FINAL RESEARCH REPORT

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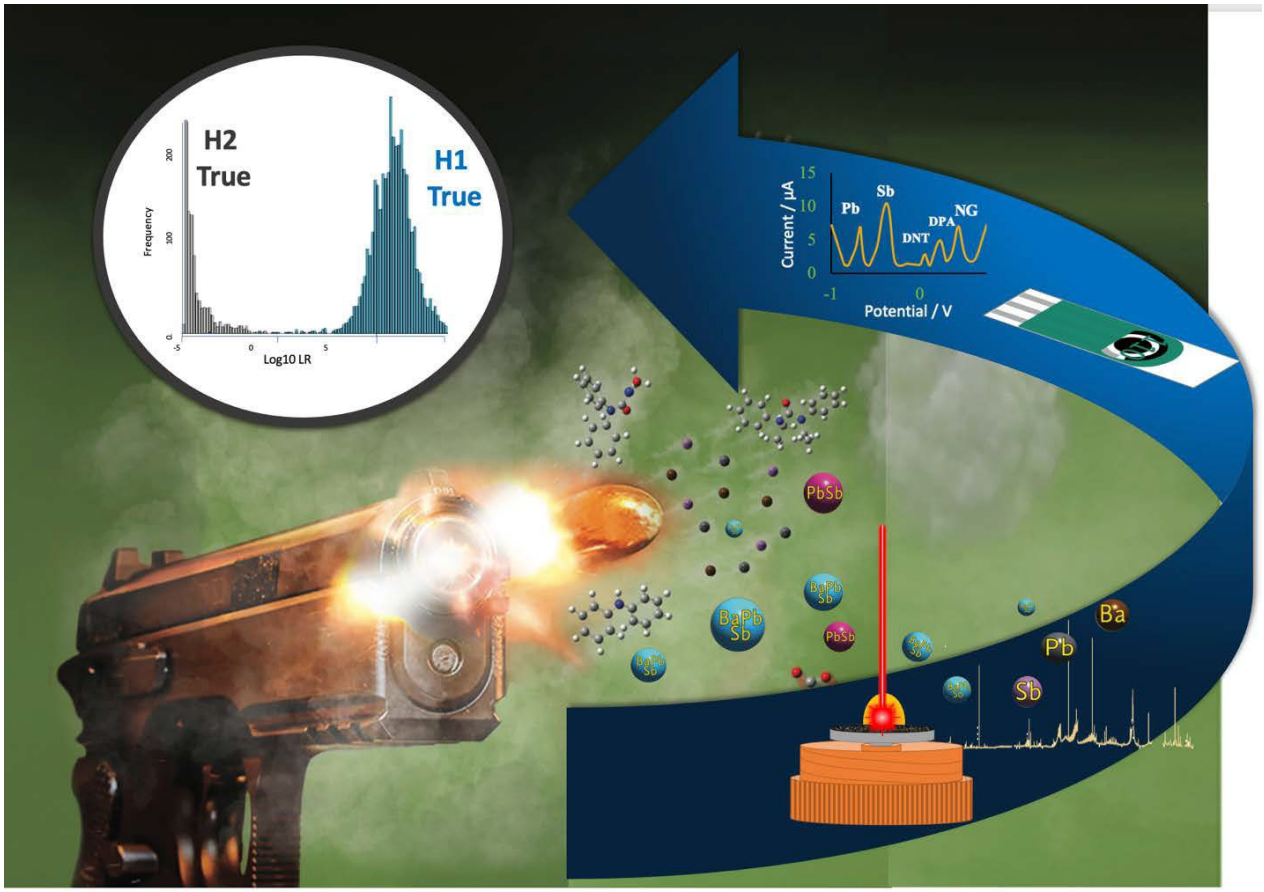
FINAL RESEARCH REPORT

Fast screening of firearm discharge residues by laser-based spectrochemical methods, electrochemical sensors, and chemometrics



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I PROJECT SUMMARY

1.1. Abstract

Gun violence is a global issue affecting public health. The alarming rise in the number of deaths by homicide, suicide, and mass shooting in the United States is becoming a pandemic.[1] Improved approaches for prevention, prediction and policy are necessary to address this growing crisis. From law enforcement and criminal justice perspective, technological advances to achieve faster responses and more efficient evidence interpretation can significantly contribute to safer communities.

The detection of gunshot residue (GSR) provides valuable information in violent crimes, accidental shootings, and terrorism. Despite the scientific validity of this discipline, there are persisting challenges regarding the speed of analysis, preservation of the evidence, and interpretation of results. Consequently, there is a critical need to improve the discipline's turnaround times and reliability.

Our overall purpose was to develop a comprehensive approach to overcome these significant concerns and enhance the criminal justice system capabilities. This project developed and validated fast and reliable tests, using LIBS and electrochemical (EC) sensors for GSR detection. Also, statistical models were applied for the quantitative interpretation of the evidence. The combination of LIBS and EC data permitted the accurate identification of organic and inorganic residues (OGSR & IGSR, accuracy ranging from 92-99% depending on the subpopulation and classification models). Our research focused on developing SMARTER (Simpler, Modern, Affordable, Rapid, Transformative, Effective, and Reliable) solutions for GSR examinations. We have achieved that through the following main contributions (see **Figure 1**):

1) *Application of universal and expanded collection methods*—our approaches used universal collection methods compatible with current practice, facilitating adoption. Moreover, the technique's utility and versatility were demonstrated for several substrates commonly found in firearm related cases, such as hands of individuals of interest, clothing, and large non-movable objects.

2) *Development of novel, ultrafast methods for dual detection of IGSR and OGSR*—this study provided, for the first time, selective and sensitive GSR screening tools (LIBS and EC) that can generate results in under 5 minutes as opposed to the 4-20 hours per sample needed with the standard methods. Another advantage of these techniques is the detection of both IGSR and OGSR compounds, while leaving the sample intact for further testing.

3) *Development of modern 3D chemical imaging for crime scene reconstruction*—LIBS identified a more substantial number of elements used in modern ammunition and generated 3D-chemical images of the spatial distribution of GSR for more objective estimations of shooting distance, identification of bullet holes, and rapid scanning of large areas. These assays are expected to modernize current methods and offer a cost-effective strategy to complement traditional tests widely utilized by forensic laboratories and law enforcement.

4) *Development of novel micro-particle GSR standards*—This study also enhanced existing capabilities by producing tailor-made microparticle GSR suspensions used for the quality control of GSR analysis, validation of existing and emerging methods, and as ground truth for some of the ammunition characterizations. To characterize the elemental composition and particle morphology, the IGSR microparticles standards were evaluated by various analytical techniques. The standard demonstrated stability in its dry and suspension forms, providing versatility for use in multiple types of analytical methods and substrates.

5) *Creation of a large population study and probabilistic interpretation framework*—This study created an extensive one-of-a-kind database of organic and inorganic gunshot residues using a multi-technique approach (SEM-EDS, EC, and LIBS) on various populations of legitimate shooters and background non-shooters. The sample sets included complex scenarios such as those individuals that may represent a high risk for occupational/environmental false positives, individuals who have conducted post-shooting activities, and shooters who have used mixed leaded and lead-free ammunition. The dataset consists of over 100,000 data files of GSR's chemical profiles. Relative occurrence of IGSR and OGSR components of modern ammunition is made available for various populations. Also, we applied ground-breaking statistical methods to interpret GSR evidence using artificial intelligence (neural networks) and likelihood ratios to estimate the weight of the evidence, proving substantial progress to conventional categorical approaches.

The results show that the use of fast emerging methods and the OGSR/OGSR data derived from this large population study, combined with probabilistic interpretation, can provide more comprehensive tools for assessing GSR evidence. These approaches not only can streamline processes but, most importantly, open new venues to responding to the court-relevant questions regarding if a person of interest fired a gun. Altogether, this research's overall framework and interconnected objectives offer a significant leap of knowledge in this field and are anticipated to promote the modernization of current practice.

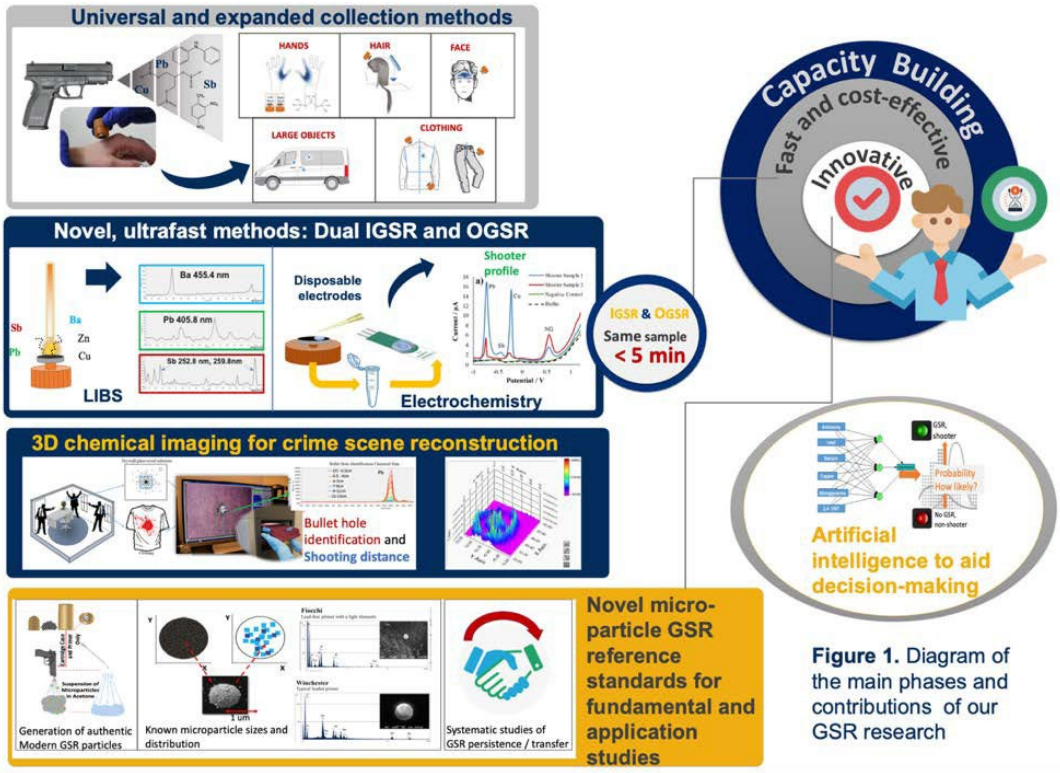

Figure 1. Diagram of the main phases and contributions of our GSR research

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1.2. Problem statement

Innovations in forensic science are crucial for advancing the administration of justice, homeland security, and public safety. From the 1980s until the early 2000s, forensic research focused on laying the scientific foundations of methods that are now widely applied across law enforcement agencies. One of the most significant advances in this field was the advent of DNA methods, which can identify a person of interest and answer questions such as who may have committed a crime or who was the victim. On the other hand, materials that are often invisible to the naked eye, also known as trace evidence, are essential to answer other relevant questions, like how and when the materials were transferred, where the sample came from, and what may have occurred during the crime.[2] Thus, trace evidence plays a significant role in criminal investigations to link places, people, and objects to a crime. One example of these relevant traces is gunshot residues (GSR), which often escape from a firearm during a shooting event, and are deposited on the target and surrounding surfaces, such as the shooter's hand and other objects.[3]

With over three-hundred thousand shooting incidents a year, gun violence affects the U.S. society like no other part of the world.[1] Thus, research advances in this field directly impact how safety and justice are maintained in our communities. Developments in mass spectrometry, spectroscopy, laser-based technology, and computer science are examples of tools that have greatly influenced our capabilities to identify these traces.[4-18]

More recently, forensic science research has shifted towards the development of technology that can lead to more cost-effective and faster solutions while increasing the data's objectivity and reliability. More than a decade ago, the National Academy of Sciences (NAS) and the President's Council of Advisors on Science and Technology (PCAST) raised awareness of the need for reporting error rates and uncertainties associated with subjective analysis in forensic science.[19,20]

Rapid and accurate detection of firearm discharge residues is highly desirable in circumstances that require fast response to protect the welfare of citizens and reliable information to make informed decisions. Quick screening methods for gunshot residue (GSR) are particularly useful, but currently difficult, in cases involving shootings of multiple victims and suspects. Regardless of the existing standardization of current analytical assays, there are still challenges in this area regarding the speed of analysis, preservation of the evidence, accuracy, and interpretation of results.[4,5,21,22]

For instance, crime-fighting technology such as real-time acoustic detection is now implemented in over 100 U.S. cities to detect, locate, and immediately alert police of a gunshot activity.[2,3] These systems have proved to be effective in providing tactical information to attend a crime scene quickly, intercept the potential shooter, and collect key evidence. However, forensic methods for the detection of gunshot residues do not match the speed of these crime-fighting tools. It currently requires days to weeks to confirm the presence or absence of GSRs and months to submit a report, limiting the efficiency of the investigations.[24]

Moreover, GSR is prone to contamination and short-term persistence. Elements such as lead, barium, and antimony are known to transfer to the hands of the shooter and target materials and are used to draw inferences about the handling of a firearm, or presence near a discharged firearm.[4,5,26] In recent years, examiners have recognized that the sole detection of inorganic species may not be sufficient to confirm the presence of gunshot residues due to the prevalence of these elements in the

environment.[27-36] For instance, these elements can be found in paints, grease, sunscreen, printing inks, batteries, matches, and solder, to mention some. Also, GSR-like particles can be formed during the deployment of vehicle airbags and brake linings. On top of this, GSR can be easily lost after common post-shooting activities and environmental conditions, and new toxic-free ammunition requires expansion of current analytical capabilities for the identification of more diverse components.[2-4]

Particle imaging and elemental analysis by SEM-EDS is the standard for confirmation of IGSR (ASTM E1588-20).[37] However, the method is limited to detection of IGSR, not apt for field analysis, and requires highly trained personnel. The SEM-EDS requires several hours to scan a $1cm^2$ area and therefore is mostly restricted to the study of hand residues because the examination of other specimens with larger areas is prohibitively time-consuming. Although the scientific validity of SEM-EDS for IGSR detection is well established, the methods for identification of decision thresholds are mostly based on binary decisions and not fully standardized. Thus, the community would benefit from data that can evaluate and enhance the accuracy of current practice.

On the other hand, in cases involving identification of bullet holes and shooting distance determinations, the pattern and distribution of the gunshot residues are typically inspected by visual examinations and color tests. Although color reactions enhance the visibility of the gunshot residues, the colorimetric tests are very subjective and have known limitations such as poor selectivity, fast fading of the color, and instability of the reagents. For instance, the detection of nitrites by Griess test and lead by the sodium rhodizonate test are susceptible to interferences that can lead to false positives and false negatives.[38,39] There are circumstances in which the color of the fabric, the degradation of the material, and the presence of dirt and blood can mask essential features. Furthermore, the rhodizonate test is ineffective to detect lead-free ammunition.

Also, the endeavor of building consensus-based guidelines and methods that demonstrable error rates is a challenging one. As a result, several scientific groups have recognized the need to merge knowledge from multiple disciplines, including forensics, statistics, mathematics, law, management, and psychology, and capturing different perspectives from practitioners, academia, and industry.

Observant of this critical trend, this project applied the combined expertise of forensic practitioners, researchers, statisticians, and industry for undertaking research that responds to fundamental demands in the field of gunshot residues. The long-term goal of this study is to develop a comprehensive approach to overcome these significant concerns in GSR detection and to improve current capabilities in the criminal justice system. This research focused on building capacity through the development of:

a) Innovative methods that are faster, more objective, informative, and applicable to laboratory and on-site crime scene settings for more effective decision-making processes;
b) Encompassing interpretation models that can better use the data to assist in a more accurate assessment of the significance of the findings at investigative stages and in the courtroom.

This study aims to address the following needs for a) complementary practical methods that allow the detection of IGSR and OGSR, b) rapid screening of GSR, c) extended capabilities detection for modern ammunition, d) sampling protocols compatible with existing standard methods, e) versatile tests for analysis of a variety of specimens, and f) the probabilistic assessment of decision thresholds and interpretation of the evidence. The sections below describe the goals, tasks, and experimental design used in this study.

1.3. Major Goals and Objectives

The overall goal of this project was to develop a comprehensive strategy to enhance the reliability of the analysis and interpretation of firearm discharge residues (FDR). Our central hypothesis was that Electrochemical Detection (EC) techniques in combination with Laser-Induced Breakdown Spectroscopy (LIBS) will provide screening methods that are faster, more selective, and more informative than current laboratory-based and field tests.

The primary aims of this project were:

1) **Objective 1:** develop versatile and fast methodologies for FDR detection on hands and other target materials, and
2) **Objective 2:** apply statistical methods for the probabilistic assessment and interpretation of the evidence.

These goals were accomplished through the following specific tasks (**Figure 2**):

1) **Task 1.** Collect a large sample set of firearm discharge residues from target materials (fabrics), hands of shooters, and background data.
2) **Task 2.** Validate LIBS and electrochemical sensors for estimation of shooting distance and FDR detection on clothing.
3) **Task 3.** Validate screening methods (LIBS and ECD) and confirmatory methods (Scanning Electron Microscopy-Energy Dispersive Spectroscopy, SEM-EDS) for Inorganic Gunshot Residue (IGSR) and Organic Gunshot Residue (OGSR) detection on hands of potential shooters.
4) **Task 4.** Validate statistical data pre-processing, multivariate analysis, and machine learning algorithms to generate decision thresholds and probabilistic approaches for interpretation of the significance of the evidence.

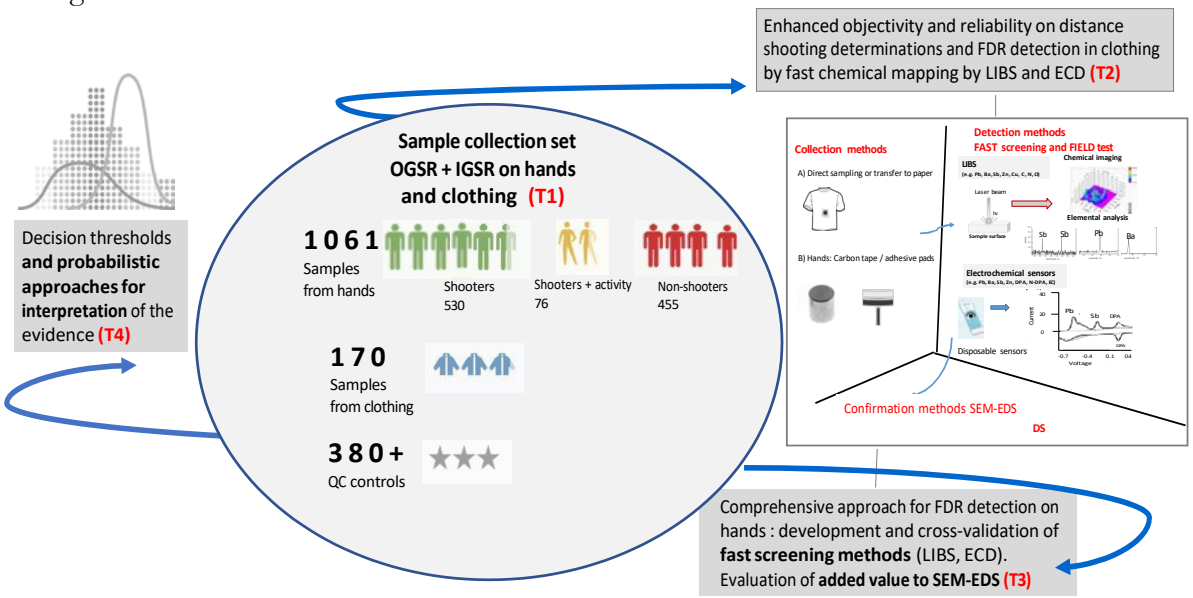

Figure 2. Overview of the primary task/outcomes of the proposed approach to enhance the reliability of FDR identification. (T=task outcome).

1.4. Research Design, Methods, Data Analysis

1.4.1. Methods of analysis

This project involved multiple analytical techniques and methods for the different tasks. First, the study developed and validated two emerging analytical tools for analyzing GSR, LIBS, and Electrochemistry-Screen-printed carbon electrodes (SPCEs). Second, alternative confirmatory methods were employed to cross-validate results, including SEM-EDS, LC-MS/MS, LA-ICP-MS, and acid digestion-ICP-MS. The instrumental parameters of each method are described below, and more details of the methodology and experimental designs can be found in the published manuscripts.[4,10-18]

1.4.1.1. Instrumental analysis by LIBS

A J200 Tandem LIBS system (Applied Spectra, CA) equipped with a 266 nm, high-power, Q-switched, Neodymium doped Yttrium Aluminum Garnet (Nd YAG) nanosecond laser was used for analysis. The instrument detector optics and sensor consisted of a six-channel Czerny-Turner spectrometer with a spectral range from 190 to 1040 nm and a Charge Coupled Device (CCD) based broadband detector, respectively.

The LIBS' versatility allowed the development of multiple applications for GSR detection. **Figure 3** illustrates the use of LIBS for GSR detection from hands (left side). The hand is stubbed using standard collection methods; then, the carbon stub is placed directly inside the ablation chamber, where the laser beam is fired at 25 different microscopic locations. Simultaneous elemental detection particle(s), such as PbBaSb, are then confirmed and visualized using micro-spatial 3D chemical mapping. The LIBS can also be coupled to ICP-MS for LA-ICP-MS mapping. The right side of the figure illustrates the use of LIBS for scanning larger surfaces such as clothing or tape lifts of non-movable objects. The ablation chamber allows the direct stretching of large surfaces on a platform. The laser beam is scanned using various patterns around the bullet hole to identify GSR and estimate shooting distances based on a statistical analysis of the chemical patterns.

Parameter's optimization was conducted following a response surface Box–Behnken design for multiple factors and levels. **Table 1** summarizes the optimized instrumental parameters used for GSR detection on carbon stubs from a person's hands, clothing, and hard objects (shooting distance estimations), and non-movable objects for bullet hole identification.

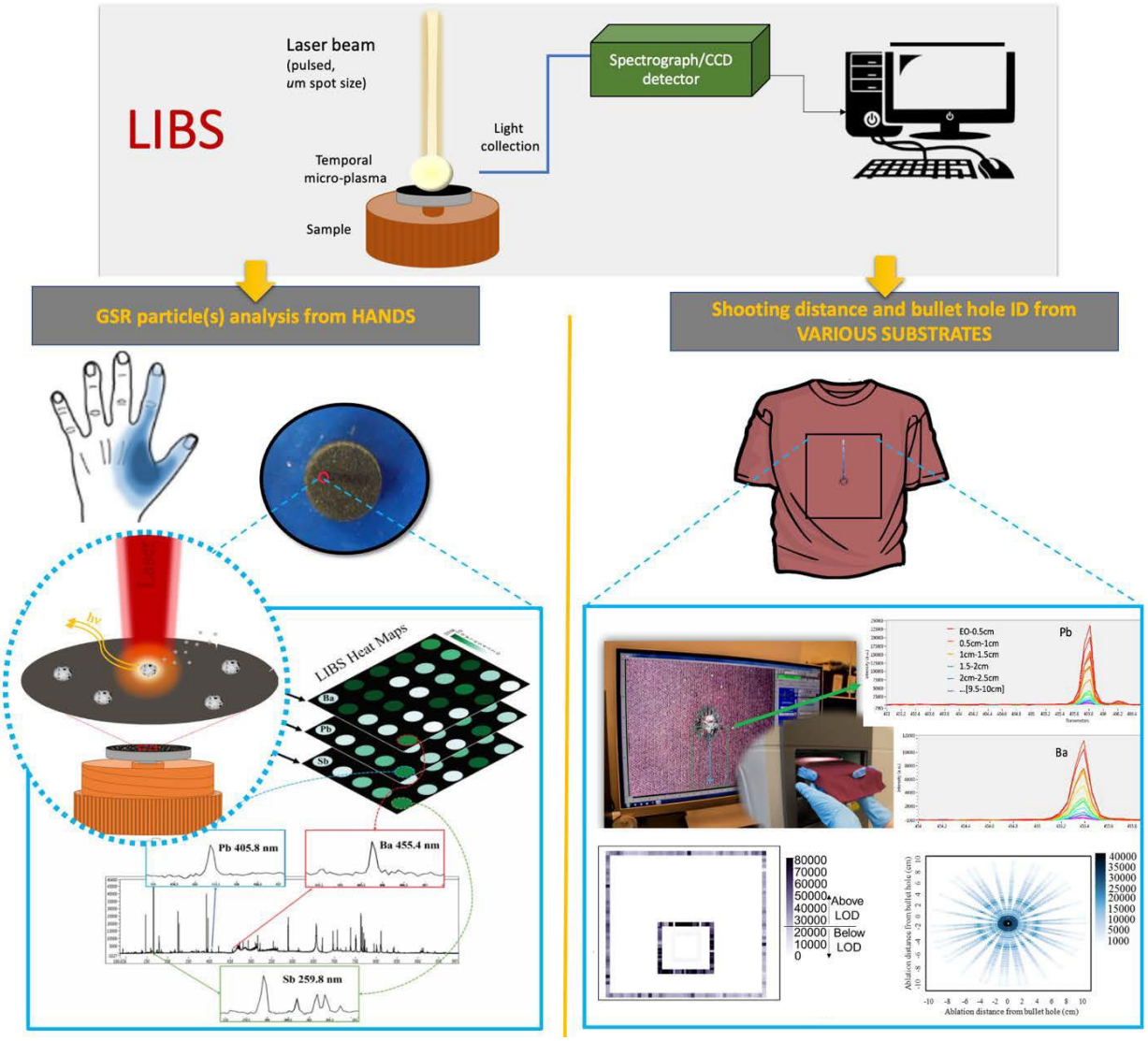

Figure 3. Schematic of LIBS methods for GSR particle(s) analysis from a person's hands using carbon stubs and fast chemical mapping at 25 micro-locations (left), and from various surfaces to identify GSR around a bullet hole and estimate shooting distances based on statistical analysis of chemical mappings of Pb, Ba and Sb around the entrance hole.

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Table 1. LIBS optimized parameters used for the ablation methods validation study

Parameter	Microbulk-Line Method for Hands' stubs	Micro-Mapping Method for Hand's Stubs	3D Mapping Method for Shooting Distance	Mapping Method for Bullet Hole Identification
Ablation mode	Line scan	Single spot	Line scan	Line Scan
Spot Size (μm)	100	100	100	50
Gate delay (μs)	0.5	0.5	0.5	0.5
Laser Pulse Frequency (Hz)	10	5	5	10
Stage Velocity (mm/s)	0.15	1	0.3	1
Line Length (mm)	7	n/a	100 (20 segments of 5mm each)	60mm, 10mm and 5mm square pattern
Average Laser Output Energy (mJ)	14.7	15.5	10	10
Gas Flow Rate (L/min)	Argon, 1	Argon, 1	n/a	n/a
Number of Shots	469	2	2 per spot, 85 per segment	2 per spot, 160, 40 and 20 per squared pattern
Accumulate Data	Yes	Yes	Yes	Yes
Total Spectra Collected	1	25	20 (1 per segment)	280 per mapping
Acquisition Time (sec)	55	91	333	320
Ablation chamber	Custom stage platform with center hole to mount the SEM stub pin, argon flow.		Custom stage platform with magnets to stretch and hold fabric or tape lifts, air environment	

1.4.1.2. Instrumental analysis by electrochemistry

Screen-printed carbon electrodes model type DRP- 110 were purchased from Metrohm DropSens, USA. Electro- chemical measurements were carried out using an Autolab PGSTAT128N potentiostat along with the NOVA software, version 2.1.4, from Metrohm USA, Inc. A Metler Toledo FiveEasy (Columbus, OH) pH meter was used for determining pH values. Analysis of samples was achieved first through the application of −0.95 V potential, used as a preconcentration step to deposit the analytes in their reduced form at the surface of the working electrode.[10,15] Following this preconcentration step, a square-wave procedure was used to sweep the potential between −1.0 and +1.2 V to strip analytes from the surface and obtain the oxidation peaks of the analytes of interest. The parameters used for the SWASV analysis can be found in **Table 2.** This procedure was utilized for the construction of calibration curves for each analyte of interest.

Several quality control (QC) samples were analyzed at the start and end of each analysis period, including blank buffer, negative stub control (a carbon stub not used or exposed during sampling), IGSR standard mix, and OGSR standard mix. **Figure 4** illustrates the main steps for the electrochemical analysis.

