alternative sample in comparison to blood from the same individual by determining the identities of individual analytes and their metabolites. Results (n=41) following routine testing has shown that for individual analytes, there is an 80-90% correlation between blood and bladder wash results. In addition, when examining both parent drug and metabolite data, this estimate correlation increases. The results have also led to interesting findings regarding specific analytes. The primary metabolite of heroin, 6-acetylmorphine (6-AM) was detected in blood in 4 cases. Surprisingly 6-AM was only detected in the BW for one of these cases. In methadone/EDDP cases, both analytes were detected in three out of five cases. In the 2 other cases, only methadone was detected in the one BW and only EDDP in the other. In 2 out of the 7 fentanyl/norfentanyl cases, both drugs were detected in the BW, but not in the blood. Three other fentanyl cases had both drugs detected in the blood, with either one or neither of the drugs detected in the BW. The most significant finding, however, is that there were several cases in which a drug was detected in the BW but not in the blood analysis. The most significant of these detected drugs was benzoylecgonine, as it indicates the involvement of previous cocaine use in the case.

While further data and statistical analyses must be performed to fully assess its significance, it currently appears that standardizing the collection and analysis of bladder washes in postmortem toxicology will assist forensic pathologists in determining the cause and manner of death in cases where urine and/or other biological specimens are not available for collection and subsequent analysis. In addition, since two different specimens from the same case can be used for

a drug confirmation, analyzing a bladder wash in addition to a blood sample can allow a forensic toxicologist to confirm drug results without requiring traditional screen and confirmation regimes.

Development of Colorimetric Methods for Marijuana Identification via Mobile Phone Imaging

Griffin Cassata, University of New Haven.

Marijuana has been at the forefront of social and political dialogue with respect to medical and recreational use. Congress recently passed the “Hemp Farming Act of 2018,” which allowed for the cultivation and sale of hemp. This law defines hemp as *Cannabis sativa L.* containing less than 0.3% Δ^9 -tetrahydrocannabinol (THC) by dry weight. Because of the precision of this definition, some level of quantitative test is now necessary to make the distinction between hemp and marijuana in field settings. In response, some law enforcement organizations are now using the 4-aminophenol (4-AP) test, which has been claimed to draw this distinction based on different color responses to differing ratios of THC and cannabidiol (CBD). However, validation studies of the 4-AP test have demonstrated its inadequacy for the purposes of quantitatively identifying THC levels.

Due to recent advances in photographic capability, cell phones are widely viewed as tools capable of objective field testing. Recent studies have also demonstrated the use of various pixel analyses to achieve quantitative results using colorimetric tests. Toward the goal of differentiating hemp and marijuana, this study proposes the use of two well-known presumptive tests (i.e., the Duquenois-Levine and Beam tests) in conjunction with cell phone photography. Tests were performed using solutions of THC and CBD standards allowing for external calibration of both the Duquenois-Levine and Beam Tests. As anticipated, issues with lighting and camera settings contributed to substantial hurdles in data acquisition from these images. Changes in the approach to data analysis and alterations to the sample medium to provide a more homogenous image can potentially mitigate these problems. Further development of these methodologies is expected to lay the foundations for a reliable field test to differentiate hemp and marijuana.

Obtaining Request Handwriting Exemplars in a Socially Distant World

Khody R. Detwiler, Lesnevich & Detwiler.

If 2020 has taught us anything, it is that many industries, including our own, need to learn how to harness new technology to assist us in completing day-to-day tasks more efficiently without jeopardizing the health and well-being of others. Due to the current state of affairs, it is clear we now live in a world that will require us to become more socially distant, necessitating the need to adapt and change the way we may have done many things for many years. Fortunately, aside from providing testimony - which can often be done remotely, much of the daily responsibilities of a Forensic Document Examiner (FDE) can be completed in an isolated and controlled environment. Depending upon the FDE, and/or the FDE laboratory, one exception may be the process by which request handwriting exemplars are obtained.

Although this presentation stems from casework, it has become more prevalent in recent months since dealing directly with the side-effects of the COVID-19 Pandemic – in particular, having to follow strict stay-at-home orders and being required to work in remote environments with limited resources. Since 2015, the author has been working directly with the New York County District Attorney’s Office (DANY) to establish a more cost-effective method for obtaining request handwriting exemplars, while also ensuring that the quality of work is not negatively impacted by any potential limitations or circumstances resulting from the process itself. This collaborative effort has resulted in a unique procedure by which an FDE can seamlessly administer a handwriting exemplar session remotely using Skype™, or a similar cloud-based peer-to-peer software platform such as Zoom. This method also allows for multiple video recordings to be obtained simultaneously at different angles of the subject writer (i.e., face and hands) as he/she

is providing the handwriting exemplars in real time. Since the session is recorded, the audio and video footage can be accessed by the FDE at any phase of the examination process; which can be especially helpful when preparing for testimony or producing the appropriate court demonstratives in cases where the exemplars were obtained months or even years prior. This presentation will take a closer look at the developments of this process over the past several years, as well as discuss some of the benefits of this method to not only the FDE but the entire court system as well.

