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Evaluation and Optimization of DNA Recovery and Amplification from Bullet Cartridge Cases

Executive Summary Report

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EXECUTIVE SUMMARY

Introduction - One of the most common types of evidence found at the scene of homicides, robberies and aggravated assaults are cartridge cases. During the process of being loaded into a magazine prior to firing, cartridges are typically touched and pressure is applied as the magazine is loaded. This allows for the deposition of epithelial cells and cell-free DNA on the surface of the metal casing. Despite the prevalence of cartridge cases and their potential to link a suspect to a crime scene, they are not routinely submitted for DNA analysis. This stands in sharp contrast to the increasing frequency with which samples are submitted for "touch type" or "trace" DNA analysis to aid the investigation of property crimes as well as crimes against persons. While it might be reasonable to anticipate that recent improvements to STR chemistries and more sensitive detection platforms would produce greater success with cartridge case evidence, this has not been borne out in practice. Rather, DNA profiling of cartridge cases typically yield minimal results with few if any amplified alleles being detected.

The overarching goal of the research completed under award 2015-DN-BX-K059 was to optimize the recovery, extraction and amplification of trace DNA from a variety of commonly encountered cartridge case types. To achieve this, a variety of theories offered to account for the generally poor results obtained with cartridge case evidence were evaluated. Both existing and novel methods for the recovery and extraction of DNA from metal cartridge cases were assessed in order to develop robust protocols and an optimized workflow. The applicability of the optimized workflow was demonstrated over a wide range of commonly encountered ammunition calibers and cartridge case types. Finally, standard operating procedures were written to facilitate adoption by casework practitioners.

Optimized Recovery and Extraction Methodologies - Metal substrates have often proven to be challenging sample types for DNA recovery. It has been hypothesized that this may be the result of active DNA degradation by reactive metal species or a higher binding affinity for DNA that impedes recovery. Recent

studies have suggested that the self-sterilizing characteristics of some metal surfaces is due to lipid membrane disruption rather than DNA degradation. Working from the hypothesis that intact DNA persists on the cartridge case surface, a variety of recovery methods were evaluated to determine if DNA recovery could be substantially improved. For these studies, pair-wise combinations of five DNA recovery methods (*i.e.,* wet:wet swabbing, wet:dry swabbing, soak and sonicate, soak and vortex and tape-lift) and three extraction methods (*i.e.*, PrepFiler®, QIAamp®, and organic [phenol:chloroform:IAA]) were used on each of four metal substrates used for the manufacturing of cartridge cases (*i.e.*, both fired and unfired brass, nickel-plated, aluminum, and steel cartridge cases). Following extraction and quantification, extracts were concentrated by lyophilization as pilot studies indicated that this approach produced improved genetic profiles with low quantity samples without an increase in stochastic effects. Statistical analyses of the results employed single factor one-way ANOVA.

Unfired Cartridges: The soak and sonicate method consistently generated the highest DNA yield and profile quality regardless of metal type. It should be noted that for effective use of the soaking methods, larger caliber cartridge cases (*e.g.*, rifle rounds), require tall but narrow tubes to keep the total volume of solubilization buffer at <1 mL. It should also be noted that the addition of BSA and copper-binding tripeptide GGH to the solubilization buffer does not appear to impact the quality of resulting electropherograms (**Figure 1**). Tape lifts generated the second highest yield except with steel cartridge cases. The graphite coating applied as a dry lubricant to the steel surface saturated the adhesive properties of the tape (**Figures 2-5**). In addition, rifle rounds may require the use of multiple tape dots to cover the entire surface. By contrast, traditional swabbing techniques consistently produced significantly lower DNA yields. In terms of metal substrates, aluminum cartridge cases consistently produced the highest DNA yields and best profile quality regardless of recovery method employed.