Table 2. SWASV parameters for analysis of GSR with SPCE

Parameter	Value
EC Technique	SWASV
Deposition potential	-0.95 V
Deposition time	120 s
Start potential	-1.0 V
End potential	1.2 V
Step	0.0004 V
Modulation amplitude	0.025 V
Frequency	8 Hz
Interval time	0.125

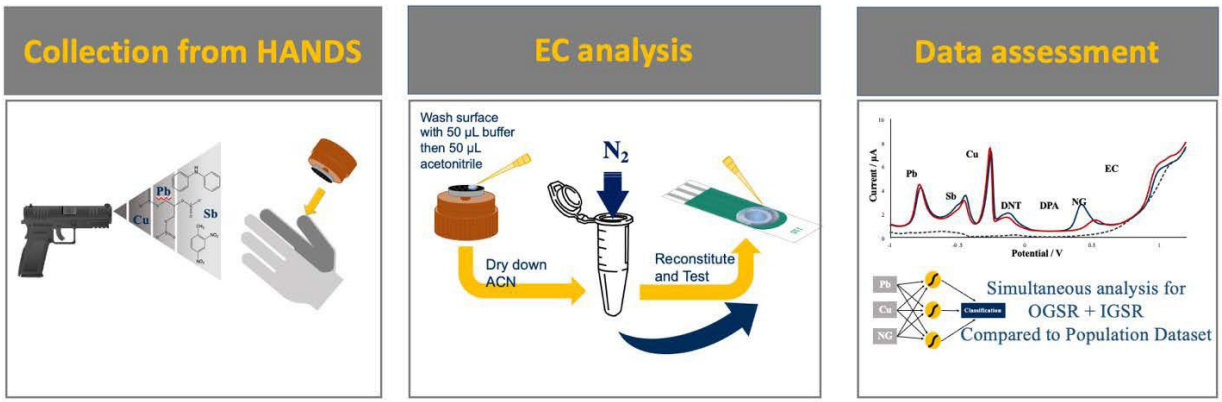

Figure 4. Main steps for EC analysis. 1) Samples are collected from the hands of a person with carbon stub, then analyzed by LIBS, 2) Some of the carbon stub sampled with micro-washes of buffer and acetonitrile, then dry down with nitrogen and reconstituted in buffer on SPCE electrode for EC analysis, 3) IGSR and OGSR components are identified, and data assessed using NN.

1.4.1.3. Instrumental analysis by acid-digestion ICP-MS

The ICP-MS instrument (Agilent 7800, Santa Clara, CA) was equipped with a MicroMist nebulizer, a double pass quartz Scott-type spray chamber, and a quartz torch with an inner diameter of 2.5mm. Automated sample introduction was controlled by an autosampler (SPS 4; Santa Clara, CA) and a peristaltic pump (PeriPump; Santa Clara, CA). The operational parameters were a radio frequency of 1550 W, an auxiliary gas flow of argon at 0.90 L/min, and a plasma gas flow of argon at 15.0 L/min. Three replicates were collected for every sample at 100 sweeps each. All the elements of interest were monitored with He in the collision cell, except for Si, which was analyzed using no gas mode. Concentrations were determined using an external calibration curve with internal standard.

The GSR digestion protocol was optimized following a Plackett Burman ruggedness test and validated for analytical performance and figures of merit. Sample preparation for ICP-MS began with digestion and then dilution of the IGSR microparticles as described in Menking-Hoggatt et. al.[16]

1.4.1.4. Instrumental analysis by LA-ICP-MS

The GSR stubs were individually placed in the ablation chamber of a Tandem J200 laser ablation unit (Applied Spectra, CA). The ablated particles were carried by helium (0.9 L/min) to the 7800 Agilent

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ICP-MS (Agilent, CA) system, using a tygon tube. The ablation parameters were adapted from the micro-spatial method previously developed by our group for the analysis of GSR from the hands of shooters by LIBS[24]. The performance of the instrumentation was monitored by conducting daily performance checks using a liquid tuning solution and glass standard reference materials NIST 612, as per the manufacture's recommendations. The signal sensitivity, peak resolution, precision (<5%) and bias (<5%) were monitored as per our established quality controls for low (^{7}Li), medium (^{140}Ce) and high (^{232}Th) mass-to-charge ratios. Likewise, oxide formation ($^{248/232}$ThO/Th; <1%), doubly charged species ($^{21/42}Ca^{++}$/ Ca^{+}; <3%), and fractionation ($^{232/238}$Th/U ratio 1 ± 0.1 for laser ablation conditions) were optimized according to our daily quality protocols using standard reference material NIST 612. Data was acquired using a transient signal while monitoring 49 isotopes that correspond to 34 individual elements, using optimized parameters listed in **table 3**.

Table 3. Instrumental parameters for LA-ICP-MS analysis[17]

LA-ICP-MS Instrumental Parameters		
Laser	Model, brand	Tandem J200, Applied Spectra
	Laser wavelength	266 nm
	Laser energy	20 mJ
	Frequency	10 Hz
	Spot size	100 μm
	Ablation pattern	Spot mode 5x5 grid (25 spots)
	Shot number per spot	2
	Pre-ablation delay	30 sec
	Ablation time	8 min 35 sec
	Carrier gas	He (0.9 L/min)
ICP-MS	Model, brand	7800 ICP-MS, Agilent
	RF power	1500 W
	Acquisition time	9 min
	Integration time/mass	0.01 s
	Makeup gas	Ar (0.9 L/min)

1.4.1.5. Instrumental analysis by SEM-EDS

SEM-EDS analysis was conducted on a JOEL 6490LV (Peabody, MA) in accordance with ASTM 1588-20 standard for the analysis of GSR.[37] The manufacturer SEM user interface software was version 8.14. The instrumental parameters used during analysis and spectra collection consisted of an accelerating voltage of 25 kV, a spot size of 60 μm, a working distance of approximately 18mm, and a magnification of 500-1000x. A backscatter and a secondary electron detector were used to image particles, while an Oxford Instrument INCAx-sight 7623 Energy Dispersive X-ray Spectroscopy (EDS, England) detector collected elemental information about the particles of interest. After consulting with our crime laboratory collaborators, a cutoff of 10 characteristic particles was included in the run to simulate protocols used by some laboratories and decrease the amount of analysis time per stub.

1.4.1.6. Instrumental analysis by LC-MS/MS

The validated LC-MS/MS was published by Feeney et. al. as part of his NIJ STEM Fellowship award.[18] The method was used to compare IGSR and OGSR results to LIBS and EC. An Agilent 1290 Infinity II liquid chromatography housing an Agilent pentafluorophenyl (PFP) Poroshell 120 column (2.7 μm

2.1 x 50 mm) separated OGSR compounds. The binary flow parameters consisted of water with 0.1% FA (*A*) and acetonitrile with 0.1% (*B*) with a flow rate of 0.350 mL/min. Initial conditions were 80%A/20%B and ramping to 5% A/95% B for nine minutes The total injection volume was 1.0 µL. For the inorganics, a Hamilton PRP-X100 cation exchange guard column (10 µm 2.1 x 33 mm) was added to the LC system. The crown ether complexes traversed the column using an isocratic flow at 90% *A*/10% *B* in positive electrospray (ESI) conditions. At 4 minutes, the source polarity switched to negative ESI (ESI-) mode, and the composition of mobile phases switched to 98%*A*/2%*B* for the tartaric acid complexes. The injection volume for the IGSR method was 10 µL.

1.4.2. Data Analysis

Data analysis in this project required using each analytical instrument's software for signal processing, such as background subtraction, smoothing, signal integration, and, when applicable, quantitative analysis. All statistical analyses were conducted using G*Power (3.1), JMP Pro 14 software (SAS, Cary, NC), and R Studio (open source, 1.2.1335). Depending on the data type and variables, various data normalization and scaling methods were applied (e.g., Min-Max normalization, Max, Z-score, MAD, TanH).

Exploratory and descriptive statistical analysis, significance tests, machine learning algorithms, and classifier methods were used in this study to evaluate their ability to predict class membership on shooter and non-shooter subpopulations (Logistic Regression (LR), Naïve Bayes (NB), and Neural Networks (NN)). ROC curves and the misclassification outputs were used to assess the method's performance (sensitivity, selectivity, error rates, and accuracy). Membership probabilities of the classifiers were used to plot Histograms, Tippet plots, Kernel Density Functions (KDF), log10 of the likelihood ratios, and the information was used as models to assess the weight of the evidence.

1.5. Expected applicability of the research

This study offered innovation and increased knowledge to the forensic examination and interpretation of gunshot residues. The ground-breaking approaches developed in this project will benefit the criminal justice system by modernizing current practice and providing resources for more streamlined processes in firearm-related investigations. This project also contributed by educating and training a future workforce, providing a leap of the body of knowledge in the field, transferring technology from laboratory to marketplace, and expanding the resources and visibility of NIJ-funded efforts nationally and internationally.

This research developed a practical, more straightforward, faster, and superior approach for the simultaneous detection of IGSR and OGSR using LIBS and electrochemical sensors. The methods are versatile as they can be applied for various purposes, such as a) the identification of IGSR and OGRS on the hands of an individual of interest, b) determination of shooting distances, and c) the identification of firearm discharge residues on bullet holes of various substrates encountered in crime scenes. All this information is critical for reconstructing events, providing investigative leads, and supporting the trier-of-fact in the decision-making process. A notable advantage of these methods is the capability to conduct both laboratory-based analysis and field detection of both inorganic and

organic components under 5-10 minutes per sample with high accuracy. The application of data-driven statistical methods adds objectivity and impartiality.

This study collected a large population set to serve as the basis for practical solutions to the forensic community by incorporating emerging fast screening techniques that complement traditional methods. The collection set of over 3,000 samples, collected from the hands of more than one thousand individuals, generated over 100,000 data files with metadata, electrochemical, and spectrochemical information derived from LIBS, ECD, and SEM-EDS. Also, 3D chemical maps were generated for 170 substrates (fabric, wood, drywall and window) to estimate the muzzle-to-target distance and rule out GSR sources. This information serves as the foundation for the reliability of these methods, as well as future research.

Finally, we applied statistical methods to our extensive database to interpret GSR evidence considering probabilistic approaches and artificial intelligence. This research addressed several needs in gunshot residues, such as the speed of analysis, detection of organic and inorganic components, increased knowledge on residues from modern ammunitions, and more overarching interpretation models.

The community's interest in this research is reflected by the broad participation in scientific venues and awareness in social media and webinars. Most importantly, practitioners and managers have approached our team to investigate the potential adoption of these technologies in their case management, something we will be exploring in more detail in the second phase of this research.

We have developed and validated the techniques that serve as the basis for a second phase project to prepare for future technology transfer (2020-DQ-BX-0010). In forensic science, the adoption of newer methods requires a process of consensus assessment of the methods, which is time consuming and requires evidence of its scientific validity. Nonetheless, when the technology becomes available to crime labs and law enforcement, the proposed approach is anticipated to provide added value to conventional methods such as:

Speed and ease of analysis: The LIBS microbeam can be scanned across a target in seconds providing simultaneous detection of multiple elements without the use of chemical reagents or sample preparation. Also, electrochemical sensors can be adapted and optimized for fast (minutes) in-situ analysis of residues and require minimally trained operators.

Superior selectivity and reliability: LIBS can detect multiple atomic, ionic, and molecular lines per element, facilitating the reduction of false positives. Electrochemical detectors can be optimized with high specificity for the redox potential of the target species.

Superior sensitivity and expanded detection capabilities: Electrochemical sensors can detect a large number of IGSR and OGSR components at trace and ultra-trace levels. LIBS can identify most of the elements with typical detection limits in the low ppm, expanding current capabilities to a more extensive suite of components associated with lead-free IGSR residues. The tests will allow fast data generation of residues from standard and non-toxic ammunition, providing a piece of knowledge that is needed, but presently limited, in the field.

Sample preservation and versatility: Target material is minimally damaged during the procedure, enabling subsequent sampling or analysis by other means. This is critical for laboratory confirmations. The methods can be applied to multiple substrates and applications, such as analysis of hand residues,

chemical mapping of questioned orifices, reconstruction of events by estimation of shooting distances and detection of residues on non-movable and large items.

Superior interpretation of evidence value: The statistical analysis and validation of the proposed approach will allow evaluation of error rates and facilitate probabilistic characterization and identification of components, strengthening the conclusions presented in court. Finally, one advantage of the analytical and statistical methods proposed here is their generalizability as they can be easily applied to other materials (e.g., identification of explosives, drug residues, and trace materials, to mention some). The proposed methods (SEM-EDS) are widely available in forensic laboratories, while LIBS and electrochemical sensors have gained a level of maturity and simplicity that would facilitate their adoption.

II OUTCOMES

2.1. Activities/accomplishments

Each of the proposed objectives and tasks was satisfactorily completed in this project. The four main tasks contained 42 specific research activities, including the following categories:

1. Sample collection
2. Sample preparation
3. Methods' development, optimization, and validation
4. Data analysis
5. Statistical analysis and data interpretation
6. Reporting results in the scientific literature
7. Disseminating findings at scientific meetings
8. Creation and curation of the database

In addition, the project management included six main activities: group meetings to discuss research results, planning meetings to monitor accountability for the main tasks and assignments, advisory meetings with practitioners, data analysis review sessions with statisticians, preparation of progress reports, and submission of manuscripts.

The substantial dissemination of this study's research findings in peer-reviewed journals and scientific forums serves as an indicator of the interest raised within the forensic community. We have **published the main results of this research in ten scientific publications in peer-review journals**, and two more are in progress. The research has been **published in journals of high impact factor** and read by a broad audience, including Talanta, Journal of Forensic Science, Journal of Forensic Chemistry, Journal of Chemometrics, Spectrochimica Acta B, Analytical Methods, and Analyst.

Among the notorious contributions are the manuscripts assessing the feasibility of the emerging methods for GSR detection and a review on Trends in Composition, Collection, Persistence, and Analysis of IGSR and OGSR. These publications have served as an essential basis for ongoing

interlaboratory studies that aim to provide uniformity and reliability between the laboratories that may adopt the techniques in the future. Also, two of our students completed and published the **master's thesis (1)**[40] **and doctoral dissertation (1)**[41] **and two more doctoral dissertations** are anticipated from this effort (expected graduations in December 2021 and Spring 2022).

The GSR research has been **disseminated at forty-three scientific meetings**, thirteen of which were invited contributions. Among the invited presentations is a webinar sponsored by the National Institute of Justice to present our GSR research results, which attracted 315 registrants. We have also received NIJ invitations to present the progress in this research at events dedicated to disseminating their funded research, like the Annual American Academy of Forensic Sciences and PITTCON 2020 and 2021 meetings. These events are broadcasted and provide valuable opportunities to publicize WVU/FIS and NIJ-funded research.

Finally, one of the PI's of this project was appointed as the Program Chair of the 2020 and 2021 Online Forensic Symposium: Current Trends in Forensic Trace Analysis, a unique scientific forum that gathered over 1,000 live attendees representing 70 countries, six continents, and over 300 additional on-demand views. In each of these symposiums, a day was dedicated to novel trends in gunshot residues. The opportunity for worldwide dissemination of this event and positive feedback from participants was overwhelming. **Our research has also been featured on several local news, social media, and podcasts**, such as the Just Science podcast, which is widely attended by crime laboratory managers and practitioners.

In the past year, we have been reached out by forensic practitioners and laboratory supervisors from four national and three international crime laboratories, who are interested in learning more about the emerging methods and how to incorporate them. We are currently working on two MOUs as part of the newly funded second phase of this project.

Moreover, we are also working with three industries interested in making the technology more easily accessible at crime scenes and laboratories. We anticipate that the foundation developed in this research will serve to transition these novel methods from the research setting to practice in the future.

Finally, **our team has been recognized with distinctions and awards for products derived from this study**, including the award of several graduate student Fellowships, student scholarships to attend meetings, first-prize student's presentations recognitions, and Dr. Trejos recently received the prestigious award from WVU' Eberly College of Arts and Science "ECAS Outstanding Researcher Award" for her research in gunshot residues.

Specific results and details of the main milestones are discussed in the following sections.

2.2. Results and findings

2.2.1. Results and Findings for Task 1: Collect a sample set of firearm discharge residues from target materials (fabrics), hands of shooters, and background data

This study created the most extensive GSR dataset reported from a single study, consisting of over 3,200 samples recovered from hands of more than one thousand individuals, and nearly 200 fabric, glass, wood, and drywall surfaces. The dataset consists of over 100,000 data files from LIBS, EC, and SEM-EDS, and the respective metadata. The dataset contains analytical information of organic and inorganic profiles on various populations and substrates.

The collection set was designed to validate the proposed methods and the statistical interpretation of the data. The dataset served as a basis for the rest of the tasks in this project. The sample size for each subpopulation was estimated based on our pilot data, the number of sensors to be tested, the number of variables anticipated from preliminary data, the significance level, the smallest main effect of scientific interest selected as the percent of positive/negative rates, and the desirable power (>0.95).

The overall collection set was initially estimated for 640 samples, 375 from hands, 165 from fabrics and glass windows, and 100 samples of positive and negative quality controls. The collection set goal was surpassed by more than double (1611 samples), including samples from 1061 individuals (>3300 stubs from hands), 170 samples from fabrics, glass, wood, and drywall, and over 380 QC controls (**Figure 5**).

The hand-residue analysis evaluated the capabilities of each of the methods to differentiate residues from shooters and non-shooters, based on the recognition of chemical patterns. The study of target materials (fabric, glass, wood) evaluated the reliability of the method for shooting distance estimates and the rapid identification of bullet entrance holes. The large sample size permitted the use of various statistical methods to interpret the data and draw conclusions.

The population study for residues from hands included samples from five main groups:

1) The random presence of residues from non-shooters (*low-risk background set*),
2) Residues from non-shooters that pose a higher contamination risk (*high-risk background set*, e.g., individuals that use firearms regularly, such as police officers, staff at ballistic laboratory, or those who fire guns for hunting or as a hobby, and individuals who pose exposure to components associated to GSR, such as electricians, mechanics, agriculture),
3) Shooters who have fired a gun with traditional ammunition (*leaded shooters set*, collected within less than two hours after shooting),
4) Shooters collected after some regular activity (*post-shooting activity set*, walking, running, placing hands on pockets, rubbing hands, driving) to simulate real situations that may affect persistence, and
5) Shooters that have shot non-toxic ammunition (*lead-free shooters set*)

It is worth noting that the proposed approach allowed sequential analysis by LIBS, ECD, and SEM-EDS on the same specimen. Human subject sampling protocol was approved and updated during the research by the Institutional Review Board (WVU IRB protocol # 1506706336).

A fundamental aspect of the database was data traceability. A unique sample labeling scheme was created for tracking the sample information, including collection date, sample number, type of firearm (e.g., pistol, revolver), collection substrate (e.g., hands, fabric, glass, etc.), ammunition class, population category (e.g., standard ammunition-shooter, non-toxic ammunition-shooter, low risk background non-shooter, high risk background non-shooter), collection area (e.g., left palm vs. right back), and instrumental replicate number (subsample).

As per the IRB protocol, all collections from donors were anonymous, and therefore, no identifiable data was included in the labeling or documented at any time. **Table 4** lists the types of primers that were characterized in this study and their respective abbreviations for the sample ID label.

Multiple collections from known shooter's hands and fabric substrates were conducted during the project, and a summary is described in **Table 5**. Each of the known shooters sets consists of 4 subsamples (left and right hands, palm and back, respectively), while each of the known backgrounds consists of 2 subsamples (one per hand). Therefore, 2,424 hands-subsamples from 606 "shooter" individuals and 910 samples from 455 "background" individuals have been collected and analyzed (**Figure 5**). Also, additional sample sets were collected for method optimization purposes.

Samples were collected at the WVU campus (Oglebay Hall indoor and outdoor areas, Mountainlair student center indoor area, and the Ballistics Laboratory indoor shooting range). Also, we coordinated a two-day sampling of over 400 individuals (shooters and backgrounds) at the World Scout Jamboree in WV. The sampling event took place on July 28th and 29th, 2019. The event provided a unique opportunity to collect samples from individuals with a diverse background, to disseminate the outcomes of this research project, and to raise awareness of the relevance of forensic science in STEM education and careers. The sampling settings at OGH and WSJ allowed to generate a more extensive collections set than the number of samples proposed in this project for shooters and low-risk background sets, which have been characterized by LIBS, ECD and SEM-EDS.

Table 4. Abbreviations for different types of primers characterized in this study.

Examples of Standard Ammunition			Examples of Nontoxic Ammunition		
Brand	**Abbreviation**	**Lead-free**	**Brand**	**Abbreviation**	**Lead-free**
CCI	CCI	No	Fiocchi	FIO	Yes
CCI Magnum	CMG	No	Hevi-shot	HEV	Yes
Winchester	WIN	No	Inceptor RNP	INC	Yes
Winchester Magnum	WNM	No	Geco Super Matrix	GEC	Yes
TulAmmo	TUL	No	Lawman Ammunition	LAW	Yes
Sellier & Bellot	SEL	No	SYNTECH Federal	SYN	Yes
Remmington	REM	No	ICC AMMO Green Elite™	ICC	Yes
Federal	FED	No	Winchester Super Clean	SUP	Yes
Federal Match	FCH	No	CCI nontoxic	CCX	Yes
Federal Magnum	FDM	No	National Police Ammunition	NPA	Yes

Table 5. Sample collection details for known shooter and low-risk background sets. (Total of 606 shooter sets, 350 low-risk background sets, and 105 high-risk background sets, collected and analyzed from Jan 2019 to May 2021)

Firearm	Type of Firearm	Type of Ammunition	Lead-free	Caliber	Sets Collected	Set #	Location (abbreviation)	Date of Collection
N/A	N/A	Background samples (low-risk)	N/A	N/A	20	001:020	Oglebay Hall (OGH)	052419
Springfield XD9	Pistol	Reloaded and unknown	**No**	9 mm	20	001:020	WVU Ballistic Lab (OGH)	052419
Springfield XD9	Pistol	Manufacturer loaded Blazer	**No**	9 mm	20	021:040	WVU Ballistic Lab (OGH)	060319
N/A	N/A	Background samples (low-risk)	N/A	N/A	10	021:030	Oglebay Hall (OGH)	060319
Springfield XD9	Pistol	Specialty loaded Remington	**No**	9 mm	40	041:080	WVU Ballistic Lab (OGH)	061119
N/A	N/A	Background samples (low-risk)	N/A	N/A	38	031:068	Outside OGH Oglebay Hall	062019
N/A	N/A	Background samples (low-risk)	N/A	N/A	32	069:100	Outside Oglebay Hall (OGH)	062419
SIGSAUE P320,Ruger Mark IV	Pistols	Manufacturer loaded Federal and SYNTECH	**Mixed**	.22 LR and 9 mm	220	081:328	World Scout Jamboree (WSJ)	072919 and 073019
N/A	N/A	Background samples (low-risk)	N/A	N/A	200	101:300	World Scout Jamboree (WSJ)	072919 and 073019
Springfield XD9	Pistol	Specialty loaded Winchester	**No**	9 mm	20	329:348	WVU Ballistic Lab(OGH)	091119
Taurus Model 608	Revolver	Specialty loaded Remington	**No**	9 mm	50	349:398	WVU Ballistic Lab (OGH)	112119
Taurus 357 Magnum	Revolver	Manufacturer loaded American Eagle Federal	**No**	38 Special	50	399:448	WVU Ballistic Lab (OGH)	121019
N/A	N/A	Background samples (low-risk)	N/A	N/A	50	301:350	Outside OGH Oglebay Hall	121219
Springfield XD9	Pistol	Specialty loaded Fiocchi lead-free	**Yes**	9 mm	20	041:060	WVU Ballistic Lab (OGH)	062220
Springfield XD9	Pistol	Specialty loaded CCI lead-free	**Yes**	9 mm	20	061:080	WVU Ballistic Lab (OGH)	062220
Springfield XD9	Pistol	Specialty loaded CCI lead-free	**Yes**	9 mm	14	090:103	WVU Ballistic Lab (OGH)	062920
Springfield XD9	Pistol	Manufacturer loaded Hevi-Shot	**Yes**	9 mm	2	031:032	WVU Ballistic Lab (OGH)	062920
Springfield XD9	Pistol	Manufacturer loaded SYNTECH	**Yes**	9 mm	30	033:040 081:089 104:116	WVU Ballistic Lab (OGH)	071620
Springfield XD9	Pistol	Specialty loaded Fiocchi lead-free	**Yes**	9 mm	14	117:130	WVU Ballistic Lab (OGH)	071620
N/A	N/A	Background samples (high-risk firearm research)	N/A	N/A	32	001:032	Oglebay Hall research lab (OGH)	030921 and 031021
N/A	N/A	Background samples (high-risk agriculture)	N/A	N/A	21	033:046 and 066:072	WVU organic farm and greenhouse	031821 and 033021
N/A	N/A	Background samples (high-risk mechanics)	N/A	N/A	19	047:065	5 mechanic garages around Morgantown	033021
N/A	N/A	Background samples (high-risk police/station)	N/A	N/A	33	073:105	Morgantown Police Department	033121

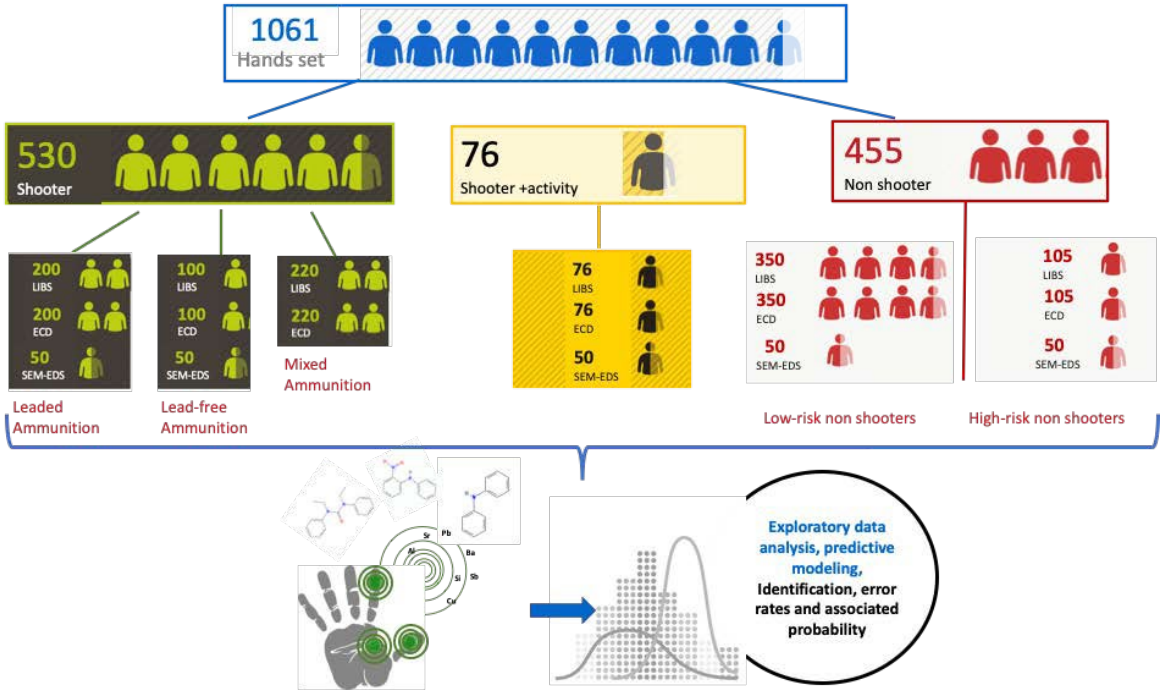

Figure 5. Diagram of number of individuals sampled for the analysis of GSR on hands, and respective sets analysis by LIBS, ECD and LIBS.

When sampling GSR from known-shooter hands, the subject washed hands between every collection and then applied hand sanitizer to reduce cross-contamination. Negative control samples were taken from the hands of the individuals who were collecting the GSR samples and the people doing the actual firing, after washing their hands both before and after collection at the beginning and end of the sampling day.

The slide and barrel of the firearm were cleaned when ammunition type changed. Also, a separate barrel is used for nontoxic ammunition. At the laboratory, the working bench near the instruments was cleaned and covered with white butcher paper before analysis. Positive controls and blank control samples are also monitored as part of the daily performance check protocol. All these steps were implemented to reduce and monitor cross-contamination.

During this study, we collected and characterized different types of ammunition. We have used specialty loaded ammunition, reloaded ammunition, and factory-made ammunition. Specialty loaded ammunition used a primer composition that we have previously characterized in our group by ICP-MS and SEM-EDS. A specific loading process was designed to ensure that the elemental composition of the GSR originates mostly from the primer. The controlled loading process consisted of a new brass cartridge case (Starline), loaded with Winchester 231 powder (4 grains), total metal jacket bullet, and the respective primer. On the other hand, reloaded ammunition is standard practice by gun users. Still, unlike the specialty loaded set-up, the chemical composition of reloaded ammunition was not controlled. Therefore, the ground truth about the formulation in reloaded ammunition was considered an "unknown" source in the population study. Lastly, factory-made ammunition was bought preloaded from a manufacturer and used without any modification.

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Finally, a set of 170 samples was collected for estimation of shooting distance and bullet hole identification. Thirty-seven samples were used for method optimization, and the remaining 133 samples were used for testing the method's performance (see figure below). Shooting distance samples were collected by firing fabrics at different muzzle-to-target distances (5 distances, from contact to 36 inches) to generate calibration standards and train the prediction models (60 calibrators). Forty-five (45) samples shot at different distances were used as blind unknowns to validate the method, including 15 dark-color fabrics, 10 patterned-fabrics, and 20 blood-stained fabrics. Another set of 28 samples consisting of fabric (7), wood (7), glass (7), and drywall (7) were fired, and GSR residues were recovered around the bullet entrance holes. A subset of fabrics was also used for detection of OGSR and IGSR via electrochemical sensors.

Additionally, a separate dataset was created from the optimization and characterization of the primer-only micro-particle GSR standards.[16] All the data has been analyzed and a curated dataset and will be archived by NIJ National Archive. A comprehensive standard operating procedure (SOP) was created to detail the data file naming, data storage, data back-up, and description of folders content and type of file formats.