Rapid Screening of 44 Drugs of Abuse in Urine by UHPLC-MS/MS

Jamie Foss, PerkinElmer.

Drugs of abuse have become an important cause of health, economic, and social problems. As such, the identification and determination of drugs of abuse in biological matrices is essential for forensic and toxicological studies. The emergence of liquid chromatography tandem mass spectrometry (LCMS/MS) as a gold standard analytical platform for quantitative method development in forensic toxicology has been well documented. This study utilizes a rapid acetonitrile extraction of urine samples which provided recoveries ranging from 83 – 120%. Excellent linearity was obtained over the concentration range of 0.5 to 80 ng/mL. Limits of quantitation (LOQs) ranged from 0.025 to 2.5 ng/mL. Analytical run time was 5 minutes with a 2-minute column equilibration in between injections. This work demonstrates a rapid and selective method for the separation and detection of 44 drugs of abuse and metabolites in urine using the PerkinElmer QSight 220 triple quadrupole mass spectrometer.

Soil Mineral Analysis by Particle Correlated Raman Spectroscopy (PCRS): Method Optimization

Hannah Garvin; Nicholas Gogola; Savannah Brown; Virginia Maxwell, Ph.D.; John A. Reffner, Ph.D.; Peter R. De Forest, D.Crim; Christopher Palenik, Ph.D.; Peter de B. Harrington, Ph.D.; Deborah Huck-Jones, Ph.D.; Bridget O'Donnell, Ph.D.; Andrew Whitley, Ph.D.; Brooke W. Kammrath, Ph.D., University of New Haven.

Soil is a valuable and powerful trace evidence that provides linkages and investigative leads. Criticisms of forensic soil analysis (e.g. subjective, labor-intensive, time-consuming) have resulted in a considerable decline in its use in forensic investigations and created countless missed opportunities within the criminal justice community. Consequently, there is a need for a statistically-supported, automated, and objective analytical method for soil analysis. Particle correlated Raman spectroscopy (PCRS), also known as particle driven or morphologically directed Raman spectroscopy (MDRS), is a novel yet reliable analytical technique that can add significant value to the forensic examination of soil evidence. It has proven easy-to-use and nondestructive, and its ability to be automated enables a large amount of data to be collected with minimal time required by the criminalist. Resulting data provides qualitative and quantitative information on a sample. PCRS obtains particle images of soil components (i.e. minerals) and produces microscopic morphological characteristics (e.g. circularity, elongation, brightness) and particle size distribution for the particles present. Particles can then be targeted randomly or based on morphological characteristics for Raman analysis to provide secure mineral identification.

The research presented here focuses on PCRS method optimization for soil mineral analysis using traditional figures of merit and response surface modeling of a multi-level experimental design. Laser wavelength, laser power, magnification, grating, and exposure time were examined for chemical identification of minerals via Raman spectroscopy. Particle size, particle destruction, and detector oversaturation influenced the final parameters to be evaluated for spectral analysis. The optimal parameters were determined based on a complex mineral mixture which reflect the diversity of minerals (based on stability, structure, Raman scattering capability, transparency, and fluorescence) that may be found in a single soil sample. The contrast/illumination method, magnification, and targeted morphological analysis were then evaluated to obtain an optimized method for automated imaging and Raman analysis. The effect of field of view and contrast on particle sensitivity and time of acquisition were important factors

considered. At this step, final parameters were set for each soil type based on the individual parameters determined necessary for minerals of that given soil type. Now that the optimal method parameters have been determined, they can be applied to the analysis of a range of soil samples to generate a robust PCRS dataset for forensic soil analysis.

The Investigation of a Chemical Reaction Between TNT and Hypochlorite

Garrett Greathouse, University of New Haven.

Improvised explosive devices (IEDs) are commonly utilized in terrorist attacks because they cause both destruction and devastation to society. If a bomb was constructed on a surface which was then cleaned to destroy remaining trace evidence, it is possible that a chemical reaction between the explosive residue and the cleaning product could occur. Current methods used in the forensic community test for the explosive residue itself. The downfall to these methods are that they exclude any possible analogues of explosives, making it possible to obtain a false negative. No research regarding how these explosives interact with cleaning reagents exists, so this study will use GC-MS to investigate the chemical reaction between TNT and hypochlorite, the active ingredient in bleach.

