



Northeastern Association  
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# Forensic Toxicology Abstracts

## **Inhibitory Effect of Ethanol on GABA-Transaminase**

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Ethanol is the primary active ingredient in alcoholic beverages, and functions in the body as a central nervous system (CNS) depressant. The overt effects ethanol has on the body, e.g. diminished cognitive ability, loss of motor control, slurred speech, impaired balance, etc., have been well documented. While the behavioral consequences of consumption are well understood, how ethanol actually functions on a mechanistic level to elicit those effects has yet to be satisfactorily elucidated.

The structural homology between ethanol and the C3 and C4 carbons of gamma- hydroxybutyric acid (GABA), the major inhibitory neurotransmitter in the mature CNS, has led us to hypothesize that the mechanism by which ethanol causes CNS depressant effects may be related in part, to the inhibition of GABA catabolic enzymes. This inhibition would be expected to increase the functional concentration of GABA in the inhibitory neuronal pre-synaptic neurotransmitter vesicles, and the inter-neuronal synaptic space following a depolarization event.

The two primary enzymes involved in GABA catabolism are GABA-Transaminase (GABA-T) and Succinyl Semialdehyde Dehydrogenase (SSADH). GABA-T exchanges an amine group from GABA with the keto group from the co-substrate  $\alpha$ -ketoglutarate to produce succinyl semialdehyde (SSA) and glutamate. SSA is subsequently oxidized to succinic acid via SSADH, with either NAD<sup>+</sup> or NADP<sup>+</sup> as the oxidizing agent. Inhibition of either, or both, enzymes would slow the catabolism of GABA, resulting in increased intracellular concentration. We have evaluated aspects of this hypothesis using a combined GABA-T/SSADH mix, and have demonstrated an overall effect of ethanol on the biphasic enzyme system. This research is focused on an effort to distinguish the effects of ethanol, if any, on each enzyme in the linked system.

To test this hypothesis, we modified the enzyme assay protocol outlined in Tsukatani, Higuchi & Matsumoto (2005). Activity of the enzyme system was measured indirectly by the amount of NADH produced using a UV-Vis spectrophotometer @ 340 nm. A series of substrate-velocity experiments were conducted with varying concentrations of GABA to evaluate the ethanol-based inhibition of the enzyme system. To isolate the effect on GABA-T, we utilized a pre-incubation period where the necessary cofactor for the oxidation by SSADH, NAD<sup>+</sup>, was initially withheld. After the pre-incubation, NAD<sup>+</sup> was added to the reaction and the absorbance was monitored. As such, if and to the extent that ethanol inhibited the GABA-T reaction, the concentration of SSA available as a substrate for the subsequent NAD<sup>+</sup> dependent reaction would be reduced.

Michaelis-Menten coefficients (KM) and maximal reaction rates (VMAX) were then determined to evaluate the type inhibition recorded. VMAX was unaffected by the addition of ethanol. KM of the enzyme system as a whole, and specifically for GABA-T, was increased upon the addition of ethanol. These findings are characteristic of competitive inhibition. Therefore, at physiologically relevant levels, (e.g. 0.1, 0.2 and 0.37 g/dL) ethanol acts as a competitive inhibitor of GABA-T, which can explain in part, the behavioral effects seen with alcohol consumption.

## **The Evaluation of Two Extraction Methods for the Quantitation of Delta-9-THC in Blood using LC/MS/MS** Michaela Tapia, West Virginia University and Ashley Tinervia, West Chester University

In February 2024, the State of New Jersey's 221st Legislature introduced Bill S2805. This bill proposes expanding the implied consent to include blood testing and a per se standard for driving under the influence of marijuana, as well as some controlled dangerous substances. A person will be considered under the influence of a narcotic, hallucinogenic, or habit-producing drug if their blood contains three nanograms or more of delta-9-THC per milliliter. If the proposal is accepted, it will be necessary for the New Jersey State Police Office of Forensic Sciences to validate and institute a procedure to quantitate delta-9-THC in blood. The quantitative method developed will need to include a reproducible, safe, and uncomplicated extraction procedure with suitable recovery. During this research project, two extraction methods were evaluated with a few variations: United Chemical Technologies' (UCT) Clean Screen THC extraction columns (1 mL and 3 mL) and Agilent's Captiva EMR-Lipid cartridges (1 mL and 3 mL). A UCT positive pressure manifold (PPM 2.0) with a nitrogen gas source was used for all extractions. The PPM was capable of uniform extractions

of up to 48 samples at a time, and 1 mL and 3 mL adapter plates were used to change the size of the cartridges easily. All the extractions' advantages and disadvantages were evaluated, including ease of use, solvent usage, reproducibility, recovery, and time. A methanol/acetonitrile crash inside and outside of the Captiva EMR-Lipid cartridges was assessed, as well as a pre-extraction acetonitrile crash for the UCT cartridges. Instrument sensitivity was an issue during the project; therefore, the sample size, solvent amounts, and instrument sample preparation were adjusted. The final evaluation focused on the two 3 mL extraction cartridges. Using an LC/MS/MS, the percent recovery of spiked matrix-matched calibrators was calculated using the response for each of the 3 mL cartridge extractions in comparison to the NEAT delta-9-THC calibrators' responses. Based on the percent recovery, a final extraction procedure was decided. Destroyed blood samples from positive blood alcohol cases were screened for THC and metabolites using ELISA. Positive cannabinoid samples were extracted and quantitated for delta-9-THC using matrix-matched calibrators and controls concurrently extracted, evaluating sample extraction reproducibility. An Agilent 1260 Infinity II with an Agilent 6470 LC/TQ was used for all recovery studies.

### **LC/MS/MS Method Development for the Separation of Cannabinoids and Quantitation of delta-9-THC in Blood**

Ashley Tinervia, West Chester University, and Michaela Tapia, West Virginia University

In 2021, the State of New Jersey legalized the sale and consumption of cannabis products for recreational use. In February 2024, New Jersey 221st Legislature was proposed to implement a 3ng/mL per se law for  $\Delta$ 9-THC in blood. If the proposal is accepted, the New Jersey State Police Office of Forensic Sciences (NJSP OFS) will need to validate and institute a procedure to quantitate  $\Delta$ 9-THC in blood. Due to the possibility of a new DUI law, a research project was initiated to develop a quantitative  $\Delta$ 9-THC method in blood using an Agilent 1260 Infinity II with an Agilent 6470 LC/TQ for all method development. Ten cannabinoids:  $\Delta$ 9-THC,  $\Delta$ 8-THC,  $\Delta$ 10-THC,  $\Delta$ 9-Carboxy-THC, 11-OH- $\Delta$ 9-THC,  $\Delta$ 9-THC-d<sub>3</sub>,  $\Delta$ 9-Carboxy-THC-d<sub>9</sub>, 11-OH- $\Delta$ 9-THC-d<sub>3</sub>, Cannabinol (CBN), and Cannabidiol (CBD) were optimized, and included in the development of a dynamic multi-stage reaction monitoring (DMRM) method. During the project, many hurdles were overcome, and many parameters were optimized while pursuing an adequate method for quantitative measurement of  $\Delta$ 9-THC. Separation of the cannabinoids was achieved using a ZORBAX Bonus RP (2.1x50mm, 1.8 $\mu$ m) Rapid Resolution HD column, with mobile phase A: Water, 5mM Ammonium Formate, and 0.1% Formic Acid, and mobile phase B: Acetonitrile, 10% Methanol, 5mM Ammonium Formate, and 0.1% Formic Acid. The gradient program started at 35% A and 65%B and the flow rate was 0.5mL/min. Afterwards, the final method was used to evaluate  $\Delta$ 9-THC blood extraction methods for future work. Once an extraction method was determined, thirty-two positive THC samples were extracted in duplicate along with the eight matrix-matched calibrators and then analyzed using the LC/QQQ quantitative method. The thirty-two positive THC blood samples were tested using the enzyme linked immunoassay (ELISA) instrument, which has a positive threshold of 10ng/mL of THC for a sample to be considered positive. The quantitative method developed was used to report the amounts of  $\Delta$ 9-THC present in each blood sample. Delta-9-THC concentrations were reported in a range in ng/mL. Due to unforeseen power complications, sixteen of the samples in duplicate along with the eight calibrators sat overnight and crystallization occurred in the final eluent. The crystallization may have contributed to variable results that needs to be further investigated.

### **Drug Recognition Expert: Rebound Dilation Response of Intrinsically Photosensitive Retinal Ganglion Cells**

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**Hypothesis:** Marijuana impairs the dopamine dependent intrinsically photosensitive retinal ganglion cells (ipRGC). ipRGC are responsible for the sustained constriction of eye pupils. These cells respond to blue wavelength light stimulus at wavelengths between 415 and 470. Part of a Drug Recognition Expert evaluation (DRE) for marijuana includes the assessment for rebound eye dilation. The pupil when not impaired stays constricted with the input from ipRGC. With acute marijuana use the ipRGC are inhibited and there is a rebound dilation.

**Methods:** We undertook a review of three IRB approved research data sets using opportunistic dosing. These studies had non standardized light stimulus measures of the pupil response. The light stimulus included medical grade transillumination. We undertook a review of two case studies utilizing controlled wavelength light stimuli, standard medical, red, blue, and green wavelengths.

**Results:** Research results related to DRE evaluations show that less than 75% of evaluations identified rebound dilation of the pupils. An IRB approved protocol using a medical grade transilluminator found a lack of rebound dilation in 100% of twenty-seven volunteers. A commercial penlight identified rebound dilation in two of four volunteers. The use of medical transilluminator, commercial penlight or LED hobby light had a variety of results in thirty-seven volunteers. Our case study of two using controlled testing with four wavelength type illuminators showed a weak response of rebound dilation with both red and blue in normal room illumination and a stronger response of rebound dilation to blue in darkened environment.

**Conclusion:** Identification of rebound dilation with marijuana use is dependent on quality of the illumination output and wavelength. Studies under more rigorous controls are warranted.

### **2F-2oxo-PCE: A Mind-Altering NPS's Potential Role in a Double Suicide**

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2-fluoro-2-oxo-phenylcyclohexylethylamine (2F-2oxo-PCE, 2F-NENDCK) is a novel synthetic dissociative/hallucinogen that was first reported in early 2022. As characteristic of many novel psychoactive substances (NPS), little pharmacological information is available for 2F-2oxo-PCE.

However, it is structurally similar to ketamine and PCP and, like classic dissociatives and hallucinogens, believed to also produce similar psychological and physiological effects. For example, classic hallucinogens and dissociatives are known to cause mood, perception, and thought alterations, as well as feelings of paranoia, suicidal ideation, depersonalization, and derealization.

Here, the authors present a companion case where 2F-2oxo-PCE was confirmed in the blood of a 28 year old male and a 24 year old female. Both cases were ruled suicides with cause of death from intraoral gunshot wounds. Initial toxicology testing was performed at NMS Labs where the specimens were screened using high performance liquid chromatography/time of flight-mass spectrometry (LC/TOF-MS). The isomeric pair of 2F-2oxo-PCE and fluorexetamine, a separate NPS dissociative/hallucinogen, was detected using a surveillance library processed concurrently with expanded testing protocols. The submitting agency was contacted regarding these findings and further confirmation testing was subsequently performed at the Center for Forensic Science Research and Education (CFSRE) to determine the specific isomer. 2F-2oxo-PCE was confirmed at 720 ng/mL and 710 ng/mL in the two patient cases using liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS) and the method of standard addition. To date, these concentrations are among the highest reported from casework. Other findings detected in these cases included delta-9 THC (plus metabolites), psilocin, and bromazolam.

2F-2oxo-PCE likely was not a pharmacological reason for death in these cases. Nonetheless, as a dissociative/hallucinogen, 2F-2oxo-PCE may have negatively altered the decedents' sense of reality, perception, and judgement. Therefore, one might speculate that the presence of psychoactive substances played a role in the decedents' mental state prior to death. Several studies have evaluated the risk of suicide and mental disorders when dissociatives/hallucinogens are recreationally used. In the adolescent population, some research suggests a significant association between hallucinogen use and suicidal ideation. Findings in the adult population, on the other hand, are currently mixed. More research is needed given these mixed findings.

Toxicology testing is an integral part of suicide investigations, even if the means of death are not related to drug toxicity (i.e. suicides by firearm, hanging). This case study highlights the need to include NPS in toxicology testing protocols, as these drugs can play a role in suicides both by pharmacological means but also reality-altering effects. The pervasiveness and role of NPS is important for suicide prevention efforts—especially within the domain of public health. NPS should be included in the scope of toxicological testing to help inform treatment efforts and understanding the role of substance use in suicide.

## Rapid and Accurate Detection of Benzodiazepines in Urine: Calibration, Validation, and Real-World Application

Dr. Terry Bates, Bruker Scientific, Alex Maggitti, DrugScan Inc., and Francois Espourteille, Bruker Scientific

**Introduction:** Immunoassay (IA) detection is commonly used for initial drug screening in urine due to its speed and ease of automation. However, it has two major limitations: a high rate of false positives and negatives, and a slow turnaround time for new assay development. These limitations directly impact the accuracy of results and hinder the adaptability of assays to emerging novel psychoactive substances (NPS).

DART-MS measurement provides selective results, significantly reducing or eliminating false positives and negatives compared to IA-based screening, while also allowing for the rapid addition of new target analytes to assays. In this work, we report the development and validation of a rapid, chromatography-free screening approach for common benzodiazepines and their metabolites in urine, including: alprazolam (1), clonazepam (2), diazepam (3), flurazepam (4), lorazepam (5), oxazolam (6), temazepam (7), triazolam (8), alpha-hydroxy alprazolam (9), alpha-hydroxy midazolam (10), N-desalkylflurazepam (11), nordiazepam (12), 7-aminoflunitrazepam (13), and 7-aminoclonazepam (14). This DART-MS screening method successfully measures 1-14 in 96 samples, with a throughput of 23 seconds per sample.

**Methods:** For method development, triplicate calibration series were prepared by spiking synthetic urine with standards 1-14 (25-1000 ng/mL) and cutoff standards at 50 ng/mL using deuterated analogues as internal standards. Hydrolysis was performed at room temperature using Kura enzyme added to 150  $\mu$ L urine samples. After hydrolysis, 500  $\mu$ L 0.1 M Borax buffer (pH=10.4) and 2.5 mL 30:70 (ethyl acetate:n-chlorobutane) were added to each sample. Samples were centrifuged and the organic layer was transferred to glass vials and evaporated to dryness followed by reconstitution in 100  $\mu$ L MeOH. 5  $\mu$ L aliquots of reconstituted sample were transferred onto a QuickStrip HTS-96 screen and dried. For analysis, the screen was loaded onto the DART TQ-Plus (Bruker Daltonics) mass spectrometer for analysis. Interference, carryover, and ion-suppression studies were performed as described by ASB-036 Standard. Inter and Intraday precision was determined using matrix matched samples at the defined decision point (50 ng/mL). Results were validated against LC-MS that was performed using authentic urine samples (n=68) confirmed as positive for one or more analytes.

**Results and Discussion:** DART and TQ-plus parameters were optimized for selectivity, sensitivity, precision, and analysis time. Unique MS/MS transitions and collision energies were identified for 1-14 followed by optimization of MS scan time (10 ms), cone temperature (275°C), and collision cell pressure (1.5 mTorr). DART temperature and grid voltage were optimized to 300°C and 50 V, respectively. DART-MS analysis resulted in a good linear correlation of  $R^2 > 0.995$  and recoveries between 92 and 103% for all 14 analytes across a linear range of 25-1000 ng/mL. Inter- and intraday precision analyses demonstrated a %CV of  $< 20\%$  for all analytes at the decision point. The lower level of quantitation (LLOQ) was defined at 25 ng/mL. Cross-validation of the samples demonstrated good correlation with LC-MS data ( $R^2 > 0.90$ , RMSE=4.34 ng/mL\*,  $p < 0.05$ ) and a slope of near unity, indicating that this rapid chromatography-free workflow is sufficient in measuring 1-14 at or below the common cutoff values with improved accuracy compared to IA based screening approaches.

### Drug Trends: Then and Now

Jolene Bierly, NMS Labs

Forensic Science has experienced a variety of drug trends in the last fifty years. Some drugs exploded on the scene, were popular for a season, and disappeared like most novel psychoactive substances or synthetic cannabinoids. While older well-known drugs have experienced recent resurgences in popularity, much like the recycled clothing trends. The goal of this presentation is to provide information concerning drugs that were popular in previous decades and are becoming popular once again. Highlighted drugs will include methamphetamine, cocaine, and nicotine. Current trends, drug formulations, and common routes of administration will be addressed.

While both used medicinally, methamphetamine and cocaine quickly became popular drugs of abuse in the 1900's. Stimulant abuse persisted into the 2000's but was overshadowed by the opioid epidemic and the proliferation of designer drugs. In the current iteration of the opioid epidemic, methamphetamine and cocaine have experienced a resurgence in prevalence as these drugs are commonly mixed with fentanyl. Drug overdose deaths involving methamphetamine or

cocaine have reached their highest rates in the United States since 1999 (1). Stimulant prevalence is increasing in the driving population as well.

Tobacco products containing the active compound, nicotine, have been around for centuries. Historically, nicotine was believed to have medicinal properties that would treat ringworm, tetanus, and a variety of other ailments. The harmful physical effects and addictive nature of the drug have caused millions of deaths worldwide. Educational campaigns aimed at preventing or quitting tobacco use have produced long term decreases in cigarette smoking rates among youth and adults. However, electronic cigarettes have given nicotine a new appeal with a variety of new colors and flavors. Electronic cigarette use increased among adults and youth with the highest increases occurring among middle school students, a 667% increase from 2011 to 2022 (2).

1. <https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates>

2. <https://www.lung.org/research/trends-in-lung-disease/tobacco-trends-brief/overall-smoking-trends>

## **Supply Chain Impact of FDA’s Warnings on an Unregulated Drug: A Case Study on Tianeptine**

Kristopher Graf and Donna Papsun, NMS Labs

Tianeptine, a misused drug in the United States (US), was given the moniker “gas-station heroin” in media stories warning the public about its availability in commercial establishments and its opioid type effects at the high doses in these unregulated products. Tianeptine is a short-acting tricyclic anti-depressant as well as an atypical opioid agonist. It is prescribed as an antidepressant in Asia, Latin America, and Europe with a typical dose of 12.5 mg/day but is not approved for medical use in the US. Nonetheless, tianeptine has been sold commercially with product names including “Neptune’s Fix” and “ZaZa,”; these products are marketed under the guise of “dietary supplements” or “mood enhancers” and include labels such as “not for human consumption” to evade certain regulations and monitoring while containing supratherapeutic doses of tianeptine compared to those studied for medicinal purposes. Adverse effects reported after supratherapeutic doses of tianeptine have included agitation, drowsiness, confusion, rapid heartbeat, and decreased respiratory function that can lead to coma and death.

There have been multipronged efforts in order to combat the misuse and commercial availability of unregulated tianeptine products, included state scheduling. In November 2023, the US Food and Drug Administration (FDA) issued a public safety alert regarding a specific brand of tianeptine products, reminding consumers that tianeptine was not an approved drug and that the product may contain additional harmful ingredients. In addition, the FDA urged all retailers to stop selling tianeptine-related products. This led to voluntary recalls of the products by the manufacturers, likely disrupting the supply of tianeptine products to potential consumers and preventing further adverse events associated with the products.

To weigh the potential impact of the FDA’s letter as well as other scheduling initiatives, all forensic blood cases (postmortem and human performance) that reported tianeptine from a large forensic toxicology reference laboratory were queried.

Between 2018 and July 2024, tianeptine was reported in 144 blood specimens, with 87.5% reported prior to 2024. Nearly all submissions were for postmortem analysis. Blood concentrations ranged from 25 -190,000 ng/mL with approximately 72% having concentrations above 500 ng/mL. Samples were submitted from 36 states within the US. Mississippi reported approximately 14% of cases (n=21); however, all cases were reported before a July 2023 scheduling of tianeptine at the state level.

A decline in tianeptine positivity has been noted, which may be a culmination of various public health and safety efforts combating the misuse of tianeptine. At least nine states have scheduled tianeptine as a level I or II substance as of late 2023, which likely plays a role separate from the FDA alert and subsequent recall. State-wide scheduling, however, is an enforcement action, while the FDA warning is considered informal and advisory. The disruption of the supply of tianeptine products after the FDA letter should be considered a victory with the use of a unique tool combatting the spread of unregulated and mislabeled drugs that may pose significant harm to the public.

## **New York State Police's Evidential Oral Fluid Testing Pilot Program**

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**Introduction:** The use of oral fluid for drug testing is applicable in many sectors of forensic toxicology, including workplace drug testing, pain management, and impaired driving. It has many advantages for driving under the influence of drugs (DUID) investigations, but its widespread implementation in the United States has been hindered by the need for legislative action in many states, as well as a lack of laboratory resources available to develop and validate the testing methods.

In the United States, blood and urine are the specimens most commonly collected for DUID. In New York, there are numerous challenges to obtaining blood draws which lead to long delays in collection, or the inability to collect it, and the use of urine in DUID cases is discouraged. Since New York has had saliva in its impaired driving statute for decades, it provides a viable alternative.