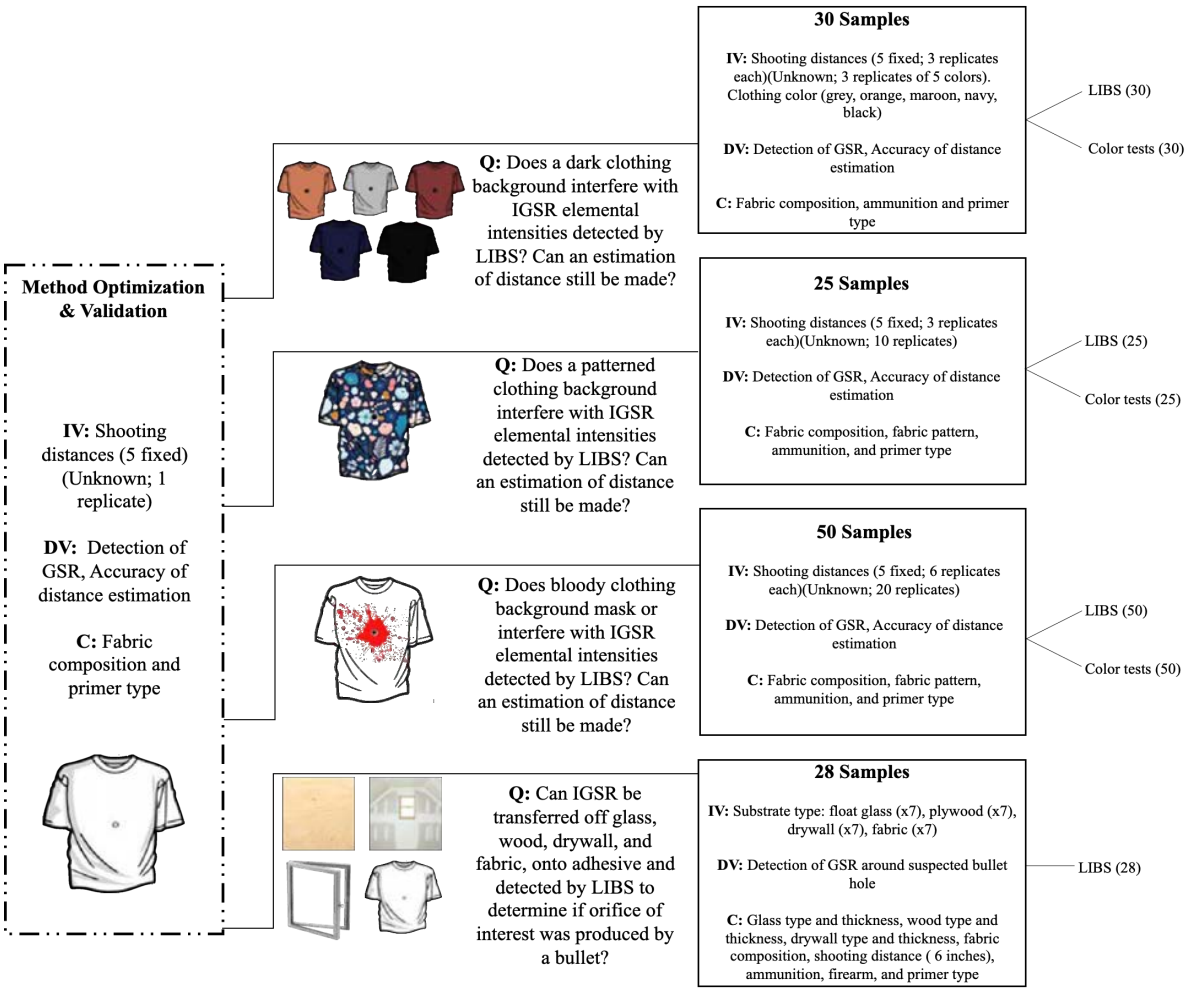

Figure 6. Experimental design for shooting distance determination and bullet hole analysis of IGSR using LIBS, including sample types, variables, and analyses performed for each sample. (IV = independent variable, DV = dependent variable, C = constant variable, Q = question of interest).

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2.2.2. Results and Findings for Task 2: Validate LIBS and electrochemical sensors for estimation of shooting distance and FDR detection on clothing.

This task aimed to validate the methods of analysis for shooting distance and identification of bullet holes and is anticipated to offer breakthrough technology to improve leading information and evidentiary value in gunshot-related investigations. The number of samples required for validation experiments was estimated based on a randomized blocked design, considering the numbers of factors, levels, and replicates.

The detection of gunshot residues can provide essential information in firearm-related investigations. For instance, when the question of suicide or murder arises, estimating the barrel to victim distance plays a critical role in the outcome of the case. Also, clothing, wounds, and other target materials are often inspected to determine if a bullet has produced an entry or exit orifice.

Currently, the most common method for distance determination and identification of bullet entrance holes is by visual inspection of FDR residue and chemical colorimetric tests that react in the presence of nitrites or lead.[38,39] Although these color tests are widely used in forensic laboratories, the major pitfall is their subjectivity and poor selectivity for gunshot residues. Dark or bloody items significantly diminish the efficacy of these assays. Also, color tests are difficult to perform on non-movable or large objects, and false positives can be derived from oil, dirt, and other common contaminants.

Therefore, there is a critical need for modernizing current methods for estimation of shooting distances. The primary aim of this task was to investigate the capabilities of LIBS for shooting distance determination and identification of FDR on substrates of forensic interest. Our central hypothesis was that LIBS would improve the scientific reliability of the detection and observation of gunshot residues. This assumption was based on the ability of LIBS to perform simultaneous multi-elemental detection at low ppm levels, LIBS' superior selectivity, and the potential for confirmation of numerous emission species per analyte.

Moreover, electrochemistry proved to be a viable emerging method for simultaneous detection of IGSR and OGSR for bullet hole identification. The simultaneous analysis was able to be completed within 10 minutes with the extraction procedure. Electrochemistry increased detection reliability by analyzing both IGSR and OSGR components like lead, antimony, copper, diphenylamine, nitroglycerin, and ethyl centralite.

The LIBS and ECD methodologies developed in this study aim to bring more objective and accurate estimations for shooting distance determination, more confidence to bullet hole identification in large surfaces commonly encountered at crime scenes, and an additional detection method for non-toxic ammunitions that are becoming markedly more popular. Incorporating these methodologies in forensic laboratories will help modernize current practices and increase the scientific validity of the detection of gunshot residues in crime scene reconstruction.

Our group has published the following articles and master's thesis describing the main results for shooting distance estimation and bullet hole reconstructions by LIBS, and an additional manuscript describing the EC results is in preparation:

1) C Vander Pyl, K Morris, L Arroyo, T Trejos. Assessing the utility of LIBS in the reconstruction of firearm related incidents, Journal of Forensic Chemistry, 19, June 2020, https://doi.org/10.1016/j.forc.2020.100251
2) C Vander Pyl, O Ovide, Ho M, Yuksel B, T Trejos. Spectrochemical Mapping Using Laser Induced Breakdown Spectroscopy as a More Objective Approach to Shooting Distance Determination, Spectrochimica Acta B, 2019, 152, 93-101. https://doi.org/10.1016/j.sab.2018.12.010
3) B Yuksel, M Ho, O Ovide, C Vander Pyl, T Trejos. Infrared Imaging as a Complementary Aid in Estimating Muzzle-to-Target Shooting Distance: An Application on Dark, Patterned and Bloody Samples. T K J Foren Sci Leg Med, 16,2, 2019, 73-80. DOI: 10.5336/forensic.2019-64837
4) Courtney Vander Pyl, MSFS, 2019, WVU Department of Forensic and Investigative Science, Chemical Analysis of Firearm Discharge Residues Using Laser Induced Breakdown Spectroscopy. https://researchrepository.wvu.edu/etd/4058

Detailed information can be found in these publications, and a summary of the major findings are listed below.

2.2.2.1 LIBS analysis for shooting distance estimations and bullet hole identification

A LIBS method was developed and validated for the analysis of substrates commonly found during firearm-related crimes. Residues from different ammunition and firearms were analyzed of 133 fabrics, glass, drywall, and wooden samples (see figure 6).

Samples shot at known muzzle-to-target distances were used as calibrators and for training the machine learning algorithms. Shooting distances for the control calibration samples were chosen to represent gunshot residue patterns that are commonly associated with a contact, close range (6 inches & 12 inches), and long-range (24 inches & 36 inches) shootings. A graph of LIBS intensity versus ablation distance from the bullet hole was built for each element of interest (Pb, Ba, Sb). The accumulated area intensity of each 5 mm increment as the laser moved away from the orifice was plotted to visualize GSR patterns (**Figure 7**). From these patterns, heat maps were created to represent the distribution of GSR around the orifice in a 3D image. The x and y-axes in the 3D chemical map represent the relative distances from the bullet hole while the z-axis represents the intensity of Pb, Ba, and Sb, respectively. A darker color indicates higher amounts of the element in a given spatial location (**Figure 7).** One advantage afforded by chemical imaging is the permanent record of the GSR pattern, which can be further used for demonstration as evidence in court, and to conduct more objective estimates of the shooting distance. On the other hand, color tests fade quickly, and estimations are very subjective as the examiner is the one making decisions based on their capability to recognize and compare patterns by eye.

One of the most common items submitted to crime labs for distance determination is the clothing that the victim or suspect was wearing during the time of the shooting. Casework clothing comes in many different designs and colors. Therefore, we decided to compare the effect of dark color and patterned designs on the accuracy of visual inspection, color tests, and LIBS analysis. Also, blood-stained items were included in the study as it is expected most bullet wounds would induce bleeding near the entrance and exit holes. Physical characteristics that provide leading information for

estimating shooting distance can be masked, making visual interpretation challenging. Additionally, the color reaction may be hidden by the blood, requiring indirect testing methods, which in turn, further reduce the sensitivity of the colorimetric assays.

Statistical methods, like Principal Component Analysis and Multivariate Discriminant Analysis, were performed to estimate shooting distances and identify the presence of GSR residues. Three-dimensional heat maps were created with the spectrochemical information of the elemental composition around a bullet hole. The pattern and intensity of the GSR around the bullet is used to predict the shooting distance (**Figure 7**). For dark-colored and patterned fabrics, color tests lead to misclassification of 9 out of 35 unknown shooting distances (26%), while the LIBS method correctly classified 100% of the unknown distance testing samples by Discriminant Analysis.

The LIBS method was also tested and validated on blood-stained items. Results show that LIBS was able to correctly predict shooting distances with no interference from blood, while visual, physical analysis and color tests were only able to accurately predict 50% and 40% of the unknown distances on bloody fabrics, respectively (**Figure 7 and Table 6**)

Another application of LIBS was developed for the rapid identification of chemical markers indicative of bullet entrance orifices. LIBS was able to correctly identify elemental profiles of gunshot residues from all standard ammunition deposited on clothing, drywall, glass, and wood. The presence of Pb, Ba, Sb, and Cu was observed on all GSR from standard ammunition by LIBS and SEM-EDS. Both LIBS and SEM-EDS confirmed elements such as Al, K, Ca, Ba, Bi, and Zn in nontoxic primers. Nonetheless, nontoxic ammunition characterization was more challenging as LIBS did not confirm all elements detected by SEM-EDS. In particular, some elements such as Si, Al, K, and Ti were also detected at low levels on background fabric samples, limiting the confirmation by LIBS in some primers. SEM-EDS permitted the analysis directly on the particle of interest, minimizing the effect of other non-GSR background occurrences in the surrounding area of the stub. Still, LIBS provided a more valid identification than color testing.

This characterization of GSR in substrates demonstrates the complexity of gunshot residue interpretation. For instance, standard ammunition is anticipated to contain the markers Pb, Ba, and Sb. Nonetheless, one of the "standard" ammunitions did not have any barium (TulAmmo), and another one had an inconsistent presence of lead in the residues (Remington). SEM-EDS, LIBS, and ICP-MS were used to characterize and confirm the composition of these residues.

One advantage afforded by the LIBS method is the versatility for sample sizes with none or minimal sample manipulation. The **figure at right** shows a schematic of the custom-made holder that allows stretching of the fabric into a flat platform without the need of damaging the material of interest. Tape lifts sampled from non-movable objects like doors or walls can also be directly placed in this ablation chamber for rapid spectrochemical mapping.

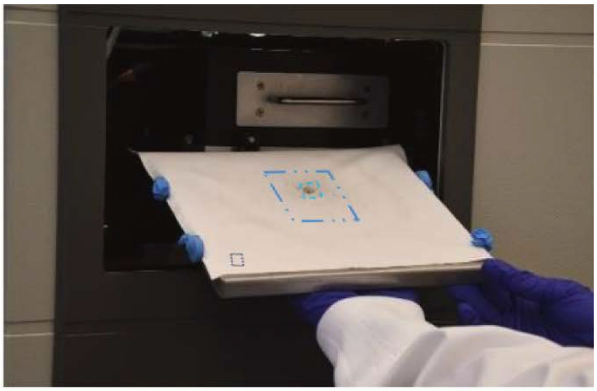

Figure 8. Photograph of the custom-made laser ablation sample holder and ablation patterns.

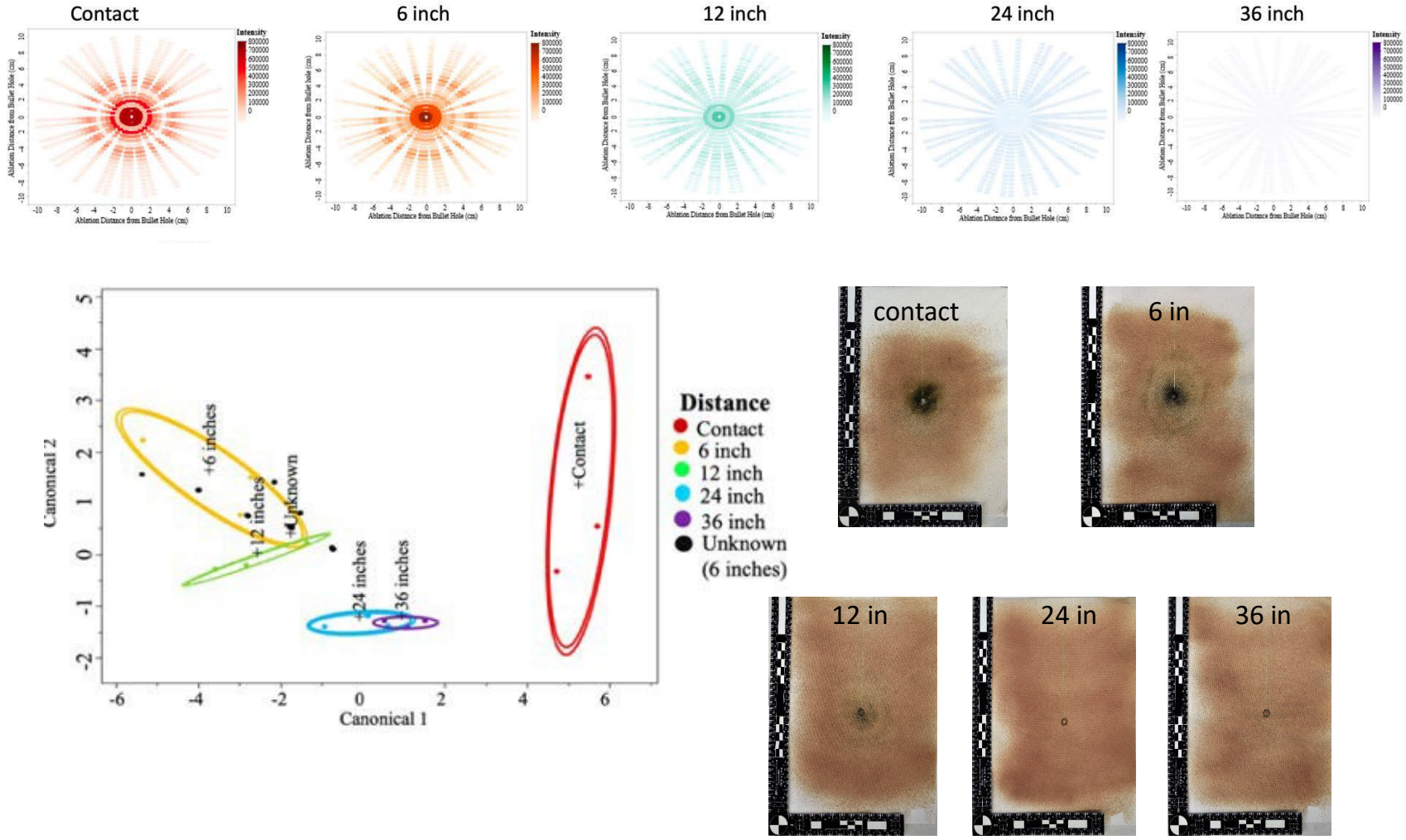

Figure 7. Top: Spectrochemical images of distribution of lead around bullet entrance orifice on fabric at contact, short (6-inch, 12 inch), and long shooting distance (24-inch, 36 inch). Bottom: Canonical plot classification of the known distance calibrators and respective predictions of unknown bloody samples and respective photographs of fabrics stained with blood fired at different muzzle to target distance.

Table 6. Predicted distance ranges by LIBS and Discriminant Analysis (DA), visual interpretation, and color tests, and respective correct classification rates for blood-stained fabrics. Number in red represents the true distance.

Unknown Sample Name	LIBS/DA Distance Range Classification	Actual Distance	Correct Distance Range by LIBS/ DA?	Visual/Physical Distance Range Classification	Correct Distance Range by Visual/Physical?	Color Tests Distance Range Classification	Correct Distance Range by Color Tests
1 Blood (P)	6-**12** inches	6 inches	Yes	6-12 inches	Yes	6-12 inches	Yes
2 Blood (P)	6-**12** inches	6 inches	Yes	6-12 inches	Yes	6-12 inches	Yes
3 Blood (P)	6-**12** inches	6 inches	Yes	6-12 inches	Yes	Contact-12 inches	Range Too Large
4 Blood (P)	6-**12** inches	6 inches	Yes	6-12 inches	Yes	Contact-24 inches	Range Too Large
5 Blood (P)	**6**-12 inches	6 inches	Yes	Contact-6 inches	No	Contact-24 inches	Range Too Large
6 Blood (P)	**6**-12 inches	6 inches	Yes	6-12 inches	Yes	Contact-12 inches	Range Too Large
7 Blood (P)	**6**-12 inches	6 inches	Yes	24-36 inches	No	Contact-12 inches	Range Too Large
8 Blood (P)	**6**-12 inches	6 inches	Yes	6-12 inches	Yes	Contact-12 inches	Range Too Large
9 Blood (P)	**6**-12 inches	6 inches	Yes	6-12 inches	Yes	Contact-24 inches	Range Too Large
10 Blood (P)	**6**-12 inches	6 inches	Yes	6-12 inches	Yes	Contact-24 inches	Range Too Large
11 Blood (P)	**24**-36 inches	24 inches	Yes	6-12 inches	No	24-36 inches	Yes
12 Blood (P)	**24**-36 inches	24 inches	Yes	6-12 inches	No	24-36 inches	Yes
13 Blood (P)	**24**-36 inches	24 inches	Yes	12-24 inches	No	24-36 inches	Yes
14 Blood (P)	24-**36** inches	24 inches	Yes	12-24 inches	No	24-36 inches	Yes
15 Blood (P)	**24**-36 inches	36 inches	Yes	24-36 inches	Yes	24-36 inches	Yes
16 Non-Blood (P)	**6**-12 inches	6 inches	Yes	6-24 inches	Range Too Large	Contact-12 inches	Range Too Large
17 Non-Blood (P)	**6**-12 inches	6 inches	Yes	12-24 inches	No	6-24 inches	Range Too Large
18 Non-Blood (P)	**6**-12 inches	6 inches	Yes	6-12 inches	Yes	Contact-24 inches	Range Too Large
19 Non-Blood (P)	**6**-12 inches	6 inches	Yes	12-24 inches	No	6-12 inches	Yes
20 Non-Blood (P)	**6**-12 inches	6 inches	Yes	12-24 inches	No	6-24 inches	Range Too Large
Correct Classification Rate		**LIBS**	**100%**	**Visual/Physical**	**50%**	**Color Test**	**40%**

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2.2.2.2. Electrochemical analysis for identification of IGSR and OGSR on bullet holes

Bullet hole identification was also analyzed using electrochemical procedures. Reagents and standards are described by Ott et al. in addition to electrodes and instrumentation.[11] Additionally, a Leica stereoscope, forceps, and scalpel were used in the collection of GSR flakes from the fabric surface after shooting. A Springfield XD9 firearm was used for the shooting and collection of the fabric samples. Three ammunitions were used in collection, CCI Magnum, Blazer 40, and Federal, with 4 to 10 samples collected per ammunition.

A total of twenty authentic clothing substrate samples were collected at West Virginia University's Ballistics Laboratory. White fabric (100% cotton) was cut into 8 in. by 11 in. rectangles and backed with manila paper and covered by white printer paper to protect the fabric from contamination before and after the shooting event. These substrates were then secured in labeled butcher paper until processing. Collection of samples was done by securing each fabric sample to a vertical wall on the firing range made of self-healing shooting block with thumb tacks. All twenty samples were then shot once from 6 inches away, measured from muzzle to target. After firing, each fabric sample was removed from the block and placed back into the appropriately labeled butcher paper.

In addition to the authentic samples, seven simulated distance determination samples that contained distributions of lead on fabric were also analyzed. The samples were simulated from firing distances of 2, 5, 10, 20, and 30 cm and processed via electrochemistry in addition to two unknown simulated distances.

Authentic fabric samples collected at WVU were used in the determination of sampling methods used for bullet hole identification and distance determination information. Two sampling methods for bullet hole identification were explored: using SEM aluminum stubs and handpicking of partially burnt flakes. These methods are depicted in **Figure 9**. The bullet hole sampling method employed a clean GSR stub used to sample the bullet wipe by lifting in the 12, 3, 6, and 9 o'clock positions around the bullet wipe (denoted as 1, 2, 3, and 4 in figure 9). The handpicking method utilized forceps and a stereo microscope to handpick partially burnt flakes from the fabric samples. The most circular flakes were chosen for analysis. Approximately three flakes were picked off the fabric and placed on a clean microscope slide. Before extraction, a scalpel was used to crush the hydrophobic outer layer of the flake which was then placed in a microcentrifuge tube with forceps. A 200 µL aliquot of acetonitrile was added to the microfuge tubes and left for roughly four hours to dissolve the flakes.

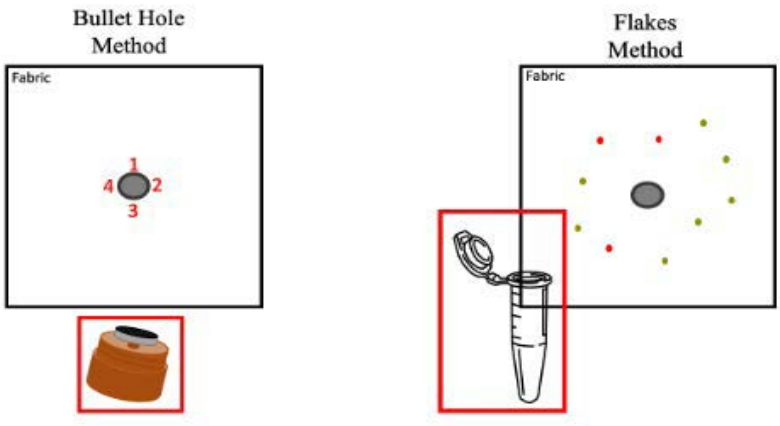

Figure 9. Sampling methods used to collect GSR particles from fabric samples for bullet hole identification.

For distance determination, various sampling approaches were explored to gain spatial information for classification of shooting distance in addition to the elements and compounds detected. In the line sampling method, a clean stub was used to lift from the bullet wipe edge to the top edge of the fabric following a straight line. The stub was lifted approximately 10-15 times in this area moving upward to not overlap with the stub surface area. The next method, referred to as the interval method, used multiple SEM aluminum stubs to collect in determined areas 0-2, 2-4, 4-6, and 6-8 cm outside the bullet wipe area and moving toward the edge of the fabric in a line. Like the interval method, the concentric circle method uses the same interval area, however, the SEM stub is used to sample in a circle around the bullet wipe instead of a line. These two methods require four stubs per one fabric sample. These sampling methods are shown in **Figures 10 and 11** below. Seven simulated samples were collected using the interval method and analyzed in duplicate so that 8 stubs were used per one sample for a total of 56 observations.

Several quality controls were run pre and post analysis, which included acetate buffer blank, negative stub, and fabric controls, collected using the bullet wipe and LIBS ablation line methods on blank, unused fabric, as well as 10 and 2.5 ppm mixtures of IGSR and OGSR standards.[15] The stubs were collected and set aside for extraction using the GSR extraction procedure from Ott et al.[15] For the handpicked GSR flakes methods, the organic extracts were dried under nitrogen and reconstituted in 200 µL acetate buffer pH 4.0 (Figure 14). Fabric controls were taken using a clean piece of fabric. From the 20 authentic samples, a total of 70 stubs from sample replicates were extracted and analyzed by the square-wave anodic stripping voltammetry method using 50 µL on a screen-printed carbon electrode.[15]

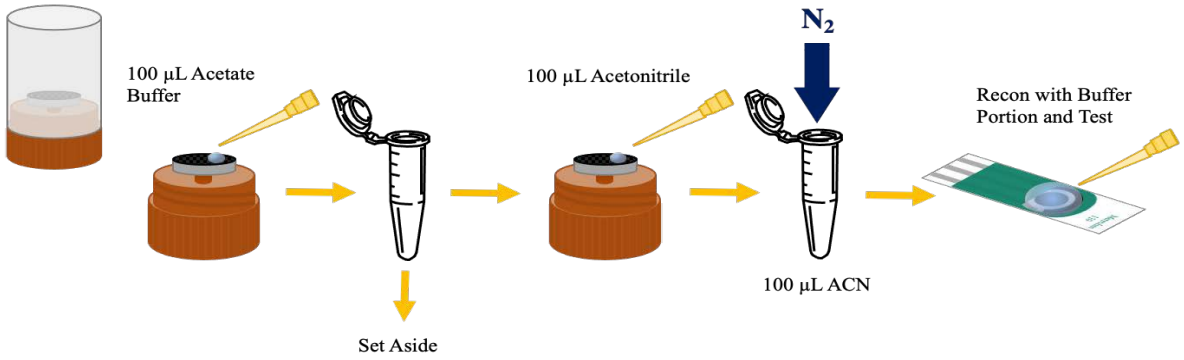

Figure 10. Extraction procedure for collection methods using the SEM Carbon stubs.

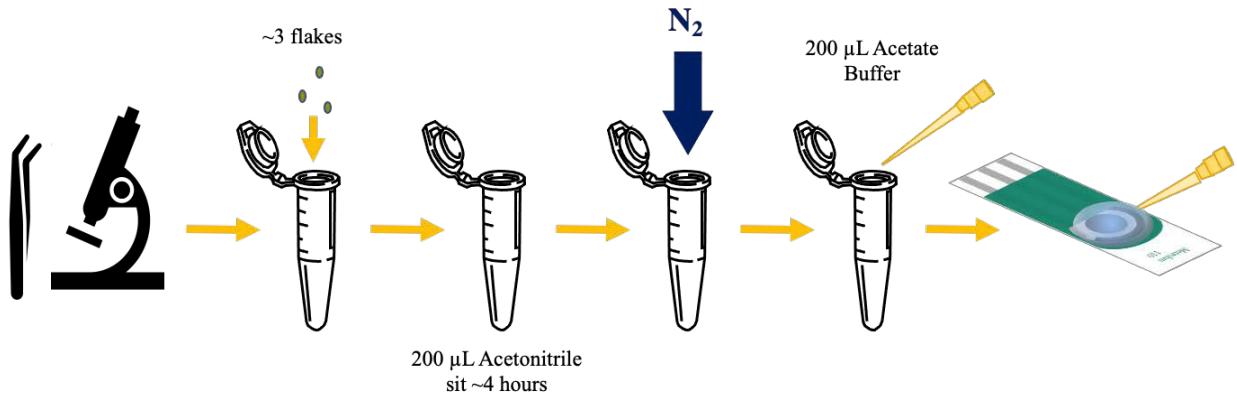

Figure 11. Extraction procedure for handpicking GSR flakes.

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Bullet hole identification currently relies on visual and physical examination, color tests, and instrumental analysis procedures that can be destructive to the sample and require long analysis time. New emerging screening methods can aid in workflow efficiency, quick analysis time, and may help reduce the backlog of firearms casework. In this study, electrochemical detection for bullet hole identification and distance determination was evaluated as a proof of concept for a rapid screening method that has the potential to be used in the field, and on difficult or immovable substrates to detect IGSR and OGSR simultaneously.

Analysis of the authentic samples on bare-carbon screen-printed electrodes was successful for the detection of common GSR analytes like lead, antimony, copper, diphenylamine, nitroglycerin, and ethyl centralite. Lead and nitroglycerin were the two most prevalent GSR markers found in all the samples and between ammunition types.

Electrochemistry was also effective for shooting distance estimations, estimating the distribution of Pb and nitroglycerine around the entrance hole. The methods were simple and quick with samples taking less than 5 minutes to collect from single fabric samples. Presented in **Figure 12,** the overlay of the GSR standard mix shows DPA and NG standard mixtures compared to the sample. The broad peak demonstrates a shoulder with low resolution; however, they can be identified as DPA and NG with comparison of peak potentials.