Starting compounds that contain a chemical structure resembling TNT will be mixed with hypochlorite to elucidate how the possible TNT and hypochlorite reaction occurs. The starting compounds chosen for this study were 1-nitropropane, toluene, 4-nitrotoluene, and dinitrotoluene. A GC-MS method was created in order to simultaneously detect 1-nitropropane, toluene, 4-nitrotoluene, dinitrotoluene, and TNT prior to beginning the reaction studies in order to identify possible formed products. The reaction between TNT and hypochlorite will be investigating by varying time, concentration, and temperature. For the time experiment, the analyte and hypochlorite will be allowed to react for 5 minutes, 1 hour, 12 hours, and 24 hours at room temperature while fixing the concentrations of the reactants. To investigate the effect of concentration, hypochlorite concentration of 50 µg/mL to 400 µg/mL will be mixed with fixed analyte concentrations at room temperature and fixed time. To study the effect of temperature, the reactant concentrations will be fixed while the temperature will vary from 10 °C to 50 °C at a fixed reaction time.

Preliminary experiments of mixing hypochlorite with all analytes resulted in a color change and release of heat; indicating a reaction is occurring. TNT displayed a red wine color when reacted with hypochlorite, while dinitrotoluene turned an orange color and 4-nitrotoluene was yellow. This suggests that the nitrate substitution of the benzene ring is correlated with the activity or products of the reaction, resulting in varying degrees of color change. Since the nitrate groups are in their most oxidized form, it is possible that hypochlorite has a reducing effect on the nitrate groups. GC-MS analysis of the reactions are needed to further investigate formation of any new products.

Analysis of DNA methylation sites used for forensic age prediction and their correlation with human aging

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When DNA left at a crime scene does not match any forensic database or reference samples, an investigation may run cold for lack of valuable information indicating suspects. In such cases, investigative approaches using advanced technologies could be used to gain valuable leads of possible suspects by describing phenotypic traits of the person who left the DNA material. It is well established that phenotypic traits are linked to genetic components of the human system through regulatory pathways and epigenetic modifications, such as DNA methylation, are major regulators in the translation of genotype to phenotype. As differential methylation patterns began being more widely described, research started focusing on understanding how variations in human DNA methylation patterns can be used to describe and predict phenotype-related features, such as age. Age-related CpG sites have been identified and have been described to have predictive value in the estimation of chronological age from biological samples. The most

current used methodology to identify CpG sites consists in using data obtained from DNA methylation arrays. CpG sites are chosen based only on statistical correlation with age, and not based on their location on the chromosome or on the function of genes where they are located. There seems to be a lack of information regarding the identified genes and if they have a direct or indirect relation to the aging process, or if the statistical correlation is more likely to be random. For this reason, this study aimed to evaluate the current progress in forensic age prediction based on DNA methylation patterns and to investigate the identified CpG sites/genes and their significance and correlation with human aging.

Comparison of Standard Operating Procedures Used for the Detection of Opioids in Blood

Ka Kiu Natalie Law, Boston University School of Medicine.

In forensic toxicology, opioids are frequently associated with drug abuse or drug- related death cases. An optimal method for use in the identification and quantification of opioids in a complex blood matrix is of paramount importance. Along with the ability to identify and quantitate opioids, this method should be accurate, sensitive, and selective. The application of sample pre-treatment and solidphase extraction are common to purify and concentrate the target analytes before analyzing with liquid chromatography-tandem mass spectrometry.

The purpose of this study was to compare the performance of two standard operating procedures, adopted by the Massachusetts State Police Crime Laboratory Toxicology and the Biomedical Forensic Sciences– Toxicology Laboratory at Boston University School of Medicine, for detecting opioids in blood. A total of eight drugs were analyzed: 6-monoacetylmorphine, codeine, fentanyl, hydrocodone, morphine, norhydrocodone, oxycodone, and oxymorphone. Comparison was performed using the parameters studied as part of method validation, including calibration model, bias, precision, carryover, interferences, ionization suppression/enhancement, and recovery.

The results indicated that the method from Massachusetts State Police provided a better performance with between-run precision, interferences from matrix and other commonly encountered drugs, matrix effect at high concentration (250 ng/mL) and matrix recovery. Meanwhile, the method from Biomedical Forensic Sciences showed less bias, within-run precision, and matrix effect at low concentrations. Carryover and internal standard interference were comparable in both standard operating procedures. The calibration models were adjusted by altering the selection of the regression model for improved quantification method performance. The volume of solvents, sample matrix, as well as time, were taken into consideration in accessing the overall performance of identification and quantitation. Both procedures were comparable yet the one from Massachusetts State Police was more beneficial in identifying the target analytes with greater sensitivity and selectivity and the one from Biomedical Forensic Sciences was more economical and efficient.