**Objectives:** In September 2023, the New York State Police (NYSP) launched an evidential oral fluid pilot program to allow stakeholders to adapt to the alternate specimen type, identify any hurdles to broader implementation, and to collect blood and oral fluid correlation data. Once the pilot program was completed, the focus for the NYSP evidential oral fluid program is to collect blood and oral fluid in all serious injury and fatal motor vehicle investigations, and to have oral fluid be a stand-alone option for non-injury misdemeanor DUID investigations.

**Methods:** The Quantisal™ oral fluid kit was used for specimen collection. The oral fluid testing scheme meets or exceeds the National Safety Council's Alcohol, Drugs and Impairment Division's recommendations for Tier 1 drugs plus phencyclidine (PCP) and delta-8-tetrahydrocannabinol (THC). Screening for all compounds is accomplished using a liquid-liquid extraction followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Drug confirmation is accomplished using solid phase extraction (SPE) and LC-MS/MS. One confirmation targets delta-9-THC and delta-8-THC; the other method targets the remaining 30 drugs and metabolites.

Blood and urine drug testing also meets or exceeds the recommendations for Tier 1 drugs plus phencyclidine (PCP). The protocol screens for 14 drug/drug classes using enzyme linked immunosorbent assay (ELISA). Drug confirmations use SPE combined with various gas chromatography/mass spectrometry (GC-MS) and LC-MS/MS analyses.

During the pilot program, NYSP Drug Recognition Experts (DREs) were instructed to collect oral fluid in addition to blood in routine non-injury DUID investigations.

**Results:** The pilot program ran from September 2023 through May 2024, and the laboratory received 243 oral fluid samples; 200 blood-oral fluid pairs, 11 urine-oral fluid pairs, and 32 oral fluid only samples were submitted. Testing was completed for 238 oral fluid samples and the most detected drugs were delta-9-THC (55%), cocaine/benzoylcegonine (41%), fentanyl (32%), and methamphetamine/amphetamine (31%).

As of July 31, 2024, testing was completed for 196 blood-oral fluid pairs. There was an overall correlation rate of 97% in which at least one drug matched in both samples; 56% had an exact match for parent and/or metabolite; oral fluid detected additional drug(s) in 40% of pairs. The most common additional drugs detected in oral fluid included cocaine (22), 6-acetylmorphine (18), morphine (16), delta-9-THC (12), methamphetamine (11), amphetamine (10), and fentanyl (10).

**Discussion:** Blood-oral fluid paired samples showed an excellent overall correlation of 97%. Detection differences were primarily around cut-offs. Some of the drugs reportable only in the oral fluid did show elevated blood ELISA screens, but there was no trend noted related to concentration or collection times. This is likely due to drug presence below the ELISA blood assay cut-off.



Feedback from the DREs involved in the pilot program was very favorable. The process was fast and easy. Challenges noted were that the indicator did not turn blue, or the subject did not follow directions (chewed on pad, removed collector).

1 “Recommendations for the Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2021 Update,” A. D’Orazio, A. Mohr, A. Chan-Hosokawa, C. Harper, M. Huestis, J. Limoges, A. Miles, C. Scarneo, S. Kerrigan, L. Liddicoat, K. Scott, B. Logan, *J Anal Tox*, 2021, 45:529-536.



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# Trace Evidence/Fire Debris & Explosives Abstracts

## **“Discrimination of Blue Denim Fibers Utilizing Physical Characteristics, Optical Properties, Fluorescence Microscopy, Ultraviolet-Visible Microspectrophotometry, and Microchemical Testing”**

Elkin Howard, B.S., Mary Keehan, M.S., and Nicole Palmer, M.S., Virginia Department of Forensic Science, and Eric Hazelrigg, M.S., Virginia Commonwealth University

Cotton, the most common natural fiber, has numerous uses in the textile industry. This fiber type is ubiquitous in forensic casework due to its prevalence, particularly in blue jeans. The abundance of blue cotton in society and its limited variations in features can diminish the probative value of its presence on items of evidence. While a pair of blue jeans may be constructed with a combination of natural and manufactured fibers, the most likely fiber type to be transferred between sources is cotton. In this study, a comprehensive analytical approach to blue jean fiber analysis was explored to determine if blue cotton fibers from different blue jean sources could be reliably discriminated. The degree of differentiation was determined through the calculation of discrimination power for each individual technique, as well as additively at each step of the analytical scheme. Finally, the discrimination power of the analytical scheme as a whole was calculated. A combination of brightfield (BF) comparison microscopy, polarized light microscopy (PLM), fluorescence, ultraviolet-visible microspectrophotometry (UV-Vis MSP), and microchemical testing were employed to determine the fiber discrimination power achieved throughout the scheme. Examiner bias was mitigated by randomizing and renumbering all fabric samples prior to conducting examinations. The analytical procedures were assessed with two duplicated samples. Additionally, verifications of all sample discriminations using PLM were performed by a qualified practitioner. Given the variability in fabric dyeing, finishing processes, and deposits from wear and laundering, the goal of the project was to reassess the evidentiary value of blue jean fiber examinations to determine if a low significance currently assigned to common source comparisons is appropriate. After comparison of fibers from 64 pairs of jeans (including two duplicated pairs), the resulting independent discrimination powers for BF/PLM, fluorescence, UV-Vis MSP, and microchemical analysis were 48%, 48%, 9%, and 6%, respectively. The comprehensive fiber discrimination power achieved was 70%. Incorporating fabric examination as the first step in the scheme yielded a combined discrimination power of 99%. The independent fabric analysis discrimination power was 98%. These results indicate that blue jean cotton common source examinations do not possess the potential to be assigned a higher degree of association than previously held. However, if the known and questioned fabrics are also compared, then a higher degree of association should be considered despite being composed of blue cotton fibers.

Keywords: Fibers, Trace analysis, Blue cotton discrimination, Microscopy, Fluorescence, Ultraviolet-visible microspectrophotometry (UV-Vis MSP), Microchemical testing, Discrimination power, Common source comparison, Association level

## **“Enhancing Discrimination through Forensic Glass Coatings Analysis: Drawing Parallels with Forensic Paint Analysis”**

Katie Favale, Trace Evidence Unit, Michigan State Police Grand Rapids Laboratory, Forensics Science Division

Paint and glass fragments often found at crime scenes are pivotal in forensic investigations. Standard forensic paint analysis meticulously examines all layers of paint, allowing for high discrimination among samples. However, traditional forensic glass examinations often overlook additional layers on glass fragments, such as coatings applied for UV protection, tinting, or energy efficiency. These coatings possess unique chemical and physical characteristics that vary with their composition and application methods. Typically, forensic glass analysis focuses solely on the glass's physical and chemical properties, like refractive index and elemental composition. This research aimed to determine whether analyzing coatings on glass fragments could enhance the discriminatory power of forensic glass analysis.

A multi-faceted approach was employed to analyze coated glass fragments. Initially, visual examinations using alternative light sources and fluorescent microscopy were conducted to observe and characterize the coatings. This was followed by elemental analyses using scanning electron microscopy with energy-dispersive spectroscopy (SEM-EDS) and X-ray fluorescence spectroscopy (XRF) to determine the coatings' elemental composition.

The findings demonstrated that glass coatings could be effectively discriminated using SEM-EDS and XRF analyses.

These techniques provided detailed elemental profiles of the coatings, facilitating the differentiation between glass fragments. SEM-EDS analysis achieved a 97.78% discrimination rate between coatings, while XRF analysis achieved a 100% discrimination rate. These results suggest that incorporating the analysis of glass coatings in forensic examinations could enhance the ability to discriminate between glass samples, thereby improving the accuracy and reliability of forensic glass analysis.

This study underscores the potential of glass coating analysis in forensic science. Integrating glass coating analysis could expand the forensic toolkit by providing additional layers of information that improve discrimination and identification processes. By incorporating this technique, forensic scientists could achieve greater accuracy and reliability in glass examinations, offering a more comprehensive and precise approach to forensic analysis.

### **“Soil Discrimination by Particle Correlated Raman Spectroscopy (PCRS)”**

Drew Kuroda, B.S., Jasmine Kaur, M.S., Joshua Christensen., and Marisia Fikiet, PhD, University of New Haven, West Haven.; Peter de B. Harrington, Ph.D., Ohio University, Athens; Peter R. De Forest, D.Crim, Forensic Consultants, Ardsley, NY; and Brooke W. Kammrath, Ph.D., University of New Haven, West Haven

**Learning Overview:** After attending this presentation, attendees will learn about the discrimination capability of Particle Correlated Raman Spectroscopy (PCRS) for soil samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating the discriminating potential of Particle Correlated Raman Spectroscopy (PCRS) for the differentiation of soil samples using varied statistical approaches, including hypothesis testing and chemometric methods.

Forensic soil analysis has had a rich history of providing valuable information for investigating criminal events. However, many modern forensic laboratories have stopped performing this analysis due to the perception that it is either too time-consuming or labor-intensive. Further complicating the issue is the view that many forensic soil analytical methods are subjective due to their reliance on feature comparisons. Particle Correlated Raman Spectroscopy (PCRS) is a new method that may address both concerns by providing automated and objective analysis and comparison of sample mixtures. PCRS is an integrated technique that combines automated image analysis with Raman spectroscopy. Particle imaging determines particle size and shape distributions for each component in a sample, yielding detailed morphological information (e.g., circularity, area). Simultaneously, Raman spectroscopy can probe the molecular chemistry of specific particles of interest. In forensic soil analysis, PCRS can non-destructively identify the types of minerals present and provide morphological information about individual mineral grains. Particle size distributions can be generated for the entire sample or for each mineral present, along with quantitative information on the relative amount of each type of particle.

Mineral counts and morphological properties are used as the basis for classification and comparison of Raman-identified particles. The discrimination potential of PCRS was explored using various statistical methods from data collected from topsoil samples collected in triplicate from 30 different locations in the Northeast United States. First, error rates were investigated using traditional comparative statistics (i.e., hypothesis testing) with eight match criteria. The match criteria tested consisted of confidence intervals based on range, range +0.0005, mean +1 standard deviation, mean +2 standard deviations, t-test at 95% confidence, t-test at 99% confidence, mean +0.0001, and mean +0.0002 (Miller criterion). The particle size and other morphological property distribution comparisons were evaluated using a two-sample Kolmogorov–Smirnov test, due to its sensitivity to differences in both location and shape of the empirical cumulative distribution functions of the two samples. Multivariate statistical methods were explored for the discrimination of PCRS mineral data, which included creating and testing models with Partial Least Squares-Discriminant Analysis (PLS-DA), Support Vector Classification (SVC), and Hierarchical Cluster Analysis (HCA). Ultimately, this research provides statistical evidence for the discriminatory power of minerals and their morphologies for the classification and source identification of soil samples.

**Keywords:** PCRS, Raman Spectroscopy, Discrimination, Forensic Soil Analysis

## **“The Effects of Adhesives on the Subsequent Instrumental Analysis of Various Types of Trace Evidence”**

Ashlyn Bartley, Ted Schwartz, M.S., ABC-GKE, and Brooke W. Kammrath, Ph.D., University of New Haven, West Haven, and Colin Upton, Assistant Forensic Scientist, Westchester County Forensic Laboratory

Technicians and analysts at both crime scenes and in the lab are reliant on a variety of adhesives to accomplish proper collection and containment of evidence. During use, adhesives come into direct contact with evidence such as fibers and paint. This contact introduces a possible risk for either physical or chemical interference. As contamination can have consequences in a forensic case, any area of risk must be investigated with the utmost sincerity.

Codified into both NIST and SWGMAT standards is the suggestion that adhesives can alter trace evidence, specifically paint, during collection and subsequent storage. However, neither committee appears to have concerns regarding the use of adhesives during fiber or other trace evidence collection. Importantly, there is very little in literature that shows any chemical or physical interactions between trace evidence and adhesives.

The goal of this project is to investigate if adhesives have a chemical or physical impact on the trace evidence, and whether these differences can be observed during laboratory examination, specifically Fourier Transform Infrared Spectroscopy. In this study, six distinct adhesives were utilized to test for their potential chemical or physical impacts on trace evidence. To simulate common trace evidence three types of fiber evidence, as well as three different types of paint evidence were placed onto the adhesives. All adhesive and evidence combinations were stored at four different storage intervals, and then chemical and physical changes were documented by the IlluminatIR FTIR microscope.

Based on preliminary results from the current work, there is an indication that some physical damage can occur upon removal of the evidence, depending on the adhesive and trace material. Additionally, some adhesive-specimen combinations show physical transfer of the adhesive to the material. However, this does not necessarily compromise the FTIR analysis, since there are techniques that can be employed to avoid the transferred adhesive. Research also shows storage conditions have a significant impact specifically during use of white gelatin lifters.

## **“Investigating Odor Signatures of Electronic Devices”**

Samuel Friday, B.S., University of New Haven, West Haven; Jon Naples, Connecticut State Police, Emergency Services Unit – K9 Division, Meriden; Pauline E. Leary, Ph.D., Noble, Stevensville, MD; Marisia Fikiet, Ph.D., Alyssa Marsico, Ph.D., and Brooke W. Kammrath, Ph.D., University of New Haven, West Haven

Electronics are integral tools for many aspects of our everyday lives, and thus may contain valuable evidence for criminal investigations. Recent developments in canine detection have shown that dogs are capable of distinguishing characteristic odor signatures/profiles of electronic mass storage devices (MSDs) such as micro cell phones, USB drives, and SD cards with levels of accuracy identical to when they detect drugs, explosives, and people. However, the specific chemistry of said detection is unknown, since the sensitivity of a dog’s nose far exceeds that of modern instrumentation.

Current research on electronic device odors is minimal and contradictory. Foundational but unpublished research from 2012 by the Connecticut State Police, Emergency Services Unit - K9 Division, in collaboration with a Connecticut state forensic chemist, used direct thermal analysis gas chromatography-mass spectrometry (GC-MS) to analyze various MSDs. Triphenylphosphine oxide (TPPO), a flame retardant which coats all electronic printed circuit boards to prevent their overheating, was identified as a target odor to train canines. TPPO has since been used successfully as a training aid for the detection of MSDs, with canines successfully uncovering hundreds of hidden devices. While the dogs are initially trained on TPPO, this is only the first stage of training since odor signatures of MSDs are complex. As training continues, a selection of real samples of MSDs are used so the canines can recognize the full odor signatures for each type of device. In 2017, room temperature headspace analysis via solid phase microextraction (SPME) with GC-MS of electronic devices found several volatile compounds common to SIM and SD cards, as well as USB drives (1). The authors concluded that MSDs do have characteristic odor profiles, making detection with minimal false alerts feasible for trained canines. However, no TPPO was detected or identified in this study, contradicting prior research and the demonstrated of TPPO in canine detection training. The lack of TPPO detection in room temperature SPME/GC-MS analysis is feasible due to TPPO’s low volatility. However, because dogs have detection capabilities at least 103-105 times greater than our chemical instrumentation, the canines may be able to detect and use TPPO. This research aims

to resolve this question through an investigation of different GC-MS sampling methods of MSDs.

In the first part of this study, GC-MS sampling and method optimization for TPPO detection and identification was performed. Different GC-MS sampling methods were investigated, including room and high temperature headspace SPME with different fiber phases, direct heated headspace analysis, and direct thermal analysis, to evaluate their capabilities and limitations with respect to the detection and identification of TPPO. In part 2, various MSDs (e.g., RAM drives, SIM cards, USB flash drives) were analyzed using the optimized methods from Part 1 to investigate their odor signatures. The results of this research demonstrated that MSDs do have a characteristic odor profile, however the specific profile detected depends on the method of sampling the volatile organic components. This information is important for consideration when determining the components of training aids for the canine detection of MSDs.

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### **“Enhancements in mobile technology and strategies to triage firearm-related evidence at the scene for more efficient investigations”**

Leah Thomas, B.S., Roger Jefferys, MSFS, Kourtney Dalzell, M.S., Luis Arroyo, Ph.D., and Tatiana Trejos, Ph.D., West Virginia University, Department of Forensic and Investigative Science

Violent crimes are one of the many unfortunate outcomes of the gun violence epidemic that must be managed through improved efficiency in the criminal justice system. In the past decade, the US has seen record numbers of gun injuries, mass shootings, suicides, and murders. With limited operating budgets, the increased criminality affects turnaround times and backlogs. For example, according to Project FORESIGHT, turnaround times of gunshot residue (GSR) evidence have nearly tripled while the cost to complete one case has increased by 171% in the past decade. [1–7] To alleviate pressures on law enforcement and forensic laboratories, solutions to improve the cost, time, and resource efficiency when processing GSR casework are highly sought after.

One approach to more streamline processes is to integrate fast screening tools into the workflow. This method has proven effective in various fields like controlled substances, explosives, and DNA, but is currently lacking for GSR. Recently, our groups have developed mobile tools to assist in triaging evidence at the scene, enabling investigators to access more forensic-related intelligence in a fraction of the time. Mobile Laser-Induced Breakdown Spectroscopy (LIBS) technology has demonstrated high analytical reliability and efficiency in simulated GSR casework items. [8] With over 98% accuracy observed in diverse population datasets, the Mobile LIBS method requires no sample preparation and offers high-quality camera magnification capabilities, enabling single-particle targeted ablation with analysis times of under two minutes. [9] Analytical confidence has been established on hand samples of persons of interest for GSR analysis and other traces inadvertently collected along with the GSR, which can provide additional links to a firing event, such as target materials, bullets, and cartridge case residues. LIBS has also proven useful in quick identification of bullet holes, GSR in various substrates, and estimating shooting distances. [8] This screening tool offers a wide range of valuable information, including color, morphology, and elemental profiles of trace residues in under 5 minutes without the need for transportation to another site. To demonstrate its applicability, this study presents performance results from over 400 hand samples collected from shooters as well as over 100 samples collected from various surfaces (glass, fabrics, plywood, painted and unpainted drywall, concrete, and several automotive parts).

The integration of this screening tool fills a gap by assisting in prioritizing evidence collection at the scene. Triaging cases and evidence can significantly reduce the number of samples requiring costly confirmatory testing at the laboratory, saving direct costs of packaging, transportation, and analyst time. Furthermore, forensic-related information will be available earlier in the investigation for subsequent decision-making, thereby reducing unnecessary judicial expenses.

This study assesses the practicality of adopting new techniques, the increased probative value gained by utilizing these tools, and the extent of efficiency through a cost-benefit analysis. Practical hesitations regarding implementing these techniques can be described as a risk versus reward scenario: will the investment in new technology provide more

probative value to the case or improve casework efficiency? Within this presentation, the principal concerns by crime laboratories are discussed.

This research is part of a NIJ-funded grant project with award number: #15PNIJ-23-GG-04218-SLFO to West Virginia University: Strengthening Scientific Foundations for Advancing Best Practices in the Collection, Storage, Analysis, and Interpretation of Organic and Inorganic Gunshot Residue.

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#### **“A pilot study for the adoption of GSR screening techniques into forensic laboratories”**

Christopher George, M.S., New Jersey State Police Office of Forensic Sciences, Hamilton, and Kourtney Dalzell, M.S., West Virginia University, Department of Forensic and Investigative Science, Leah Thomas, B.S., Tatiana Trejos, Ph.D., and Luis Arroyo, Ph.D., West Virginia University, Department of Forensic and Investigative Science

The current state of gunshot residue (GSR) analysis relies on the reliable consensus-based confirmatory method, scanning electron microscopy energy dispersive spectrometry (SEM-EDS) for its superior detection of morphology and elemental composition of single inorganic GSR (pGSR) particles. However, research has surged in the past decade to assess the potential for complementary mobile and fast methods of analysis to benefit on-site safety, triage from crime scene to laboratory, and sample prioritization similar to screening tools used in forensic disciplines like seized drugs, DNA, and explosives. The capability for on-site GSR detection can support decision-making during evidence collection, preservation, and analysis and streamline the turnaround times in the criminal justice system. While techniques such as LIBS, electrochemistry, and Raman have shown great potential for GSR screening, there are often stalemates when the developed methods are transitioned from research to forensic laboratories. The adoption of new technology faces many challenges cited by the Forensic Technology Center of Excellence, including the lack of clear expectations from the research, interaction between researchers and end users, lack of feedback mechanisms, no standard approach for transitioning technology, and lack of awareness of successful implementation, which often leads to hesitation to be the first to adopt new technology. Finally, laboratories, often do not have the time, manpower, or resources to execute the technology transfer.

Our research group has investigated electrochemical detection (ECD) and laser-induced breakdown spectroscopy (LIBS) as a powerful duo for the detection of inorganic and organic gunshot residue both in laboratory and on-site settings. Studies have been performed to determine the capabilities and limitations of the method through large population studies, transfer and persistence, and preservation and storage studies. To move into the implementation step, through an NIJ-funded project, we have established a collaboration between West Virginia University and the New Jersey State Police Office of Forensic Sciences to initiate a pilot study to evaluate the adoption of novel screening methods. This presentation demonstrates how researchers can provide scientific basis, business metrics, and time and manpower to overcome the limited resources of forensic laboratories during the adoption process. Additionally, process mapping and economic metrics can be used prior to adoptions to estimate return on investment, assess risks, identify potential bottlenecks, and aid in sample prioritization. Finally, we illustrate success on mock and real casework samples using the screening technology and how it fits into the current analytical workflows using SEM-EDS. The pilot study's progress so far has shown how the collaboration of researchers and forensic laboratories can lead to an effective

adoption process with a mutual understanding of capabilities and limitations, shared time and resources, and feedback mechanisms.