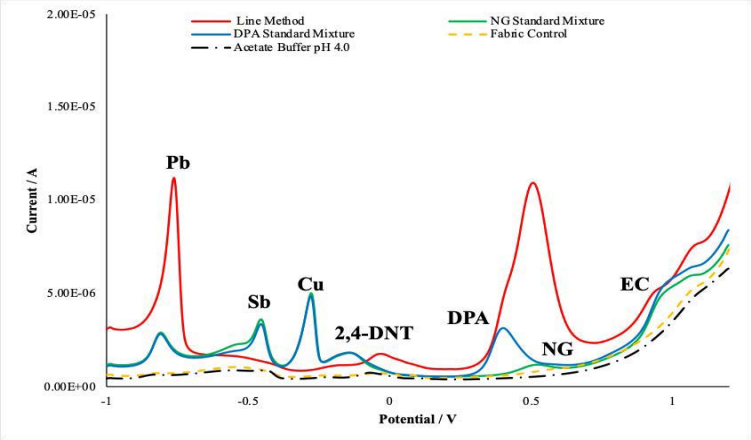

Figure 12. Comparison voltammograms for peak potential for an authentic sample overlaid with GSR standard mixtures, acetate buffer, and fabric control.

Distance determination analysis was completed for lead only as a proof of principle. The shooting distances 2, 5, 10, 20, and 30 cm demonstrated a decrease in lead with farther shooting distances. Observations from voltammograms with respective heat maps were created to visualize the concentrations of Pb at different intervals collected, depicted in **Figure 13.** The same observations were collected and shown for the two unknown samples in **Figure 14.** Unknown A was estimated between 10 and 20 cm while unknown B had an estimated distance range of approximately 20-30 cm, which corresponded to the ground truth.

As a proof of concept, the presented results provide a preliminary study to bullet hole identification and distance determination using electrochemical methods as a rapid screening analysis and show much promise for electrochemical analysis of difficult substrates and potential use for shooting distance estimations. This demonstrates an important concept for GSR detection, as the crime scene

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may dictate analysis of substrates other than the hands of an individual. Future work will focus on increasing the number of samples, varying the shooting distance range, and testing the portability and applicability of statistical analysis for identification and classification.

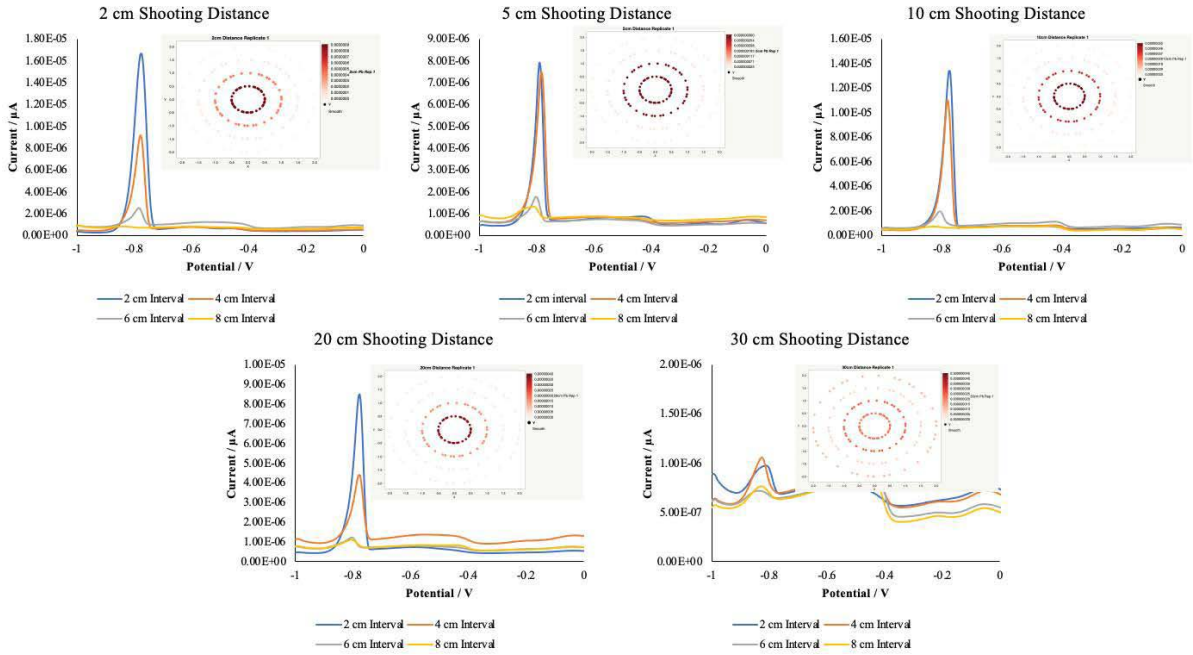

Figure 13. Shooting distance voltammograms and heat maps analyzed by electrochemical detection for lead in known distance samples.

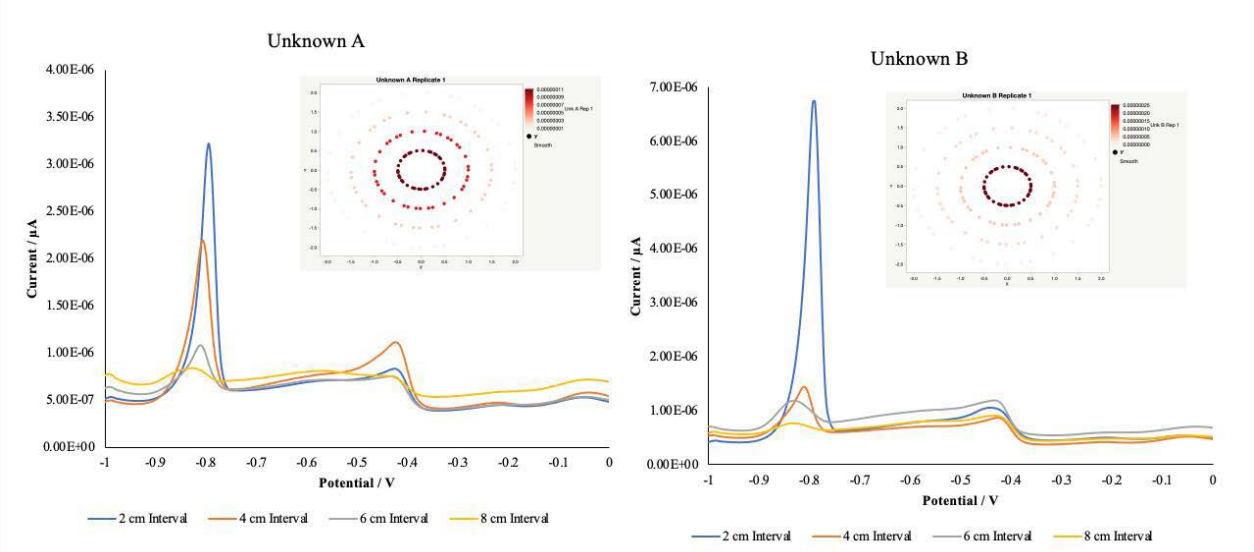

Figure 14. Shooting distance voltammograms and heat maps analyzed by electrochemical detection for lead in unknown distance samples.

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Further testing of the LIBS and EC method for shooting distance determination was completed on simulated GSR samples provided by the ENFSI Proficiency Test on Shot Range Estimation. The study provided twelve known shooting distance samples (2cm, 5cm, 10cm, 15cm, 20cm, 30cm, 40cm, 60cm, 80cm, 100cm, 150cm, 200cm) created by screen printing a lead paste onto dark brown fabric squares. Additionally, two unknown shooting samples, labeled "Case A" and "Case B" were also tested. Each fabric sample was first processed by LIBS, followed by Sodium Rhodizonate colorimetric testing, then electrochemistry. Correct distance ranges were determined for Case A and Case B unknown items based on LIBS and EC, as compared to color testing reported by the remaining participants.

2.2.3. Results and Findings for Task 3: Validate screening methods (LIBS and ECD) and confirmatory methods (SEM-EDS) for IGSR and OGSR detection on hands of potential shooters

The premise of task 3 was that combinations of the proposed methods will allow efficient detection of IGSR and OGSR on the hands of shooters and enhance the overall reliability of these examinations. This premise was demonstrated through thorough optimization and validation of the methodologies using standards and large populations of authentic samples that simulated casework specimens.

Our team focused efforts on collection devices that permit further analysis by standard methods (e.g., SEM-EDS). Carbon conductive tabs were chosen as substrate, as they are commercially available at relatively low cost, offer a low background for the compounds of interest, and are compatible with the range of techniques proposed in this study, including SEM-EDS.

This study developed a reliable laser-induced breakdown spectroscopy (LIBS) screening approach to detect IGSR in just a few minutes with minimal damage to the sample, high specificity, and sensitivity. The laser beam permits micro-sampling on the stub's surface to gather three-dimensional data of the simultaneous occurrence of IGSR markers from a discrete space. The micro-spatial chemical analysis is possible from just two laser shots fired at an area of 100-μm diameter. A benefit afforded by this approach is the use of the universal hand's collection method currently used by practitioners while leaving over 99% of the stub left unaltered for further analysis.

The study further demonstrated the usefulness of electrochemical testing for the identification of IGSR and OGSR components. The increasing demand for rapid methods to identify both inorganic and organic gunshot residues make electrochemical methods an attractive screening tool to modernize current practice. Moreover, electrochemical screening of GSR samples delivers a simple, inexpensive, and sensitive analytical solution capable of detecting IGSR and OGSR in less than 5 min per sample. Utilizing bare screen-printed carbon electrodes, the detection and resolution of seven components (IGSR; lead, antimony, and copper, and OGSR; nitroglycerin, 2,4-dinitrotoluene, diphenylamine, and ethyl centralite) was achieved with limits of detection (LODs) below 1 μg/mL.

Machine learning algorithms were used to classify samples derived from shooters' hands versus non-shooters hands, based on their electrochemical profiles and LIBS spectrochemical data. Four different approaches—critical threshold, logistic regression, Naïve Bayes, and Neural Networks—were applied to examine the methods' performance and accuracy. Logistic regression and neural network models showed a similar predictive ability to distinguish between shooter and non-shooter classes, and they were relatively easy to apply. One advantage of these statistical methods is that they provide

probabilistic outputs that can be utilized to interpret the weight of the evidence further. The accuracy of each method, and when combined, was evaluated using large data sets, and a comprehensive discussion of the performance rates is provided under the task 4 section.

Figure 15 illustrates the main steps of the proposed approach. Incorporating this rapid screening and statistical decision-making approach could offer more efficient case management in firearm-related investigations. The combined analysis of LIBS and ECD can be done in the same sample in just 5-10 minutes, unprecedented in this type of evidence. They represent a remarkable analytical resource that is anticipated to offer, for the first-time, reliable screening complementary to SEM-EDS, increasing confidence in the results, and assisting with sampling strategies at the laboratory and the scene.

The analysis of over 200 stubs after LIBS and ECD analysis proved that the methods were minimally destructive and didn't prevent GSR particles' confirmation via SEM-EDS. Likewise, complementary testing by LA-ICPM-MS or LC-MS/MS was possible on the same stub after LIBS and EC analysis. These findings add a critical value to the methods. It demonstrates the feasibility of incorporating LIBS and ECD in future case workflow as an effective screening to simplify, strengthen, and provide a more streamlined process.

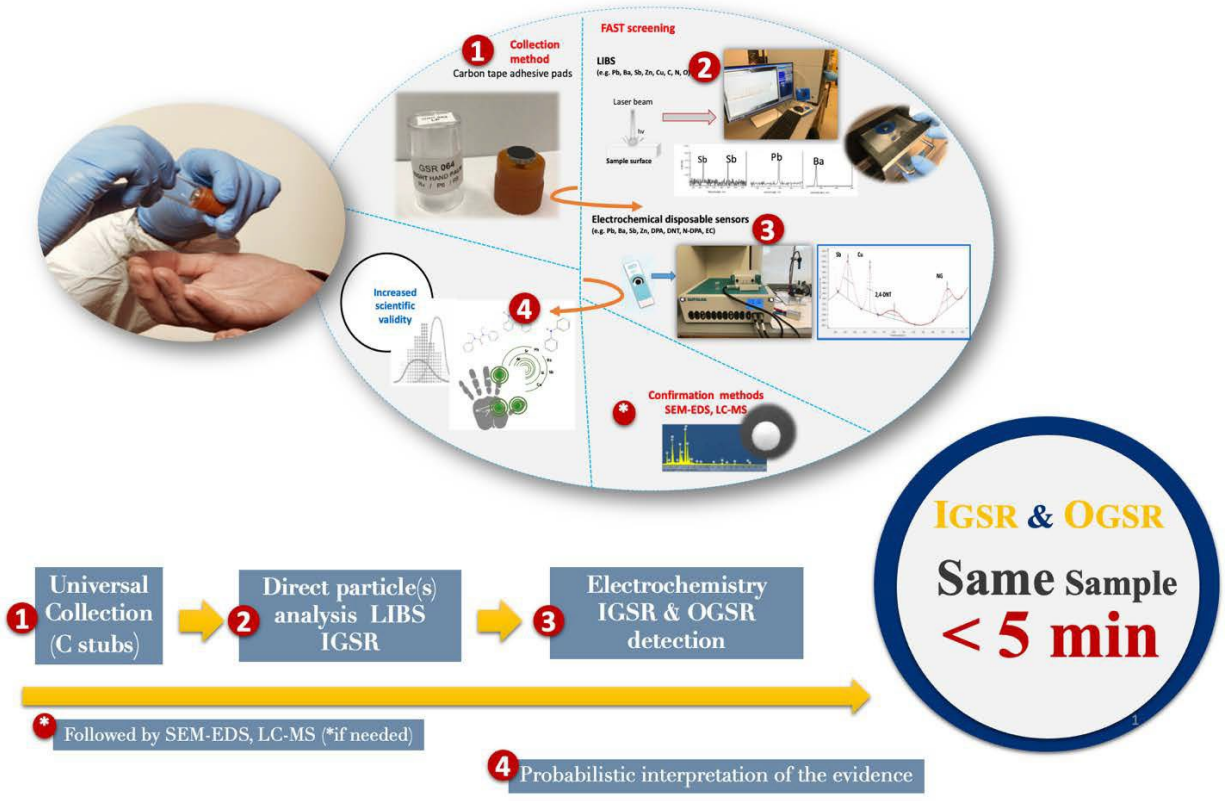

Figure 15. Schematic of the main steps for the characterization of IGSR and OGSR in hands: 1) collection with universal carbon stub, 2) direct IGSR particle(s) analysis with LIBS without sample preparation needed, 3) electrochemical analysis for IGSR/OGSR detection, 4) probabilistic interpretation using neural networks and likelihood ratios. All analyses are made in the same sample and SEM-EDS can be used for additional confirmation, when needed.

The following manuscripts and dissertation describe in detail the main findings of LIBS and EC applications to hands' residues:

1. Korina Menking Hoggatt, Ph.D, Spring 2021. WVU Department of Forensic and Investigative Science, Characterization of modern ammunition and background profiles: A novel approach and probabilistic interpretation of inorganic gunshot residue. Graduate Theses, Dissertations, and Problem Reports. 8336. https://researchrepository.wvu.edu/etd/8336

2. C Vander Pyl, C Martinez-Lopez, K Menking-Hoggatt, T Trejos. Analysis of primer gunshot residue particles by Laser Induced Breakdown Spectroscopy and Laser Ablation Inductively Coupled Plasma Mass Spectrometry. *Analyst.* 2021. https://doi.org/10.1039/D1AN00689D

3. W Feeney, K Menking-Hoggatt, C Vander Pyl, C Ott, S Bell, L Arroyo, T Trejos. Detection of organic and inorganic gunshot residues from hands using complexing agents and LC-MS/MS. *Analytical Methods.* 2021, 13, 3024-3039, https://doi.org/10.1039/D1AY00778E (Journal COVER PAGE and hot article)

4. Ott, K Dalzell, PJ Calderon, AL Alvarado-Gomez, T Trejos, L Arroyo. Evaluation of the Simultaneous Analysis of Organic and Organic Gunshot Residues within a Large Population Data Set Using Electrochemical Sensors, Journal of Forensic Sciences, 2020, https://doi.org/10.1016/j.forc.2018.02.006

5. K Menking-Hoggatt, C Martinez, C Vander Pyl, E Heller, E Pollock, L Arroyo, and T Trejos. Development of Tailor-Made Inorganic Gunshot Residue (IGSR) Microparticle Standards and Characterization with a Multi-technique Approach. Talanta. Published online December 2020, hardcopy publication April 2021, 225, https://doi.org/10.1016/j.talanta.2020.121984

6. W Feeney, C Vander Pyl, S Bell, T. Trejos. Trends in Composition, Collection, Persistence, and Analysis of IGSR and OGSR: A Review. Journal of Forensic Chemistry, 19, 2020, https://doi.org/10.1016/j.forc.2020.100250

7. K Menking-Hoggatt, L Arroyo, J Curran and T Trejos. Novel LIBS method for chemical micro-mapping of inorganic gunshot residue collected from hand samples, Journal of Chemometrics, Dec 2019. https://doi.org/10.1002/cem.3208

8. T Trejos, C Vander Pyl, K Menking-Hoggatt, AL Alvarado, L Arroyo. Fast Identification of Inorganic and Organic Gunshot Residues by LIBS and Electrochemical Methods, *Forensic Chemistry, Elsevier*, 2018, 8, 146-156. https://doi.org/10.1016/j.forc.2018.02.006 (published before grant start date, but served as basis for this project)

2.2.3.1. Task 3.1. Validation of LIBS method for GSR detection from hands

Two ablation patterns were evaluated during the method optimization, a bulk-line and a micro-spot mapping. In the bulk-line method, a single accumulated spectrum of 469 shots was collected from a line pattern 100 μm wide by 7 mm long. This bulk method demonstrated accuracy better than 87% for GSR classification. Although the bulk-line method was efficient, we decided to push further the capabilities of LIBS by moving away from a "micro-bulk" ablation line model to a "micro-mapping" model capable of producing simultaneous multielement information from only 2-shot ablations in a reduced space. This mapping method allows simultaneous detection of GSR markers (i.e., Pb, Ba, and Sb) on a fixed spatial area of only 100 μm in diameter, producing 25 individual spectra from different locations, and yielding an accuracy better than 93.7% for the evaluated datasets.[11]

A central aspect of GSR evidence analysis is the ability to find the combined characteristic elements within a single small particle. So, from a forensic perspective, chemical micro-mapping has several benefits. First, when we can narrow down the area from where the signal is produced, this increases the confidence that all inorganic markers are originating from a single particle or an isolated group of particles in a reduced area. On the other hand, the bulk analysis could result from the sum of numerous particles, or worse, the product of contaminants in the sample that mimic the IGSR composition. In the line pattern we do not have the added benefit of knowing where a combination of the GSR markers was simultaneously detected. The use of a micro-mapping pattern provided a viable solution to particle(s) analysis.

Second, since GSR particles are randomly distributed on the substrate during collection, micro-mapping allows for a more comprehensive analysis on the surface while still maintaining the integrity of the sample. The micro-mapping is done by ablating a 5 by 5 grid pattern. The 25 ablation spots result in a smaller amount of the stub being ablated—about 0.2%, as opposed to the bulk method at about 0.6%—and cause less damage to the surface of the stub, since the grid pattern only requires two laser shots per spot versus the 469 shots of the bulk method. A comparison of the optimized parameters for each method was summarized in **Table 1**.

The study indicates that the micro-mapping LIBS method is fit for purpose and produces reliable results similar, or superior, to the bulk-line ablation. A set of over 300 samples originating from 56 shooters and 51 non-shooters were used for validation purposes. Four different approaches—critical threshold, logistic regression, Naïve Bayes, and Neural Networks—were applied to examine the performance and accuracy of two different ablation patterns. The validation set resulted in an overall accuracy between 87-100%, depending on the ablation pattern and the type of prediction model applied (Table 9).

Additional shooter and background subpopulation sets were completed to validate the micro-mapping LIBS method. A comprehensive description of the statistical analysis and the performance rates of these sets are discussed below under task 4.

Table 7. Comparison of the statistical analysis and performance measures for LIBS ablation methods (bulk-line vs. micro-mapping) on a subset of 300 samples from shooters and non-shooters' hands.[11]

	Critical Threshold		Logistic Regression		Naïve Bayes		Neural Networks	
Performance measure	**Bulk-line**	**Micro-mapping**	**Bulk-line**	**Micro-mapping**	**Bulk-line**	**Micro-mapping**	**Bulk-line**	**Micro-mapping**
False positive	0.0%	0.0%	5.5%	0.3%	11.5%	0.2%	0.0%	0.0%
False negative	27.0%	0.0%	2.7%	0.3%	3.6%	0.9%	0.0%	0.0%
True negative (Specificity)	100.0%	100.0%	94.5%	99.7%	88.5%	99.8%	100.0%	100.0%
True positive (Sensitivity)	73.0%	100.0%	97.3%	99.7%	96.4%	99.1%	100.0%	100.0%
Accuracy	87.0%	100.0%	96.4%%	99.7%	93.7%	99.5%	100.0%	100.0%

2.2.3.2. Task 3.2. Validation of electrochemical method for GSR detection from hands

Optimization of the Square-wave Anodic Stripping Voltammetry (SWASV) method was conducted using Box Behnken designs and robustness tests. A list of the optimal parameters using screen-printed carbon electrodes was listed on **Table 2**. As part of a robustness study, the factor that had most impact on efficiency was the buffer solution freshness. It was found that false negatives decreased when the buffer was prepared the same day of analysis.

Sample preparation for this method involved the washing of a portion of the adhesive collection stub first with 50 µL of 0.1 M sodium acetate buffer pH 4.5. The buffer portion is then removed, and a 50 µL drop of acetonitrile is used for a second wash of the same area. The organic was evaporated at room temperature and using nitrogen. The buffer portion was then used to reconstitute the sample before analysis.

This method detected various leaded IGSR components (lead, antimony, copper), lead-free IGSR elements (Cu, Bi, Zn) and OGSR compounds (2,4-dinitrotoluene (DNT), diphenylamine (DPA), nitroglycerin (NG), ethyl centralite (EC), Arkadite II (AK2), 2-NDPA and 4-NDPA). A combination of inorganic and organic GSR makers was detected in authentic samples from the hand-skin of individuals that have fired a gun (**Figure 16**). An advantage of the combined detection of IGSR/OGSR is that increases confidence that traces originated from GSR as opposed to other non-GSR environmental sources. The limits of detection obtained for the target materials allowed a semi-quantitative approach for the analysis of samples (see **Table 8**).

Table 8. Summary of peak potential, limits of detection (LOD), linearity, and repeatability for target analytes in GSR using bare carbon electrode and SWASV.

IGSR	Potential (V)	Linear Range (µg/mL)	R^2	Repeatability (%RSD, n=3)	LOD (µg/mL)
Lead	-0.784 ± 0.035	0.10 to 2.0	0.999	4.4	0.055 ± 0.01
Antimony	-0.401 ± 0.027	0.75 to 7.5	0.986	10	0.183 ± 0.07
Copper	-0.292 ± 0.053	0.05 to 1.0	0.990	2.3	0.012 ± 0.001
Titanium	0.796 ± 0.015	5 to 20	0.977	7.1	4.72 ± 1.61
Bismuth	-0.431 ± 0.017	0.2 to10	0.999	4.7	0.262 ± 0.04
OGSR	**Potential (V)**	**Linear Range (µg/mL)**	**R^2**	**Repeatability (%RSD, n=3)**	**LOD (µg/mL)**
2,4-Dinitrotoluene*	−0.132 ± 0.032	1.0 to 20	0.982	5.6	0.200 ± 0.03
Diphenylamine	0.406 ± 0.018	1.0 to 8.0	0.987	6.2	0.462 ± 0.06
Nitroglycerin	0.509 ± 0.010	0.50 to 8.0	0.998	10	0.147 ± 0.08
Ethyl centralite	1.03 ± 0.045	0.50 to 8.0	0.998	8.0	0.450 ± 0.09
Akardite II	1. 01 ± 0.011	2.0 to 25	0.998	6.9	0.842 ± 0.13
2-Nitrosodiphenylamine	0.612 ± 0.010	2.0 to 12	0.954	9.9	0.557 ± 0.51
	0.233 ± 0.014	4.0 to 12	0.984	14	0.946 ± 0.24
	0.73 ± 0.015	2.0 to 12	0.998	8.3	1.13 ± 0.32
	-0.201 ± 0.015	4.0 to 15	0.846	55	1.39 ± 0.50
4-Nitrosodiphenylamine	0.002 ± 0.005	2.0 to 15	0.999	18	5.71 ± 4.11
	0.578 ± 0.010	4.0 to 15	0.964	9.5	0.254 ± 0.05

** 2,4-DNT was assessed as peak current height whereas all other analytes are assessed as peak current area.*

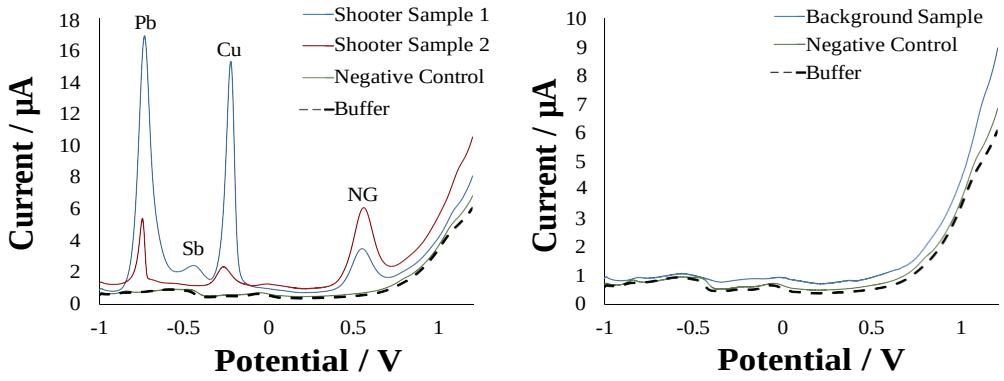

Figure 16. Example voltammograms for the analysis of shooter samples from standard ammunitions (left) and their comparison to background samples (right).

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Figure 17 demonstrates typical voltammograms and an average calibration curve obtained for the analysis of copper after the preconcentration step (reduction of Cu^{2+} to Cu^{0}) and later oxidation.

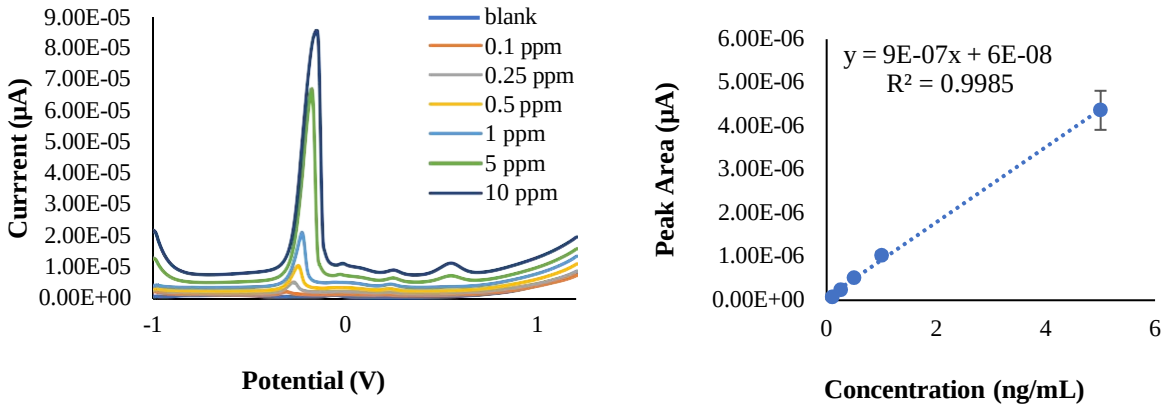

Figure 17. SWASV detection of copper using SPCE in buffer solution (left) and typical calibration curve for copper using the bare carbon SPCE (right).

One of the advantages of EC is its speed of analysis and non-destructive nature, allowing for combination with LIBS to further increase the accuracy of GSR detection. **Table 9** illustrates the individual performance of each method, as well as combined data in the identification of GSR in shooters samples and differentiation from non-shooter sets. Overall, neural networks showed superior performance and therefore was selected as the classifier method of choice for the additional population study.

Table 9. Summary of the performance rates for GSR by LIBS and EC on a population of 350 low risk backgrounds (non-shooters) and 520 shooters (200 leaded-ammunition, 100 lead-free and 220 mixed ammunition)

	Critical Threshold			Naïve Bayes			Logistic Regression			Neural Networks		
Performance measure (%)	LIBS	EC	**LIBS + EC**	LIBS	EC	**LIBS + EC**	LIBS	EC	**LIBS + EC**	LIBS	EC	**LIBS + EC**
False positive	0.0	0.9	**0.9**	8.7	50.4	**8.7**	25.2	0.0	**22.8**	26.1	0.0	**12.3**
False negative	8.1	41.5	**2.9**	10.0	18.9	**10.8**	2.2	4.9	**2.4**	2.0	5.3	**1.7**
True negative (Specificity)	100.0	99.1	**99.1**	91.3	49.6	**91.3**	74.8	100.0	**77.2**	73.9	100.0	**87.7**
True positive (Sensitivity)	91.9	58.5	**97.1**	90.0	81.1	**89.2**	97.8	95.1	**97.6**	98.0	94.7	**98.3**
Accuracy	95.2	74.8	**97.9**	90.2	78.2	**89.4**	95.6	95.5	**95.9**	95.8	95.2	**97.4**

2.2.3.3. Confirmation of GSR hand samples by SEM-EDS after LIBS and EC screening

As part of the cross-validation of the methods, a subset of each population was measured by SEM-EDS after LIBS and EC analysis in the same sample through a randomly generated chart. A six-sided rolling die model was used to determine which hand sample in the set would be analyzed (1-right hand, 2-left hand, 3-right back, 4-right palm, 5-left back, and 6-left palm). A total of 239 stubs collected from volunteers' hands were analyzed from the following populations: 56 stubs low-risk background, 52 shooters-leaded ammunition, 54 shooters post-activity (leaded ammunition), 50 shooters-lead free ammunition, and 17 for the high-risk background. A publication describing these results is currently in preparation, and a summary follows.