Screen Your Unknown Samples in a Minute or Less using “Classical” EI GCMS Spectra and Agilent’s QuickProbe™ Technology

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Routine screening of unknown powders, granules, tablets, and liquids can be time consuming and if not screened properly can send samples to the wrong section or department. Trace evidence can be especially difficult to screen due to the nature of limited sample being lost in consumption of the screening technique. Pre-screening of possible explosive compounds, dangerous drugs, and chemical agents has become a prerequisite for most laboratory analysis and presents several challenges to the analytical process. In this research, a new technique is demonstrated for fast GCMS analysis in under a minute and requires minimal to no sample preparation prior to analysis. This technique can

be achieved by using the Agilent QuickProbe™ GC/MS system and classical EI NIST library searches. The purpose of this research is to demonstrate the ability of the QuickProbe™ to be successfully incorporated into an existing Agilent GCMS system with a conventional capillary split/splitless inlet using Agilent's Capillary Flow Technology (CFT) that provides the ability to screen (QuickProbe™) and confirm (Conventional GCMS) on the same instrument. This work seeks to illustrate how the QuickProbe™ can be incorporated as a fast-qualitative screening tool for explosives, drugs, and chemical agent simulants on an existing 597X/7890 or 5977/8890 GCMS system. Overall control of the QuickProbe™ and the conventional GCMS system is based on Agilent's MassHunter software for streamlined access to acquisition parameters, qualitative analysis, unknown's deconvolution, or ChemStation Data Analysis.

Rapid In-Field Screening for Fentanyl Analogs Using Coiled Microextraction and Portable GC/MS

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In recent years there has been an increase in fatalities related to the use and consumption of opioids, a class of synthetically manufactured pain-relieving drugs similar to naturally derived opiates like morphine, opium, codeine, etc. The Torion T-9 (Coiled MicroExtraction) CME-GC/MS method successfully collected, analyzed and identified the compounds of interest in relevant forensics scenarios involving adulterated heroin and synthesized fentanyl analogs. The Torion T-9 GC-TMS features a low thermal mass capillary gas chromatograph (GC) with high speed temperature programming (>2 °C/sec) and a miniature toroidal ion trap mass spectrometer (TMS) with a nominal unit mass resolution over a mass range of 45 to 500 Daltons. The system is totally self-contained and has an on-board helium GC carrier gas supply cartridge (2500 psig, 90 cc). The Torion T-9 GC-TMS features electronic pressure control (EPC) of the helium GC carrier gas. New additions to the Torion T-9 include expanded on-board library search capabilities, calibration and performance validation routines, addition of the NIOSH database, quantitative results, and the addition of two new sampling accessories.



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Proceedings of the
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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

A

Albino, Alaina E33
Ambers, Angie F40
Appley, Meghan C14
Arabio, A C14, F41

B

Beidler, Katlyn D22
Belec, Andrea A3
Berger, Jane D22
Bianco, Christina C15
Botch-Jones, Sabra A3
Buffolino, P C15

C

Candela, Kylie F41
Carter, Brady D23
Cassata, Griffin F42
Chany, Christopher B7

D

Detwiler, Khody F42
DiEmma, Gabrielle C16
Dimino, Erin E33

E

Echternach, Victoria B7

F

Fiorentin, Tais D24
Foss, Jamie A4, F43

G

Garvin, Hannah B8, F43
Glynn, Claire E34
Greathouse, Garrett F44

H

Hane, Kimberly E35

K

Kammrathm Brooke B9
Karantenisli, Georgia F44
Kurth, Jake C17

L

Law, Ka Kiu Natalie F45
LiCausi, Jamie B9
Lokits, Kirk F45

M

Mancuso, Tom D26, F46
McCall, Holly A4
Millard, Rebecca B10
Minero, Stephanie D26
Mistek-Morabito, Ewelina C17
Musah, Rabi D23

N

Nagle, Mary E35
Negri, Nicholas D27
Nick, Rebecca C18

O

Osborne, Amy C18

P

Paris, Jennifer D27
Pietrangelo, Julia B11
Prusinowski, Meghan B12

R

Ribadeneyra, Tiffany D28
Richey, Stephen E36

R

Rose, Amber D28
Roseman, Sarah D29
Roy, Tiffany C19, E36
Rubinovitz, Ron D29

S

Sguela, Joanne E36
Snider Leonhauser, Sarah D25
Starck, Abigail C19

T

Trapani, Genevieve E37

V

Ventura, Monica D30

W

Weeden, Rebecca E37

Y

Yount, Paul C20



Northeastern Association of Forensic Scientists
Proceedings of the
October 2020 Annual Meeting