### **“Investigating the Use of Amino Acid Ratios Obtained from GC-MS Analysis to Aid in the Differentiation of Hair from Individuals with Similar Hair Color”**

Alyssa Marisco, Ph.D., Forensic Science Department, University of New Haven, West Haven

Hair trace evidence is currently analyzed using microscopic hair comparison (MHC) and DNA analysis. Both nuclear and mitochondrial analysis can be done, but only nuclear DNA analysis can individualize samples. Traditional MHC is subjective, and DNA cannot be performed on inadequate samples. Recently, single nucleotide polymorphisms, which result in non-synonymous amino acid changes in the hairs protein sequences, are being explored as a potential alternative method for forensic hair analysis. Genetically variant peptides have been used in proteomic analysis for non-related individuals, but amino acids may provide a less complicated method of analysis. This method has been used for differentiating between plants, bee propolis and humans. In humans, amino acids have been shown to differentiate individuals based on their demographic and geographic characteristics. A study by Yaroshuk evaluated the discriminating power of amino acid ratios in mainly dark-haired individuals using gas chromatography-mass spectrometry (GC-MS). Expanding on this study, this research included alternative hair colors to further evaluate the use of amino acid ratios to differentiate between individuals using GC-MS analysis. This continued research aims to develop a method that can be used to supplement traditional MHC when DNA analysis cannot be conducted.

Samples were collected from 17 individuals. Microscopic hair images were obtained for RGB analysis in order to group individuals by similar hair color. Samples were divided into 4 RGB groupings. The peak areas obtained from GC-MS analysis were used to calculate amino acid ratios for comparison between individuals and, out of 45 possible amino acid ratios, 21 were used for comparison. Of those ratios, a total of 14 could be used to differentiate hair of similar colors. More specifically, 5 were determined to be highly variable between individuals with similar hair color and another 9 were variable between select individuals, but could still be used for differentiation. T-tests showed that the differences in amino acid ratios between the individuals with similar hair color were statistically significant.

### **“On the characterization and differentiation of clearcoats in automotive paint using UV-Vis microspectrophotometry, FTIR spectroscopy, and Raman spectroscopy”**

Swathi Murali, M.S., Morgan Carpenter, B.S., and Patrick Buzzini, Ph.D., Sam Houston State University, Houston, TX

Automotive paints are routinely examined in forensic laboratories in connection with hit-and-run accidents and traffic collisions. The forensic examination of paint begins with macroscopical and microscopical examinations followed by different instrumental analysis methods. Typical paint systems are multilayered and are mainly composed of a clearcoat, basecoat, primer, and primer surfacer. While a vast body of literature exists on the analysis of the colored basecoat layers, research on clearcoats has been limited. Clearcoats are the topmost protective layer of an automotive finish system and are most likely to be transferred during contact thereby establishing their forensic relevance. This layer is primarily composed of a binder or resin dispersed with additives such as light stabilizers and UV absorbers. While the binder is typically characterized using Fourier transform infrared (FTIR) spectroscopy and pyrolysis gas-chromatography-mass spectrometry (py-GC-MS), UV absorbers tend to absorb energy in the 240-400 nm wavelength range of the electromagnetic spectrum and hence can be studied using UV-Vis microspectrophotometry (UV-Vis MSP). The goal of this project was to compare the discriminating capabilities of UV-Vis MSP with those of FTIR spectroscopy and Raman spectroscopy in the analysis of clearcoat layers of automotive paint. Twenty-one automotive paint samples were examined using UV-Vis MSP, FTIR spectroscopy, and Raman spectroscopy. The sample set consisted of twenty-one automotive paint samples from various manufacturers including different models, colors, and years. The acquired data were qualitatively assessed to determine the chemical properties of clearcoats. Then, their discriminating powers were calculated. Based on UV-Vis MSP spectra, samples could be assigned to 8 groups resulting in a discriminating power of ~86%. In comparison, both FTIR spectroscopy and Raman spectroscopy could discriminate ~87% of the samples. Upon combining the three methods in sequence, a total discriminating power of ~99% was achieved. The use of UV-Vis MSP followed by FTIR yielded a discriminating power of ~98% while applying Raman spectroscopy in conjunction with UV-Vis MSP, a discriminating power of ~97% was obtained. For any technique and their joint use, no discernable grouping pattern was observed in terms of the make, model, color, and year of the paint sample. The



outcomes of this study highlight the importance of UV-Vis MSP in the analytical scheme for discrimination of automotive clearcoats. In combination with orthogonal techniques such as FTIR and/or Raman spectroscopy, discriminating capabilities increased substantially.



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# Criminalistics/Crime Scene & Digital Evidence Abstracts

## **Smokeless Powder Additive Profiles for Potential Brand Identification and Sample Discrimination**

Jack Hietpas, PhD., John Jay College of Criminal Justice Claire Harper, M.S., Oak Ridge Institute of Science and Education, Otyllia Vercelletto, M.S., Microtrace, LLC., Wayne Moorehead, ForensicTRACE, LLC . Oak Ridge Institute of Science and Education

Small arms propellants (SAP) are readily accessible and cost-effective materials that firearms enthusiasts can acquire for the legitimate assembly of ammunition. Unfortunately, the ease of access and low cost of these materials is advantageous for their utilization in the construction of improvised explosive devices (IEDs). Thus, there is a need to develop robust metrics for the characterization of propellants that are used as explosives as well as for comparisons between exemplar and recovered explosive residues. The goals of this presented research are to investigate GC/MS additive profiles of SAP for potential brand identification and sample discrimination. 204 one-pound canisters of SAP were purchased from local firearms stores and online dealers. The samples represent a wide selection of distributors (n=9) and brands (n=154). For GC/MS additive profiling, aliquots of ~30 mg for each sample were extracted with 5 mL of 3:1 methanol: n-butanol, following the method described by Reardon and MacCrehan (2001). Samples were analyzed using two GC instruments equipped with RTX-1 columns. Samples were prepared and analyzed in triplicate, thus approximately 600 samples were characterized in terms of their additive profiles.

In total, 14 different additives were identified in the samples analyzed. Each component was categorized on the basis of their peak area percentage of the whole: not detected (n.d.), trace (<1%), minor (1-10%), and major (>10%). This process of reducing the data to categorical values allowed better comparison of the data collected on the two instruments. Additionally, the data were also examined to look for additive profile trends associated with the SAP distributors. These trends will be presented and may provide caseworkers with a framework in which to interpret the strength of a comparison between questioned and known samples using additive profiles.

The goal of this work aims to provide a robust method for sample differentiation and comparison through the fusion of data from multiple analytical methods (i.e., micromorphometry and GC-MS). By combining propellant morphology and GC/MS additive profiles, a large number of unique combinations were recognized. From micromorphometry comparisons previously performed on this sample set, 32 SAP misclassifications occurred. These misclassified pairs were further assessed using GC/MS additive profiles. The results of this combined analysis show that 18 of the 32 could be differentiated using additive profiling.

## **Study of Casing Markings of Semi-Automatic Firearms Modified with a Glock Switch**

Nicole Sachman, M.S., Lawrence Quarino, PhD. Carol J. Ritter, M.S., Cedar Crest College Peter Diaczuk, PhD., John Jay College of Criminal Justice.

After attending this presentation, attendees will understand the difference between casing markings fired in the modified and unmodified fashion of semi-automatic firearms with the addition of a Glock switch. A Glock switch is an illegal modification that allows the firearm to alternate between semi-automatic and fully automatic fire.

This presentation will impact the forensic science community by bringing awareness to the increased use of Glock switches on semi-automatic firearms and its potential effect on firearms examinations.

This report will present research completed involving three modified firearms. The firearms used included two Glock 17's and one Glock 19. Each firearm fired three magazines (17 cartridges per magazine): one in the semi-automatic mode and two in the fully automatic mode. Standard manual examination of the 153 casings in the study was performed using a LEEDS comparison microscope at 11x magnification. Side by side comparisons were made of semi-automatic casings and fully automatic casings fired from the same firearm to compare striation patterns on the breech face. No discernible differences were found in the striation patterns fired from the same firearm with and without the Glock switch. Once manual comparison on casings was completed, the LEEDS comparison microscope was used in conjunction with the SPOT Imaging Solutions application to evaluate firing pin drag mark length, ejector marks, and drag mark width on each casing at 22x magnification. The increased magnification was needed to ensure sufficient detail. Statistical analysis was completed on measurements using a two-tail t-test for two dependent means at 95% confidence to compare markings made in the semi-automatic mode with markings made in fully automatic

mode on casings from the same firearm.

Unlike the comparison of striations, results of the two-tail t-tests from this portion of the study showed that in two firearms, the drag mark length was significantly different between the semi-automatic and fully automatic casings fired from the same firearm. Furthermore, in one firearm involved in this portion of the study, the ejector mark location was significantly different between the two modes.

Although the chance of an incorrect conclusion reached by a trained firearms examiner is likely rare, the results of this study indicate that firearm examiners must nonetheless be aware that markings may change on casings due to the use of a Glock switch.

**Keywords:** Firearm Examination, Modified Firearms, Glock Switch

### **The Missing Puzzle Piece: A Single DNA Match**

Ashley Bonomi, B.S., Tobi Kirschmann, Siena College

Forensic investigative genetic genealogy (FIGG) employs familial DNA to identify unknown DNA samples. These DNA samples are from homicide, rape, and unidentified remain cases that were not identified through the CODIS database. This technique also aids in resolving cases of unknown parentage such as adoptions and unknown affairs allowing for individuals to know their heritage.

An example of this is the case of DET, a man who was raised by his biological mother that passed away without revealing the identity of his father. To identify a potential father, a list of familial DNA matches and an overview of DET's ethnicity were produced through a SNP analysis of the entire genome. The results showed that the father's ethnicity is about half Irish and half Armenian. Nearly all the DNA matches were of Irish heritage and connected to the surname of Lovelace. This presented an important question, where was the Armenian half of the father's heritage?

The solution was a single DNA match, with the surname of Keurjian, with 135 centimorgans which represents a possible second cousin once removed relationship to DET. This match was not related to any of the Irish Lovelace relatives within the constructed family tree suggesting that she represented part of the missing Armenian half. Through record searching, including census and immigration records from the Armenian region as well as the identification of a short-lived marriage between a Lovelace and Keurjian. The product of this marriage was the birth of a male Lovelace who had the correct age, location, and ethnicity as the prediction of the unknown father which confirmed that this person was in fact the father. This result would not have been achieved without the Keurjian DNA match, showing the significance of a single DNA match with FIGG.

### **Enhancing Forensic Lab Readiness: Aligning Education with Practical Laboratory Needs**

Peter Valentin, PhD., University of New Haven

While forensic science programs and forensic service providers have their respective accreditation bodies, it has become increasingly clear that accreditation often fulfills objectives beyond simply enhancing outcomes. This disconnect between expectations and results is particularly troubling for forensic science programs, laboratory supervisors, and hiring professionals because institutions adhering to FEPAC standards may feel confident that they are meeting the benchmarks to prepare graduates for careers. However, there is growing acknowledgment that more can be done, and many of you who are involved in onboarding have likely noticed shifts in the level of preparedness among new hires.

This talk seeks to foster a discussion on how we can collaborate more effectively to develop insights into the specific technical and soft skills that must be prioritized so graduates can succeed in their first career positions.

For many of us, this information is shared through personal relationships and our own experiences. However, a consensus document developed with laboratory personnel would guide programs in making informed curriculum adjustments that better align with industry needs.

(A moderated discussion will follow.)

## **2D examination of natural aging and depletion processes of latent fingerprints**

Alexzandra Buscarello, M.S., Josep De Alcaraz-Fossaul, PhD., University of New Haven

As fingerprints age, their three-dimensional ridge structures undergo time-dependent topographical changes, making it difficult to accurately estimate their time-since-deposition. Traditional methods for visualizing and assessing these topographical changes often involve the application of powders with brushes, such as titanium dioxide, which can be semi-destructive due to the need for direct contact between the applicator and the fingerprint. Black magnetic powder, which adheres to sweat secretions without direct contact, offers a non-destructive alternative for nonporous surfaces, though it still risks over-development and potential damage. Recent studies have focused on aging patterns, including the loss of color contrast between ridges and furrows after development and image processing. However, standardized optical methods for determining the natural age of latent fingerprints, alongside the chronological sequence of post-deposition depletions, have not yet been established. These two temporal mechanisms may play distinct roles in fingerprint aging, highlighting the need to simultaneously investigate the topographical changes that occur during each process for determining the timing of a crime while identifying suspects.

This experiment aims to evaluate the physical changes of 1,440 fingerprints developed in a "cassette" container with black magnetic powder at four specific time points: days 0, 5, 13, and 27 post-deposition. Sebaceous-rich fingerprints from the index finger and thumb of the dominant hand were collected in triplicate from four healthy adult males, with 15 depletions at each time point. The samples were deposited on glass microscope slides, stored in complete darkness, and examined under controlled environmental and lighting conditions. The 2D examination provided morphometric data, including levels of color contrast between ridges and furrows (CC), color-coded maps of ridge clarity (BG), and fingerprint visual quality scores (QS). Further, the CC metric represents the ratio of the average color (mean intensity, MI) and the spread of color intensities (intensity amplitude, IA). For the BG metric, areas of high ridge clarity are indicated by blue and green color-coded areas, while red and yellow color-coded areas indicate low ridge clarity. It is hypothesized that 2D time-dependent changes in ridges occurred due to a combination of natural aging and depletion processes. However, it is expected that depletion data will reveal more minute changes over time.

Univariate and multivariate ANOVA analyses for each 2D metric were conducted with IBM® SPSS® Statistics 25. Principal Component Analysis (PCA) identified correlations between variables (CC, BG, QS) and reduced dataset dimensionality. Preliminary regression analysis of the depletion mechanism revealed similar patterns among donors. A positive linear relationship was observed for the CC metric, and the BG metric data revealed a linear decrease over 27 days. Regarding the natural aging mechanism, preliminary regression analysis indicated that the CC metric follows a second-order polynomial model, with an initial steady increase in the MI/IA ratio. A negative linear trend was observed for the BG metric. This preliminary comparison of aging mechanisms suggests that while there are differences in the topographical changes of fingerprints over time between the two temporal mechanisms, similar trends were observed across all donors.

## **Solving a Crime in Reverse: IGG Provides a New Path to Discovering Perpetrators**

Tobi Kirschmann, M.S., Siena College Nora Cheek, Kevin Sullivan, and Don Carola of DNA Finders, Inc.

Investigative Genetic Genealogy (IGG) appeared first in 2018 with the arrest of Joseph James DeAngelo, aka The Golden State Killer, who was sentenced to life in prison for multiple burglaries, rapes, and murders that spanned four decades in the state of California. Now IGG is becoming a tool used nationwide by law enforcement agencies and medical examiners to identify perpetrators and human remains. As IGG becomes a common tool for human identification, we are encountering new ways of looking at old cases. One such topic is the discovery of crimes that likely occurred in the past and are only confirmed now with DNA technology and IGG. These new discoveries question standard Criminal Justice views of posthumous prosecution and the statute of limitations.

## **Communicating Forensic Findings: Are we making complex Science Accessible for Recipients?**

Sandra Koch, Ph.D., National Institute of Standards and Technology- NIST

This presentation will provide a review of the discussions heard during a 2-day NIST-hosted workshop on Communicating Forensic Findings: Current Practices and Future Directions (<https://www.nist.gov/news-events/events/2024/06/communicating-forensic-findings-current-practices-and-future-directions> ). The goal for presenting this information at NEAFS is to broaden NIST's engagement with forensic practitioners and to foster greater dialog with the forensic science community on how to improve the communication of forensic findings. The June workshop presentations and discussions focused on: 1) how forensic findings are currently expressed in reports and testimony, 2) the use of likelihood ratios and verbal scales among a subset of forensic disciplines, 3) the strengths, limitation, and clarity of these approaches to communicate the weight of forensic evidence, 4) potential gaps in research or base knowledge that may limit an end user's ability to use or understand the findings, and 5) what the forensic community can do to improve communication and increase recipient understanding of forensic findings. NIST is looking to engage the broader forensic community in discussions on the potential for evaluative reporting to be adopted in the US. Through this outreach we hope to gain input from the NEAFS and ASTEE membership on how the forensic science community can improve the clarity of forensic findings in reports and testimony as well as explanations of limitations to interpretations. NIST plans to use the information and insights gained from the June workshop and outreach at forensic meetings to inform an upcoming Foundation Review.

## **The Current State of the Forensic Science Standards Landscape in the United States**

Vincent Desiderio, National Institute of Standards and Technology –NIST

From the classroom to the crime scene, through the laboratory to the courtroom, forensic standards play an increasingly important role in the improvement and harmonization of forensic practice. Although an often difficult and time-consuming undertaking, the development, publication, and implementation of consensus built standards have many benefits including:

- Ensuring a minimum level of practice with respect to the application and use of forensic methods,
- Ensuring consistent practice within and between jurisdictions,
- Gradually raising the level of practice through refinement and continual improvement over time,
- Building trust between stakeholders and the general public,
- Helping to identify areas of need and obtaining resources to meet those needs.

The Organization of Scientific Area Committees (OSAC) for Forensic Science, in conjunction with their critical standards development organization partners have been working diligently to develop and improve forensic science standards covering a wide range of interdisciplinary topics and meet discipline specific needs. Over the past few years, OSAC has also increased its efforts to measure the impact of forensic standards. Through its annual Open Enrollment initiative, OSAC has gathered a significant amount of data to determine the extent of standards implementation across the forensic sciences and better direct resources to areas of need.

This presentation will provide a brief overview of the forensic science standards development landscape and highlight the ongoing efforts to develop new standards, improve existing standards, and measure the impact of standards through implementation related initiatives. In order to gain broader awareness and participation, and further encourage implementation, various available resources will be discussed and an analysis of the aforementioned implementation related data will be provided.

## **Development of a Mass Spectrometry-Based Species Identification Prediction Model for Entomological Samples for Postmortem Interval Estimation**

Alexa Figueroa, Louisiana State University, Jennifer Y. Rosati, Ph.D., John Jay College of Criminal Justice, Rabi A. Musah, Ph.D., Louisiana State University

Forensic entomology leverages the patterns associated with insect colonization of remains to estimate time since

death, or postmortem interval (PMI), which is a crucial aspect of death investigations. Blow flies (Family: Calliphoridae), are early colonizers of corpses which can facilitate PMI estimation through a "back-calculation" method. This approach correlates insect species identity, environmental conditions, decomposition stage, and species-specific life cycles to determine time since egg-laying based on the assumption that the eggs were laid within a time period shortly after the death occurred. Accurate PMI estimation hinges on precise species identification, as developmental rates vary significantly among blow fly species as a function of temperature and weather conditions. Additionally, retrieved entomological evidence can reveal where the death occurred by indicating whether the collected specimens are insect species endemic to the area in which the remains were found. As a result, accurate knowledge of the insect species present is essential.

Traditional methods of insect identification based on morphological characterization are time-consuming and prone to errors, especially for immature life stages (i.e., eggs, larvae and pupae) due to their visual similarity across multiple species. Thus, if the specimens are viable, an experienced entomologist may rear them to adulthood to assess species identification based on the visually apparent gross morphological features of the emergent adults.

Reported here is an alternative novel approach that mitigates the aforementioned issues through the utilization of mass spectral analyses and chemometrics for rapid blow fly egg species identification, which can be used to facilitate estimation of PMI. By analyzing 70% aqueous ethanol suspensions of egg specimens from multiple species representing the genera *Calliphora*, *Chrysomya*, and *Lucilia* using direct analysis in real time — high-resolution mass spectrometry (DART-HRMS), unique mass spectral profiles were obtained for each species. Application of Kernel Discriminant Analyses (KDA) to the MS profiles of each genus successfully clustered each profile, demonstrating the potential for genus identification with accuracies of 88.75%, 95%, and 92.50% respectively. Moreover, application of KDA to the mass spectral data acquired from egg specimens of *Cynomya cadaverina* from 3 regions (Louisiana, USA; Manhattan, USA; and Sauble Falls, Canada) showed the potential to determine geographic provenance for samples of the same species from different regions with an accuracy of 95.56%. Future research will focus on developing a database of the species-specific metabolome profile signatures for species identification of entomological evidence that will increase the evidentiary value of juvenile life stages in the determination of PMI, and enable assessment of whether the remains were moved from one location to another.