Samples were measured following ASTM 1588-20 standard for GSR analysis and protocols were established to maintain the traceability and quality control of the acquisition and documentation of the data:

1. The location of the samples in the SEM chamber was carefully selected to prevent contamination between samples, and the process documented through a QC diagram sheet (SEM-EDS Sample State Setup for GSR diagram sheet)
2. Samples were maintained caped until analysis and always manipulated with gloves and set into the stub holder with specialized SEM tweezers to transfer the stubs without touching the adhesive surface.
3. As part of our quality control program, the date and time are recorded when the filament is turned on and off to monitor the filament life and instrument use.
4. A QC standard (GSR_QC_check, ENFSI Duo GSR proficiency stub) and blank control are analyzed daily between each hand sample.
5. Each automated batch is reviewed to ensure the software is set appropriately, check the hand samples and QC controls' order, and check that each sample has the correct position selected.
6. Acquired data is manually reviewed by at least two examiners following standardized criteria.

The collected hand samples were reviewed and recorded for the total number of features, their classification, and the number of features detected for the "characteristic," "consistent with," and "commonly associated particles" categories. The percentage of the stub that completed the particle discovery was also recorded. The recipe was edited to stop finding particles once it reaches 10 characteristic features for leaded ammunition and 20 characteristic features for lead-free ammunition. This cut-off was somehow arbitrary as there is no standardized threshold within the SEM-EDS GSR users' community. Some laboratories use the cut off at ten characteristic particles, some monitor the whole stub, and others have different intermediate thresholds, depending on instrument configurations, personnel and instruments, and casework load. We selected this cut-off as a compromise between the speed of analysis and the results' confirmatory value.

The "Review features" in the workflow were also selected to review the morphology of the samples. Metadata concerning the morphology for characteristic particles were recorded, such as spheroid versus irregular particles. A schematic of the workflow is depicted below.

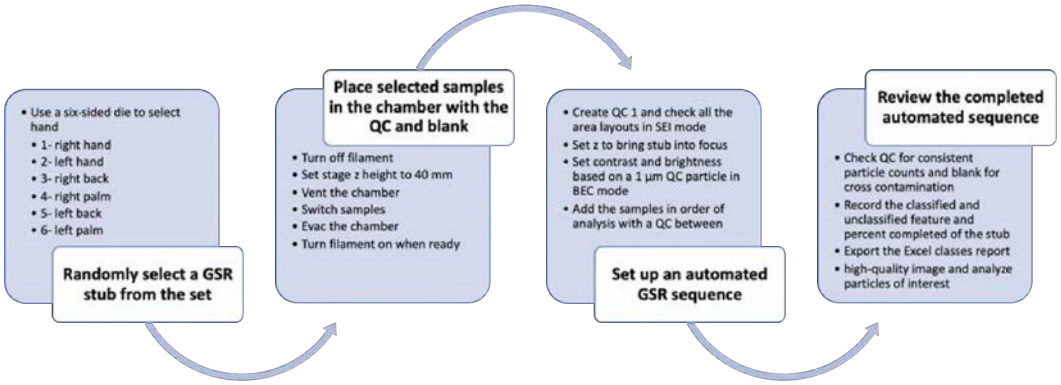

Figure 18. Workflow of the SEM-EDS sample selection and automated GSR particles discovery procedure.

The ASTM standard establishes that characteristic or consistent GSR particles are often spheroid and ranging from 0.5 µm to 5 µm in diameter.[37] The elemental composition of those particles provides additional confidence for GSR identification. The standard practice classifies the elemental information for IGSR particles into three categories depending on the confidence to assign the sources as GSR (characteristic, consistent with, or commonly associated with)

Samples that are "characteristic" for IGSR particles contain lead, barium, and antimony.[37] This combination is rarely found in particles from any source other than GSR. Characteristic particles demonstrate the presence of IGSR, but do not indicate that the sampled individual had fired the weapon, only that they could have been in the vicinity of a shooting event. Samples that are classified as "consistent with GSR" contain particles of two of the characteristic elements, with silicon or calcium also sometimes being detected. Samples that are classified as "consistent with GSR" particles may be attributed to sources other than firearm ammunition, so morphology must also be considered during analysis. Commonly associated samples contain one of the characteristic elements, in addition to the presence of other more common elements such as potassium, zinc, or aluminum. [14] This classification is the weakest ranking for GSR identification since these particles can derive from numerous environmental sources.

Research of modern, lead-free ammunitions using tailor-made IGSR suspension that were characterized by our research group allowed for the creation of custom classifications for lead-free ammunition and elements not included in the ASTM method.[12] Lead-free ammunition is challenging because it does not contain the same elements of interest as leaded ammunition and the ASTM standard does not contain a comprehensive list of elements for identification of these IGSR. As a result, categorization was based on a tailor-made microparticle standard, which composition was previously characterized using three different instruments (ICP-MS, LIBS, and SEM-EDS). Lead-free ammunition brands that were utilized in this research study were Fiocchi, SYNTECH, and CCI.

Elements of interest targeted for classification in the Fiocchi ammunition were potassium, copper, and sometimes bismuth. When characterizing this ammunition though, bismuth was not consistently detected by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Laser Induced Breakdown Spectroscopy (LIBS) since it was too low in concentration (< 1 ppm). Bismuth was the only consistently characteristic element in the SYNTECH ammunition, while CCI was classified based only on barium. The presence of these 'known" elements GSR from the respective lead-free ammunition

was therefore deemed characteristic for the lead-free set. While the ASTM standard does not even list bismuth as a possible GSR element, it does define an elemental combination of titanium and zinc as "consistent with" classification for lead-free ammunition. The Ti/Zn combination can be indicative of the elemental composition of the cartridge case, and this was also included in the automated IGSR classification recipe for lead-free ammunition. In the study, only a few particles contained this combination, and the elements of interest were more consistent with the elements identified by ICP-MS and listed above for each type of ammunition.

SEM-EDS results for low-risk background population

In congruence with LIBS and EC analysis, the incidence of false positive particles on background samples was very low. **Figure 19** displays the total number of particles identified by SEM-EDS for the low-risk population, originating from 56 stubs. While five particles were originally classified as characteristic and numerous other particles were given "consistent with" rankings, further data analysis excluded the particles as they were not indicative of GSR. Moreover, the few particles considered characteristic had a combination of SbSnBa or TiZnCu that was established in our protocol, to include leaded free GSR, but are not considered characteristic according to the newest ASTM standard.[37]

The background samples contained several particles of TiZn, Sr, SbBa, SbPb, and BaPb; all these compositions classify as the "consistent" category. These additional elemental combinations were observed in relatively similar frequency in the low-risk background samples and the leaded shooter samples (**Figure 21**), and the leaded shooter activity samples (**Figure 22**). In addition, the morphology of these particles was recorded and none of the particles were spheroid, so the particles were not confirmed as GSR. The exception can be found in **Figure 20**. While the particle contained the typical characteristic elements, this was the only particle indicative of GSR found on the entire stub, which could weaken the evidential value of a sample due to the low particle count.

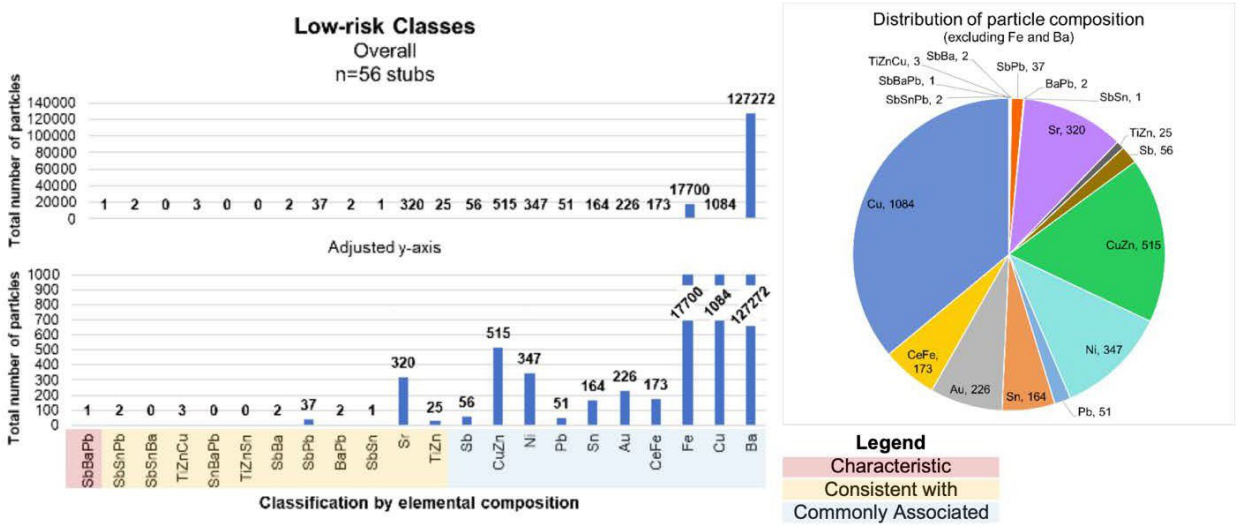

Figure 19. Low-risk background population and the classification with rankings for the total number of particles found on all the stubs analyzed (n=56). The characteristic particle's category includes leaded and lead-free elemental profiles.

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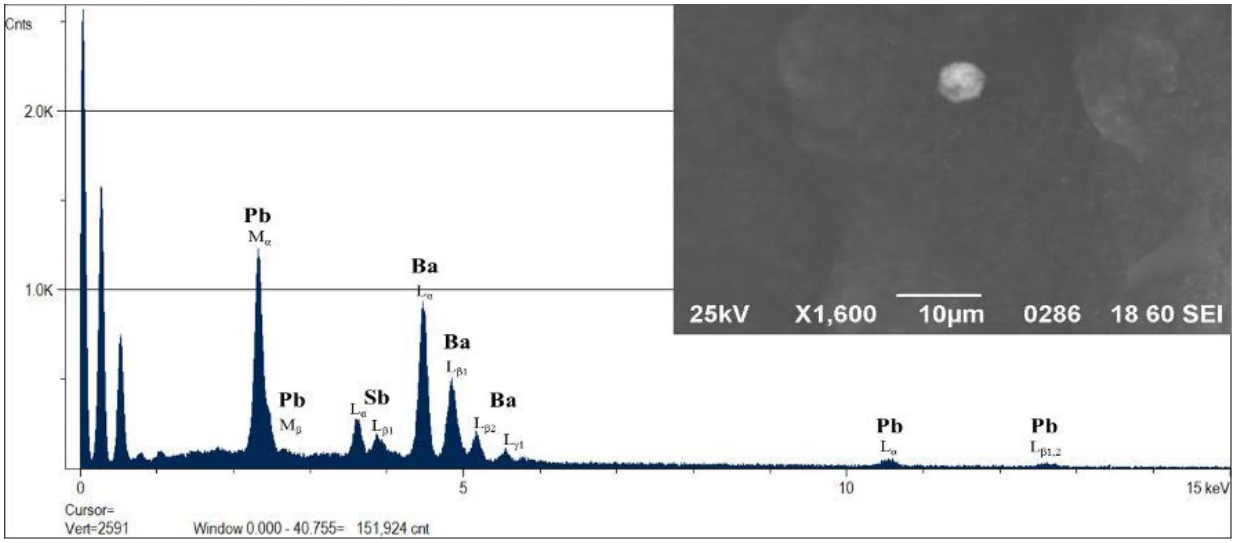

Figure 20. SEM-EDS spectrum and image of the only characteristic GSR particle identified during the confirmation of low-risk background hand samples.

SEM-EDS results for leaded-shooter population and post-activity population

The discovery of GSR particles on the leaded shooter population was straightforward, allowing GSR confirmation on all samples, even after analyzing the same stub by LIBS and EC. All leaded shooter samples had at least 6 characteristic particles, with a dominant typical particle composition of SbBaPb and spheroid morphology. In the "consistent with" category, most particles were combinations of SbPb, SbBa, or BaPb. Finally, in the commonly associated class, the most prominent were particles containing only Ba or Fe. A summary of all the elemental combinations found during confirmation can be found in Figure 50. Also, out of the 52 stubs analyzed, 47 reach the threshold of 10 potential characteristic particles not requiring monitoring the entire stub's surface. Only five stubs required scanning the whole stub because the software did not flag 10 GSR characteristic particles.

In the shooter's post-activity set, characteristic spheroid particles were still detected. Like the pre-activity samples, the incidence of particles consistent or commonly associated with GSR was observed after activity. The ten characteristic particle threshold was reached in this population, with only one stub needed 100% mapping. In this sample, no characteristic particles were found but two particles that were consistent with IGSR were flagged and identified as IGSR. **Figure 22** provides a graph of the elemental combinations and total number of particles, which is comparable between the two leaded sets (with and without activity), except the amount of barium found in the leaded shooter population is 100 times higher. Still, both sets were considered positive for presence IGSR according to the ASTM.

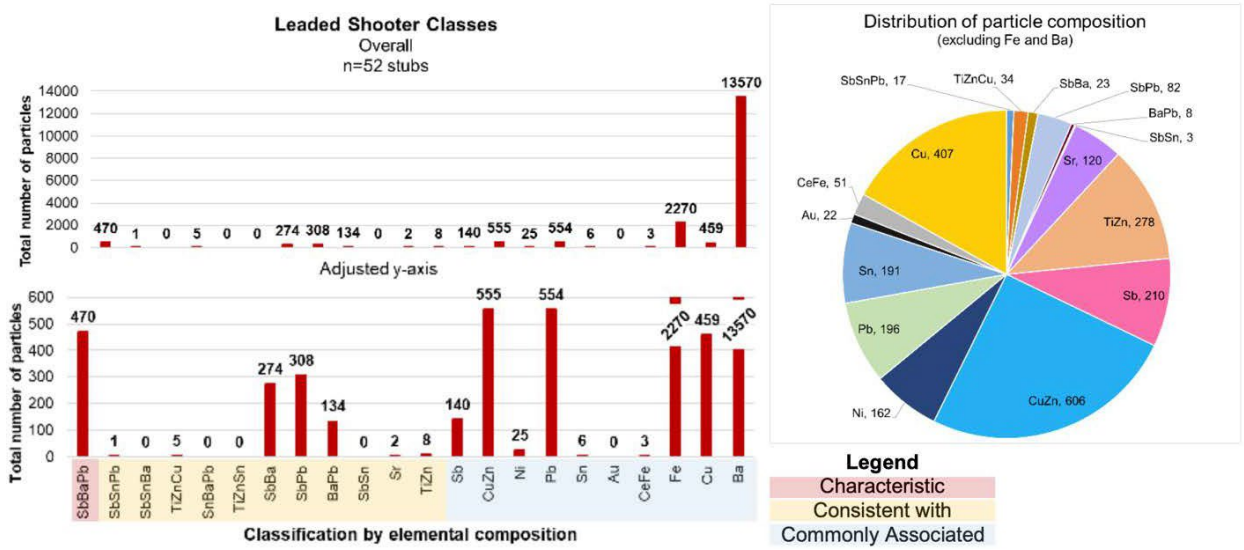

Figure 21. Leaded known shooter population and the classification with rankings for the total number of particles found on all the stubs analyzed (n=52)

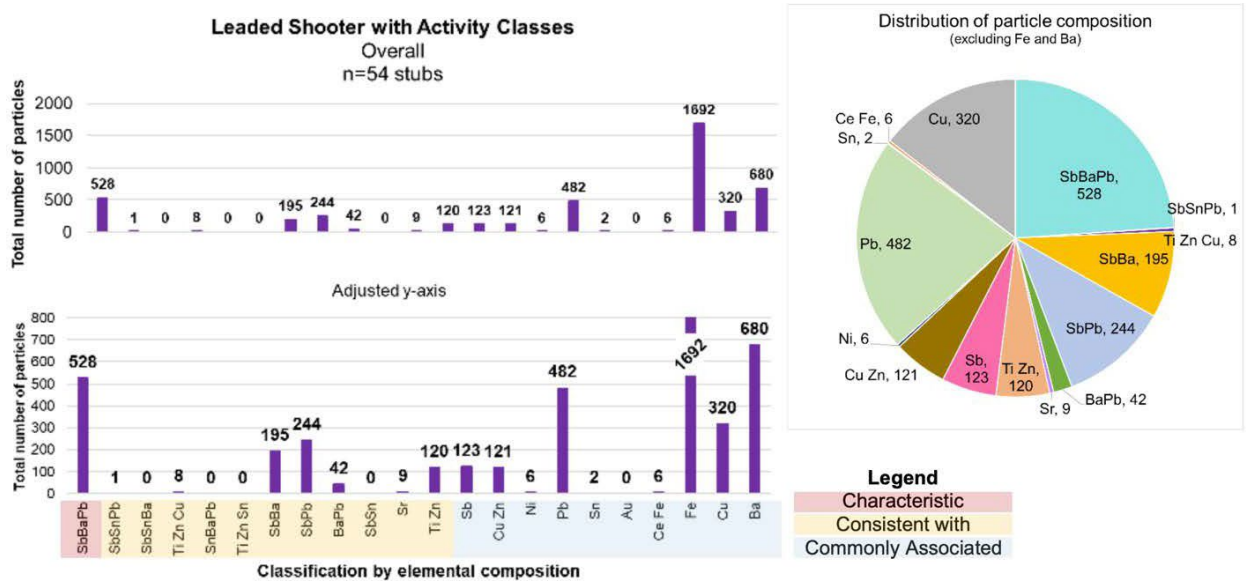

Figure 22. Leaded known shooter with activity population and the classification with rankings for the total number of particles found on all the stubs analyzed (n=52).

SEM-EDS results for leaded-free shooter population

A challenge during the confirmation of hand samples was the amount of "only barium" particles observed in the different populations. Barium is considered a commonly associated element in the ASTM guidelines. Figures 19 and Figure 21 show a high particle count for barium present in the shooter's samples and the backgrounds. What makes this tricky is that Ba is one of the few elements that permit elemental identification of a GSR for some lead-free ammunition. For instance, the CCI

ammunition determined barium to be the only IGSR element of interest in the primer (**Figure 23**). This is problematic for GSR identification because the ASTM standard classifies the presence of Ba as a commonly associated feature, so identifying an IGSR particle from a CCI primer could be quite challenging for interpretation and not exclusive of lead-free formulations. Upon analysis of the CCI ammunition, the morphology was analyzed to determine if particles containing barium had spherical or irregular morphology to classify characteristic GSR. Only then was the sample considered positive if the particles were spheroid. Both types of morphology were observed by the analyst (**Figure 24**).

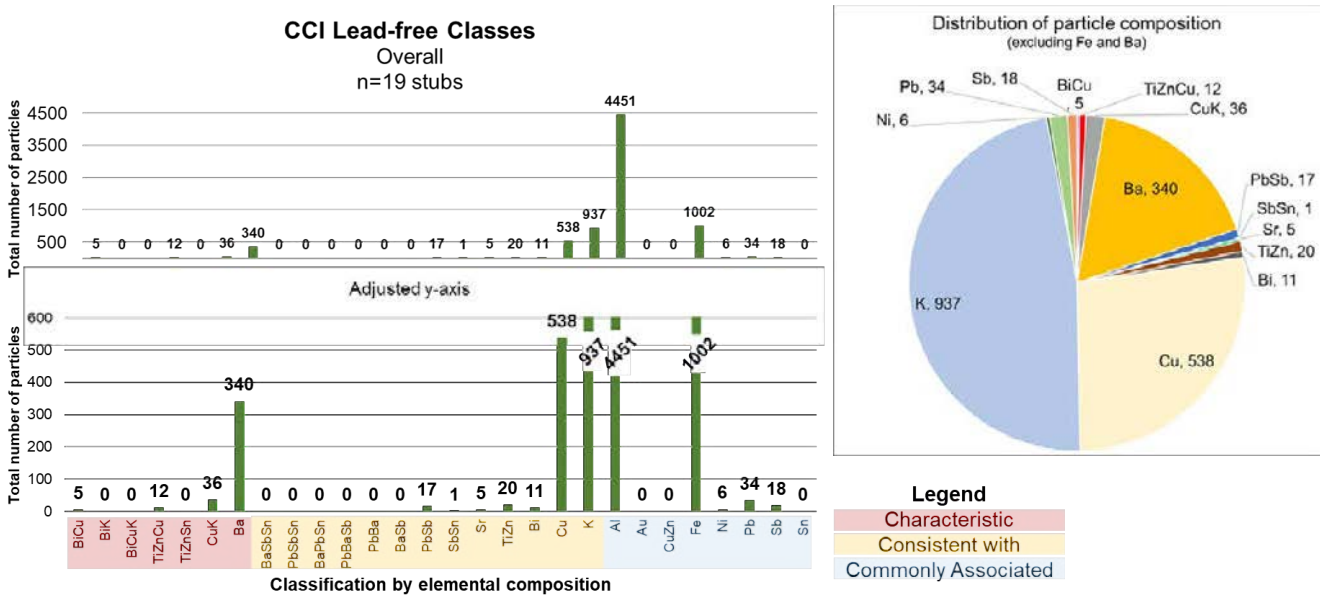

Figure 23. Lead-free known shooter from CCI ammunition and the classification with rankings for the total number of particles found on all the stubs analyzed (n=19)

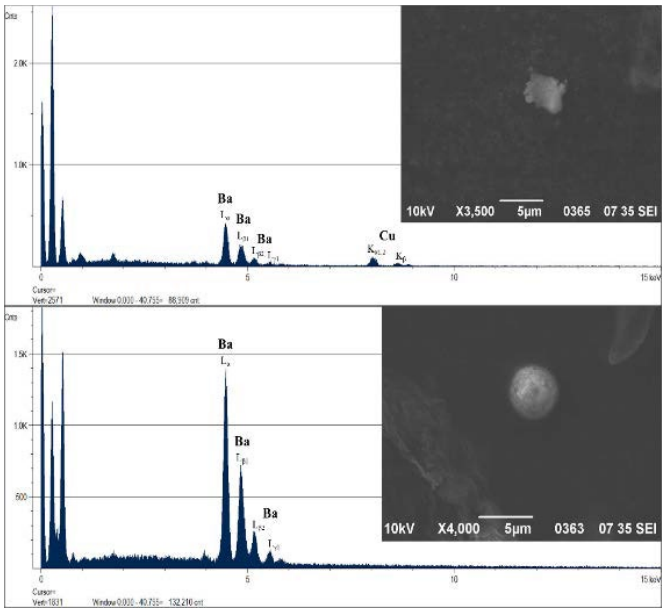

Figure 24. SEM-EDS spectrum and image of irregular (top) and spherical (bottom) CCI GSR particles identified during the confirmation of lead-free known shooter hand samples

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The other two types of lead-free ammunition analyzed were SYNTECH with only bismuth present as an IGSR element of interest (**Figure 25**) and Fiocchi with copper and potassium, both lighter atomic weight elements (**Figure 26**). Particles that were considered characteristic were identified with customized elemental combinations based on the characterized tailor-made IGSR primer suspensions and the ASTM standard guideline. Despite the challenges of only one GSR marker of interest in the SYNTECH ammunition and lighter elements in the Fiocchi ammunition, the automated SEM-EDS discovery for IGSR analysis was able to identify the particles based on these adapted combinations correctly. So, despite the changing elemental composition of modern ammunition, it is possible to identify IGSR by SEM-EDS when using custom-made classification recipes.

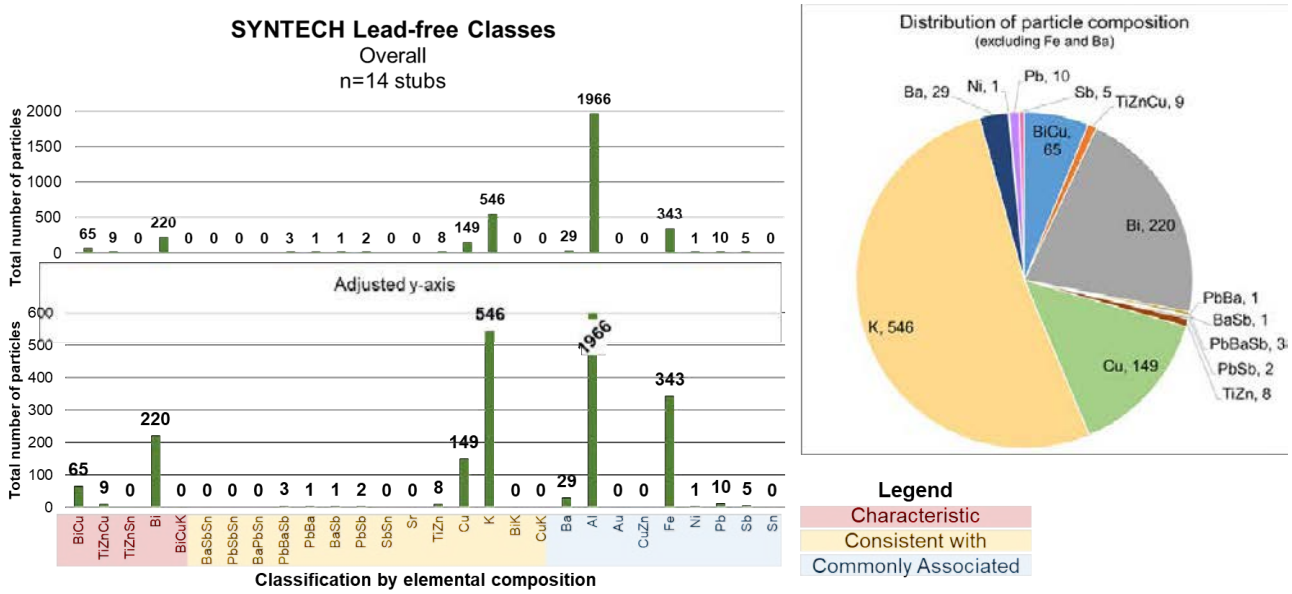

Figure 25. Lead-free known shooter from SYNTECH ammunition and the classification with rankings for the total number of particles found on all the stubs analyzed (n=14).

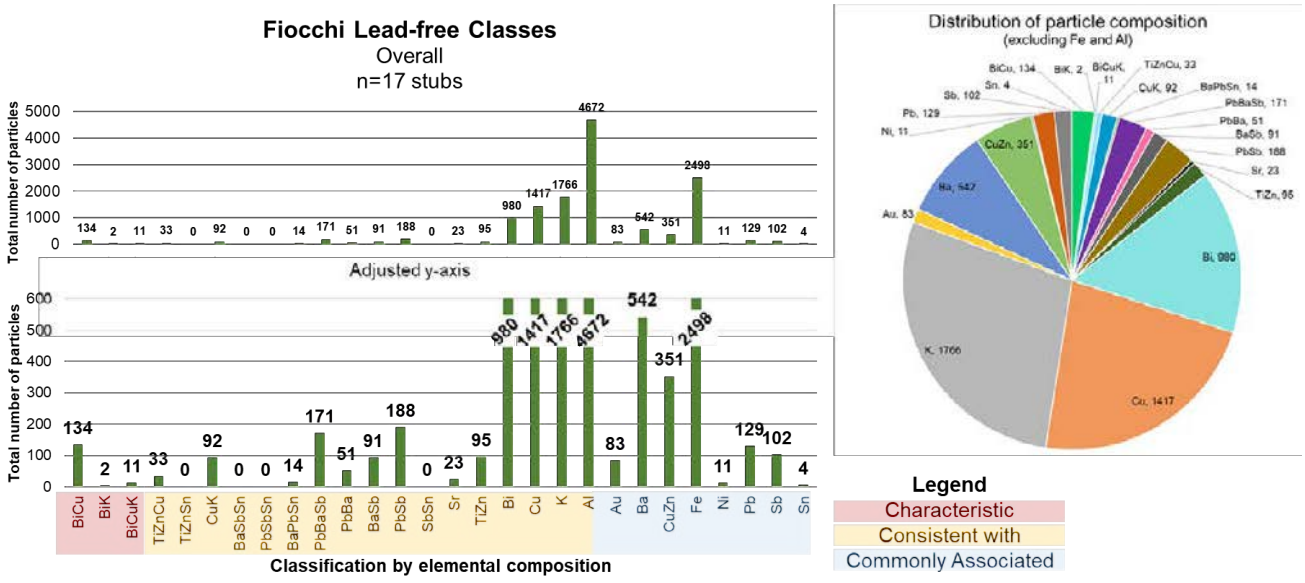

Figure 26. Lead-free known shooter from Fiocchi ammunition and the classification with rankings for the total number of particles found on all the stubs analyzed (n=17).