### **380 ACP Caliber Cartridge Fired from a 9 mm Luger Caliber Pistol**

Peter Diaczuk, Ph.D., John Jay College of Criminal Justice

This research was inspired by a habeas case where the accused was found guilty of homicide. Two individuals were shooting at a third. One of the shooters had a firearm chambered in .380 ACP and the other had a firearm chambered in 9mm Luger. The fellow with the .380 ACP firearm was convicted. The victim sustained a penetrating bullet wound that hit bone, mangling the bullet so extensively that it had no value to compare with test fires from the firearm. The deformed bullet had the weight and size characteristics of a .380 ACP bullet. Several spent cases were scattered around the crime scene, consisting of both .380 ACP and 9mm Luger, suggesting that both men had discharged their firearms. The fellow with the .380 ACP firearm insisted that he purposely shot over the head of the victim so as not to hit him. His claim was that the other fellow, who was sporting the 9mm Luger caliber pistol, chambered and fired a .380 ACP cartridge that struck the victim, killing him. Unfortunately, the firearm examiner testified at trial that it is impossible to fire a .380 ACP cartridge from a firearm chambered for 9mm Luger. This is blatantly false. The goal of this research was not only to show that a .380 ACP cartridge could be fired from a 9mm Luger firearm, which is no secret, but whether it is possible to determine if a .380 ACP case was discharged from a 9mm Luger firearm. Many, but not all, .380 ACP caliber firearms use a simple blowback principle to operate (as did the firearm used by the accused), whereas many, but not all, 9mm Luger caliber firearms use a locked breech principle to operate (as did the firearm used by the co-defendant). Since the locked breech principle in the firearm involved always resulted in a firing pin drag mark, the initial research involved looking for a drag mark on .380 ACP cases fired from it. This quest failed. The reason was revealed by high-speed video. However, rather unexpectedly, a distinct difference was present in .380 ACP cartridges fired from a 9mm Luger pistol.

## **A Study of Nine Millimeter Parabellum Bullet Ricochet from Common Substrates**

Peter Diaczuk, Ph.D., John Jay College of Criminal Justice

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





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# Drug Chemistry Abstracts

## **Mass Spectral Analysis of Used Test Strips for Multilayer Drug Identification**

Dr. Meghan Appley, Elise Pyfrom, Edward Sisco, National Institute of Standards and Technology

With the constantly evolving drug landscape and near all-time high fatal and nonfatal drug overdoses, there remains a need for rapid drug analysis techniques. These analysis techniques can either be done onsite by law enforcement using lateral flow immunoassays (i.e., test strips) or in a laboratory setting using mass spectral techniques. Each of these techniques present their own sets of challenges including high specificity and lengthy turnaround times. To address these limitations, it was proposed that compounds could be extracted from used test strips for post-collection analyses using laboratory-based techniques. This proposed method would allow for multilayer drug identification through rapid onsite analysis followed by comprehensive laboratory analyses.

There were four components to the method development. The first was to determine the optimal extraction method using a design of experiment approach. The optimal extraction method was to extract a cut test strip using 0.5 mL methanol while vortexing for 10 sec. The next component was to determine the feasibility of using direct analysis in real time – mass spectrometry (DART-MS) to analyze the used test strip extracts. This involved the successful identification of compounds of interest using existing protocols and identifying potential false positive identifications from the chemical background produced from the extracted test strip. The third component was to identify the limits of detection for a range of compounds. The limit of detections ranged from 0.5 % to 5 % by weight in a mixture. The final component was to evaluate the extraction approach using authentic samples. Used test strips were submitted by several harm reduction sites along with authentic drug collection samples (drug product or trace residue). The concordance between test strip results and used test strip extracts was 96%. The compound identification agreement between used test strips and authentic drug collection samples was ~80%. The results were similar for different test strip types and preparation methods.

To keep up with the changing drug landscape, there is a need for rapid, comprehensive drug analyses. This work demonstrates that low-barrier test strips can be analyzed using mass spectral techniques to provide comprehensive chemical information. This information can then be used by law enforcement officials to gain added insight into the drug landscape.

## **GCMS Low Energy Ionization to Determine Structural Information on Fentanyl and Nitazene Analogs**

Alexis Willey, Agilent Technologies

This work compiles low energy ionization spectra of fentanyl and nitazene analogs to assist in the identification of isobaric spectra generated under 70 eV ionization energy. Low energy ionization spectra of nitazene fentanyl analog standards were generated at 10, 12, 15, and 17 eV ionization energies and optimized for the formation of a molecular ion, molecular ion abundance, and high spectral fidelity (isotopic ratios) of the molecular ion patterns. The spectral patterns were then compared to street drug case samples and identified nitazene analogs and other controlled substances based on the creation of their respective molecular ion and isotopic patterns. The data illustrates the power and ability of the Agilent 7250 QTOF to produce the molecular ions of difficult to identify drugs due to similar or isobaric spectra that do not produce characteristic spectral fragmentation and or a molecular ion under conventional ionization energies of 70 eV.

## **Differentiation of Cocaine Salt and Cocaine Base Using PY-GC/MS**

Dr. Heather Harris, Dominic Bierwisch, Arcadia University; Chuck Ahrens, CDS Analytical

Abstract: This project is intended to develop and validate a non-standard method for the differentiation of cocaine salt and cocaine base using GC/MS with a pre-injection pyrolysis separation. The intended outcome of this project is a qualitative method capable of confirming the identification of the specific form of cocaine in a solid sample. The base form of cocaine will volatilize at a significantly lower temperature than the salt form. The pyroprobe sampling apparatus allows for the exploitation of this temperature difference to volatilize the specific form of cocaine prior to injection on the GC/MS. Thus, the purpose of this project is to validate the use of the pyroprobe as a separation sampling apparatus and to demonstrate its capabilities to provide additional sample information to an existing GC/MS method.

## **Drug Chemistry and Prosecution of Crimes**

Rick McKelvey, Atlantic County (NJ) Prosecutor's Office

Abstract: I am the Executive Assistant Atlantic County Prosecutor and here to speak as part of the Scientific Session on Drug Chemistry on Wednesday, October 23. My presentation will focus on the role of the prosecutor concerning drug chemistry in the criminal justice system.

I plan to speak for approximately 15 minutes, beginning with an introduction to crimes related to controlled dangerous substances, such as possession offenses, distribution offenses, drug-induced deaths, manufacturing facilities, and leaders of trafficking organizations. I will also address the use of drug chemistry in investigations and prosecutions, covering interactions with laboratories, discovery requests, and circumstances requiring expedited responses.

Additionally, I will discuss the testimony of chemists and relevant Rules of Evidence, including subpoenas, witness preparation, expert testimony, and certification or hearsay exceptions. I will touch on common issues such as court scheduling, chain of custody, mixtures, and speedy trial or detention concerns.

While my experience is primarily based on New Jersey criminal prosecutions, I will strive to make the presentation relevant to practitioners nationwide. There will also be a Q&A session at the end.

## **What is Marihuana? - A Validation of an HPLC Method for Hemp versus Marijuana Differentiation**

Peter Murphy, New Jersey State Police - OFS - South Regional Lab

The Agriculture Improvement Act of 2018 was signed into law on December 20, 2018. Also known as the Farm Bill, this act de-scheduled some cannabis products from the Controlled Substances Act. Hemp legalization is restricted to plants with low levels of delta-9-THC. Hemp is defined as cannabis with less than 0.3% THC. My presentation will provide insight to the validation process of a straightforward method to analyze Marijuana and Marijuana products and to determine the concentration of Total THC in order to differentiate Hemp from Marijuana.

## **Training and Validation of an Electronic Index for the Comparison of Street-Level Fentanyl Samples**

Dr. Joshua DeBord, Barry K Logan, PhD, F-ABFT, Alex J Krotulski, PhD, Center for Forensic Science Research and Education

The ability to accurately assess the composition and variability of fentanyl in local and regional drug markets is critical for public health and forensic investigations. In this study, we utilized a customized data processing algorithm in conjunction with quantitative chemical analysis to perform pairwise comparisons of fentanyl samples collected from the Philadelphia area in 2021 and 2022. The algorithm streamlined the profiling, enabling rapid comparison of sample compositions based on defined metrics of equivalence, including standard deviation and absolute deviations from the mean.

Authentic samples of street-level fentanyl were used to train the algorithm and establish the metrics of equivalence, based upon the quantitative analysis of fentanyl and other commonly observed substances such as 4-ANPP, xylazine, lidocaine, and caffeine. The training sample set was comprised of two bundles of street fentanyl obtained with a high probability of association within each group as well as discrimination between groups, both of which were confirmed by the analytical results. The validation set of samples consisted of over 200 authentic fentanyl samples, with test groups defined by their packaging and branded markings. The automated processing algorithm played a crucial role in identifying, associating, and discriminating between samples within test groups. In one case, two test groups with different packaging stamps showed close quantitative similarity, with fentanyl concentrations of  $10.29 \pm 0.25\%$  and  $10.15 \pm 0.31\%$ , respectively, as well as consistency in all other analytes. Conversely, another group of identically branded samples indicated the presence of two very different sub-groups, one with mean fentanyl concentrations of 2.71% and the other 17.57%, highlighting the elevated risk posed by inconsistent drug composition.

The custom algorithm automated the indexing of chemical profiles, significantly improving the efficiency of pairwise comparisons between samples. By enabling rapid, systematic comparisons, the data processing method facilitated the

identification of health-critical differences among similarly branded drug products. This approach advances traditional forensic drug analysis by extending its scope from mere identification to detailed quantitative assessments, which can be used to inform public health initiatives and aid forensic investigations by linking or distinguishing drug batches based on chemical composition. The results of the validation demonstrate the utility of automated data processing in uncovering trends in drug adulteration and distribution. Integrating applied algorithms into forensic drug profiling enhances the ability to detect variability in drug composition, ultimately aiding efforts to mitigate overdose risks and disrupt illicit drug distribution networks.

### **Improving Liquid Chromatography-Mass Spectrometry Sensitivity for Barbiturates**

Ethan Khandaker, Pennsylvania State University

The goal of this project is to increase the sensitivity of barbiturates in both negative and positive ion mode. The specific drugs used were barbital, phenobarbital, butalbital, butabarbital, pentobarbital, amobarbital, and secobarbital. The first step of this project involved performing infusions on each of the drugs to determine ideal mass spectrometer parameters. Despite their structural similarities, each barbiturate had varying optimal values for vaporizer temperature, spray voltage, and capillary temperature. A median value was selected to provide satisfactory results for all drugs. Ideal values for the S-lens, collision energy, and product ion were also obtained for each drug. With optimal mass spectrometry conditions in place, a gradient of 20% to 35% ACN over 8 minutes was used to achieve baseline separation. Mobile phase A was chosen to be a 2.5 mm ammonium acetate buffer to enhance the sensitivity of barbiturates. Amobarbital and pentobarbital have identical masses of 226.27 g/mol and very similar structures, causing them to co-elute. Since the focus of this project is sensitivity, amobarbital was excluded from the panel when generating standard curves, though it was still run on each column to assess the impact of column geometry and stationary phase on separation.

Several columns with varying stationary phases, lengths, silica particles, and internal diameters were tested. While column length did not significantly affect sensitivity, it did enhance selectivity between the co-eluting peaks. Superficially porous silica particles significantly improved sensitivity and selectivity compared to fully porous silica particles. Reducing the internal diameter from 2.1 mm to 1.5 mm provided a near two-fold sensitivity enhancement and provided near-baseline separation of the co-eluting peaks. Comparisons were primarily made using C18 reverse-phase material, but a biphenyl phase and a high pH phase were also tested. The biphenyl phase reduced sensitivity and selectivity between the co-eluting peaks compared to the C18. Adjusting the mobile phase pH from 4.22 to 9.52 using ammonium hydroxide was expected to enhance ionization and sensitivity but instead halved the sensitivity on the high pH phase column. While these tests were conducted in negative ion mode, positive ion mode might yield better results, achievable via derivatization; an experiment using tetramethylammonium hydroxide as the derivatizing agent is planned for the near future.

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

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

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

This presentation will provide attendees with an update on SWGDRUG activities during the year 2023 and currently in 2024. Recent publications include revisions to the SWGDRUG Recommendations Parts II: Education, Training, and Continuing Professional Development, IIIA: Methods of Analysis/Sampling Seized Drugs for Qualitative Analysis, IVB: Quality Assurance/Validation of Analytical Methods and Annex A: SWGDRUG Glossary of Terms

and Definitions as well as Supplemental Document SD-7: Construction of an Analytical Scheme. Recent activities include revising Supplemental Document SD-2: Validation of Analytical Methods and Supplemental Document SD-6: Uncertainty Examples of Measurement Uncertainty for Extrapolations of Net Weight and Unit Count. Subcommittees have also been devoted to training and outreach and developing new recommendations for structural elucidation and cease analysis procedures. Lastly, the current state of SWGDRUG as well as future initiatives will be reviewed.

### **Synthetic Cannabinoid Structure Classification using the Bridge Carbonyl Frequency in Vapor Phase**

Lewis Smith, Cape May County Forensic Laboratory

Forensic examination for the majority of seized drug samples involves a sample dilution followed by Gas Chromatography due to the limited quantity and/or impurity of the evidence. Over the years, the “gold label” standard of analyzing the gas effluents from a GC column was by Electron Impact Quadrupole Mass Spectrometry (EIMS). In some cases, the mass spectra produced from this technique results in either no discernable Molecular Ion identity and/or poor fragmentation yielding few ions of significant abundance. For these special circumstances, coupling Gas Chromatography with Fourier Transform Infrared Spectrometry (GC/FTIR) provides an alternative means of identification.

Synthetic Cannabinoids have become the largest class of designer drugs encountered in forensic chemistry. With the absence of intermolecular hydrogen bonding, Infrared analysis of these molecules in the vapor phase has made it possible to identify every single carbonyl band present. A relationship was discovered within this band cluster that could systematically organize Synthetic Cannabinoids into 64 categories. An overview of this classification system will be given and its applications as well as the unique properties of Infrared vapor phase itself which made this all possible.

### **The Concerns of Using Chloroform for the Analysis of Seized Drug Evidence**

Joshua Rosenthal, New York City Police Department Time

Chloroform is a common solvent that is used for the analysis of Seized Drug evidence using Gas Chromatography/Mass Spectrometry. Chloroform naturally breaks down into dichlorocarbene, which reacts with oxygen to form phosgene, a toxic gas. Chloroform manufacturers add a stabilizer (typically ethanol) to the chloroform to prevent the buildup of phosgene. Ethanol will react with the phosgene to form ethylchloroformate (ECF) intermediate, which reacts further with ethanol to form diethylcarbonate.

If enough ECF is present in the chloroform, it has the possibility to react with seized drug evidence that have a free hydrogen. The mechanism is driven by removing chlorine atoms from phosgene and its resultants downstream, forming hydrochloric acid every time this occurs. This makes the reaction occur faster in basic environments, because the basic environment can better accept the HCl. Primary and secondary amines have a free hydrogen, making them susceptible to reacting with ECF to form an ethyl carbamate. Because tertiary amines do not have a free hydrogen, they are not known to react. Additionally, some alcohol functional groups could react with ECF to form an ethyl carbonate.

Common primary and secondary amines observed in seized drug casework include 4-anilino-N-phenethylpiperidine (4-ANPP) and substituted phenethylamines such as amphetamine, methamphetamine, and MDMA. At the New York City Police Department Police Laboratory, these substances are typically prepared in a chloroform basic extraction using distilled water and sodium hydroxide. In chloroform with an excess of ECF, these substances have been observed converting both partially and completely to an ethyl carbamate compound. Tramadol and tapentadol, both tertiary amines but containing a benzylic alcohol group, are also be prepared in a chloroform basic extraction at the NYPD laboratory and have been observed converting to an ethyl carbonate.

While the majority of these new compounds that are formed are not controlled, 4-ANPP can react with ECF to form fentanyl carbamate, and federally controlled Schedule I substance. If chloroform is used as a solvent in the analysis of seized drug evidence, caution must be taken to ensure that it is not causing any inadvertent reactions with the compounds present in the evidence.

## **GCMS Analysis of Street Drugs Utilizing Hydrogen Carrier Gas in Combination with a HydroInert EI Source**

Alexis Willey, Agilent Technologies

Analysis of street drugs in the forensic realm has routinely utilized capillary chromatography with mass selective detectors (MSD). The MSD provides sensitivity, selectivity, and permits structural identification of the specific compounds found in forensic street drug samples. The purpose of this research is to demonstrate that several recent advances in inert coatings on the mass spec source assembly, found in the Agilent Technologies HydroInert™ Source, can be successfully incorporated into utilizing hydrogen as an alternative carrier gas in the current screening methods involving street drug samples. This work seeks to demonstrate the improvements in source reactivity, increases in analyte response, spectral fidelity, and speed of analysis when using the HydroInert™ source in combination with hydrogen as the carrier gas. This study applied Method Translation software to convert a conventional street drug screening method without changing peak elution patterns or negatively affecting peak resolution. The advancement of the HydroInert™ Source design facilitates the GCMS solution, utilizing hydrogen as the carrier gas, and generating spectral library matches from commercial libraries and or the generation of custom libraries for targeted drug compounds.

## **Demonstrating the Benefits of Pairing Laboratory-Based Testing with Community Drug Checking Methods**

Max Denn, Joshua S. DeBord, PhD., Alexis D. Quinter, M.S., Angel McDowell, B.S., Alex J. Krotulski, PhD., Barry K. Logan, PhD, Center for Forensic Science Research & Education

Deaths stemming from the opioid crisis have created enormous public and governmental interest in understanding recreational drug markets across the United States. Public safety surveillance and seizure activities along with public health reports of overdose outbreaks currently serve as methods by which officials may gather information on regional drug supplies. However, information gathered through these methods is inherently retroactive and offer no possibility of achieving a safer drug supply. Additionally, information gathered through law enforcement activity is often not available to the public. Community-based drug checking programs help fill these information gaps by allowing individuals to submit samples for analytical testing. Field efforts quickly test for hazardous drugs like fentanyl and xylazine, and more detailed results can be obtained following chemical confirmation by a laboratory.

The Center for Forensic Science Research and Education (CFSRE) has partnered with harm reduction drug checking organizations that provide point of service drug screening at sites across the Northeastern United States. Confirmatory testing is accomplished using an Agilent 6890N/5975B gas chromatograph mass spectrometer (GC-MS) and a SCIEX TripleTOF 5600+ liquid chromatograph time-of-flight mass spectrometer (LC-QTOF-MS). Since the beginning of 2024, 1,035 samples have been analyzed, 702 (68%) of which contain fentanyl. Alarming, 99 (14%) of the fentanyl-containing samples were suspected by the submitter to contain drugs other than fentanyl or an opioid, illustrating the danger posed by untested recreational drug products.

In addition to occurrences of well-known drugs, drug checking efforts are useful in identifying the occurrence of toxic adulterants and novel psychoactive substances (NPS) in local drug supplies. 24 tablets suspected to contain Xanax (alprazolam) contained bromazolam, a NPS benzodiazepine. In August of 2024, our laboratory began detecting bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate (BTMPS) in fentanyl-containing samples from several states. BTMPS is an industrial chemical used in plastics that has recently emerged in drug markets across the country at an unprecedented rate. No research has been performed to date on the effects of BTMPS in humans but reports from individuals who have used drug material containing BTMPS have indicated negative effects like headaches, blurred vision, nausea, and more. BTMPS is not routinely in the testing scope of any crime lab or hospital lab so, without real-time drug checking agencies, this substance may have continued to covertly permeate the US opioid market.

Real-time laboratory testing gives public health and safety agencies the ability to detect potentially hazardous substances in drug supplies before they appear in an overdose or a postmortem investigation. This allows public health intervention strategies to mitigate the potential harm a substance might cause. In the rapidly evolving recreational drug landscape, drug checking programs can interrogate samples containing potentially unknown, harmful substances before they take root in the drug supply, contributing to harm-reduction through a safer supply and more informed public.



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# Forensic Biology/DNA Abstracts

**Investigative single-cell genetics: Determining the probability of a genotype after observing single-cell data enables robust database searches regardless of mixture complexity.**

Qhawe A. Bhembe M.S., Rutgers University; Nidhi C. Sheth M.S., Rutgers University; Leah O'Donnell M.S., Maynooth University, Ireland; Ken R. Duffy Ph.D., Maynooth University & Northeastern University; Desmond S. Lun Ph.D., Rutgers University; Catherine M. Grgicak Ph.D., Rutgers University.

In scientific deduction, one of the primary goals of the analyst is to collect data that will discriminate candidate hypotheses. The traditional method of procuring the data is by way of bulk treatments, where the DNA of all contributors is extracted in a single vessel resulting in equally distributed DNA fragments in solution. If the concentration of any one contributor is low, then allele drop-out can result. If the number of donors is large, allele overlap results. With all contributors' DNA being sampled together, the evidence – i.e., the likelihood ratio – approaches one as the number of contributors to a mixture increases since these data are comprised of many peaks which are a superposition of peaks from an unknown number of, potentially, partially represented individuals. These issues mean that there is scope to improve the robustness of mixture interpretation.

With profiles derived from traditional bulk pipelines encumbered by numerous peaks from many, possibly partial, contributors, interpretation can become arduous, sometimes requiring numerous propositions across multiple numbers of contributors and taking hours to complete.