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Another interesting observation during the confirmation of hands samples by SEM-EDS was discovering micrometer-sized particles that were spherical (**Figure 27**). While they lack the typical leaded characteristic elements, a particle such as this could be mistaken for a GSR particle originating from lead-free ammunition (**Figure 28**). Ongoing research into the changing composition of modern ammunition is essential, but equally important is the background population's characterization. These spherical microparticles are a perfect example of how important this research is to the forensic community.

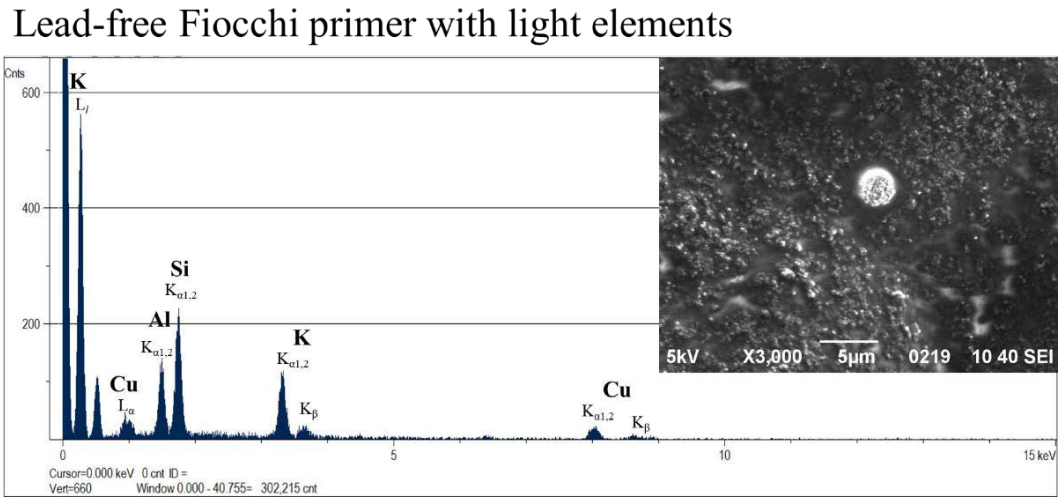

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Figure 27. SEM-EDS spectrum and image of a Fiocchi shooter sample observed during the confirmation of the lead-free known shooter samples.

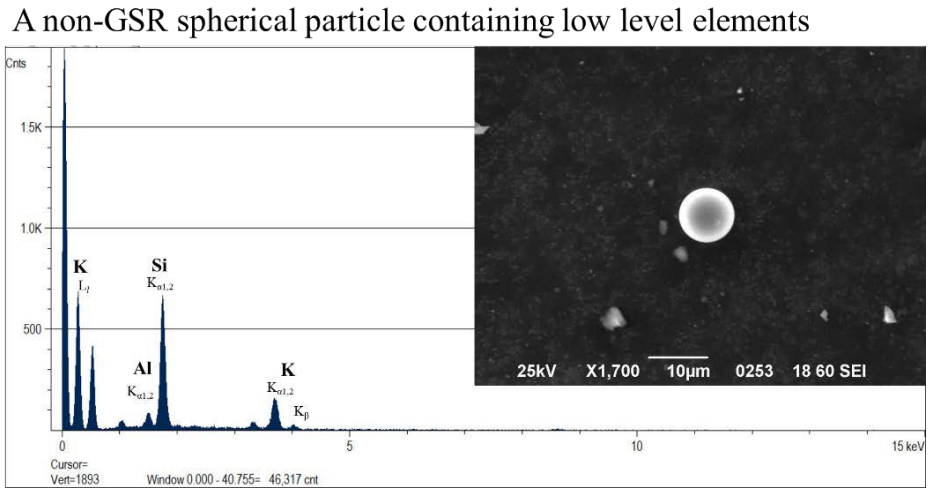

Figure 28. SEM-EDS spectrum and image of a spherical microparticle observed on a low-risk background samples during confirmation.

2.2.4. Results and Findings for Task 4: Validate statistical data pre-processing, multivariate analysis, and machine learning algorithms to generate decision thresholds and probabilistic approaches for interpretation of the significance of the evidence

This task was focused on the development and validation of statistical methods to evaluate data obtained from multiple sensors, to improve the objectivity of the determinations and to provide a probabilistic output that increases the evidentiary value of the evidence. The statistical analysis and validation of the proposed methodologies is a critical stage to enhance the current interpretation of criminal cases involving GSR evidence.

As is typical with this type of project, data analysis involved data exploration (box plots, critical thresholds, and PCA) and predictive modeling (discriminant analysis, neural networks, logistic regression, and Naïve Bayes). Standard multivariate statistical methods have been applied to the LIBS and EC data to identify and understand any underlying patterns in the data. These models have been well suited for the sensors proposed in this study, as they perform well for modeling complex relationships among large data sets and multivariate data and do not rely on normal distributions or correlations among variables. The correct classification rates from validation sets have been generally over 90% depending on the method applied (**Table 9**)

We established data-driven thresholds to differentiate a positive result from a negative outcome. A variety of methods can establish these cut-off levels. In this project, we applied machine learning outputs from control samples to estimate probability distributions and likelihood ratios. A publication describing the findings of this extensive population study is ready for submission.

In this study, we report the prevalence of OGSR and IGSR in various populations and evaluates the feasibility of using combined organic and inorganic data for the probabilistic interpretation of GSR evidence. The survey included over 3,200 samples and 104,000 spectral data files from six subpopulations. The known shooter samples originated from firing pistols and revolvers using various ammunition types: leaded (set 1), lead-free (set 2), and a mixture of both (set 3) collected shortly after discharging the gun or after conducting various post-shooting activities such as running, running in the rain, using hand sanitizer, and vigorously rubbing the hands together (set 4). Background samples originated from individuals who had not fired a gun in the past 24-hours, separated by low-risk (set 5) and high-risk (set 6). Low-risk samples were collected from volunteers who did not have hobbies or professions that could mimic GSR, while high-risk included firearm researchers, agriculture workers, mechanics, and police officers/administrators. The figures below illustrate the breakdown of the different subgroups.

Samples were first analyzed by LIBS in under two minutes, which detected multiple elements of interest and provided 25 spectra per sample with spatial information. Then, electrochemistry was completed on the same stub, simultaneously detecting IGSR and OGSR (Pb, Cu, Sb, 2.4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), nitroglycerin (NG), and ethyl centralite (EC)) in under 3 minutes. Lastly, a subset from each population was analyzed by SEM-EDS for morphological and elemental information, demonstrating that SEM-EDS could confirm GSR following screening with these novel methods. SEM-EDS elemental profiles corresponded to those obtained by LIBS, further demonstrating the benefit of using LIBS for fast learning of composition of large populations.

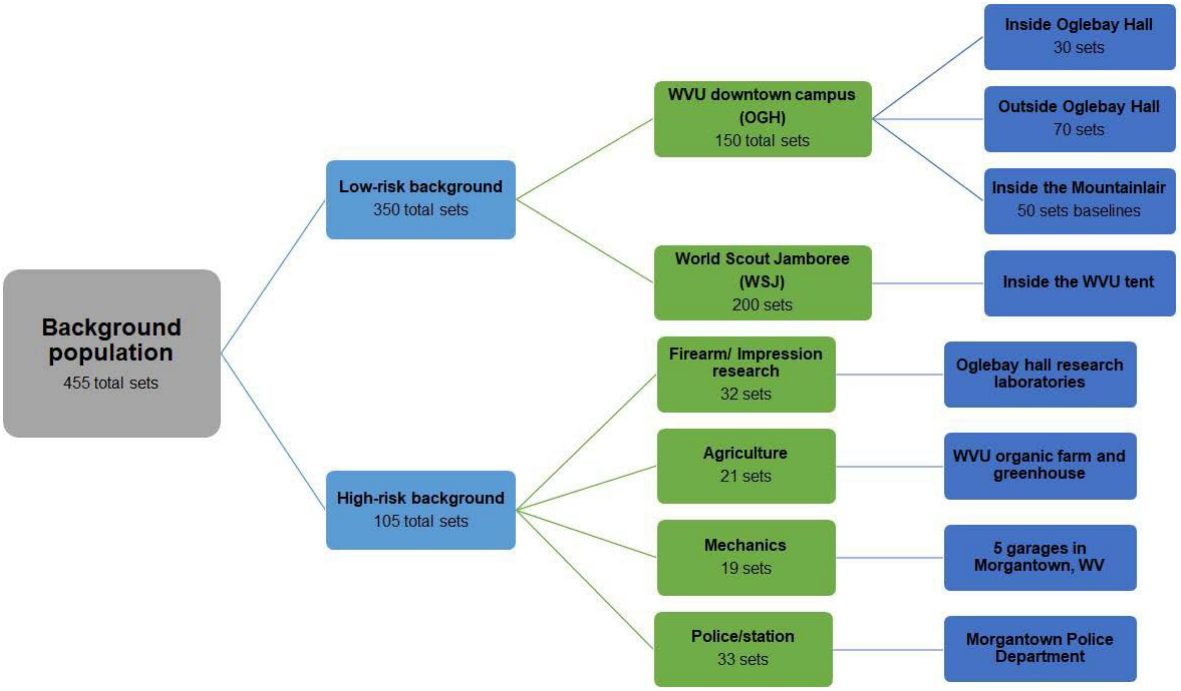

Figure 29. Flowchart of the low-risk background samples collected for the GSR hands population study.

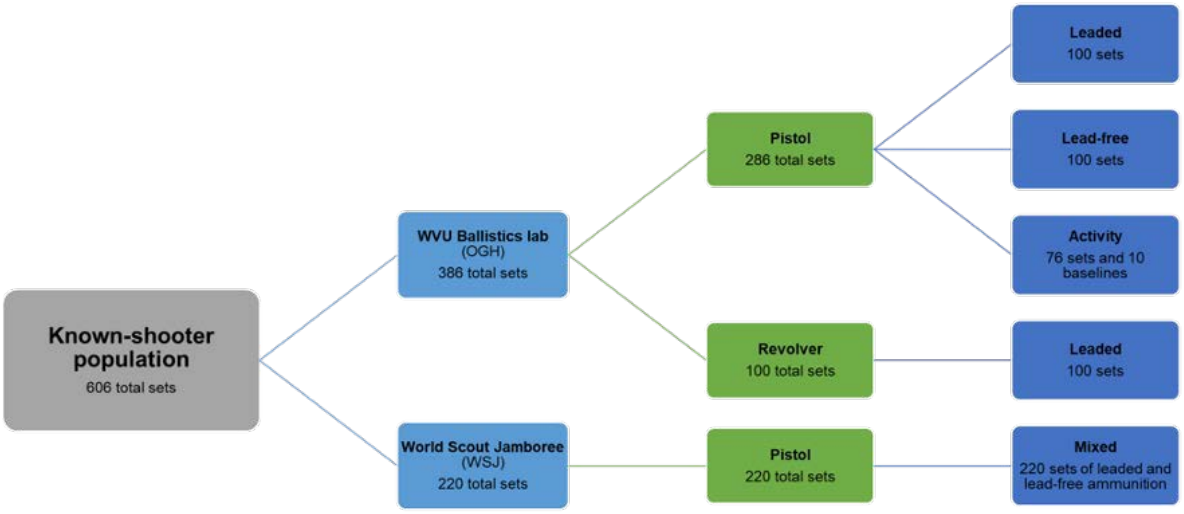

Figure 30. Flowchart of the known shooter samples collected for the GSR hands population study.

2.2.4.1. Trends and prevalence of IGSR/OGSR compounds in the sampled subpopulations

Prevalence of possible GSR elements and compounds was evaluated for the different background and shooter populations. The single presence of Pb was found in the low-risk, high-risk, and leaded shooter populations at occurrences of 26.8%, 64%, and 83% using LIBS, respectively. Barium was highly prevalent (94% in low risk, 100% high risk, 100% in shooters). Conversely, some GSR elemental combinations, such as PbBaSb were present in 100% of the leaded shooter samples, 16% of the high-risk, and only 1.8% in the low-risk background. Lead-free elements were more frequent in background sets (up to 64% TiZn or 92% Cu). Finally, using the electrochemical method, OGSR compounds such as NG were detected in shooter sets but remained undetected in the background populations. Combinations of OGSR and IGSR were not detected in background sets by EC, indicating enhanced certainty when observed in known shooter samples.

To truly understand the influence of observing potential IGSR elements of interest in a single particle, we must also examine the presence of these elements and combinations in the background population. For simplicity's sake, this study focused on eight elements and their major emission lines based on previous studies conducted in our group, where we determined major IGSR elements of interest using tailor-made *p*GSR standards as a ground truth for the IGSR elements in ammunition used in this study and can be found in the legends of **Figure 31** and **Figure 32**.[16] The relative distribution of elements within and between the different subgroups is presented as boxplots. Also, to compare the occurrence of specific elemental compositions by subpopulation, we report the relative percent of samples that presented positive detections (above an established detection threshold). These percent metrics represent the occurrence of certain elements or combinations but do not necessarily represent false positive rates for GSR. A positive GSR call considers other categorical classification and criteria and is out of the scope of this section's discussion.[11,15]

Low-risk background —All the elements monitored were present at some extent in the low-risk individuals, indicating the need to establish thresholds above which a residue would be considered GSR. However, as expected, the low-risk background presented lower SNR and less incidents above the maximum boxplot's whisker than the other populations, for all the elements (**Figures 31 and 32**). This population only had 4.3% of the samples test positive for the elemental combination of PbBaSb, which was reduced to 1.8% when confirmed by SEM-EDS. The lead-free IGSR elements of interest were Bi, Cu, Ti, and Zn, with addition to elements also found in leaded ammunition, Al and Ba. In the low-risk population, a combination of any 3 lead-free elements of interest was observed in 12.3% of the samples, and a combination of 4 elements was even lower at 3.1%. For 3 lead-free elements present, this was reduced to 1.8% when confirmed by particle analysis by SEM-EDS and we did not observe any combinations of 4 element's particles.

High-risk background —The other background population of interest was the high-risk set collected from individuals that performed activities at risk of being confused for GSR (false positives). When compared to the low-risk population, we observed comparable spread of the box plots, but numerous samples presented values above the maximum whisker in comparison to the other populations (**Figure 31** and **Figure 32**). It is important to note that although the box plots identified as outliers samples above the estimated maximum, in the context of this study, they do not represent true outliers as they are a result of the random distribution of GSR during a firing event. The spread is further

increased by the variation of professions and hobbies considered in this set. LIBS analysis of the overall high-risk population found 16.8% of samples were positive for the combination of leaded elements (PbBaSb) and 45.7% were positive for combinations of 3 lead-free elements. If we breakdown this population by the different high-risk groups, the positive samples in the ballistics researchers and police officers/administrators were 42.4% (leaded) and 5.1% (lead-free) respectively, in mechanics were 89.5% (leaded) and 57.9% (lead-free) respectively, and in agriculture workers were 9.5% (both leaded and lead-free). This observation shows that mechanic groups are the most at risk for false positives for the presence of IGSR elements. The percent of positive stubs was also comparable to the results seen by SEM-EDS. For this population, most common single-element particles in the high-risk sets were Sb (74.9%), followed by Ba (51.5%), and lastly Pb (17.2%).

Leaded and lead-free shooter populations —As can be seen in **Figure 31** and **Figure 32**, there is a large increase in the presence for Ba and Cu, and a relatively smaller increase in Pb for the leaded shooter population as compared to the background populations. Conversely, a comparable amount of Pb is seen between the low-risk and lead-free shooter population. Interestingly, aluminum seems to be more prevalent in background samples as compared to the leaded and lead-free shooter samples. For this reason, the presence of Al with only one major GSR element (e.g., Pb, Ba, Sb, Zn) does not assist in GSR identification and presence of Al with multiple elements does not significantly add to interpretation of the evidence.

In addition, titanium appears to be more prevalent in the background than aluminum. LIBS analysis determined 27.1% of the low-risk, 33.3% of the high-risk, 56.9% of the lead-free shooters, and 18.5% of the leaded shooter samples contained Ti, leading us to conclude that Ti is present in the environment, but not at the relative high levels it is present in some lead-free shooter samples.

As for the presence of the 3 leaded elements PbBaSb in the leaded shooter population, 62.5% were positive for all three. For the lead-free population, for combination of three lead-free elements, 45.7% were positive by LIBS analysis. When looking at combinations of only 2 elements, this increased to 95.0% for leaded elements and 73.8%, for lead-free profiles, respectively. When confirmed by SEM-EDS, this increased to 100% detection of GSR particles in both leaded and lead-free shooter populations.

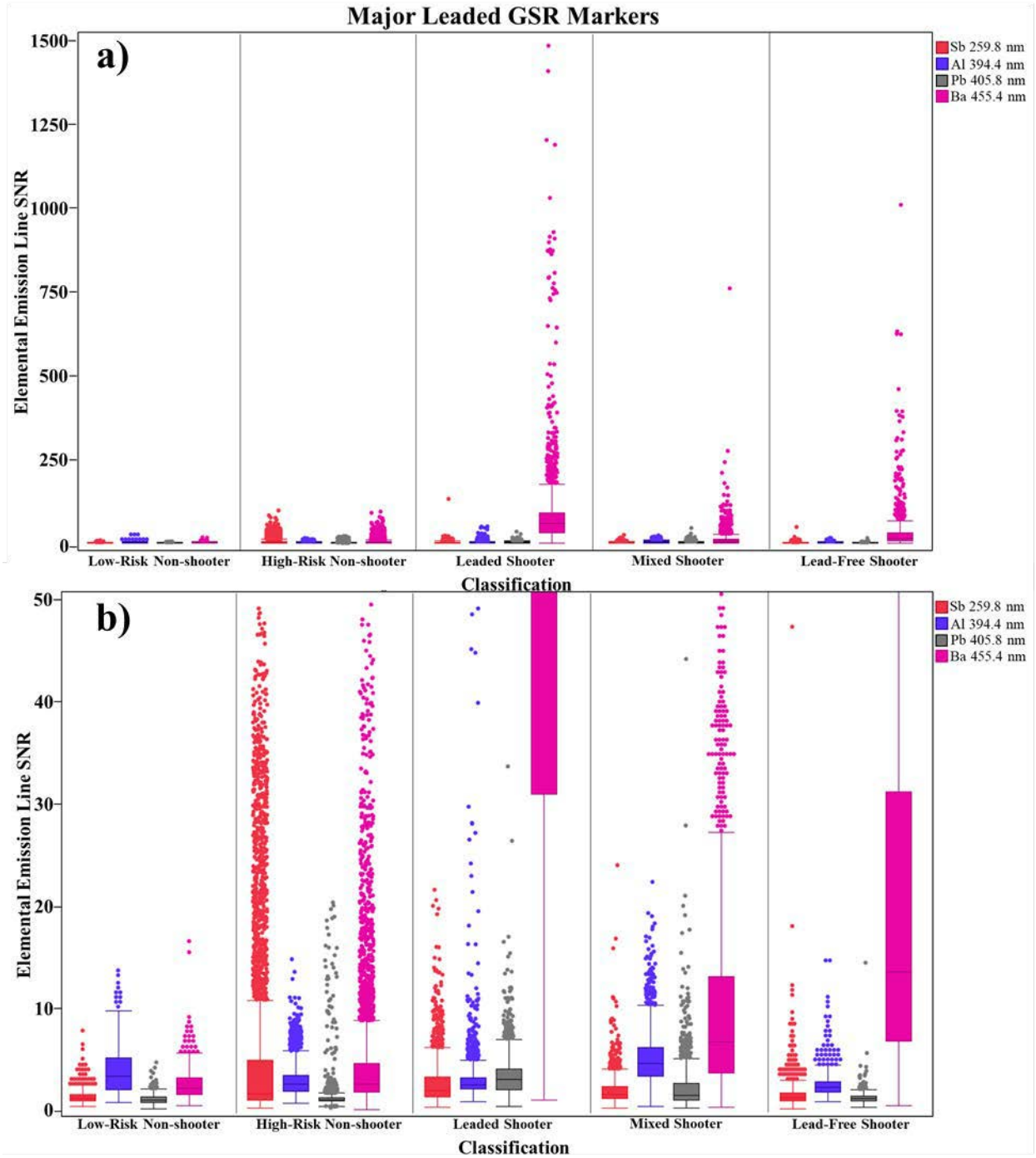

Figure 31. LIBS comparison box plots for the signal-to-noise ratio for leaded GSR elements lead, barium, antimony, and aluminum for selected subpopulations. a) overall b) zoomed.

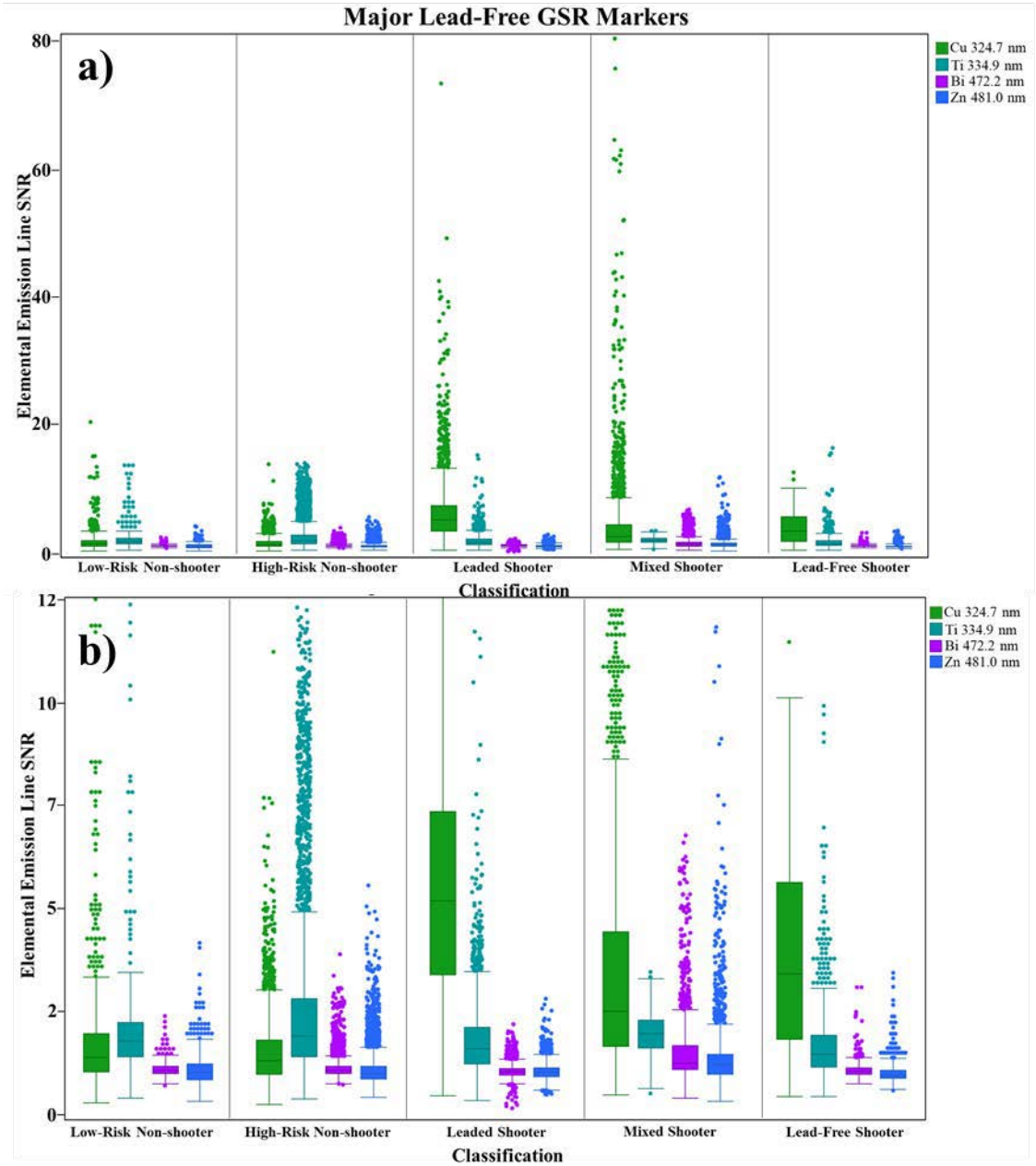

Figure 32. LIBS comparison box plots for the signal-to-noise ratio for lead-free GSR markers bismuth, copper, titanium, and zinc for selected subpopulations. a) overall b) zoomed.

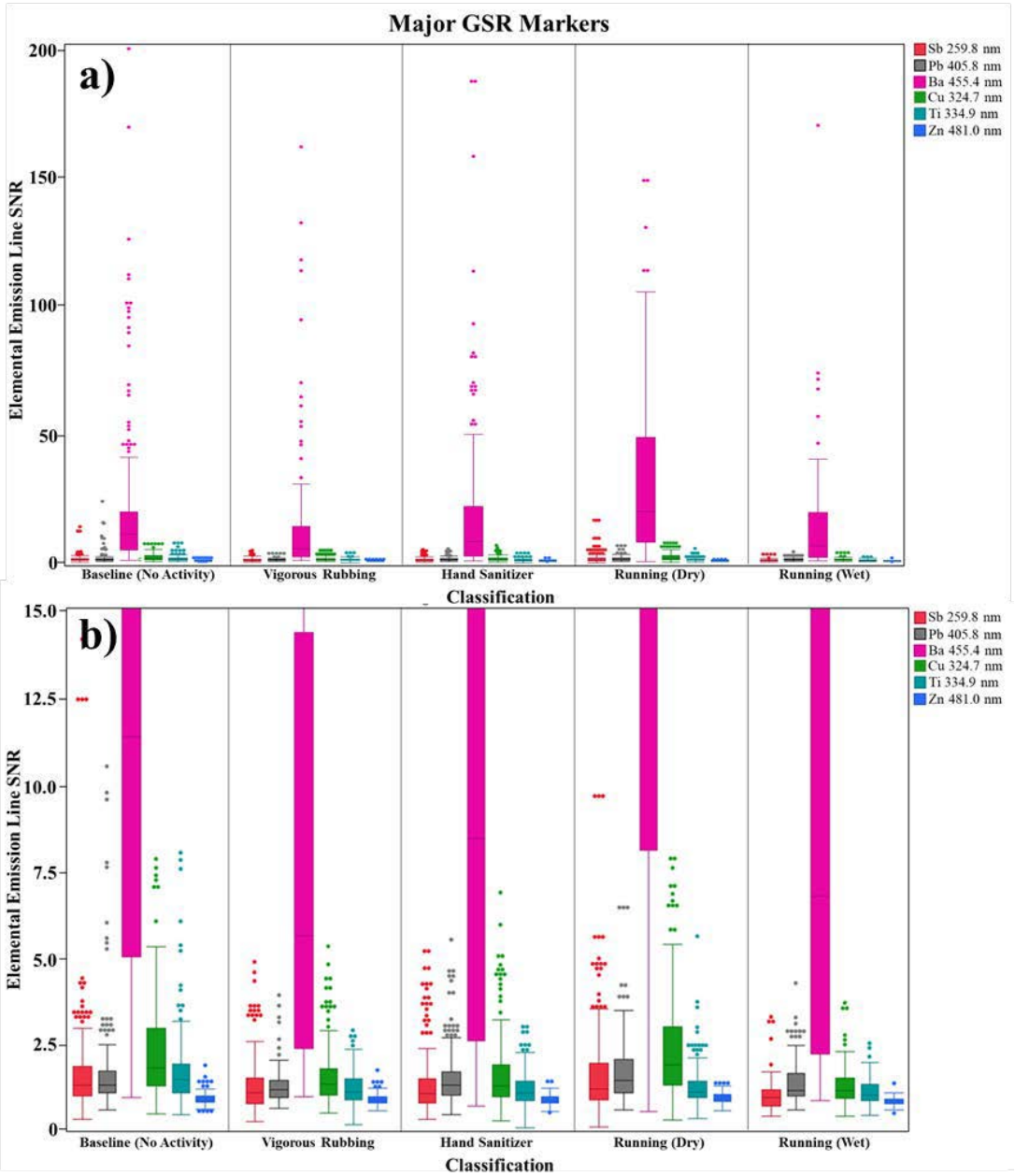

Figure 33. LIBS comparison box plots for the signal-to-noise ratio for both leaded and lead-free GSR elements for the leaded activity subpopulation broken down by different activities and compared to a baseline of no activity. a) overall b) zoomed.

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More complex shooter populations—The additional sets of leaded activity and mixed shooter samples represented samples that might be submitted to the laboratory under more challenging situations. The post-shooting activity samples recreated possible scenarios taking place after a firing event and in **Figure 33** the distribution of the data of the different activities are comparable to the baseline shooter samples with no activity. When looking at the percent occurrence per sample, LIBS analysis found that 21.8% of activity samples were positive for PbBaSb, and 55.1% when only looking for a combination of 2 out 3 elements. While 16.2% of the activity samples were positive for 3 lead-free elements. When we confirmed these samples by SEM-EDS, the subset was 98% positive for PbBaSb and comparable to the samples positive for lead-free elements at about 15%. These results illustrate a decrease in detection of inorganic particles when post-shooting activities are performed and problems of classification of GSR particles by SEM-EDS when lead-free ammunitions are used during the firing of the weapon.