One path forward is by way of single-cell analysis, which is defined as a system of laboratory procedures that: i) isolate each cell, and ii) extracts and directly amplifies the DNA in the same vessel to which the cell was added. By applying single-cell treatments, alleles of each contributor remain coupled and variability due to extract fractionation is circumvented, leading to m single-cell, single-source contributor electropherograms (scEPGs). The interpretation of this highly resolved signal, therefore, changes from one of determining the weights of multifarious genotype combinations to one that relies on dependable clustering. For non-suspect casework, an evaluation that estimates the probability of genotype  $g$  given the data of a cluster can potentially provide significant gains in investigative genetics. It is for these reasons we expound the legitimacy of single-cell data by evaluating the robustness of the system for investigative purposes. Specifically, we take 630 admixtures containing anywhere from 17 to 75 scEPGs from any of 2- to 5- contributors. We cluster the scEPGs into groups, based on similarity, using a package named `mclust` in R. We then use EESCI<sup>TM</sup> to estimate the probability of these scEPGs in a cluster,  $C$ , given  $g$ , where  $g$  is a given genotype.

EESCI<sup>TM</sup> then applies the traditional form of Bayes' rule to estimate the  $P(G=g|C)$  for each locus allowing a decision threshold, such as 0.998, to define the credible set of genotypes explaining the cluster of cells.

To test the performance of the method, we report the proportion of loci for which only one genotype is in the credible set and that genotype is the true genotype. This occurred 84% of the time. Further explorations demonstrated when more than one genotype belonged to the credible set, it was affiliated with low peak heights. Specifically, the median total intensity was 11,432 RFU for clusters with one credible genotype, while the median was 1,825 RFU for clusters where there were two credible genotypes. Mosaic plots demonstrate that this is affiliated with those clusters with 2 or fewer cells. With highly concentrated probability masses on only one or two genotypes for nearly all clusters, we demonstrate the potential of single-cell analysis to the forensic domain.

This project was partially supported by NIJ2018-DU-BX-K0185 and NIJ2014-DN-BX-K026 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not reflect those of the Department of Justice.

**Keywords:** Single-Cell Genetics, Database searching, Investigative Genetics.

**The need for team collaboration and mutual education between law enforcement and forensic labs.**

Detective First Grade Christy F. Girard, Cold Case Detective, Greenwich CT Police Department.

Law enforcement is using DNA more than ever for criminal cases; however, there continues to be a sizable gap for both police and prosecutors in the understanding of lab reports, new technologies and testing, CODIS restrictions



and how to apply the DNA results into their investigations. There is another gap with how forensic labs present their results for law enforcement understanding.

A team-based approach to investigations that use DNA testing can address this issue. However, it starts by creating a curriculum to educate law enforcement, labs and prosecutors on the newest capabilities of forensic science, along with how these results can lead to other paths of investigation.

This presentation will discuss the necessary team-based collaborative approach I use when incorporating DNA investigations--both cold and current. It will also describe how my Master's Certificate in FIGG has changed my approach to STR capabilities and how to think out of the box for these cases. I will give examples of why I am pushing for an overlapping education with the necessary stakeholders in criminal investigations.

### **Investigation of DNA methylation of cellular senescence genes and their correlation with human age.**

Dr. Debra Silva, Adriana Medina, Ella Pickell, Chemistry Department, Hofstra University.

In recent years, Forensic DNA Phenotyping (FDP) has garnered significant attention from forensic researchers, particularly in estimating externally visible characteristics (EVCs) based on DNA from biological samples. It is well established that phenotypic traits are linked to genetic components through regulatory pathways, with epigenetic modifications playing a crucial role in the translation of genotype to phenotype. DNA methylation is the most well-characterized epigenetic modification and is usually associated with transcriptional repression. 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. Recent developments in the analysis of epigenetic DNA methylation patterns have demonstrated that certain genetic loci show a linear correlation with chronological age. However, methylation levels of genes that are associated with cellular senescence have not been analyzed for their correlation with human age. The goal of this study was to investigate the methylation patterns of genes involved in the process of cellular senescence and their potential to be used in forensic investigations. First, the list of CpG sites interrogated by the Infinium HumanMethylation450 BeadChip array was investigated to compile a list of sites from cellular senescence genes. Then a study on DNA methylation analysis on individuals with different ages available in the Gene Expression Omnibus (GEO) database was selected, and the data available from this study was used in our investigation. A bioinformatic analysis pipeline was built to identify and select CpG sites correlated with human age. Through statistical analysis, these selected sites were ranked based on their potential for predicting age. This work contributes to the discovery of new DNA methylation markers for age estimation, a critical component of reconstructing biological profiles in forensic investigations.

**Keywords:** Forensic DNA Phenotyping, Age Prediction, DNA Methylation, Bioinformatics

### **Examining the Presence of Foreign DNA on Neck Swabs.**

Alexa Gonzalez Morales, Duquesne University, Lyndsie Ferrara, Ph.D.: Forensic Science & Law Program, Duquesne University, L. Kathleen Sekula, Ph.D.: School of Nursing, Duquesne University, Sara E. Walker, M.S.: Astrea Forensics.

**Learning Objective:** This poster will determine if extraneous DNA is present on necks after performing daily activities. Attendees will learn about the factors that impact the presence of DNA on necks and at what concentration DNA is present in this area. They will also be able to view the preliminary results of genotyped samples and see if there is extraneous DNA present in the profiles.

**Impact Statement:** This poster will impact the forensic science community by determining the validity of neck swabs as forensic evidence. It is important to know what could be present on a victim's neck before testing for touch DNA and how daily activities could impact the presence of DNA.

Strangulation is the compressing of blood vessels and/or air passages through external pressure on the neck. Growing

research and anecdotal information suggest the increase of strangulation in sexual assault cases. Across four studies, an average of 9.7% of survivors of sexual assault experienced strangulation.<sup>1-4</sup> In a study conducted by the Maine Coalition to End Domestic Violence with 151 survivors, 72.8% of participants had been previously strangled.<sup>5</sup> In a similar study conducted by the Georgia Coalition Against Domestic Violence with 115 participants, 80% had been previously strangled.<sup>6</sup> In suspected strangulation cases, it may be possible to retrieve touch DNA from a survivor's neck and detect the DNA profile of the perpetrator. However, it is imperative to first establish if foreign DNA is present on an individual's neck even without a strangulation event. This study looks to determine the presence of foreign DNA on a subject's neck after exposure to everyday activities. The goal is to determine if there is a buildup of foreign DNA on our necks even without direct contact. Reference and neck swabs were obtained from numerous volunteers across three separate visits to a DNA lab. DNA from the swabs was extracted with a Qiagen QIAamp® DNA Mini and Blood Mini kit, quantified using a QuantiFiler™ HP kit, amplified with the PCR GlobalFiler™ kit, and genotyped on the SeqStudio™ Genetic Analyzer. Volunteers also filled out a questionnaire related to their daily life and activities they had performed in the 24 hours prior to the collection of the swabs. This questionnaire included their relationship status, living arrangements, social activities performed, and the time since their last shower. Reference profiles were compared to the DNA profiles obtained from the neck swabs to identify the presence of foreign DNA. Preliminary results show that DNA is present in very low quantities in the neck area. Genotyped DNA samples displayed a variety of results including single-source profiles matching the reference, two-person mixtures, and incomplete profiles. The relationship status, diversity of living arrangements, social activities performed, and the time since last shower does not seem to affect the quantity of DNA present in the neck area.

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### **NIJ funding target at public crime laboratories - A historical look at over the last eight years.**

Rachel Wendt, Tracey Johnson, NIJ-OIFS.

Starting in 2015, NIJ initiated an annual solicitation specifically aimed at providing funding to public forensic laboratories titled Research and Evaluation for the Testing and Interpretation of Physical Evidence in Publicly Funded Forensic Laboratories. The goal of this solicitation is to support research to evaluate current laboratory methods or emerging methods. Awards under this solicitation produce deliverables that include best practices to improve efficiency, accuracy, reliability, and cost-effectiveness as well as protocols that can be adopted by the community. Over the last eight years, this solicitation has provided funding for thirty-eight awards across multiple disciplines including DNA, impression and pattern evidence, seized drugs, and toxicology. Historic output from these awards will be highlighted as part of presentation. In addition, NIJ will recommend and provide resources to encourage public laboratories engagement with research and contributions toward building a positive research culture within the forensic sciences. NIJ will explain how these activities can assure improvements to both scientific integrity and quality within the forensic DNA discipline.

### **Calibrated likelihood ratios for DNA evidence without assuming the number of contributors.**

Dr. Desmond Lun, Catherine Grgicak, Ph.D., Rutgers University.

Likelihood ratios (LRs) that over- or understate the strength of evidence lose their meaning as Bayes Factors. It is for this reason that confirming that reported LR's are calibrated is a necessity. We discuss two recently proposed tests that

query LR calibration discrepancy: fiducial calibration plots and devPAV. We discuss how these tests are calculated, the inputs required, and how to interpret their outputs.

We show the result of applying these calibration discrepancy tests to LRs produced by the probabilistic genotyping software CEESIt applied to all 766 GlobalFiler profiles in the PROVEDIt dataset. These profiles are comprised of mixtures of up to five contributors of varying quality. A key design principle in CEESIt is to improve consistency in the overall LR system and evaluate the data using a complete set of hypotheses. Thus, CEESIt does not require a number of contributors (NoC) assignment. Instead, it directly calculates the LR, treating the NoC as a random variable with a given prior distribution. The LR obtained by assuming varying values of the NoC as well as the posterior distribution on the NoC are provided by CEESIt as additional, auxiliary outputs. Both fiducial calibration plots and devPAV indicate that the LRs produced by CEESIt are well-calibrated, supporting its operational use.

### **Practical and Ethical considerations for the responsible use of Forensic Investigative Genetic Genealogy (FIGG).**

Dr. Claire Glynn, University of New Haven, Henry C. Lee Institute of Forensic Science.

Forensic Investigative Genetic Genealogy (FIGG) combines the fields of forensic genetics with genetic genealogy and traditional genealogical research to generate investigative leads in criminal investigations, namely violent crimes (homicide and sexual assault) and the identification of Unidentified Human Remains (UHRs). It is estimated that FIGG has helped generate investigative leads in at least 1,000 case investigations in the United States (US) in recent years.

FIGG traverses both the public sector and the private sector, with federal and state agencies and local departments developing in-house FIGG units/programs, and private companies offering FIGG services. Robust training and education in FIGG should equip analysts/practitioners with the core competencies necessary to interpret complex genetic data, understand genealogical methodologies, and navigate the ethical and legal implications involved. As this novel tool makes use of consumer DNA databases and goes beyond routine Short Tandem Repeat (STR) analysis of evidence samples, appropriate precautions, policies, and procedures must be developed and followed to safeguard individual privacy and uphold ethical standards when FIGG is implemented in forensic workflows. The intersection of privacy, ethics, and legal boundaries demands rigorous scrutiny, particularly as the field of FIGG continually evolves rapidly. A balance between leveraging genetic data to advance criminal investigations and respecting the privacy rights of all parties is attainable if all stakeholders make a commitment to adhering to best practices for maintaining transparency and accountability in FIGG investigations.

This presentation will address key steps in the FIGG workflow to include, but are not limited to, case qualification and triaging, use of public genetic genealogy databases, third-party DNA testing, privacy and protection of data, case reporting, and oversight. This presentation will highlight the importance of comprehensive training and education, continuous professional development, and interdisciplinary collaboration to enhance the accuracy and integrity of FIGG investigations. In addition, existing policy (e.g., the US DOJ interim policy), guidance documents (e.g., from the NTVIC FIGG working group), and state legislation (e.g., Maryland, etc.), will be discussed. Attendees will gain insights into developing policies and procedures that protect both individual privacy and the integrity of the investigative process, ensuring that the application of FIGG adheres to sound science, legal requirements, and ethical principles.

### **Investigation and Detection Methods for Digital and Penile Penetration Without Ejaculation.**

Brianna Gregory, Cedar Crest College, Janine Kishbaugh, M.S.FS, Cedar Crest College, Amrita Lal-Paterson, M.S.FS, Division of Forensic Science, Wilmington, DE, Lawrence Quarino, Ph.D., Cedar Crest College.

In sexual assault cases, the forensic scientist is often looking to identify seminal fluid as a biological matrix housing male cellular material. In cases devoid of seminal fluid, the analysis can be challenging because the analyst is reliant on the typically small amount of male epithelial cells present in a highly concentrated female DNA sample. As a consequence, sexual assault kits may not be examined if case histories suggest that body fluids from a male source

will not be present, as when digital penetration assault is alleged to have occurred due to the belief that male DNA will be insufficient in quantity. There have been studies that review old sexual assault casework samples and have reported successful detection of male epithelial cells after penile penetration up to 48 hours post deposition (1,2). A further study used Y-STRs to identify that the window of survival and detection of DNA from male epithelial cells deposited from digital penetration can extend as far as 72 hours (3). The objective of this research is to study the collection of non-sperm samples deposited in the vaginal cavity via digital and penile penetration, as well as external genitalia swabs, 24 and 72 hours after deposition. Samples were created by couples who abstained from sexual activity prior to collection. The samples collected from the couples were digital penetration samples with and without saliva as lubricant and penile penetration samples with no ejaculation. All collections were performed at a controlled time interval and before each collection control swabs were taken. Post-penetration, female participants documented any hygiene activity as well as time of their menstrual cycle. All Y-STR profiles were entered in YHRD where it was determined that 70% of the samples from both 24 and 72 hours had no matches. Overall, successful DNA profiles were obtained for all categories of samples included in the study.

**Keywords:** Forensic science, sexual assault, digital penetration, Y-STRs

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**Advances and Challenges with Forensic DNA in Central America.**

Mr. Carlos Morales - International Coordinator, Center for Human Identification (CHI), Erika Ziemak - Director of Special Projects, Center for Human Identification (CHI), University of North Texas, Health Science Center (UNTHSC).

Reducing Human Trafficking through Forensics in Central America, commonly referred to as “The Project”, is a grant funded by the U.S. State Department, specifically the Bureau of International Narcotics and Law Enforcement Affairs (INL). The purpose of The Project is to support DNA laboratories in Central America as well as the establishment and strengthening of DNA databases. This support takes many forms, including training in DNA analysis concepts, validation plans, CODIS software training, provision of hardware and software, equipment, capacity enhancement, and best practice recommendations in quality management.

The center for Human Identification (CHI) at the University of North Texas, Health Science Center (UNTHSC) is a leader in the forensic community because of the advanced array of testing it offers. To date CHI is the only university that has access to the CODIS software, and it is the Texas State Missing Persons Laboratory. That means when unidentified remains are discovered in the State of Texas, the law dictates a portion of those remains are sent to CHI to generate a DNA profile. DNA profiles can be used to help identify remains in cases where other forms of identification (e.g., fingerprints) are not viable. This is done by entering the DNA profiles into CHI's CODIS database. Once entered, the DNA profiles can be searched against family reference samples; other unidentified human remains; and offender samples throughout Texas and nationally.

However, there are times when these profiles do not result in a hit in the CODIS database. The hope of The Project is to establish international sharing of DNA data for humanitarian purposes. With permission from the Federal Bureau of Investigation, CHI has created a Humanitarian Database which is a clone of their local CODIS database. The Humanitarian Database contains profiles from unidentified human remains that did not result in associations after being uploaded into the CODIS software, including those likely developed from missing migrants. CHI has the ability to search DNA profiles from family reference samples developed at laboratories outside of the United States against CHI's Humanitarian Database (not criminals, not crime scene evidence) the hope is to be able to use DNA to identify these remains and be able to provide closure to families with missing loved ones.

In addition to sharing DNA data with CHI, The Project is actively working towards building an infrastructure to facilitate DNA data sharing throughout the Central American region (Panama, Costa Rica, El Salvador, Honduras, and Guatemala), borrowing from the Prüm Treaty in Europe as a model for automated data sharing. However, we recognize that achieving this goal requires more than scientific expertise; it also demands legislative support. Subsequently, CHI has sub-contracted with a governmental relations firm specializing in DNA legislation. Together, we are working to continue building regional collaboration within Central America and leverage DNA testing as a tool to combat the heinous crime of human trafficking.

### **Results of an Anonymous DNA Survey for Non-Practitioners**

Susan Horan, DNA Specialist, Kings County District Attorney's Office.

This presentation will discuss the results of a 21 question, anonymous survey completed by 467 non-practitioners regarding some common DNA testimony phrases and activity level concepts. The survey also discusses participants' consumption of true crime and fictional crime media as well as their participation in genetic testing, court observations, and jury duty. This talk will give attendees a snapshot of how some common DNA testimony concepts may be interpreted (or misinterpreted) by a jury. The survey also asked questions of the participants of some activity level concepts regarding direct and indirect transfer. It may help inform DNA practitioners of a potential juror's prior understanding of the transfer and persistence of crime scene DNA, and some common misconceptions that may regard clarification during trial testimony.

### **Enhanced performance from a prototype eight-dye, CODIS focused STR system.**

Danielle Brownell M.S., MBA, Senior Forensic Regional Account Manager, Anupama Gopalakrishnan, Nick Courtney, Promega Corporation.

Traditional capillary electrophoresis (CE) is widely used for forensic DNA typing due to its time- and cost-effectiveness. Eight-color STR Systems on the Spectrum CE and Spectrum Compact CE platforms enhance performance by distributing loci across more dye channels, thereby effectively reducing amplicon size. This presentation will showcase the newest eight-color STR System in development. Suitable for both casework and direct amplification samples, the new kit simultaneously amplifies the 20 CODIS core loci along with Penta D, Penta E and SE33 to increase discrimination and allow for wider database searching. Amelogenin and DYS391 are included for gender determination, as well as two rapidly mutating Y-STR loci (DYS570 and DYS576) and two Quality Indicators (QI). By focusing on the core CODIS loci and utilizing 8 colors, this advanced STR system can improve the success rate of generating CODIS-eligible profiles from challenging samples, such as those with degraded or low input DNA.

### **Preparation and Extraction of Petrous Bones from Medieval Slovenia**

Madalynn Martino - Armed Forces DNA Identification Laboratory, Jayne-Leigh Thomas - Indiana University, Kimberly Sturk-Andreaggi - Armed Forces DNA Identification Laboratory, Charla Marshall - Armed Forces DNA Identification Laboratory.

In this project, DNA was extracted from 20 petrous bones from remains dated to late 8th, early 9th century Slovenia. The petrous, a component of the temporal bone, is believed to have better preservation of DNA than other bones in the human body. This is thought to be attributed to the bone initially containing more DNA, thus it is more likely to be preserved in ancient remains (Ilbrahim et al. 2022) or is due to the high density of the bone directly correlating to higher DNA preservation (Gaudio et al. 2019).

DNA was extracted using the non-organic method described in Taylor et al. 2023. DNA was then repaired using a USER treatment and libraries were created using the KAPA Hyper Prep Kit and IDT Unique Dual Indexes. Three separate myBaits hybridization capture enrichment panels were used. The Expert Mito – Human, Modern Global panel was used to target the whole mitochondrial genome (mitogenome). The FORensic Capture Enrichment (FORCE) panel and the 95K Capture Enrichment panel were utilized to target nuclear SNPs to gain additional information about the samples. The FORCE panel targets approximately 5500 SNPs including identity, ancestry,

phenotype, X- and Y- chromosomal SNPs, as well as nearly 4000 autosomal SNPs for extended kinship analyses (Tillmar et al. 2021). The 95K panel targets approximately 95000 SNPs for extended kinship analyses (Gorden et al. 2022).

Data were generated on the MiSeq FGx System and analyzed using the CLC Genomics Workbench for the mitogenome panel or Parabon Fx Forensic Analysis Platform for the SNP panels.

Full mitogenome profiles were obtained for 18 individuals with an average coverage of 7317X across the samples. 16 unique haplotypes were identified, and two pairs of individuals had shared haplotypes. The 20 samples also obtained an average SNP recovery of 3970X for the FORCE panel and 71432X for the 95K panel. These SNP recoveries were sufficient to provide a prediction of sex, ancestry, and phenotype for most samples. No data was generated for two individuals due to insufficient human DNA being present.

This data will allow for kinship and lineage marker analysis to assess population demographics of the Medieval Slavs.

Disclaimer: The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, or the Armed Forces Medical Examiner System.

### **Let's catch up on the latest innovations from Thermo Fisher Scientific.**

Jeremy Boone, Senior Field Application Scientist – IID, Thermo Fisher Scientific.

Join us for a presentation on the latest innovations from Thermo Fisher Scientific. This session will provide comprehensive updates on several cutting-edge products and technologies designed to enhance forensic work. We will delve into the advancements of the RapidHIT™ ID System, highlighting its updates. Attendees will also learn about the new RapidINTEL™ Plus Sample cartridge, engineered for superior sample processing. Further, we will introduce the SeqStudio Flex, our latest capillary electrophoresis system, which offers enhanced flexibility and performance for forensic analysis. Additionally, we will present the IID NIMBUS Presto QNA system, which combines purification, quantification setup, and normalization/amplification setup in one easy to use system. Lastly, we will discuss the new features and improvements in GeneMapper™ ID-X version 1.7.

### **A Confirmatory Forensic Body Fluid Assay Using Proteomic Mass Spectrometry.**

Iyman Almubarak, The New York City Office of Chief Medical Examiner.

While DNA STR testing can identify an individual in a forensic case, currently there are no confirmatory tests able to identify the source of that person's DNA – for example, blood, saliva and semen. Consequently, a suspect may reasonably claim that their DNA profile at a crime scene could be from shed epithelial cells or DNA transfer. With grant support from the National Institute of Justice, the New York City Office of Chief Medical Examiner has developed, validated and brought into routine casework a confirmatory body fluid assay. Using liquid chromatography and mass spectrometry, multiple marker proteins from each body fluid can be identified to confirm the presence of a body fluid or a mixture of body fluids in a forensic sample. For each body fluid, three or four proteins have been selected as markers based on their essential functions in those body fluids, for example hemoglobin carries oxygen in blood, semenogelin aids sperm in reproduction, and amylase helps digest carbohydrates in saliva. For each protein, three peptides (with four transition ions) have been selected to identify a protein. The assay has been accredited by ANAB and approved by the New York State Commission on forensic Science. The OCME has started using this assay on forensic samples of juvenile sexual assault cases since July 2023. The OCME is currently working on improving the assay by adding additional controls as well as testing new instruments. New body fluid markers will be added in the future to identify different body fluid such as urine, vaginal fluid, and menstrual blood.

### **The Status of New DNA Technologies in the Courtroom.**

Dr. Amanda Murray, DNA Labs International.

The last 6 years have seen a dramatic boom in DNA innovation from Rapid DNA, Next Generation Sequencing (NGS)/Massively Parallel Sequencing (MPS), Forensic Investigative Genetic Genealogy (FIGG), Phenotyping, X-STRs, identity single nucleotide polymorphisms (SNPs), evaluative reporting, and more. From 2014 to 2015 probabilistic genotyping began being implemented throughout the United States and quickly admissibility challenges in the courtroom started playing out across the country. However, with more recent technologies we have seen a slow movement of admissibility motions in the courtroom. The lack of challenges can give a false sense of security that future evidentiary challenges may not be just around the corner. This presentation will explore the delays in admissibility challenges because of the COVID pandemic, slow adoption of some technologies, and legal reasoning behind some of the lack of challenges. A peek at what the future may hold including the importance of internal validation, documentation, training, and accreditation will be discussed. Finally, the status of current technologies in the courtroom on national and global level will be summarized.

## **An Overview of the NIST/NIJ Expert Working Group on Human Factors in Forensic DNA Interpretation Report.**

Tracey Johnson, National Institute of Justice.

Studying human factors is essential to inform our understanding of humans' interactions with the systems they use. This is especially important in forensic science, where the outcomes of these interactions can have a direct impact on an individual life or liberty. The National Institute of Standards and Technology (NIST)/National Institute of Justice (NIJ) Expert Working Group on Human Factors in Forensic DNA Interpretation was charged with conducting a scientific assessment on the effects of human factors in forensic DNA analysis and interpretation with the goal of recommending approaches to improve its practice and reduce the likelihood of errors. The Expert Working Group developed a report containing these recommendations based on relevant bodies of scientific literature and technical knowledge. This evaluation serves to educate member of forensic DNA laboratories and allied criminal justice partners (e.g., attorneys, investigators, parent organization leadership) alike and includes topics related to:

- Interpretation
- Quantitative and Qualitative Expressions of DNA Results
- Reporting
- Pre-Trial Preparation and Testimony
- How and When Question in DNA Analysis
- Quality assurance/Quality control
- Education, Training, and Professional Credentialing
- Management
- Work Environment
- Research Culture and Research Needs

This three-year effort follows the success of two previous reports produced in the Human Factors Expert Working Group series that investigated the role of human factors in Latent Print Examination and Forensic Handwriting Examination.

During this presentation, material will be presented that highlights key findings and recommendations focusing on research needs, education, training and professional credentialing.



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# Poster Session Abstracts



## **P1. DSI-GC-MS Analysis of Designer Cannabis Products**

Hannah LaVena; Ling Huang, Ph.D.; Kevin Bisceglia, Ph.D., Hofstra University

Designer cannabis products are sold in a wide variety of matrices, including gummies, vapes, oils, and capsules. They can also be deliberately augmented by distributors by combining unregulated semi-synthetic cannabinoids with currently available strains in order to generate a product that is both higher in quality and potency, containing a vast array of cannabinoids such as delta-8 THC, CBD, and THC-A.

These products are then sold through private sellers, online retailers, or in head shops to circumvent the law and to maintain a low profile to law enforcement officials.

Further improvement of the scope and accuracy of designer product analysis can result from the evaluation of sugar, oil, and flavor matrix components, taking into account the unique and custom formulations across products in addition to their manufacturers and suppliers, thus allowing the linkage of samples to specific distributors.

As compared to traditional auto-sampler GC-MS, DSI-GC-MS has the ability to separate and detect target analytes in the presence of a complex, viscous, and often “dirty” sample matrix. It employs a programmable-temperature vaporization (PTV) injector in tandem with a ChromatoProbe™ accessory, in which a solid or liquid sample in a disposable glass vial is heated with its resulting vapors ushered into the GC column for separation.

DSI-GC-MS is proposed as an analytical technique utilizing temperature programming, rapid analysis times, smaller sample sizes, and “dirty” sample matrices as an adjunct to the investigation of seized designer cannabis products.

The presented DSI-GC-MS method allows for the rapid analysis and identification of cannabinoids in designer products with minimal sample preparation and in real time in order to maximize extraction of designer components and cannabinoids. Cannabinoids not identified to be constituents were detected, indicating that this method can be used for quality assurance and the identification of contaminants in samples, thus informing consumers of the actual composition of these popular designer products.

This report will demonstrate the benefits of DSI-GC-MS as compared to traditional autosampler GC-MS as it can differentiate, identify, and quantify cannabinoids and their isomers rapidly with minimal sample preparation in addition to identifying components within the sample matrix.

## **P2. Real Bullets, Plastic Guns- Evaluating the Strength of 3-D Printed Gun Parts**

Maria Mayol; Peter J. Diaczuk, Ph.D., John Jay College of Criminal Justice

Privately made firearms, also popularly referred to as “ghost guns” are firearms created and assembled by an individual; these firearms are not manufactured or marketed by a corporation or government and as such many of them lack serial number and are unregulated. “Ghost guns” refers to guns without a serial number, which actually only comprises a small portion of the true umbrella term, privately made firearms. Privately made firearms can be entirely 3-D printed, assembled from “buy build shoot” kits (can be purchased with a firearm already up to 80% assembled), or be composed of 3-D printed parts retrofitted onto an existing gun. With the accessibility of 3-D printing and the freely shared files of 3-D printed guns and gun parts, the appearance of privately made firearms in any of the three forms has increased in the past years in several countries. 3-D printing gun parts, except for the lower receiver, is legal according to the language of some state laws. Because firearm parts are designed to be interchangeable to allow for customization or repairs, it is possible to exchange an original manufactured gun part for a 3-D printed part, this is a problem in a legal sense when used either for concealment or forensic evasion. The identification of

firearms based on fired bullets is founded on the microscopic agreement of individual marks left on a fired bullet to the interior of the questioned barrel; 3D printing a functional gun barrel would successfully fire a projectile and leave no rifling on the bullet being that the barrel would be printed out of plastic and lack the hardness to leave marks on a metal projectile.

The goal of the experiment was to determine if a 3-D printed barrel is capable of producing a lethal projectile when retrofitted onto the original firearm. A commercially available 3-D printer (Makerbot Replicator+) was used to print an exact replica of the SBS barrel (a side by side barrel) of a Derringer Cobray Model DD in .38 Special/.357 Magnum modified to be chambered for a .25 ACP cartridge using PLA Tough filament (the strongest filament compatible with the original extruder of the 3-D printer). The modification to accommodate a smaller cartridge allows for more plastic material in an effort to maintain the integrity of the barrel when the gun is fired. The results showed that the 3-D printed barrel did not have the strength to survive multiple test fires and that the bullet was not capable of penetrating ballistic gelatin deep enough to cause lethal damage. The lack of accuracy and penetrating power of the bullet can be attributed to the lack of rifling on the 3-D printed barrel which is essential for spin-stabilization of a bullet.

### **P3. Analysis of Substituted Cathinones and Fentanyl Analogs by Gas Chromatography-Infrared Spectroscopy (GC-IRD) Using Nitrogen Carrier Gas**

Marcus Warner; Ilene Alford; Steven Williams, Palm Beach County Sheriff's Office

Substituted cathinones and fentanyl analogs that are positional isomers can produce similar mass spectra and retention times, which poses challenges for their identification. A gas chromatography-infrared spectroscopy (GC-IRD) method used as a complimentary technique to gas chromatography-mass spectroscopy (GC-MS) for the accurate identification of positional isomers was developed using nitrogen carrier gas. Nitrogen was selected due to the global helium shortage and also to pursue a helium conservation approach that included the installation of Agilent helium (nitrogen switch) conservation modules on all GC-MS instruments. With the goal of optimizing analysis time while maintaining acceptable resolution, a total method run time of 26 minutes for the analysis of 29 certified reference materials comprised of substituted cathinones and fentanyl analogs was achieved. A mixture containing prevalent drugs was also analyzed to evaluate the resolution and retention time of drugs potentially occurring in combination with positional isomers. Standards were prepared at concentrations of 100 µg/mL, 1 mg/mL and 2 mg/mL and analyzed over a 3-day period, where the 2 mg/mL concentrations of the standards were run in triplicate on day 1 and once on days 2 and 3. Twenty-seven of the standards were identified with a library match of 0.98 or higher for the 1 mg/mL concentration. Each standard had a retention time (RT) difference less than 0.05 minutes and a coefficient of variation (%CV) less than 3%. For the drug mixture peak resolution exceeded 1.5 for each component. GC-IRD was found to be suitable as a complementary analysis technique to GC-MS for analyzing the validated substituted cathinones and fentanyl analogs by the repeatable and reproducible RTs and library matches obtained during method validation.

### **P4. Look Before you LEAP**

Laura Tramontin; Charles Foster, ASCLD Forensic Research Committee

The ASCLD Forensic Research Committee has expanded its LEAP capabilities to better facilitate collaboration between academia, forensic science laboratories and industry partners. We look to highlight improvements to the platform to help align parties with mutual research interests and desired capabilities. We have incorporated Microsoft Power BI dashboard for sorting LEAP data according to organization, category, research interests, and possible internships by region making it easier to take the LEAP in expanding your organization's network of strategic partners and collaborators.

## **P5. Comparative Study of Different Charcoal Strips to Determine LOD and Resolution Using GC-MS**

Andrew Zeblisky; Deepika Das, Forensic and National Security Sciences Institute- Syracuse University

Fire and arson investigation is centered around determining the nature of fire as accidental or intentional based on usage of ignitable fluids to spread a fire. Fire debris analysis often uses headspace adsorption technique to perform this analysis. One of the primary tools that crime labs in the U.S. use in this analysis is charcoal strips. Charcoal strips function to reliably capture vapors released from burnt samples through adsorption. These vapors can originate from the sample itself as well from the presence of an ignitable liquid. Hence, it is crucial to run appropriate controls to determine the presence of ignitable liquids. Recently, Albrayco (the primary company producing charcoal strips used by crime labs) started manufacturing a smaller sized charcoal strip. This necessitates the need for a comparison and validation of the new strips to the old ones to ensure the comparable effectiveness of both the strips. This is especially important since half of the strip is not used for analysis by the technician, but rather saved for the defense in case they wish to use it. Hence, the small size of the new strips could impact its limit of detection (LOD) and resolution. This project performs a comparative study with both strips to compare the effect that variables have on the functionality of these strips such as size of container, temperature of container, and storage duration. The study also uses different classes of compounds to test the LOD of the strips. Multiple classes of compounds that were included in the testing were various single component ignitable liquids, such as dodecane, toluene, and acetone since they represent commonly observed compounds that are found in many types of ignitable liquids, commercially available ignitable liquids (that are mixtures of several compounds) like gasoline, a medium hydrocarbon filled compound, and kerosine, a heavy petroleum distillate. This study will provide necessary information that determines if the different types of strips can be interchangeably used in crime labs without compromising data accuracy. This research is a good reminder that standards and assays are constantly changing within forensics, and there needs to be a continued effort in maintaining quality assurance.

## **P6. Impact of Temperature and Storage Duration on Naloxone Stability in Narcan Spray Using LC-MS**

Rasmita Suwal; Deepika Das, Syracuse University

Opioid overdose has been an alarming social problem in the USA, and even more concerning is the increasing number of deaths per year because of such overdoses. Naloxone, an opioid antagonist, in the form of nasal spray labeled as Narcan has been a successful emergency medication for such overdoses. However, the stability of naloxone determines whether the medication works at the very moment of emergency. Because it is an emergency drug, advised to be always carried, there is a high chance that the Naloxone product is not stored as per the instructions. People might carry the antidote in their vehicles particularly cars, and the temperature of the vehicle could reach extremely high during torrid summer and extremely low during freezing winters, or they might use the product after the expiry date. The extreme temperatures of the vehicle could affect the stability of naloxone products, both unexpired and expired, which is of great concern for public health.

This project will use Narcan spray (from Adapt Pharma, Inc.) to simulate the effect of cold temperature (-20 °C) and hot temperature (75 °C) on both unexpired and expired products carried in cars for short as well as extended period. The temperatures -20 °C and 75 °C represent the extreme internal temperatures of the vehicles during freezing winter and torrid summer in the USA. The intranasal spray contains naloxone hydrochloride in an aqueous solution. The study will focus on the change in concentration of the naloxone using LC-MS. Different extraction techniques will be evaluated to efficiently isolate naloxone compound from the Narcan solution. In addition to naloxone concentration, changes if any, in the inactive agents (such as benzalkonium chloride) in the spray solution will be examined. LC-MS (Liquid chromatography mass spectroscopy) technique with ESI (Electrospray ionization) mode will be used for analysis. The lower concentration of naloxone in any of the samples, if observed, will likely be due to structural change or decomposition, and further analysis will be performed to identify the products. The study is significant as

degradation of naloxone molecule can affect Narcan's metabolism inside a human body, its safety as well as effectiveness in treating patient under opioid overdose.

There has been similar thermal stability research on intravenous naloxone ampoules (from Sandoz) in Canada using HPLC. Another study of chemical stability of naloxone was performed on expired Naloxone nasal spray and injections in the USA using HPLC. This study is unique as it utilizes LC-MS technique, and the nasal spray samples from Adapt Pharma, Inc., an OTC (over the counter) naloxone product in the USA have not been subjected to such thermal stability analyses yet. The results from this study will provide insights on impact of temperature on Adapt Narcan spray storage and hence, updated advice regarding its storage as well as usage of expired Adapt Narcan spray.

## **P7. Evaluation of GSR Preservation Via Confirmatory Analysis Workflows to Facilitate the Adoption of OGSR Analysis**

Kourtney A. Dalzell; Tatiana Trejos, Ph.D.; Luis E. Arroyo, Ph.D., Department of Forensic and Investigative Science, West Virginia University; Thomas Ledergerber, C. Eugene Bennett Department of Chemistry, West Virginia University

Gunshot residue plays a vital part in firearm-related investigations to answering questions regarding the person(s) of interest (POI) and crime scene reconstruction. This evidentiary information originates from the production of inorganic (IGSR) and organic gunshot residues (OGSR) expelled at the discharge of a firearm due to the high temperature and pressure environment of the ammunition confined within the barrel of the firearm. Traditionally, gunshot residue evidence utilizes scanning electron microscopy energy dispersive x-ray spectrometry (SEM-EDS) to determine the presence of characteristic particles containing components of the primer such as lead, barium, and antimony. This method is proficient in singular particle elemental and morphological identification and classification. However, analysis of IGSR provides information on only part of the residue. More recent research has been conducted on complementary methods for OGSR analysis by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MSMS). These methods target organic compounds commonly incorporated into propellant formulations such as plasticizers, stabilizers, and flash suppressors. Although there is a growing interest in incorporating OGSR analysis into practice, there are pressing questions surrounding the preservation and storage of IGSR and OGSR when both residues are analyzed.

This study targets these questions by investigating the effects of variables of time since collection, storage conditions, and the sequence of analytical workflow in the preservation of the evidence. A total of 114 samples from the hands of shooters were collected and split into two groups to evaluate storage conditions under freezer (N=84) and room temperature (N=30). Freezer samples were analyzed at six different times of analysis (n=14) including immediate, 2 days, 1 week, 2 weeks, and 1 month and 2 months after collection. At each time of analysis, two analytical workflows were followed, where seven of the fourteen samples would undergo SEM-EDS analysis first, followed by LC-MSMS, and the other half of the samples would undergo LC-MSMS analysis first, then by SEM-EDS. A similar process was repeated for the room temperature samples at 2 days, 1 week, and 2 weeks after collection. Results have found the detection of IGSR and OGSR shows no significant difference based on the storage conditions or time from collection to analysis. These findings may alleviate some concerns regarding the feasibility of conducting OGSR examination on samples that cannot be submitted to the laboratory right after collection at the scene and can assist the adopting agencies in establishing criteria for time windows for accepting GSR evidence. The average amount of pGSR particles was not significantly affected by the sequence of the analytical workflow. Thus, when following appropriate protocols, the integrity of IGSR evidence is not compromised by the incorporation of OGSR testing. On the other hand, a trend was noticed for OGSR where a decrease in concentration is found when SEM is performed first, however, the detection of three main analytes was still achieved in most samples. The findings of this study provide crucial information on the preservation and storage of GSR for future adoption of OGSR analysis.

## **P8. A Qualitative and Quantitative Analysis of Heavy Metals in Topical Cannabis Pain Relief Products**

Sarah Murphy; Marisia Fikiet Ph.D.; Alyssa Marsico, Ph.D., University of New Haven

In 2018, the United States passed the Farm Bill, which removed cannabidiol (CBD) from the list of controlled substances, where it was previously considered a schedule 1 drug, and legalized any cannabis-derived products containing less than 0.3% tetrahydrocannabinol (THC)<sup>1</sup>. As a result of this bill, there has been an increase in sales and an influx of matrices for cannabis-based products. One subsection of cannabis products that has gained interest in recent years is the topical use of CBD. Research has shown that topical CBD products may be useful to stimulate wound healing, treat non-melanoma skin cancer, and reduce inflammation, itching, and pain<sup>2</sup>. However, since CBD has no accepted medical or recreational use, with the expectation of Epidiolex to treat epilepsy<sup>3</sup>, any cannabis-based products on the market are largely unregulated by The Food and Drug Administration (FDA). Due to the lack of regulations and limited analysis of CBD products before they enter the market, there are concerns about label accuracy and potential product contamination. Heavy metal contamination in CBD products is an area of concern, partially due to processing cross-contamination and post-processing adulteration, but also due to the natural bioaccumulative capacity of the cannabis plant, which removes heavy metals from substrate soils and deposits them in the plant tissues<sup>4</sup>. Heavy metals are classified as carcinogens and have been shown to aid cancer progression or reduce sensitivity to treatment<sup>5</sup>. Topical exposure to heavy metals was previously thought to only have local effects on the body, however recent studies have shown that they are capable of penetrating deep into the skin, reaching the blood circulatory system, and causing exposure to internal organs<sup>6</sup>. In this study, 20 topical CBD products sold online were analyzed for the potential presence of lead and cadmium. Samples consisted of cream, gel, or balm topicals that were classified as isolate, broad spectrum, or full spectrum CBD. A method using Atomic Absorption Spectroscopy (AAS) was developed to investigate the presence of heavy metals in lotion. Standard addition calibration curves were created for each sample and analyzed with the AAS to determine lead or cadmium concentrations.

## **P9. Post-Mortem Exosome Dynamics: A Novel Approach to Time and Cause of Death Estimation in Forensic Investigations**

Abraham Boadu; Qiaochu Zhang; Basirat Rufai; Eric Klein, Jinglin Fu; Youwen Zhang, Rutgers University- Camden

Estimating the accurate time and cause of death remains a formidable challenge in forensic science. Recent advancements in molecular assessments, such as the utilization of biomarkers for post-mortem examinations, have led to significant improvements in determining the cause of death. However, the rapid degradation of bioactive materials such as DNA, RNA, and proteins post-mortem presents a significant hurdle for forensic applications. Recently, the potential of exosomes—extracellular vesicles ranging from 30-200nm in size—has been recognized in forensic investigations.

Due to their stable double-membrane structure, exosomes maintain high integrity and stability, making them ideal candidates for circulating biomarkers in forensic bioanalytical procedures. They are present in various biological fluids including blood, saliva, and vitreous humor, providing crucial insights as pathophysiological conditions and tissue damage are reflected in the stability and specificity of exosomes. Our research proposes to investigate the quantifiable changes in exosome characteristics after death, which could offer a more precise and robust alternative to traditional methods for estimating the time and cause of death. Central to this approach is the development of a nanoporous membrane-based exosome isolation method, optimized for minute post-mortem samples. Our goal is to establish a protocol that efficiently recovers exosomes from various biological fluids, enhancing the reliability of death estimations. The exosome characterization strategy will involve a comprehensive analysis of concentration, protein degradation kinetics, and microRNA identification to monitor predictable changes over the post-mortem interval. These metrics could serve as molecular evidence for determining the time and cause of death, providing essential evidence in criminal investigations. This study aims to establish the foundations for a novel forensic tool that could

significantly improve the accuracy of death determinations. Future efforts will focus on conducting large-scale validation studies, refining our estimation models, and developing standardized protocols for field applications.