The mixed shooter samples represented uncontrolled combinations of leaded and lead-free ammunition with no specification of the number of rounds fired and collected at an outdoor setting. For this reason, we can expect to see higher background introduction, for example, 56.4% of the samples were positive for Al as opposed to the second next highest at 27.1% in the low-risk population when analyzed by LIBS. For the combined 3 leaded elements (PbBaSb), 66.4% were positive, and for lead-free elements, 85.0% were positive. If we only examine 2 out of 3 elements for either leaded or lead-free, this raised to over 96% for both groups. Despite the various conditions and ammunition mix, the rapid LIBS method can handle the screening of the various types of possible GSR sample, and it was further confirmed by SEM-EDS with comparable elemental compositions.

2.2.4.2. Trends in sampled populations by Electrochemistry

Background populations—Low relative levels of IGSR and OGSR compounds were observed in the low and high-risk background populations, with less than 5% of the samples containing Pb, and none of the low-risks presenting OGSR above the method's detection capabilities. The mechanics group from the high-risk subpopulation skew the data towards higher copper detection, as 42.1% were positive for copper, compared to 9.1% for firearms researchers, and 0% for police and agriculture staff. Also, within the high-risk subpopulation, only the ballistic researchers were found to be positive for lead in 22.7% of the samples, whereas no other high-risk group tested positive for lead.

Leaded and leaded-free shooter populations —Several differences between subpopulations can be noted within the box plots for the electrochemical data (**Figures 34 and 35**). Unsurprisingly, the levels of lead in the shooter samples were higher than in the non-shooter sets. There was a very large difference seen in the leaded shooter versus the other subpopulations as demonstrated by a 91.0% positive occurrence of lead above the critical threshold versus the next closest of 30.0% for lead-free shooters, 4.9% for low-risk, and 4.8% for high-risk backgrounds. In comparison to LIBS, our electrochemical method is not as sensitive for species like Sb, and Ba has an oxidation potential outside of our operational parameters. As a result, the occurrence of tri-component PbBaSb species was not possible. On the other hand, LIBS was able to detect multiple elements and, for example, in the leaded shooter population, Ba was detected in 99.0% of the set, lead was in 87.5%, and Sb was in 70.5%. Instead, EC was capable to detect Pb, Cu, nitroglycerine and DPA on the stubs.

When considering copper levels in the shooter subpopulations, the higher levels were observed in the lead-free samples, then the mixed shooter, and lastly the leaded shooter. However, when looking closer, the median value of the high-risk group is lower than the lead-free shooters. Additionally, the occurrence of positive copper signals in the lead-free group was 74.0%, followed by the mixed shooter (62.7%), leaded shooter (40.5%), and high-risk (13.3%). This contrasts with the 6.3% positive for Cu in the low-risk non-shooter samples.

Several interesting trends can be seen with nitroglycerin within the subpopulations and their respective groups. Figures 34 and 35 demonstrate that the presence of nitroglycerin was linked with shooting events. The highest levels of nitroglycerin were obtained in the shooter samples and the shooting with activity samples. An interesting note is that the type of shooter (leaded or lead-free) did not appear to affect the median value of nitroglycerin; however, the lead-free shooter did appear to have a larger spread of data above the median value, indicating instances of larger levels of nitroglycerin. This trend is also evident in the occurrence of positive nitroglycerin levels above the critical threshold, which were similar at 36.5%, 43.2%, and 47.0% for leaded, mixed, and lead-free shooter samples, respectively. This was in stark contrast to the non-shooter samples where less than 4.3% were positive for nitroglycerin in the low-risk background and none in the high-risk set. The slightly higher occurrence of NG in the low-risk set resulted from the sampling at the WSJ since there were many people conducting various types of outdoor activities.

This may have resulted in transfers to the hands of non-shooters or participation in activities around fireworks. Breaking down the low-risk non-shooter data set into OGH and WSJ collection, only 2.0% of the samples were positive for NG in the OGH sets while 6.0% were positive in the WSJ sets, indicating a higher prevalence of NG in the background samples collected during the WSJ.

More complex shooter populations—The activity subpopulation also demonstrated interesting results. The levels of lead were affected by the type of activity and the environmental conditions. Running did not drastically affect the relative level of lead, and recovery of this element from the hands was achieved. However, when this same task was performed in the rain, a significant decrease in the amount of detected lead was observed. Small decreases were seen for the use of hand sanitizer and rubbing hands together, although lead was still detected in the samples. These trends are also noticed in the occurrence of positive lead results for the activity samples when calculated as percent of samples with Pb, where 100% of the running samples were positive for lead, followed by 69.2% when hand sanitizer and vigorous rubber was done, and only 15.4% of the samples were positive for lead when running was performed in the rain.

The same trend as lead can be seen in the samples for copper, where copper was easily recovered and detected in the running samples, but the rain and the actions of rubbing and using sanitizer effectively eliminated the presence of copper from the hands as evidenced by the occurrence of positive copper results of 76.9% in the running samples and 0% in the remaining activities.

When considering the activity subpopulation as a whole, 33.3% of samples were positive for NG. The effect of the rain was again seen, where nitroglycerin was effectively absent from all the samples except one instance of a large amount, seen in Figure 35. In addition to being higher than the non-shooter samples, the median values of nitroglycerin were similar between the other three activities, as well as the spread of the data. The most instances of positive nitroglycerin were found in the running samples (46.2%), followed by vigorous rubbing (38.5%), hand sanitizer (34.6%), and then running in the rain (7.7%).

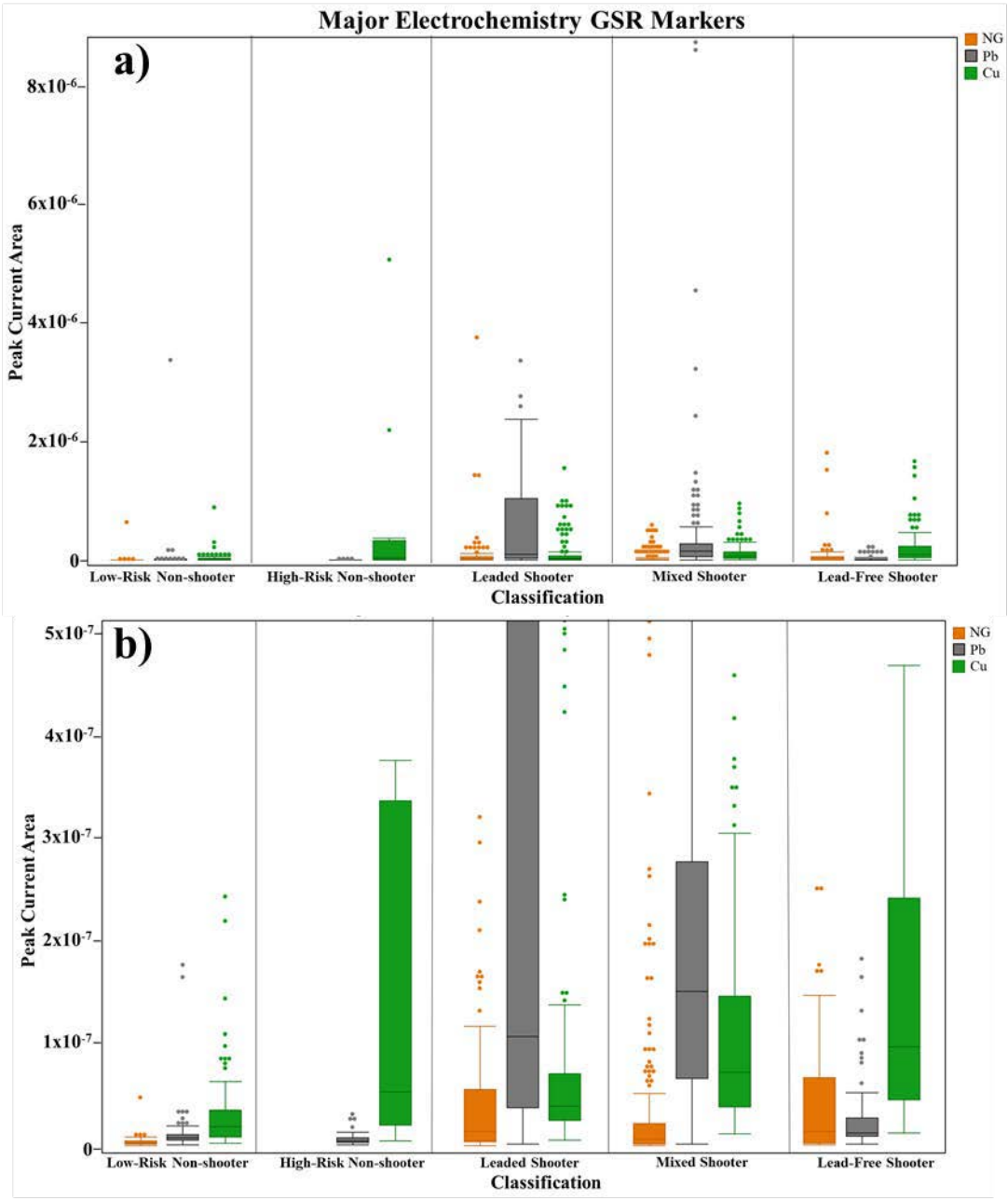

Figure 34. Electrochemistry box plots demonstrating the comparison between subpopulations for the electrochemistry markers of lead, copper, and nitroglycerin. a) overall b) zoomed.

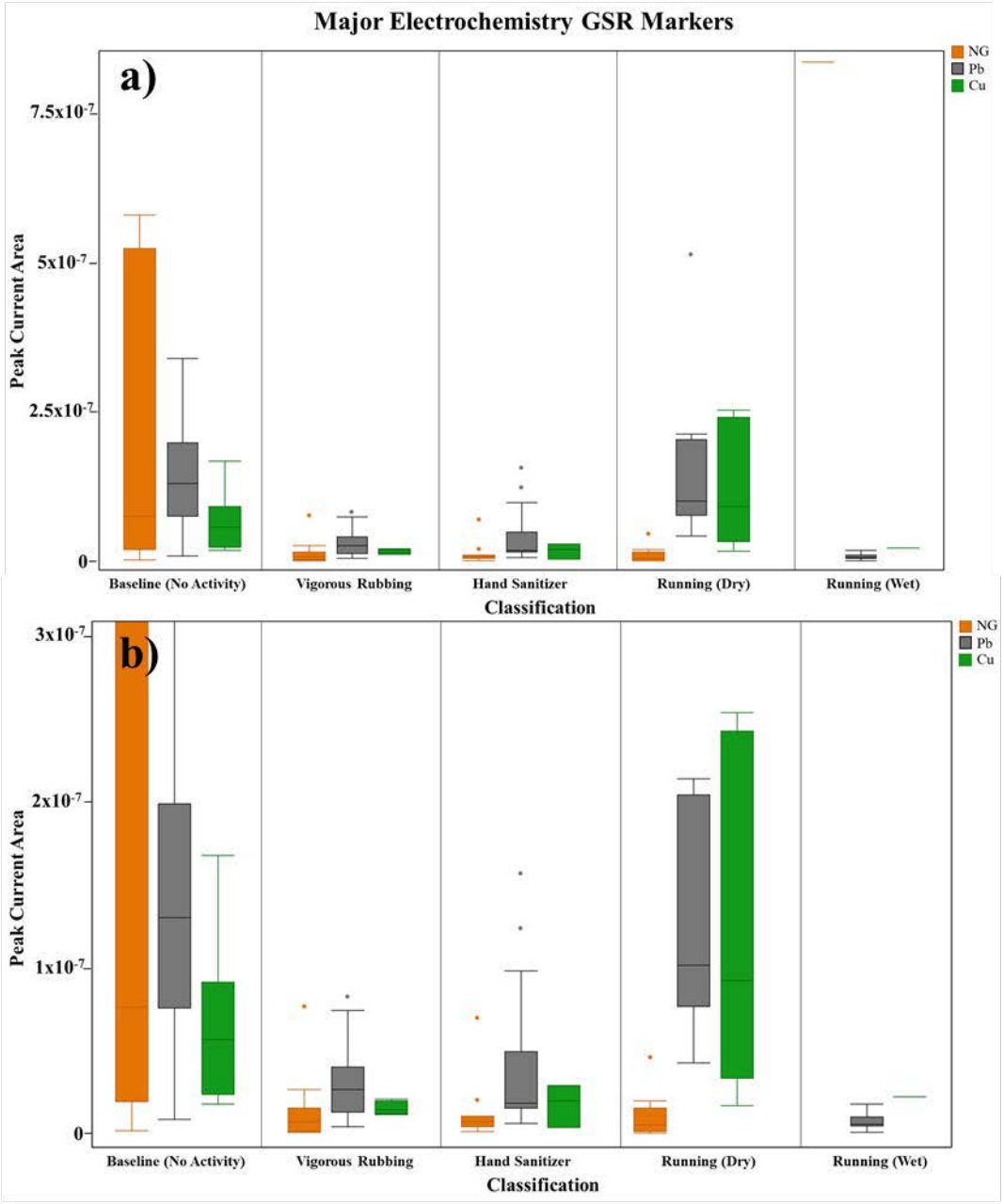

Figure 35. Electrochemistry comparison box plots for the signal-to-noise ratio for both leaded and lead-free GSR markers for the leaded activity subpopulation broken down by different activities and compared to a baseline of no activity. a) overall b) zoomed.

Other analytes of interest, including Sb and other OGSR compounds, were rarely observed in samples or at very low levels, preventing the generation of an accurate critical threshold value and reduced confidence in the identification. In addition, our research group reported in Feeney et al. OGSR by LC-MS/MS analysis on a subset of these authentic shooter samples.[18] When comparing the reported mean and range values for OGSR with the electrochemistry's LOD values reported by our group in Ott et al., the reported values fall below the LOD of our current electrochemical method.15 Therefore,

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the statistical analyses were based off the three analytes most often encountered: Pb, Cu, and NG as stated in the previous discussion.

Combined IGSR/OGSR electrochemical data—The combination of IGSR and OGSR has been shown to provide improved accuracy and reliability in GSR analysis.[4,10,11] Therefore, it is interesting to understand the combinations of compounds observed in the electrochemistry data. Perhaps the most interesting of which is the combination of nitroglycerin with either lead or copper. Within the shooter populations, the combination of positive IGSR+OGSR was seen in 35.5%, 42.7%, and 34.0% of samples in the leaded, mixed, and lead-free subpopulations. When this is compared to the non-shooter groups, both the low-risk and the high-risk subpopulations had a 0% occurrence of IGSR+OGSR. In fact, the only combination of electrochemistry GSR markers observed in the non-shooter populations (low-risk and high-risk) was lead+copper at an occurrence of 0.6% for low-risk and 1.0% for high-risk, indicating that detecting combinations of IGSR and OGSR is critical in differentiating shooter samples from non-shooter samples.

Also, of importance, several differences were noted between leaded shooter and lead-free shooter subpopulations. The most common combination within the lead-free samples was copper+nitroglycerin at 34.0%, followed by lead+copper (29.0%) and lead+nitroglycerin (13.0%). This differed from the leaded shooter where the most common combination was lead+copper at 39.5%, followed by lead+nitroglycerin (35.5%) and copper+nitroglycerin (21.0%). In general, the mixed shooter subpopulation demonstrated higher percent-occurrence for all combinations. The combination of all three components was observed 27.4% of the time for the combined leaded and mixed shooter subpopulations and 13.0% of the time for the lead-free subpopulation.

2.2.4.3. Probabilistic Interpretation of the Populations

A categorical exploratory method (critical threshold) was used to identify population characteristics and differences between the various subgroups and estimate performance rates. Moreover, machine learning algorithms (Neural Networks) were used to recognize underlying patterns in the data for group classifications. Both methods demonstrated 87% or higher accuracy for individual analytical techniques and 92% or higher when LIBS and EC were combined. Moreover, the machine learning probabilistic outputs were used to calculate log10 likelihood ratios (LR) and evaluate their distribution on the subgroups. The log10 LR were typically between -2.5 to -5 for non-shooters and >5 for shooters, demonstrating good discrimination between the overall populations and the viability of using LR as a metric for reporting the weight of the evidence. Tippet plots were used to evaluate LR and the rates of misleading evidence (RME <3.7%).

The Bayesian framework provides a means by which to assess the weight of the evidence. The odds form of the Bayes equation is given as:

$$\underbrace{\frac{p(H_p|E)}{p(H_d|E)}}_{\text{Posterior Odds}} = \underbrace{\frac{p(E|H_p)}{p(E|H_d)}}_{\text{Likelihood Ratio}} \times \underbrace{\frac{p(H_p)}{p(H_d)}}_{\text{Prior Odds}}$$

where the two hypotheses are formulated in terms of the prosecutor hypothesis (Hp or H1) and the defense hypothesis (Hd or H2), which are mutually exclusive. The odds form of the Bayes equation

allows for the updating of prior "beliefs" as evidence is assessed. Of most interest to forensic science is the likelihood ratio, or the ratio of the probabilities of the evidence given each hypothesis. Interpretation of forensic evidence generally takes three levels following the hierarchy of propositions: offense level, activity level, and source level. Due to the nature of our current study and the resulting data obtained from our analytical methods, we can speak only to the source level.

Since the previous test demonstrated that the neural network models provided better classification for our data set, these models were used to assess the usefulness of probabilistic interpretation and the calculated likelihood ratios.

2.5.5.1. Probabilistic interpretation of populations using combined LIBS and EC data

At the source level, we want to assess the probability of observing the evidence (e.g., chemical profile) given the trace came from a firearm discharge ($P[E/H_p]$) versus the probability of the evidence given the trace came from a non-related environmental source or is not GSR ($P[E/H_d]$). This can be numerically assessed using the Likelihood Ratio (LR), considering the probability of the evidence as a ratio using the two competing hypotheses.[42-46] From this calculation, we can speak to the LR for the evidence analyzed and use the ground truth knowledge (H_p true, or H_d true) to determine the distribution of the LR values for the different populations.

Histograms of the different LR calculations were created to visualize the LRs and separation between the populations when H_p or H_d are true. Histograms provide the frequency at which specific LRs were observed and places them into bins to see the distribution of the data considering a given hypothesis. The taller a bin, the more that specific LR was observed in the known population.[46] **Figure 36** shows a comparison of the leaded shooter and low-risk non-shooter populations. The distributions demonstrate support for H_p, or H_1, when the log10 LR is above zero, and the opposite for H_d, or H_2, with greater support for the respective hypothesis as the log10LR moves farther away from zero. The area of overlap evaluates the discriminating power. The more overlap between the histograms, the less discrimination power is observed. The leaded shooter has an evenly spread distribution between logLR 1 and 9. The low-risk non-shooter LR is less than 0 and the bin clustering increases until about -5, with a concentration of the frequency being around -4. Low frequencies were observed between -1 and 2.5 Log10 LR indicating good discrimination between the two alternative sets. While the range of frequencies and Log10 LR was slightly different between the different populations plotted, all histograms displayed good discrimination.

Another way to visualize and empirically measure the performance of LRs is in the form of Tippet plots.[46] **Figure 36** shows a Tippet plot for the known low risk background and shooter sets. In the graph, we can observe both LR values for when H_p (H_1) and H_d (H_2) is true and evaluate the rate of misleading (RoME) evidence. Where the line plotting the LR of the H_p/H_1 crosses the dotted line at log LR 0, the integrated area between the line and dotted line at the top represents the rate of misleading evidence for that hypothesis, or also called the false negative rate. The opposing case is where the line plotting the LR of the H_d (H_2) crosses the dotted line at 0, the integrated area between the line and dotted line at the bottom is the rate of misleading evidence for that hypothesis, or also called the false positive rate. The NN-based LR showed low rates of misleading evidence (false negative 0.15% and false positive 0.62%). The gap between the two lines in the Tippet plot is narrow, once again confirming a good discriminating power of the algorithm and subsequent LRs to distinguish shooters versus non-shooter sets.

Since our data could not be simply modeled using a parametric model, we applied a Kernel Density Function (KDF) to the histogram distributions to model this data using an estimation. The result is a continuous curve for the distributions similar in shape to the histograms and displays both distributions in the same graph. **Figure 37** is an example of all histograms and corresponding KDF using the neural network outputs for the LR calculations for the three different shooter populations (leaded, lead-free and mixed) and the one LR calculation using all shooter populations combined. The differences in the distribution of Hp (H_1) logLRs and the relative peak heights is due to the differences in the number of data points and samples within each population considered.

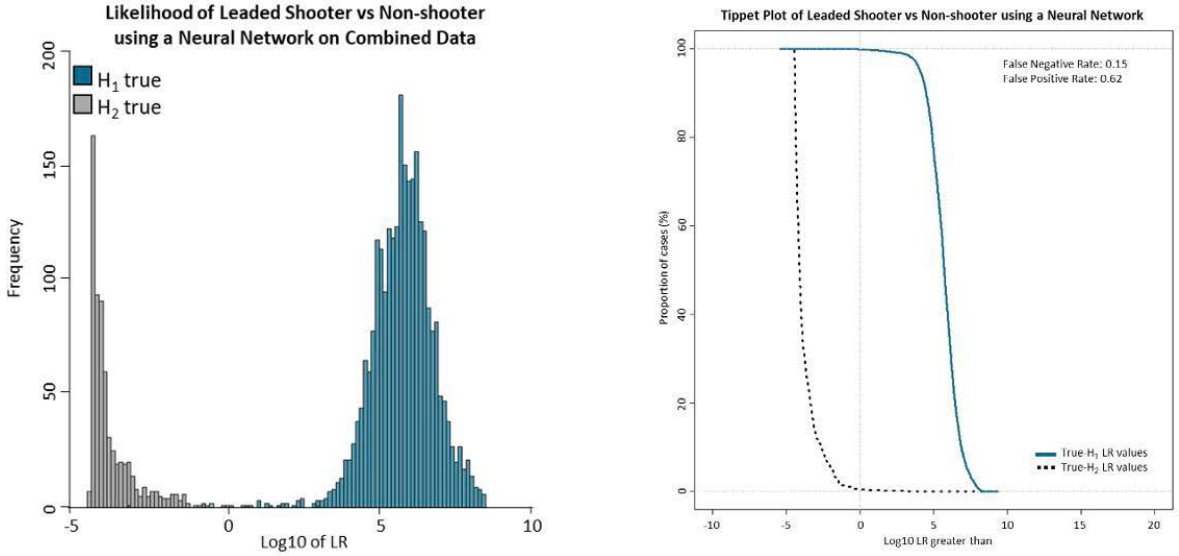

Figure 36. **Left:** Overlayed histograms displaying the distribution of log likelihood ratio calculated from a neural network for the leaded shooter and low risk background populations. H_1 is the prosecutor's hypothesis and H_2 is the defense hypothesis. **Right:** Tippet plot displaying the rate of misleading evidence.

Another interesting observation is the differences in the shapes and distributions of the populations analyzed and LR calculation equation. As expected, the leaded versus low-risk background has the least amount of overlap between the two. The logLR of a non-shooter sample was mostly between -5 to 0 and a leaded shooter mostly between 1 to 9, with a minimal overlap between -1 and 1. The lead-free shooter set had the least separation, showing an overlap logLR from -2 to 1. The last interesting observation was the distribution of the LR, where all three shooter populations are considered together. We can see two clearly defined peaks in the H1 side of the data, one for the mixed shooter and one for the leaded shooter, with lead-free falling in either area. Our data shows the more distinctive the elemental profiles and presence of characteristic particles in a sample, the larger the LR, demonstrating LRs can serve to inform the uncertainty in reporting GSR on an item of interest.

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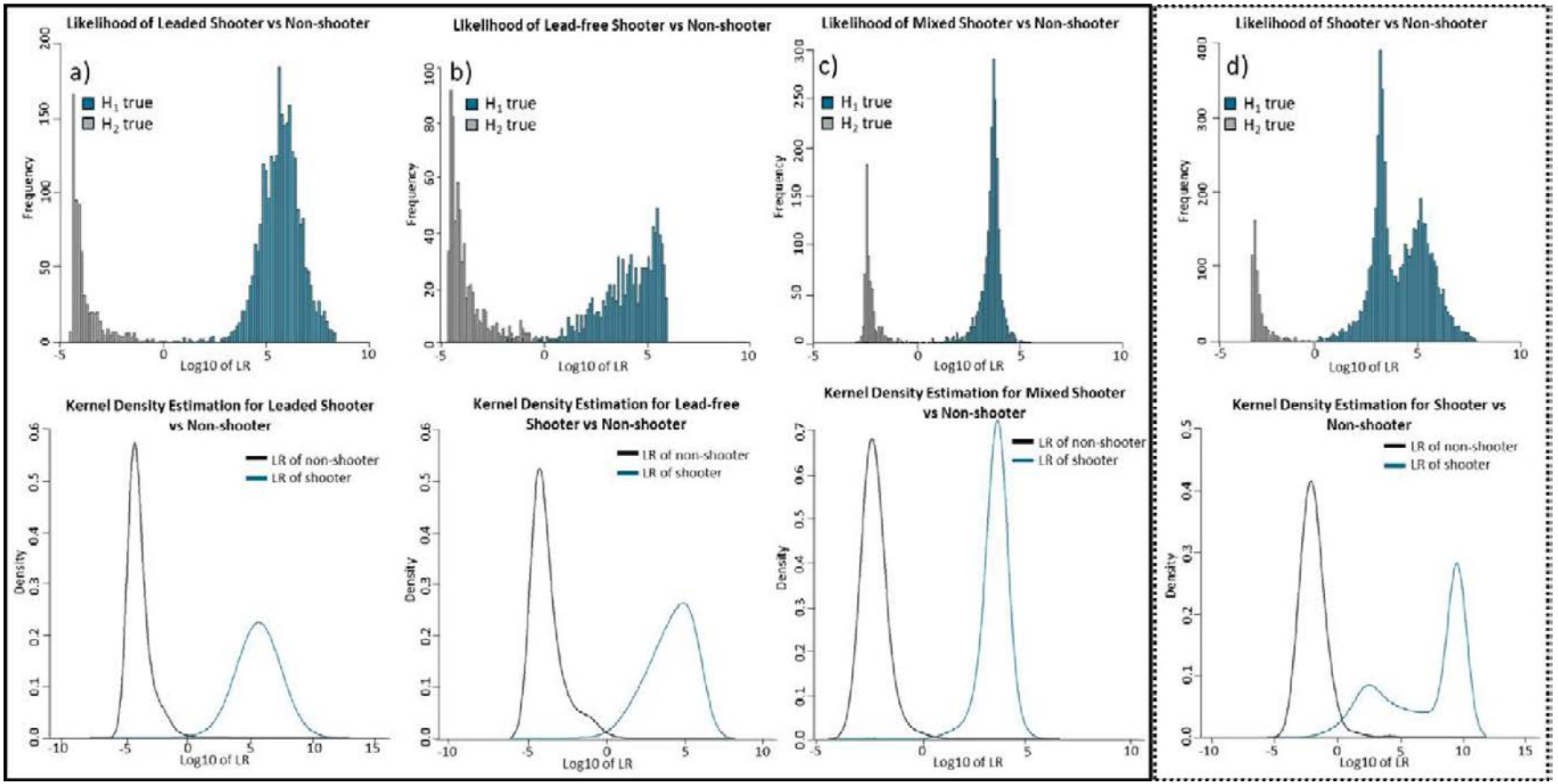

Figure 37. Histograms (top) with the corresponding KDF below. a) leaded shooter and low risk populations, b) lead free and low risk populations, c) mixed shooter and low risk populations, and d) combined shooter sets and low risk populations.

Summary of population study findings

The results from this large population have served to demonstrate LIBS and electrochemistry's utility for the rapid classification of samples based on the presence or absence of IGSR and OGSR profiles. The method demonstrated fit-for-purpose, detecting GSR from leaded and non-leaded ammunition fired with various firearms. The strength of machine learning methods is that they can effectively identify different patterns in the elemental data of subpopulations and provide probabilistic outputs that can be used to evaluate the weight of the evidence. An advantage afforded by LIBS and electrochemistry is their speed of analysis, taking only two minutes to collect rich spectrochemical information from 25 different micro-areas for LIBS and dual IGSR and OGSR data in three minutes for electrochemistry. After analysis, we were also able to successfully confirm the samples by SEM-EDS since LIBS analysis consumes less than 0.5% of the sample and the extraction area for electrochemistry was contained to approximately the same ablated area, taking only a third to half of the stub. Elemental profiles observed by LIBS were successfully corroborated by SEM-EDS, demonstrating the utility of LIBS to evaluate elemental compositions of GSR particles in a quick and accurate approach. The addition of electrochemistry data further increased the confidence of the presence or absence of GSR.

This study addresses a need in the forensic science community to understand the background prevalence of the GSR or GSR-like elements and compounds, along with assessment of modern ammunition that lacks the leaded elements (Pb, Ba, Sb). Use of a large population of samples arising from multiple types of individuals and ammunition has expanded our knowledge of not only modern, lead-free ammunition, but also the background levels of possible GSR compounds of interest. A greater understanding of the frequency of these elements on the hands of individuals who were not involved in a firing event improves our ability to differentiate between actual GSR created from a firing event and GSR-like elements commonly present in the background population, which will hopefully reduce the false detection. In turn, this will improve the error rates and increase confidence in the results. All these steps will provide the forensic community with modern alternatives for interpretation and assistance to the trier of fact.

We also addressed the need for new interpretation schemes and strengthening the presentation of the weight of evidence to the court and tiers of fact. Successful classification of the samples using machine learning techniques, the probabilistic outputs were exploited for determining the LRs for the collected data. Histograms and KDF demonstrated a reasonable separation between the two different ground truth hypotheses and their respective populations of non-shooter and shooter samples (leaded, lead-free, and mixed).