### **P10. The Impact of Dissolvable Swab Technology for Single-Cell DNA Extraction on Forensic Evidence**

Assa John; Creston Singer; David Salas-de la Cruz, Rutgers University- Camden

In recent years, forensic science has made substantial progress, but challenges remain in collecting DNA evidence from crime scenes. There is a growing need for a more effective approach to forensic evidence collection, one that can better identify individuals involved in crimes and thereby aid in crime prevention and resolution. Although cotton swabs are commonly used due to their accessibility and versatility across different surfaces, they often fail to collect a significant amount of DNA evidence. Additionally, current DNA extraction methods, which typically involve cell lysis, can destroy the evidence, preventing the extraction of individual cells for analysis. This research aims to overcome the limitations of existing DNA collection methods by introducing an innovative solution: a dissolvable swab made from calcium alginate. This new swab is designed to improve DNA recovery from individual cells, providing a non-destructive technique for single-cell forensic analysis. Single-cell DNA analysis offers a major advancement over traditional methods, delivering more precise and interpretable results, especially in cases with multiple contributors. The research is divided into two main phases. The first phase focuses on creating a dissolvable material by combining sodium alginate and calcium chloride, which is then used to coat cotton fibers and form a composite swab. The second phase involves comparing the performance of these composite swabs with that of conventional cotton swabs. The materials chemistry of the new swab is characterized using Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) with Energy- Dispersive X-ray Spectroscopy (EDS) for atomic analysis, and X-ray scattering. The material's performance, including cell uptake, release, and cell integrity, will be evaluated using fluorescence microscopy and confocal laser scanning. Results indicate that calcium-alginate can be easily dissolved from its primary components, facilitating the removal of various cell-to-matrix anchor points and providing a straightforward pathway for cell release. This study represents a significant advancement in forensic science, providing a novel approach to the ongoing challenges of DNA evidence collection.

### **P11. Evaluation of Interferences Between $\Delta 9$ -THC and CBNA in Marijuana Plant Samples by LC-PDA and DART-MS**

Megan I. Chambers Ph.D.; Walter B. Wilson, Ph.D, Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD

Over the last decade, Cannabis sativa products have become increasingly popular. Forensic laboratories often use liquid-chromatography (LC)-based approaches to analyze the cannabinoid content in these products. Our laboratory previously used the Cannabis Analyzer (LC with photodiode array (PDA) detection) to quantify 11 cannabinoids in 20 seized marijuana plant samples. During this analysis, it was found that  $\Delta 9$ -tetrahydrocannabinol ( $\Delta 9$ -THC) and cannabinolic acid (CBNA) had coeluting peaks. This complicated integration efforts and prevented the ability to accurately quantify  $\Delta 9$ -THC in samples that contained both compounds. Therefore, the focus of the current study was to further evaluate the interference between these cannabinoids using an ambient ionization mass spectrometry (AIMS) technique such as direct analysis in real time – high-resolution mass spectrometry (DART-HRMS).

A DART ion source coupled to a high-resolution ion trap mass spectrometer was used to analyze cannabinoid standards (e.g.,  $\Delta 9$ -THC, CBNA, cannabinal (CBN), and tetrahydrocannabinolic acid (THCA)) and 20 marijuana plant samples after methanol extraction. Mass spectral features such as m/z values, relative intensities, ion counts, and isotope patterns were examined to identify differences between plant extracts that contained CBNA and those

that did not. According to the LC-PDA data previously collected, 18 of the 20 samples had detectable levels of CBNA. When analyzed by DART-MS in negative-ion mode, each of these 18 extracts yielded a spectrum containing a peak consistent with the deprotonated [M-H]<sup>-</sup> mass of CBNA at m/z 353 and its respective isotope peak at m/z 354. It is important to note that the peak at m/z 354 was detected at an approximate relative abundance that reflects the elemental composition of CBNA. Although the two remaining samples produced a peak at m/z 353, they did not yield a peak at m/z 354. Therefore, these samples were identified as not containing CBNA because the correct isotope pattern was not obtained. In summary, DART-MS data for each of the 20 marijuana samples agreed with the results obtained by LC-PDA. The results of this project provide a DART-MS-based method to determine whether samples have CBNA levels that may interfere with the LC-PDA analysis of seized Cannabis plant. This approach is especially of interest because AIMS methods are becoming more prevalent in forensic science laboratories.

### **P12. Value Assignment of NIST Reference Material 8210 Hemp Plant**

Megan I. Chambers Ph.D.; Walter B. Wilson, Ph.D.; Hugh V. Hayes, Ph.D.; Monique Johnson, Ph.D.; Catherine A. Rimmer, Ph.D.; Andrea A. Yarberry, Ph.D., Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD; Colleen E. Bryan, Ph.D.; John L. Molloy, Ph.D., Chemical Sciences Division, National Institute of Standards and Technology, Charleston, SC

The National Institute of Standards and Technology (NIST) has prepared a hemp plant reference material (RM 8210) to aid Cannabis and forensic laboratories in the validation of their methods, support the development of new analytical methods, and as a quality control material for routine analysis. RMs play an important role in promoting compliance with current and future legislation, labeling accuracy, and good manufacturing processes. However, RMs are a critical measurement service that is presently lacking for forensic laboratories to use. RM 8210 has non-certified mass fractions for cannabinoids, total  $\Delta^9$ -THC, total CBD, and toxic elements on a dry-mass basis. The need to accurately measure cannabinoids in seized Cannabis plant samples became significantly important after the passage of the 2018 Farm Bill. New legislation legalized hemp in the US by removing hemp from the DEA Scheduled 1 controlled substance list and defined it as Cannabis sativa with a total  $\Delta^9$ -THC mass fraction of less than or equal to 0.3 % on a dry-weight basis. Mass fractions (%) were assigned for eight cannabinoids by NIST using LC-PDA. Cannabinoids were identified using retention times, absorbance spectra, and peak purity evaluation using a PDA detector to compare absorbance spectra across the entire peak. Contaminants such as toxic elements are the next largest analytical measurements required by Cannabis laboratories for hemp plant samples to ensure materials are safe. Mass fractions for both cannabinoids and toxic elements are assigned to the hemp plant material on a dry-mass basis in accordance with requirements outlined in the 2018 Farm Bill. The initial versions of this material were included in the second exercise of the Cannabis Laboratory Quality Assurance Program administered by NIST. Community results will be provided for comparison to NIST results.

### **P13. The Effects of DNA Longevity**

Mckenzie Lamm; Mark Flood; Kristy Henson Ph.D., Fairmont State University

The longevity of DNA is dependent on many factors. Quality control and laboratory storage can influence DNA longevity. The assurance of high standards of laboratory protocols aids in ensuring viable, repeatable results of DNA samples. Poor genotyping results not only decrease morale but can influence forensic case success by creating nonviable evidence. The purpose of this project was to sequence pre-collected DNA and determine if a genotype could be sequenced. Thirty-six individuals submitted a buccal wash for DNA analysis in 2023, DNA were extracted, and stored at -18°C. These samples were amplified by PCR in 2024. After PCR amplification, DNA quantity was measured via Nanodrop before and after dilution. Once samples were diluted to 4 ng/ $\mu$ L they were prepped and placed in SeqStudio genetic analyzer. Results were inconclusive. Remaining samples were reprocessed and tested again.

Again, results did not yield profiles. Preliminary troubleshooting indicates that proper storage and handling of the DNA samples influenced the ability to sequence the profiles. More data needs to be collected on DNA storage, DNA extraction, and reagent longevity to increase profiling success in forensic cases.

#### **P14. Skeletal DNA Analysis of Bone Submerged in Stagnant Blackwater**

Jessica Behn; Ashley Morgan, Ph.D., University of New Haven

The genetic identification of human remains is a unique challenge. This sentiment is only emphasized when considering those submerged in aqueous environments. Due to a large dependence on environmental factors, and consequential decomposition variations, investigators can easily become reliant on bone for human identification. As a source of DNA, bone is more resilient to these influences than softer tissues. However, the range of studies investigating the interaction of DNA degradation and skeletal remains is quite limited. This is especially true when considering skeletal elements under a variety of conditions – like those submerged in water. Moreover, most of these studies have focused on evaluating DNA from remains submerged in marine environments or those in aqueous bodies of higher velocity. Such conditions pose a distinctive difficulty to investigators as moisture and chemical composition can alter both the rate of degradation within skeletal elements and its quantity. Thus, this research aimed to determine the DNA quantity and rate of degradation of submerged skeletal elements in stagnant blackwater over a 4-month period. To mimic human remains in a New England Pond environment, 5 porcine femurs were submerged in an artificial pond aquarium and allowed to soak for 3 weeks, 6 weeks, 9 weeks, 12 weeks, and 15 weeks respectively. This aquarium was a tannin-rich, stagnant body of water, supporting plant life with a slightly acidic pH. A control sample was left in dry, ambient room conditions and was analyzed in tandem with the submerged samples at the end of each period. Bone samples were powdered, and DNA was extracted using the Prepfil<sup>TM</sup> BTA Forensic DNA Extraction Kit. The following two methods were used for this extraction: BTA and Updated BTA protocols. Following extraction, samples were then quantified using the QuantStudio<sup>TM</sup> 5 Real-Time PCR System, and metrics were analyzed.

#### **P15. Creation of a Nanoparticle Functionalized Adhesive for LIBS-Based Detection of Gunshot Residue**

Benjamin Wheeler; Alyssa Marsico, Ph.D., University of New Haven

Traditionally, forensic investigations of gunshot residue (GSR) particles utilize scanning electron microscopy (SEM) for identification and analysis to determine significant facts, such as whether a firearm was discharged. New methods such as laser induced breakdown spectroscopy (LIBS) have emerged that offer the potential for rapid sample analysis. LIBS can be enhanced through using nanoparticles. Studies such as one performed by Baytekin et al. have successfully created an adhesive tape with nanoparticles adhered to the surface of the tape<sup>1</sup>. No studies have researched the use of a nanoparticle functionalized adhesive for analysis of GSR. Therefore, this study investigates if a nanoparticle functionalized adhesive can be created using the determined optimal nanoparticle for GSR for use in nanoparticle enhanced LIBS.

To address this purpose, LIBS spectra from GSR samples with and without nanoparticles were created. Burnt gunpowder was collected and placed onto a microscope slide, onto which an aqueous solution containing a selected nanoparticle was applied. After being analyzed using LIBS, the peaks on the emission spectra that correspond to the main GSR elements of lead, barium, and antimony were located and their intensities were compared between nanoparticle-enhanced and non-enhanced samples, and is still ongoing. A similar methodology was used for GSR samples collected from a gun range, where traditional adhesive stubs were used to collect GSR present on the hands of a shooter. Nanoparticles were applied to the stubs and analyzed using LIBS. Analysis of the resulting emission spectra is the same as above. The optimal nanoparticle will be determined by which element and size of nanoparticle



produced the greatest increase in peak intensity for lead, barium, and antimony in both the burned gunpowder and shooting range samples. Currently, increased LIBS signals have been observed for the characteristic GSR elements using gold and silver nanoparticles, but the optimal has not yet been determined.

In order to create a nanoparticle-functionalized adhesive, a modified version of the procedure from a study by Baytekin et al. was followed. Various types of tape were manually peeled off and immersed in either a gold, silver, or copper salt solution for several hours to days under different parameters to create these tapes<sup>1</sup>. LIBS was used to confirm the procedure was successful in functionalizing the tape with NPs, with continued analysis still ongoing. A GSR sample was applied to the created adhesive and analyzed using LIBS, which is still ongoing.

A nanoparticle functionalized adhesive for LIBS analysis will be useful as a screening method for GSR samples. Reducing the samples run on a SEM to only those with a high probability of containing GSR particles will save labs time and money. Portable LIBS technologies are being researched and nanoparticle functionalized adhesives could be used for rapid, on-scene analysis on GSR samples in the future.

#### **P16. Discovering the Influence of Surface Roughness on Area of Origin Determination of Impact Spatter**

Autumn Reynolds; Carol Ritter, Cedar Crest College; Dr. Paul V. Quinn Sr., Cedar Crest College and Kutztown University

Impact spatter bloodstain patterns are created when a source of liquid blood receives a force resulting in the dispersion of smaller stains at various angles onto nearby surfaces. Physical characteristics including measurements of the stains can be used to determine the approximate location of this blood shedding event through various models that calculate the area of origin. Area of origin impact calculations are known to estimate the location of a blood shedding event, while not accounting for a due to the number of variables. One such variable is the interaction of the blood with various types of surfaces since current models only exist for smooth, non-porous surfaces that don't account for surface interactions. Most surfaces at crime scenes are not smooth or non-porous but rather, have variations of roughness and absorption characteristics. The lack of applicable models for the wide variety of rough and porous surfaces at a crime scene can result in an inaccurate bloodstain pattern analysis. This research creates an empirical model to account for a variation in the roughness of various surfaces which can increase the accuracy of any conclusions made when analyzing impact spatter at a crime scene. A blood alternative was used to interact with tiles of varying roughness values, producing graphs that implicitly suggest the existence of a friction coefficient between the blood and the surfaces interacting with it. These empirical results were used to explore a potential roughness factor which can be applied to previous models of smooth surface interactions with blood by accounting for the roughness. This allows for a more accurate modeling of the interaction between blood and the surface on which it flows, and therefore, leading to more accurate conclusions in impact spatter pattern analysis.

#### **P17. Trace Evidence Recovery from Handgun Discharge of Federal Syntech Total Synthetic Jacket Polymer-Coated Bullets**

Ethan A. Frazer; Peter J. Diaczuk, Ph.D., John Jay College of Criminal Justice

The goal of this study is to determine alternative ways to connect a polymer-coated bullet to the handgun from which it was fired. Polymer-coated bullets are projectiles that have had a synthetic layer applied to the outside of the usual lead core in an effort to reduce barrel friction and heavy metal exposure. The polymer coating on these types of bullets, which varies between brands and/or models, presents unique challenges to firearms examiners as it does not retain the individualizing striations found in traditional unjacketed or metal-jacketed bullets. A popular, modern brand of polymer-coated ammunition is the Syntech Total Synthetic Jacket (TSJ) line produced by Federal Ammunition.

Previous research has found that one method of connecting polymer-coated bullets, such as Nyclad, to the origin firearm is by recovering residue of the coating from the interior of the barrel post-firing. Unfortunately, this is only true for certain brands of ammunition and Federal Syntech TSJ residue has not been found to be recoverable this way.

In this study, 9mm TSJ ammunition was fired through a number of aftermarket traditionally rifled Glock 19 and Glock 43 barrels. Cloth targets were set up at varying distances in an attempt to capture ejected residue and determine at what distances the residue can be observed on fabric targets. After shooting, the firearm was field stripped, and the barrel was removed for examination. A bore brush was used to clean the residue from the interior of the barrel onto a watch plate. The barrel interior residue, feed ramp, and targets were then examined under a stereomicroscope for the characteristic “lipstick” red polymer.

Examination of the feed ramp found notable deposits of red residue, consistent with TSJ bullet shavings, in 67% of trials, with the remaining trials split evenly between inconclusive and negative findings. When the barrel interior was inspected, polymer residue was only found in one-third of examinations. These positive results were not strongly conclusive as they only contained one to two fragments of red residue. These findings could be explained by the bullet scraping up the feed ramp and depositing residue while being pushed into the chamber. The feed ramp is largely protected from the explosion required to launch the bullet, thus preserving this residue while the remainder in the barrel is ejected out of the muzzle. It is not clear whether the makeup of the polymer has any direct effect on this phenomenon.

The distance at which ejected particles on target surfaces can be observed varies, but one trial has found polymer residue up to 18 inches away from the muzzle. Additional trials should be performed before a conclusion is made on distance determination.

Thus far, this research has found that the feed ramp of a firearm barrel is not only a viable target for polymer residue collection but is also more consistent in recovery than the barrel interior for this brand of ammunition. This may have implications for other types of polymer-coated ammunition as well and can be beneficial in shooting investigations.

### **P18. Quantitative Method for the Estimation of the Postmortem Interval by Micro-computed Tomography in Pig and Human Bones**

Emily Curtis; William Campbell, Ph.D.; Jason Brooks, VMD, Ph.D., DACVP, Pennsylvania State University; Linda Spurlock, Ph.D., Kent State University

This presentation will provide attendees with an understanding of the difficulties forensic investigators face when determining the time since death, or postmortem interval (PMI). The PMI is a critical component in any investigation involving human remains. Attendees will hear about the weaknesses of traditional methods of estimation and the importance of exploring new techniques for the accurate determination of the PMI. The research to be presented has the potential to greatly impact the forensic community as there has been little such investigation into the analysis of bones which are a persistent and versatile analytical tool for estimating the PMI. These data are expected to accelerate the investigative process, particularly in difficult cases involving mass graves, potential archaeological remains, skeletonized human and animal remains, and cases where determining the PMI as quickly as possible is paramount. This research aims to investigate the postmortem changes in bone composition and structure from death onward using micro-computed tomography (micro-CT) scanning. Micro-CT scanning has shown the potential to be an accurate method for quantitative analysis of bone attrition markers that may correlate with increasing PMI, as well as being a non-destructive method [1,2]. This presentation will review the analysis of both human and pig samples with variable PMIs via micro-CT in conjunction with a deep learning approach. In comparing these data between samples

and between species, this study may highlight quantitative changes in the bones as the PMI increases. These data will aid in the understanding of patterns of bone diagenesis and the potential for these patterns of attrition to be used to predict PMI in forensic casework.

### **P19. Forensic Discrimination of Monozygotic Twins Using a DNA Methylation Marker**

Sydney Arnold; Dino Robinson; Lissette Delgado-Cruzata, Ph.D., John Jay College of Criminal Justice

Monozygotic (MZ) twins possess identical DNA sequences and this poses a problem for forensic scientists, as they are incapable of distinguishing MZ twins from each other using traditional DNA identification methods. However, the epigenomes of MZ twins will differ and might be used for their discrimination in forensic settings. In this research, we explored a previously identified DNA methylation site on chromosome 3, cg18562578, that showed the highest difference in DNA methylation levels in trace amounts of saliva of one twin pair. To test whether this marker could be used to discriminate MZ twins using saliva DNA, we developed a methodology and determined DNA methylation levels in cg18562578 for 166 individuals, 129 MZ or identical and 37 dizygotic (DZ) or non-identical twin pairs. DNA extracted from saliva samples underwent bisulfite conversion and a 2-step qPCR method to quantify DNA methylation levels at cg18562578. To analyze the data, we determined the relative DNA methylation percentage using commercially available enzymatically methylated human DNA and the  $\Delta\Delta C_t$  method, and we calculated the absolute difference in percent of cg18562578 DNA methylation between twins in a pair. We found that MZ and DZ twin pair absolute differences were not statistically significant ( $0.39 \pm 0.29$  vs  $0.46 \pm 0.41$ ,  $p$ -value=0.38) suggesting that DNA methylation at cg18562578 is not directly related to genetics. In addition, DNA methylation differences between MZ twins, using the mean of the lower DNA methylation value in a pair versus the mean of the larger, were found to be statistically significant ( $0.39 \pm 0.30$  vs  $0.78 \pm 0.29$ ,  $p$ -value=0.00001) indicating that differences exist in DNA methylation within a MZ twin pair on cg18562578. In future studies, we will determine the levels of cg18562578 DNA methylation in a larger sample and assess the predictive value of this marker in identifying an individual within a twin pair. Our findings suggest this site has potential to be used in MZ discrimination.

### **P20. Investigating Gunshot Distance Determination on Skin as a Substrate and Possible Substitutions by Gunshot Residue (GSR) Patterns**

Jade Marshall; Carol Ritter; Lawrence Quarino Ph.D., Cedar Crest College; Peter J. Diaczuk, Ph.D., John Jay College of Criminal Justice

Distance determination from a gunshot residue pattern is valuable for both forensic reconstructionists and forensic pathologists. The determination of the distance between a target and the firearm's muzzle allows for a better understanding of the events that may have occurred at a scene involving gunshots. While there are no standardized methods for distance determination, crime laboratories typically use white cotton substrates to create exemplars when textiles such as clothing are shot. However, if the gunshot residue pattern is deposited on the skin, forensic scientists are not comfortable conducting distance determination because they have not been trained in skin substrates. A pathologist may be able to give a description like near-contact or distance, but typically do not create exemplars, resulting in no quantitative determination for distance.

The purpose of this study was to investigate the interaction of gunshot residue particles on skin compared to gunshot residue particles with other substrates with the hypothesis that substrate should not matter when analyzing a gunshot pattern to determine distance. Four shots at twelve inches with either 9 mm or .45 caliber ammunition were used to create GSR patterns at a constant distance of 12 inches from the substrate target. To simulate human skin, pig skin was one of the chosen substrates. In addition, 100% white cotton twill was used as it is the substrate utilized by most laboratories. Furthermore, faux silicon skin, deer skin, and sheep skin were used to see if they were suitable

substitutions for the skin. For examination of the patterns, manual measurements and image analysis were implemented. Manual measurements included the measurement of the outer diameter from the densest part of the pattern and the measurement of the furthest spread particles were taken for each sample. For image analysis, Image J particle analysis was utilized. To determine the best practice for Image J particle analysis DSLR, Dino-Lite Edge Digital Microscope, and Crime-Lite ML2 images were used and compared. Each image was cropped to have the similar field of view to keep the area of particles consistent. ANOVA and pairwise testing were utilized to be able to determine any significant differences between substrates and for any significant differences in images used. Most of the ANOVA tests showed significant differences for both how the substrate interacts with the gunshot residue and the image type used for particle analysis. The pairwise testing did not show a common trend of why significant differences were observed between substrates. All substrates showed a level of variance between them which may place current guidelines into question.

## **P21. Improving Presumptive Color Tests for Cannabis Products**

Marie Lemay; Ryan A. Smith; Nanette Wachter, Ph.D.; Ling Huang, Ph.D., Hofstra University

After attending this presentation, attendees will hear about modifications made to two of the presumptive color tests for cannabis products. The Duquenois-Levine test is the primary presumptive test used to indicate the presence or absence of cannabinoids in plant material with a blue/purple color, and the Fast Blue B test, found in the KN-S NIK Kit indicates the presence of THC with a deep red color. Both tests were optimized by substituting or removing toxic reagents.

The classical Duquenois-Levine test, which has been the preferred presumptive color test for cannabis products for over 50 years, utilizes various harmful chemicals. It was determined that the Levine step, adding chloroform (a suspected human carcinogen) was not necessary in obtaining adequate results. The Duquenois reagent was also modified to produce a different color result in the presence of cannabinoids. The original reagent is composed of three components: ethanol, acetaldehyde, and vanillin. The modified reagent substituted 4-hydroxy-3-methoxycinnamaldehyde for vanillin. After the addition of concentrated hydrochloric acid, the modified Duquenois test produced a deep green as opposed to the classical purple color. The Fast Blue B test was optimized by using either dimethylsulfoxide (DMSO) or water as the solvent for the Fast Blue B salt.

The interpretation of results from both tests was made semi-quantitative using UV-Vis spectroscopy. Using the squeeze-droplet design on the Nanophotometer NP80, only 0.3  $\mu\text{L}$  of the colored matrix was necessary to develop adequate absorbance peaks. This design minimizes the sample's contact with the environment during analysis and prevents evaporation.

Both tests were performed on a variety of sample matrixes containing different cannabinoids in addition to a positive control, marijuana plant material. Both tests successfully identified the presence of cannabinoids in samples such as gummies, oils, capsules, and beverages. The Fast Blue B test can differentiate cannabinoids in these samples using the UV-Vis data. It was found that THC absorbs at 490nm, CBD has an absorbance peak of 505nm, and HHC has an absorbance of 495nm. Both Duquenois tests were less effective in differentiating cannabinoids via their absorbance peaks but were more stable when compared to the optimized Fast Blue B test.

## **P22. Illicit Drug Desorption and Chemical Profiling of Fingerprints Using SICRIT Ion Source: A Rapid Analysis Approach**

Dr. Taylor Hayward; Ciara Conway; Jan-Christoph Wolf, Plasmion GmbH

Forensic toxicology is a multi-disciplinary field that encompasses a variety of analyses, from biological sample differentiation to illicit analyte identification. In order to analyze the variety, there are various sample preparation techniques that can be time-consuming, requiring multiple analytical instruments. The focus of this study centers around fingerprints and how to conduct targeted and non-targeted analysis of analytes on this complex matrix by combining novel instrumental and computational approaches. With recent advancements in ambient ionization mass spectrometry (MS), plasma-based dielectric barrier discharge ionization (DBDI) sources, such as the SICRIT Ion Source, have been demonstrated to cover a wide range of these analytes. This ionization source, in combination with thermal desorption sampling allows for a rapid analysis while minimizing sample preparation.

The study employed a high-resolution mass spectrometer to identify unknown compounds based on exact mass, focusing on three drugs (Fentanyl, Heroin, Cocaine) in varying absolute amounts. Direct thermal desorption of samples, completed in just 2 minutes, revealed ionization of all three compounds as protonated molecules. The limit of detection (LOD) for pure substances and spiked fingerprints, even with a complex matrix of lipids and amino acids, demonstrated sensitivity suitable for detecting trace amounts found in forensic samples. Although manual sample introduction and instrument limitations led to relatively high relative standard deviations (RSDs), the sensitivity was deemed sufficient for qualitative or semi-quantitative measurements. The technology's forensic potential expanded to differentiate individuals based on chemical fingerprint profiles, proving effective even for non-volatile compounds like lipids. Principal component analysis (PCA) and a machine learning pipeline demonstrated the ability to distinguish fingerprints from different individuals, even across multiple days, with promising accuracy. The study concludes that this ambient ionization technique has potential forensic applications, offering rapid and effective differentiation of individuals based on chemical composition, particularly in cases involving smeared fingerprints on crime scenes. Further investigation with diverse demographics and extended time frames is recommended to explore its suitability for broader forensic use and comparisons with public databases. The ability to assign smeared fingerprints to potential suspects presents a valuable tool for forensic applications, showcasing the technique's potential impact in crime scene analysis.

The study introduces a thermal desorption SICRIT Ionization setup for rapid forensic sample analysis, identifying drugs in fingerprints within 2 min. The method is suitable for mobile systems, offers low power consumption, and enables personalized fingerprint identification, beneficial for smeared prints. Thermal desorption SICRIT-MS provides comprehensive forensic information without complex preparation.

### **P23. Using Historical Biocultural Variables and Vitamin D Deficiency to Assist with Biological Profiles**

Kristy Henson Ph.D, Fairmont State University/University of Leicester; Jo Appleby, University of Leicester

The biocultural approach uses biological, environmental, and cultural variables to help identify where an individual spent their childhood or their adult life. When a skeleton is identified DNA may not be sufficient in identification and complete osteoprofiles are needed. This research examines historic skeletal, genealogical, and biocultural information to determine if there is a correlation with the presence of vitamin D deficiency. One hundred seventy-seven individuals living between the 1830s and 1940s were analyzed and traced through the historical record. Preliminary results indicate that there were trends in determining where an individual spent their childhood and the likelihood of vitamin D deficiency (chi-square  $p = 0.006$ ; Spearman RS 0.92,  $p = -0.008$ ), year of death (chi-square 0.005; Spearman RS 0.87,  $p = -0.01$ ), presence of spinal pathology (chi-square  $p = 0.05$ ; Spearman RS 0.72,  $p = -0.03$ ), and trending toward age ( $p = 0.08$ ). More research is warranted but completing biological profiles using biocultural variables may help identify where someone is from and how they lived their lives which in turn will assist with identification.

## **P24. Detection of Diethylene Glycol and Ethylene Glycol Using HPTLC**

Gabrielle Marzigliano, Cedar Crest College

Diethylene glycol (DEG) and ethylene glycol (EG) are clear, odorless, and sweet tasting liquids. Both DEG and EG are physiochemically similar to nontoxic and more expensive glycols, such as glycerol and propylene glycol (PPG). Due to this, DEG and EG are sometimes used as a cheaper alternative for PPG in pharmaceutical products such as toothpaste, injectable drugs, antipyretic medication, and cough syrups. Due to their high levels of toxicity, there is no approved use for DEG and/or EG in these products and illegal use of these glycols in these products has led to many incidents of human poisonings in underdeveloped countries where lapses in quality control often occur. Currently there is no cost-effective method of detection for DEG or EG to determine whether a batch of products is safe for human consumption or not. There also is not a method that distinguishes between DEG and EG. The goal for this project is to find a cost-effective and easy way to detect these compounds in these household and pharmaceutical products using the HPTLC (high performance thin-layer chromatography) and to find a solvent system that distinguishes between DEG and EG. A successful separation of the DEG and EG occurred using a mobile phase system of ethyl acetate, methanol and ammonium hydroxide (85:10:5) with potassium permanganate as the spray reagent. Further research is currently being conducted to determine if potassium permanganate is the best spray reagent to use for detection. The successfulness of this experiment could prove helpful for poorer countries in preventing further poisoning and death due to diethylene and ethylene glycol consumption.

## **P25. Considerations in Using Teaching Skeletons to Train Forensic Practitioners**

Jay Bow; Kristy Henson, Ph.D., Fairmont State University

Human skeletal remains have been used for teaching human anatomy for centuries, giving students a hands-on, individualized experience in human osteological analysis. Due to high demand, millions of individuals (primarily from India) have been prepared and used as teaching skeletons. Usually, these individuals' skeletons are obtained unethically due to the high demand driving the sale price of skeletons up. Many countries have since limited the sale of human remains due to these ethical issues, with three US states restricting their sale: Louisiana, Georgia, and Tennessee. All other states have no restrictions other than the federal NAGPRA statute that applies to Native remains. Today, teaching institutions have increased the use of plastic models and replicas to replace unethically sourced antique skeletons. However, these models are criticized for failing to reproduce features important to osteological identification, profiling, and pathology. In addition, these institutions often still keep their antique skeletal collections due to the lack of knowledge regarding how to dispose of or repatriate them. Overall, forensic field practitioners may need the skills only obtained with the study of actual skeletal remains even though the unethical history of most teaching skeletons complicates their use, and other alternatives, such as archeological and modern donated skeletal remains, are often inaccessible. For some forensic practitioners, the ability to recognize bone and identify it as human or not is an important skill for field work which is usually obtained using teaching skeletons. Despite being overlooked and anatomized, teaching skeletons are the remains of actual individuals, and therefore deserve more consideration by those who benefit from their use and previous mistreatment.

## **P26. Effects of Environmental Factors on Rapid Shear in Fibers of a Polyester-Cotton Blend**

Daniel Oh; Peter J. Diaczuk, Ph.D., John Jay College of Criminal Justice

Rapid shear is a process that occurs in thermoplastic materials, such as synthetic fibers, caused by a high-velocity impact. The impact causes a phenomenon, due to heat generated from the friction, by forming a mushroom-shaped bulb at the damaged end in such fibers. In forensics, a common cause of such high-velocity impacts might be that of a bullet. An experiment was performed where polyester-cotton sheets were subject to various simulated

environmental factors, such as varied temperatures and different levels of wetness, and shot using an air rifle to undergo rapid shear. Polyester-cotton was selected as it is one of the most common fabric materials used in clothing not only separately in their specific categories, but also as a blend of synthetic and natural fibers.

Three sets of variables were done along with one control set. Each set consisted of two sheets of fabric, each sheet being shot five times. One set consisted of a sheet soaked in water in a ratio of 1:1.5 fabric to water by weight, another set used a 1:1 ratio of fabric to water, and the last set was a chilled sample.

A sheet of polyester-cotton would be cut out to fit within a mount that was placed 15 feet away from the end of the air rifle's barrel. A chronograph was set 5 feet away from the barrel's end to measure the velocity of the projectile before it hit the sample. Before every shot, the room temperature was recorded with a thermometer, and the fabric's temperature was measured using an IR thermometer. A laser was attached to the rifle before every shot to accurately aim where the projectile would hit the fabric to make sure the damage wasn't too close to each other, preventing fibers from being pulled out. The shots were fired from top left, top right, middle, bottom left, and bottom right.

The wet samples were first cut to size and weighed on a balance before weighing water within a beaker that was proportional to 1:1 and 1:1.5. Each sheet would then be placed into the beaker to soak in the water. The sample was then transported to the mount and the sample was immediately mounted to prevent the sample from drying too fast. The chilled samples were soaked in water, wrung out before being stored in ziplock bags, and placed into the freezer for two hours. When the samples were ready, the bag was moved directly into a styrofoam icebox before transporting it directly to the mount to be set up and shot to prevent the sample from rising too much in temperature.

After the samples were shot, they were dried for 2 hours, if needed, folded to protect the damaged areas, and stored within ziplock bags (left open to keep dry). The samples were then observed under a stereomicroscope and polarized light microscope to find signs of the mushroom-like structures on the damaged ends of the fiber and identify which fibers these structures occurred on.

## **P27. Nuclear DNA Analysis From Contact Lenses in Crime Scenes**

Hannah Calista; Caitlyn Martinez; Ryan Smith; Deborah Silva, Ph.D., Chemistry Department, Hofstra University; Georgiana Gibson-Daw, Department of Arts and Sciences, Western New England University

DNA is often extracted from biological material transferred from a donor to an object during physical contact, commonly referred to as "touch DNA." These samples typically yield low quantities of low-quality DNA, yet they are vital tools in forensic investigations. Successful collection and processing of touch DNA are essential for effective downstream analysis. Touch DNA can present challenges, such as low copy numbers when the contact duration is brief or when the surface of the item hinders cell deposition. Additionally, these samples are susceptible to environmental degradation. Samples such as used contact lenses are examples of touch DNA samples but they are rarely analyzed due to the difficulties in processing and obtaining viable DNA profiles. While numerous studies have focused on improving collection techniques for various touch DNA samples, research on contact lenses remains limited. This study aimed to evaluate the effectiveness of different collection methods for extracting nuclear DNA from contact lenses. For this experiment, one volunteer wore their contact lenses for approximately 12–14 hours. Afterward, the lenses were placed in a mock crime scene on a solid surface at room temperature and natural light. After 24 hours, the lenses were collected using sterile forceps and placed in microtubes with various solutions. The samples were processed in the laboratory, and the results were analyzed. Sufficient DNA quantities were recovered, allowing for the amplification of short tandem repeat (STR) markers. The findings suggest that contact lenses can serve as valuable evidence, as they are a potential source of DNA to generate informative profiles in forensic investigations.

## **P28. Discovery of False Positive Illicit Drug Identification with Portable Surface Enhanced Raman Spectroscopy (SERS)**

Ella Galvan; Desmond Brown; Brooke W. Kamrath, Ph.D., University of New Haven, Don Ostrowski; Pauline E. Leary, Ph.D., Noble; Richard Crocombe, Ph.D., Crocombe Spectroscopic Consulting

For several decades, the standard procedure for testing suspected controlled substances in the field involved the use of color-based or colorimetric presumptive tests. Although color tests are quite sensitive with low limits of detection, they have several recorded limitations, including a lack of specificity which means they suffer from false positives. A recent publication by the Quattrone Institute<sup>1</sup> reported:

“Each year approximately 773,000 drug-related arrests involve the use of presumptive tests. Although the true error rate of these tests remains unknown, estimates based on the imperfect data that are available suggest that around 30,000 arrests each year involve people who do not possess illegal substances but who are nonetheless falsely implicated by color-based presumptive tests.”

The availability of robust portable spectrometers has great promise for addressing some of the limitations of field color tests. The majority of instrumental methods used in the forensic analysis of illicit drugs in the laboratory have field portable versions, which includes the SWGDRUG and ASTM identified confirmatory methods of Raman spectroscopy, Infrared spectroscopy and mass spectrometry<sup>2,3</sup>. However, portable spectrometers may have meaningful differences in their performance characteristics when compared to their benchtop counterparts, which must be evaluated and understood prior to deployment of these instruments in the forensic field.

The major advantages of Raman spectroscopy for the identification of illicit drugs are its rapid, non-destructive, non-contact (e.g. through glass and plastic containers) analysis which generates a reviewable record (i.e., a spectrum) with very high discriminating capabilities. When paired with surface enhanced Raman spectroscopy (SERS), a technique which enhances Raman scattering through molecular interactions with rough metal surfaces or nanostructures, there can be a meaningful improvement in the limits of detection for illicit drugs to include trace-level detection for analytes of interest like fentanyl, heroin and cocaine.

While evaluating a commercially available handheld narcotics analyzer (a Raman spectrometer equipped with a 785-nm laser) in SERS mode for the detection and identification of low dose fentanyl mixtures, a false positive for cocaine was identified when the fentanyl was dispersed in an acetaminophen matrix at low concentrations. Other matrices evaluated in the same manner did not generate this false identification. The SERS kit uses a gold nanoparticle infused substrate in a polycarbonate holder, and a solvent for dissolving a sample of interest. It is critical for police officers and other first responders who are using this commercially available technique for the field identification of suspected controlled substances to be aware of this reproducible false positive identification of cocaine, and its potentially hazardous repercussions. This resulting false positive for cocaine and absence of identification for fentanyl will inevitably complicate subsequent laboratory testing, has implications for safety, and may have important legal ramifications.

## **P29. Chromatographic Separation of Eleven Antidepressants and Antipsychotics Utilizing LC-MS/MS**

Tyler Vidal; Meghan Smith; Ashley Tinervia; Madelyne Salgado; Christopher Pelc; Constantinos Pistos, West Chester University of Pennsylvania, Department of Chemistry; Garrett Fong, West Chester University of Pennsylvania, Department of Biology; Lisa Mundy, Philadelphia Medical Examiner's Office

Mental health disorders increasingly contribute to the world's mortality, and as a result, antidepressant and antipsychotic drugs are commonly involved in fatal cases. This has led the forensic laboratories to the need for a rapid



method for the determination of the above drugs, to facilitate the investigation of toxicological cases.

The aim of this study was to develop a rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method, for the separation and simultaneous determination of eleven selected first/second generation antidepressants (amitriptyline, citalopram, cyclobenzaprine, doxepin, fluoxetine, mirtazapine, nortriptyline, sertraline), one of their metabolites (nordoxepin), and second-generation antipsychotics (olanzapine, quetiapine). Target precursor and product ions (MRM ion transitions) were optimized by injecting standard reference materials into the LC-MS/MS system and analyzing via Flow Injection Analysis (FIA). The detection was achieved using an Agilent Jet Spray Ionization source operating in the positive mode. The separation was optimized by examining various isocratic and gradient programs, using ammonium formate 10 mM/formic acid 0.1% (solvent A), and acetonitrile or methanol with 0.1% formic acid (solvent B), different temperatures of the analytical column, and different analytical columns (biphenyl, 2.7 $\mu$ m, 50 x 3.0mm; SelectraCore AD 2.7 $\mu$ m, 50 x 2.1mm; Poroshell 2.7 $\mu$ m, 100 x 2.1mm).

The method demonstrates simplicity, sufficient separation, and specificity of the analytes which makes it suitable for further development. The next steps will include the optimization of the extraction method from whole blood, and the method validation. Using this method, forensic laboratories may take advantage of the high throughput analysis of LC-MS/MS, instead of using GC-MS, assisting the reduction of backlog cases, at comparable cost, and providing shorter time of analysis.

### **P30. Evaluation of DNA Extraction Methods**

Katie Long; Mark Flood; Kristy Henson, Ph.D., Fairmont State University

Three forms of DNA extraction types include solution-based, column-based, and magnetic bead-based methodologies, with the latter being the accepted methodology used in forensic laboratories. Magnetic bead-based DNA extraction uses the negative charge of DNA and the positive charge of the magnetic beads to isolate the DNA strands and produce a purified sample. However, solution and column-based extraction methods use the same techniques of washing to purify DNA and typically have comparable DNA yields. Cheek swabs were obtained from ten individuals and the three extraction methods were performed to determine the quantitation of double-stranded DNA and the quality of the gel electrophoresis profile. Preliminary results indicate that solution-based extraction produces a slightly higher yield of double-stranded DNA having a 3.92% difference between the average quantitation of 26.29 ng/ $\mu$ L from solution-based and 25.28 ng/ $\mu$ L from magnetic bead-based methods. However, it is expected that the magnetic bead-based extraction will produce higher quality gel electrophoresis results. Overall, the methods examined produced similar DNA extraction efficiencies.

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### **P31. The Effects of Morphology and Intermediate Targets on Bullet Path**

Natalia Aguilar, Peter Diaczuk, Ph.D.; John Jay College of Criminal Justice

The estimation of a bullet's path is an incredibly valuable tool for the reconstruction of shooting events. As such, a successful reconstruction depends on several variables including, and not limited to, bullet grain, morphology, and intermediate substrates. Current studies focus majorly on sheet metal as a target material due to its frequency, particularly in urban shooting scenes. Few studies investigated the changes in behavior due to differences in bullet morphologies and, of those, only one considered hollow point bullets (Liscio & Imran, 2020).

This investigation explores the difference in behavior between 9mm full metal jacket (FMJ) and jacketed hollow point (JHP) bullets through ½” drywall targets. Half of the targets were made with two 2x4” studs between panels of drywall. Targets were shot at varying angles, from 90 - 50 degrees, at 10 degree increments. Data was gathered by measuring the distance between reference bullet holes and those made after bullets had passed through a target. Preliminary results show that the standard deviation of the distance between bullet holes and their respective reference point increased by more than 100% for all but one angle measured. While the angle of the drywall seems to have no discernible effect on bullet path, the imposition of studs has significantly affected the standard deviation of distance from the reference shot making the bullets’ paths more challenging to track. Further research will focus on investigating other angles (40, 30, and 20 degrees) as well as how bullet morphology (FMJ vs. JHP) alter a bullet’s path through interior walls.



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