The minimal histogram overlaps, and the outputs of the tippet plots provided a preliminary assessment of the LR in terms of the distribution of LR values within populations, their discrimination power, and misleading evidence rates. The results show that incorporating fast emerging methods and the information derived from this large population study of IGSR and OGSR, combined with probabilistic interpretation, can provide more comprehensive tools for assessing GSR evidence.

2.3. Limitations

Two main limitations were encountered in this study. First, the COVID pandemic prevented for several months sampling hands from volunteers. Second, the SEM-EDS mapping and particle discovery sessions was more time consuming than anticipated, particularly with complex samples such as those collected from leaded-free shootings, as well as backgrounds from some of the high-risk populations that were relatively dirty and required long acquisition times. These combined external factors required the solicitation of a no-cost extension to complete the totality of the proposed tasks.

III ARTFACTS

3.1 List of products

3.1.1 Publications at scientific peer-reviewed journals and dissertations

1. Korina Menking Hoggatt, Ph.D, Spring 2021. WVU Department of Forensic and Investigative Science, Characterization of modern ammunition and background profiles: A novel approach and probabilistic interpretation of inorganic gunshot residue. Graduate Theses, Dissertations, and Problem Reports. 8336. https://researchrepository.wvu.edu/etd/8336

2. C Vander Pyl, C Martinez-Lopez, K Menking-Hoggatt, and T Trejos. Analysis of Primer Residue Particle by Laser Induced Breakdown Spectroscopy and Laser Ablation Inductively Coupled Plasma Mass Spectrometry. *Analyst.* **146**, 5389-5402, 2021. https://doi.org/10.1039/D1AN00689D

3. Feeney, W; Menking-Hoggatt, K; Vander Pyl, C, Ott, C, Arroyo, L; Bell, S; Trejos, T. (2021) Detection of Organic and Inorganic Gunshot Residues from Hands using Complexing Agents and LCMSMS. Accepted *Analytical Methods.* 2021, 13, 3024-3039, https://doi.org/10.1039/D1AY00778E (Journal COVER PAGE and hot article)

4. K Menking-Hoggatt, C Martinez, C Vander Pyl, E Heller, E Pollock, L Arroyo, and T Trejos. Development of Tailor-Made Inorganic Gunshot Residue (IGSR) Microparticle Standards and Characterization with a Multi-technique Approach. Talanta. Published online December 2020, hardcopy publication April 2021, 225, https://doi.org/10.1016/j.talanta.2020.121984

5. C Ott, K Dalzell, PJ Calderon, AL Alvarado-Gomez, T Trejos, L Arroyo. Evaluation of the Simultaneous Analysis of Organic and Organic Gunshot Residues within a Large Population Data Set Using Electrochemical Sensors, Journal of Forensic Sciences, 65, 6, 1935-1944, 2020, https://doi.org/10.1111/1556-4029.14548

6. C Vander Pyl, K Morris, L Arroyo, T Trejos. Assessing the utility of LIBS in the reconstruction of firearm related incidents, Journal of Forensic Chemistry, 19, June 2020, https://doi.org/10.1016/j.forc.2020.100251

7. W Feeney, C Vander Pyl, S Bell, T. Trejos. Trends in Composition, Collection, Persistence, and Analysis of IGSR and OGSR: A Review. Journal of Forensic Chemistry, 19, 2020, https://doi.org/10.1016/j.forc.2020.100250

8. K Menking-Hoggatt, L Arroyo, J Curran and T Trejos. Novel LIBS method for chemical micro-mapping of inorganic gunshot residue collected from hand samples, Journal of Chemometrics, Dec 2019. https://doi.org/10.1002/cem.3208

9. C Vander Pyl, O Ovide, Ho M, Yuksel B, T Trejos. Spectrochemical Mapping Using Laser Induced Breakdown Spectroscopy as a More Objective Approach to Shooting Distance Determination, Spectrochimica Acta B, 2019, 152, 93-101. https://doi.org/10.1016/j.sab.2018.12.010

10. B Yuksel, M Ho, O Ovide, C Vander Pyl, T Trejos. Infrared Imaging as a Complementary Aid in Estimating Muzzle-to-Target Shooting Distance: An Application on Dark, Patterned and Bloody Samples. T K J Foren Sci Leg Med, 16,2, 2019, 73-80. DOI: 10.5336/forensic.2019-64837

11. Courtney Vander Pyl, MSFS, 2019, WVU Department of Forensic and Investigative Science, Chemical Analysis of Firearm Discharge Residues Using Laser Induced Breakdown Spectroscopy. https://researchrepository.wvu.edu/etd/4058

12. T Trejos, C Vander Pyl, K Menking-Hoggatt, AL Alvarado, L Arroyo. Fast Identification of Inorganic and Organic Gunshot Residues by LIBS and Electrochemical Methods, *Forensic Chemistry, Elsevier*, 2018, 8, 146-156. https://doi.org/10.1016/j.forc.2018.02.006 (published prior the award, served as basis for the study)

3.1.2. Presentations at Scientific Meetings

1) September 23, 2021, Korina Menking-Hoggatt, Edward "Chip" Pollock, Emily Heller, Courtney Vander Pyl, Claudia Martinez, Tatiana Trejos. Inorganic Gunshot Residue (IGSR) Micro-particle Standard with Application to Method Development and Understanding Modern Ammunition. Mid-Atlantic Association of Forensic Scientist and ASTEE joint meeting (oral, MAAFS Annual Scholarship Winner)
2) September 23, 2021. Courtney Vander Pyl, Korina Menking-Hoggatt, Tatiana Trejos. Rapid Analytical Screening Methods for the Investigation of Firearm Related Crimes. Mid-Atlantic Association of Forensic Scientist and ASTEE joint meeting (oral)
3) July 29th, 2021. Declan Revenew, Courtney Vander Pyl, Bill Feeney, Tatiana Trejos. Evaluating GC-MS and LC-MS/MS Efficacy for Characterization of a Developed Organic

Gunshot Residue Standard**.** 13th Annual summer undergraduate research symposium, Morgantown, WV https://www.youtube.com/watch?v=1yszuB1nH60

4) July 28th, 2021. Jessica Friedel, Korina Menking-Hoggatt, and Tatiana Trejos. Prevalence of Particles Characteristic and Consistent with GSR Found in A Background Population Study. Current Trends in Forensic Trace Analysis 2021 Online Forensic Symposium. (poster)
5) July 28th, 2021. Courtney Vander Pyl, Claudia Martinez-Lopez, Korina Menking-Hoggatt, Tatiana Trejos. Rapid Laser-Based Methods for the Detection of Modern Gunshot Residues. Current Trends in Forensic Trace Analysis 2021 Online Forensic Symposium. (Poster, BEST POSTER ELSERVIER FORENSIC CHEMISTRY AWARD)
6) July 28th, 2021. Bill Feeney, Suzanne Bell, Tatiana Trejos. Exploring the probabilistic interpretation of gunshot residue in various populations using LC-MS/MS. Current Trends in Forensic Trace Analysis 2021 Online Forensic Symposium. (poster)
7) April 2021. William Feeney. Furthering the understanding and analysis of gunshot residue with host-guest chemistry and mass spectrometry techniques. Friday Science Short Division 643. NIST 2021. (Webinar Presentation, invited speaker).
8) March 2021, Luis Arroyo, Korina Menking Hoggatt, Colby Ott, Courttey Vander Pyl, Kourtney Dalzell, Bill Feeney. Detection of gunshot residues from leaded and non-leaded ammunition by electrochemical sensors and LIBS, PITTCON 2021 (invited speaker)
9) March 2021, Tatiana Trejos, Luis Arroyo, Korina Menking Hoggatt and Courtney Vander Pyl. LIBS as an emerging method for the detection of firearm discharge residues, NIJ (National Institute of Justice) -Emerging Analytical Methods for Chemical and Biological Forensic Evidence Session, PITTCON 2021 (invited speaker)
10) February 2021, Courtney Vander Pyl, Korina Menking-Hoggatt, Claudia Martinez,
11) Tatiana Trejos. Application of Laser-Based Methods for the Analysis of Gunshot Residue Originating from Modern Ammunition. AAFS 2021
12) February 2021, Kourtney A. Dalzell, Korina Menking-Hoggatt, Colby E. Ott, Tatiana Trejos, and Luis Arroyo. Detection of Lead-Free Inorganic and Organic Gunshot Residue Using LIBS, Electrochemistry, and Machine Learning. AAFS 2021
13) February 2021. Courtney Vander Pyl, Korina Menking-Hoggatt, Claudia Martinez-Lopez, Tatiana Trejos. Application of Laser-Based Methods for the Analysis of Gunshot Residues Originating from Modern Ammunition. 73rd AAFS Annual Scientific Meeting. (Virtual, poster)
14) January 2021, Bill Feeney, Tatiana Trejos. Detection of OGSR and IGSR from the same collection stub using complexing agents and LC/MS/MS, 4th event Global Lecture Series, Crossing Forensic Borders (invited speaker)
15) January 2021, Korina Menking-Hoggatt, C. Ott, Tatiana Trejos, Luis Arroyo. Novel rapid detection of inorganic and organic gunshot residues using LIBS and electrochemistry: a population study, 4th event Global Lecture Series, Crossing Forensic Borders. (invited speakers)
16) December 2020. William Feeney. American Society of Crime Laboratory Directors (ASCLD) Forensic Research Committee "Lightning Talks" series: Emerging Techniques and Applications for Gunshot Residues (webinar, invited speaker).
17) November 2020. Korina Menking-Hoggatt, Luis Arroyo, and Tatiana Trejos. Feasibility Study of Rapid Emerging Methods for the Analysis of Inorganic and Organic Gunshot Residues. 7º National Meeting of Forensic Chemistry / 4º Meeting of the Brazilian Society of Forensic Sciences (ENQFor/SBCF) online Joint Congress. (Virtual, oral, invited speaker)

18) October 2020. Tatiana Trejos, Luis Arroyo, Colby Ott, Courtney Vander Pyl, Korina Menking Hoggatt and Kourtney Dalzell. Investigative leads using LIBS and orthogonal methods in crime laboratories and in the field, FACSS SCIX 2020, virtual conference.
19) September 2020. Korina Menking-Hoggatt, Eduard Pollock, Emilly Heller, Courtney Vander Pyl, Claudia Martinez, Tatiana Trejos. Inorganic Gunshot Residues (IGSR) Microparticles Standard with Application to Method Development and Understanding Modern Ammunition. MAAFS Annual Meeting (Oral presentation, virtual, recipient of 2020 MAAFS Scholarship Award)
20) July 2020. Luis Arroyo, Tatiana Trejos. Development of a Versatile IGSR Microparticle Standard. 2020 Online Symposium: Current Trends in Forensic Trace Analysis (oral presentation, presenter and Symposium Program Chair)
21) July 2020. Courtney Vander Pyl, Oriana Ovide, Colby Ott, Luis Arroyo and Tatiana Trejos. Quick Spectrochemical Methods for Detecting Gunshot Residues on Crime Scene Samples. Current Trends in Trace Analysis: 2020 Online Forensic Symposium Series (virtual, poster).
22) July 2020. Korina Menking-Hoggatt, Luis Arroyo, Colby Ott, and Tatiana Trejos. Characterizing Inorganic and Organic Gunshot Residue by Laser Induced Breakdown Spectroscopy and Electrochemistry. Current Trends in Forensic Trace Analysis online forensic symposium. (Poster online presentation)
23) May 2020. Courtney Vander Pyl, Korina Menking-Hoggatt, Claudia Martinez, Tatiana Trejos. Fast Spectrochemical Methods and Micro-Particle Standards to Facilitate Transfer and Persistence Studies of Inorganic and Organic Gunshot Residues. First Online Forensic Graduate Symposium, Morgantown, WV (poster, symposium organizer) Award for "Best Research e-Poster Presentation-First Place
24) May 2020. Korina Menking-Hoggatt: Characterization of Modern Inorganic Gunshot Residue Micro-Particles for Enhancement of Forensic Analysis. First Online Forensic Graduate Symposium, Morgantown, WV (oral, symposium organizer)
25) May 2020. Colby E. Ott, Pedro J. Calderón-Arce, Kourtney A. Dalzell, Hugo Cunha-Silva, Ana L. Alvarado-Gámez, M. Julia Arcos-Martínez, Tatiana Trejos, Luis E. Arroyo. Electrochemistry: A Powerful Analytical Technique for the Analysis of Forensic Evidence. First Online Forensic Graduate Symposium, Morgantown, WV (poster, symposium organizer)
26) April 2020. Emily Heller, Korina Menking-Hoggatt, Claudia Martinez-Lopez, and Tatiana Trejos. Analysis of Inorganic GSR Microparticles using Laser Induced Breakdown Spectroscopy (LIBS), Annual Undergraduate Symposium, Morgantown, WV (e-video poster)
27) March 2020. Korina Menking-Hoggatt, Emily Heller, Edward M. Pollock, Claudia Martinez, Tatiana Trejos. Development and Characterization of Inorganic Gunshot Residue (IGSR) Standard Micro-particles to Enhance Understanding of Modern Ammunition. The NIJ Forensic Science Symposium at Pittcon, Chicago, IL (poster)
28) February 2020. Tatiana Trejos and Luis Arroyo. Rapid Detection of Inorganic and Organic Firearm Discharge Residues by Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemical Sensors. 2020 National Institute of Justice Forensic Science Research and Development Symposium, Anaheim, CA (oral, invited)
29) February 2020. Korina Menking-Hoggatt, Colby E. Ott, Courtney H. Vander Pyl, Tatiana Trejos, James Curran. The Power of Statistics and Machine Learning Applied to Orthogonal Rapid Methods for the Identification of Inorganic Gunshot Residue (IGSR) and Organic Gunshot Residue(OGSR) Markers. 72nd AAFS Annual Scientific Meeting, Anaheim, CA (oral)

30) February 2020. Korina Menking-Hoggatt, Edward M. Pollock, Tatiana Trejos. A Novel Approach for the Collection and Characterization of Inorganic Gunshot Residue (IGSR) Standards, 72nd AAFS Annual Scientific Meeting, Anaheim, CA (poster)
31) February 2020. Colby E. Ott, Pedro Calderón-Arce, Korina Menking-Hoggatt, Courtney H. Vander Pyl, Ana L. Alvarado-Gámez, Tatiana Trejos, Luis E. Arroyo. An Analysis of Organic and Inorganic Gunshot Residues (OGSR and IGSR) Via Electrochemical Methods with Screen- Printed Carbon Electrodes and Nanoparticle Modifications, 72nd AAFS Annual Scientific Meeting, Anaheim, CA (poster)
32) February 2020. Courtney H. Vander Pyl, Oriana Ovide, Colby E. Ott, Luis E. Arroyo, Tatiana Trejos. A Chemical Analysis of Gunshot Residues (GSRs) for Investigative Leads and Reconstruction of Firearm- Related Incidents , 72nd AAFS Annual Scientific Meeting, Anaheim, CA (poster)
33) February 2020. William Feeney, Suzanne Bell, Luis E. Arroyo, Tatiana Trejos. The Characterization and Detection of Organic and Inorganic Firearm Discharge Residue (FDR) Using High-Performance Liquid Chromatography-Triple Quadrupole (HPLC-QQQ) and Host- Guest Chemistry, 72nd AAFS Annual Scientific Meeting, Anaheim, CA (oral)
34) November 2019. Tatiana Trejos, Luis Arroyo and Suzanne Bell. Rapid Identification of Organic and Inorganic Gunshot Residues. Webinar Sponsored by NIJ and organized by FTCOE and RTI international, November 20th, 2019. (Invited speakers, webinar live streamed and available on demand https://forensiccoe.org/webinar/rapid-and-effective-identification-of- organic-and-inorganic-gunshot-residues/)
35) October 2019. Using LIBS for Elemental Signature Discovery in Forensic Applications. Tatiana Trejos, Luis Arroyo, Emily Haase, Courtney Vander Pyl, Korina Menking-Hoggatt. SCIX Annual meeting, Palm Springs, CA (invited speaker)
36) October 2019. Korina Menking-Hoggatt, Colby Ott, Luis Arroyo, and Tatiana Trejos. Characterizing Inorganic and Organic Gunshot Residue by Laser Induced Breakdown Spectroscopy and Electrochemistry, SCIX conference, Palm Springs, CA (poster)
37) October 2019. Courtney Vander Pyl, Oriana Ovide, and Tatiana Trejos. Application of Laser Induced Breakdown Spectroscopy in the Reconstruction of Firearm Related Incidents, SciX conference, Palm Springs, CA (poster)
38) May 2019. Tatiana Trejos. Trace Evidence: Then, Now and Moving Forward. MAAFS meeting, Morgantown WV.
39) May 2019. Courtney Vander-Pyl, Oriana Ovide, Tatiana Trejos. A Novel Approach for Increased Objectivity in Detecting Gunshot Residues Around Bullet Orifices, MAAFS meeting, Morgantown WV.
40) May 2019. Korina Menking-Hoggatt, Luis Arroyo, Tatiana Trejos. Modern Fast Screening of Inorganic and Organic Gunshot Residue (GSR) by Laser-Induced Breakdown Spectroscopy (LIBS) and Electrochemistry (EC), MAAFS meeting, Morgantown WV.
41) February 2019. Luis Arroyo, Korina Menking-Hoggatt and Tatiana Trejos. The Fusion of Electrochemical and Spectrochemical Data for the Detection of Organic and Inorganic Gunshot Residues (GSR), AAFS meeting, Baltimore
42) February 2019. Courtney Vander Pyl, Oriana Ovide, Bayram Yuksel and Tatiana Trejos. Increased objectivity of shooting distance determinations by Spectrochemical Mapping, AAFS meeting, Baltimore
43) February 2019. Korina Menking-Hoggatt and Tatiana Trejos. Laser-Induced Breakdown Spectroscopy as a Rapid Detection Technique for Gunshot Residue, AAFS meeting, Baltimore

3.1.3. Website(s) or other Internet site(s)

1. RTI/FTCoE webinar, announced in the following sites:

- At RTI/FTCoE website: https://forensiccoe.org/webinar/rapid-and-effective-identification-of-organic-and-inorganic-gunshot-residues/
- At WVU FIS website: https://forensics.wvu.edu/news-and-events/events/gsr-webinar
- At Dr. Trejos LinkedIn site: https://www.linkedin.com/in/tatiana-trejos-48a31492/detail/recent-activity/shares/

2. Just Science podcast was streamed and available on demand
Just Quick Screening Methods for Firearm Discharge Residues.

3. Our publication "Evaluation of the Simultaneous Analysis of Organic and Organic Gunshot Residues within a Large Population Data Set Using Electrochemical Sensors, Journal of Forensic Sciences" was featured at the Journal of Forensic Science- twitter account postings for our publication:
   - AAFS Twitter: https://twitter.com/AAFSorg
   - AAFS Facebook: https://www.facebook.com/AAFS.org/
   - AAFS LinkedIn: https://www.linkedin.com/company/american-academy-of-forensic-sciences

4. The presentations on GSR at the 2020 Online Forensic Symposium was announced in websites and social media (LinkedIn, Twitter, Facebook), an example below:
https://www.forensicscienceeducation.org/forensic-education/courses-and-workshops/2020-online-forensic-symposium-current-trends-in-forensic-trace-analysis/

3.2. Data sets generated

According to our data management plan, the data resulting from all the instrumental analysis was curated and compiled into a centralized dataset. The dataset consists of three main folders: 1) GSR hands data (containing data analyzed from hands residues), 2) GSR primer only data (containing data from the tailor-made microparticle standards and characterization of 20 ammunition types), and GSR substrates data (data from GSR from fabrics and rigid substrates). **Figure 38** describes the structure for the data storage divided by substrate/application and analytical technique. Within each subfolder, raw and processed data is provided with README text files. Overall, the dataset contains over 200,000 datafiles from the hands of individuals (>3200 samples), custom-made microparticle standards (>20 ammunition types), and various substrates for distance determination and bullet hole identification (>200 items). To the best of our knowledge, this study created the most extensive dataset on IGSR and OGSR data from standard materials and authentic specimens.

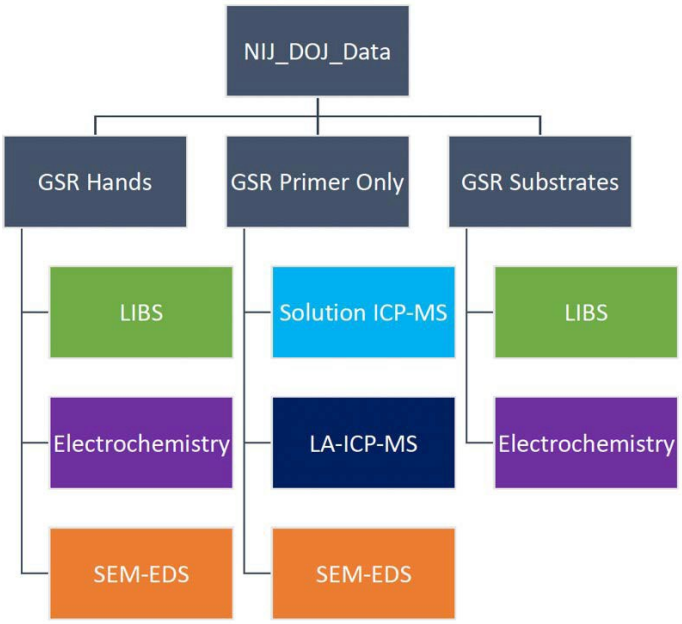

Figure 38. NIJ-DOJ dataset folder structure diagram for the GSR data storage

3.3. Dissemination activities

To date, the main dissemination routes have been the publication of manuscripts in scientific journals and presentation of research results at scientific meetings. Moreover, we collaborated with the Sacramento Crime Laboratory to evaluate the utility of the proposed approaches. The laboratory has a LIBS system and is interested in the future adoption of the methodologies. They have shown great interest in utilizing the electrochemical detection to attend crime scenes, particularly in those cases where acoustic systems are used by law enforcement to identify gunshot sounds to participate in the scene in a matter of minutes after a shooting has occurred. One of our graduate students visited the Sacramento Crime Laboratory and spent a week receiving training in SEM-EDS interpretation and providing training on LIBS analysis of GSR, as well as presenting our preliminary results to trace and firearm examiners. Also, a collaboration with Footlabs was critical to evaluate cheap portable solutions. Footlabs personnel visited WVU in June 2019 to install the demo electrochemistry that was used to test some specimens.

Also, we established networking with the NIST-OSAC GSR Subcommittee to collaborate and participate in an interlaboratory study for the examination of OGSR. We anticipate that the in-house standards being developed in our group would serve for similar purposes in the future.

Finally, the PIs participated in an NIJ-sponsored webinar organized by FTCOE and RTI International (November 20, 2019, Identification of Organic and Inorganic Gunshot Residues). The webinar was well attended, with 315 people registered and 182 attending the life-online event. The webinar was available on-demand, The organizers shared the feedback provided by the attendees. Out of 52 attendees that completed the survey 93 to 96% rated the technical quality, instructors, objectives, and

interest in the topic as good to excellent. This webinar was a unique opportunity to disseminate the research to practitioners and forensic managers.

IV PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

This research has provided a robust platform for training the next generations of forensic scientists in current and emerging methods to collect, examine, and interpret firearm-related evidence. This research has provided research opportunities for twelve students and one post-doctoral fellow. During this project, seven undergraduate students, one master's student, and four doctoral students have conducted independent research. The project has also provided opportunities for a post-doctoral scientist, who joined the workforce shortly after their residence period within our team. Table 10 lists the main participants and collaborators.

Moreover, this project's resources and research settings have provided all undergraduate and graduate students the unique opportunity to present their results at scientific venues. The opportunities provided to undergraduate researchers, some of the first-generation university students or minority students, have served as an essential foundation to their professional development. Five of our undergraduate researchers joined graduate school 1, and two of them joined the workforce. These student's achievements and STEM professional preparation are, in our opinion, the most valuable product of NIJ-funded efforts like this one.

Table 10. List of main participants and collaborating organizations

Participant Name	Affiliation	Role	Funding support	Contributions
Tatiana Trejos	West Virginia University	Principal investigator	Yes	Managed the project and directly supervised students on sample collection, the analysis by LIBS, SEM-EDS, LA-ICP-MS, ICP-MS, and LC-MS and statistical interpretation of the data. Supervised data curation and management plans.
Luis Arroyo	West Virginia University	Co-Principal investigator	Yes	Supervised research related with electrochemistry detection and LC-MS/MS. Managed data collection plans and assisted with reports, presentations, and manuscripts.
Claudia Martínez-Lopez	West Virginia University	Postdoctoral scientist	Yes (1 year)	Assisted with the supervision of graduate and undergraduate students, sample collection logistics, method validation, and statistical analysis.
James Curran	University of Auckland	Statistician Collaborator	Yes (subaward)	Collaborated as expert in statistical analysis of forensic materials and probabilistic assessments of evidence. Dr. Curran provided key support in the statistical analysis and interpretation of the data and co-author of manuscripts.

Participant Name	Affiliation	Role	Funding support	Contributions
Stacey Culp	West Virginia University	Statistician Collaborator	Yes	Provided key support during the preliminary experimental design and statistical analysis in this study.
Korina Menking-Hoggatt	West Virginia University	Graduate Student (PhD)	No	Ph.D. graduate student working at the Trejos's group (graduated in spring 2021). Korina's main contribution was in the development and optimization of LIBS and SEM-EDS methods for the detection of GSR (task 3). Korina also coordinated all sampling collections (task 1) and participates in the data analysis and interpretation (task 4)
Courtney Vander Pyl	West Virginia University	Graduate Student (MSFS and PhD)	Yes (partially, 8 months)	Courtney is a graduate student at WVU-FIS Department, who completed her MS degree in May 2019, and started her Ph.D. in Fall 2019. Courtney worked under Dr. Trejos' group. Courtney main contribution was in the development and optimization of LIBS and LAICPMS method for the estimation of shooting distances and bullet hole identifications (task 2). Courtney also participates actively in sampling collection (task 1), analysis of hands-samples by LIBS and SEM-EDS (tasks 3) and statistical analysis (task 4)
Colby Ott	West Virginia University	Graduate Student (PhD)	Yes (partially 8 months)	Colby worked under Dr. Arroyo's group. His main contribution was in the development and optimization of electrochemical sensors for the detection of GSR (task 3). Colby also participates in sampling collection (task 1) and data analysis.
Kourtney Dalzell	West Virginia University	Graduate Student (MSFS)	Yes (18 months)	Kourtney was an undergraduate student who joined the Arroyo's group in Fall 2019. She joined the group as graduate MS student in Fall 2020 and contributed to the optimization of IGSR markers by EC (task 3) and data collection (task 1)
William Feeney	West Virginia University	Graduate Student (PhD)	No	William is a PhD student in the Trejos' group. He indirectly contributed to this research since his project is an important confirmatory tool for additional cross-validation of LIBS and ECD (LC-MS/MS). He contributed with GSR collection (task 1)
Jessica Friedel	West Virginia University	Undergraduate student	No	Jessica Friedel worked during the Spring of 2021 as part of fulfillment of RAP program and 40 hours per week in the summer of 2021 in fulfillment of her internship for the

Participant Name	Affiliation	Role	Funding support	Contributions
				WVU forensic chemistry program. Her most important contribution was analysis and data organization from the SEM-EDS (task 2 and 3)
Hannah Simmerly	West Virginia University	Undergraduate student	No	Hannah contributed to the analysis and interpretation of GSR by SEM-EDS (task 3)
Emily Heller	West Virginia University	Undergraduate student	No	Contributed on ICP-MS analyses (task 3).
Oriana Ovide	West Virginia University	Undergraduate student	No	Oriana's main contribution was with sampling collections (task 1) and the estimation of shooting distances by LIBS and color tests (task 2)
Katie Speirs	West Virginia University	Undergraduate student	No	Katie's main contribution was in the method development of electrochemical quantitative analysis of OGSR markers (task 3).
Sean McIntosh	Foothold Labs	Industry Collaborator	No	Footholds and WVU signed a MOA from March 2019 until December 2020 to cooperate on development on sensors and handheld deployable electrochemical devices. In this MOA Foothold Labs provided a portable electrochemical unit called FLStat, controlled by Microsoft Surface Go.
Jong Yoo, Jhanis Gonzalez	Applied Spectra	Industry Collaborator	No	ASI assisted with custom made ablation platforms for the project and evaluated samples with ICCD detector
Chip Pollock	Sacramento County District Attorney's Crime Laboratory	Crime Lab Collaborator	No	The laboratory provided training to the graduate student, Korina, on using the SEM-EDS and the analysis of GSR evidence for forensic casework. They also provide support in the form of processing samples for interlaboratory studies and collaboration of results at scientific conferences in the form of co-authoring posters. We are working closely with the lab to evaluate the potential adoption of the rapid LIBS method.
Ana Lorena Alvarado and Pedro Calderón	University of Costa Rica, Center of Electrochemistry and Chemical Energy	Academia Collaborator	No	UCR/CELEQ provided funding to one of his master's students, Pedro Calderon, to complete a technical exchange visit at WVU Pedro was under supervision of Dr. Arroyo. Pedro's expertise in electrochemistry has been useful to our project as he trained our students on modern techniques for the modification of electrode surfaces with nanoparticles, and a method was optimized for GSR detection

V CHANGES IN APPROACH

Nothing to report

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