With respect to DNA extraction, the use of the organic extraction method consistently

produced the best results (*i.e.*, DNA yield and profile quality) for all metal substrates. Dextran derivative capture technologies (*e.g.*, PrepFiler® Forensic Extraction Kit) outperformed silica-based capture extraction methods (*e.g.*, Qiagen EZ1® and QIAamp® DNA Investigator Kit) (**Figures 2-5**). Chelex® extraction was consistently associated with the lowest yields and profile quality. As a result, further assessments of Chelex® were eliminated from the study. When assessing differences between metal types within any recovery-extraction pairing no statistically significant difference in the ability to generate donor profiles were detected. This result does not support the hypothesis that highly reactive metal species (*e.g.*, copper) present in brass cartridge cases disproportionately impedes DNA recovery (**Figure 6**).

In summary, sufficient quantities of DNA can transfer from a handler during the manual loading of a magazine to support the generation of interpretable DNA profiles from unfired rounds. Using a soak and sonicate recovery method paired with organic extraction, the average total DNA yield ranged from 430-930pg. Interpretable profiles were subsequently obtained for >95% of cartridge cases regardless of the metal composition.

Fired Cartridges: The DNA recovery and extraction results obtained with fired cartridges followed the same pattern as unfired cartridges. Specifically, the soak and sonicate method was the optimal recovery technique followed by the tape lift method. In terms of DNA isolation, organic extraction provided the best quantitative and qualitative results. While the overall pattern of performance with regard to the relative efficiency of the recovery and extraction methods evaluated was the same, it is important to note that fired cartridges were associated with significantly lower DNA recovery yields and lower profile quality. The average percentage decrease in DNA quantity relative to unfired cartridges was 67.85% (range 63.49-75.37%) with a corresponding loss in allelic peak height (including allelic and locus drop out) (**Figure 7**).

Factors Impacting DNA on Fired Cartridges - Proposed hypotheses to account for the lower

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DNA recovery from fired cartridge cases include DNA destruction by the heat/pressure of firing; PCR inhibition/DNA degradation from reactive metallic species in GSR or the cartridge case itself; and transfer of DNA from the cartridge case to the chamber during firing. Each of these were evaluated under controlled conditions.

Thermal Degradation: Published studies on the direct measure of heat at the cartridge case surface suggest that the heat generated during the firing process is insufficient to degrade DNA. The chamber and cartridge temperatures reached during firing is a function of the thermal diffusive properties of the material from which the cartridge is made. Aluminum cartridge cases showed the greatest surface temperature reaching 98.85 ºC for 1.2 ms[1].

While the current study focused on brass as the most commonly used metal for cartridge cases, an additional set of thirty 9 mm aluminum cartridge cases were also handled and sequentially fired to determine whether greater degradation/loss would be observed. All cartridge cases were processed using the soak and sonicate recovery method paired with the organic extraction. Analysis of the quantitative DNA yield data for the fired aluminum 9 mm rounds showed a 90.68% decrease compared to unfired aluminum 9 mm rounds. This was higher than the 75.37% decrease exhibited by fired vs. unfired brass cartridge cases (**Figure 8**). It is unclear from this limited sample set whether the thermal diffusive properties, expansive properties, or other physical characteristics of aluminum are responsible for this loss. Regardless of the mechanism, this higher rate of loss is offset by the higher average quantity of DNA present on/recoverable from unfired rounds.

Metal Co-Elution/Inhibition: The potential for gunshot residue and other reactive metallic species to inhibit PCR and/or degrade DNA was also evaluated. As GSR is insoluble in non-acidified aqueous

[1] Gashi, B., *et al.* (2010) "Measurement of 9 mm cartridge case external temperatures and its forensic application." Forensic Science International 200(1-3), 21-27.

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solutions, rounds of ammunition were fired at close range targeting filter paper and punches equidistant to the entrance hole were collected. This made it possible to standardize the amount of GSR added to replicate aliquots of epithelial cell slurry. Batches of ten replicate samples that underwent one of the five grant identified extraction protocols were pooled together to create a homogenous solution for metals analysis using ICP-MS. Separate batches both with and without GSR were generated. An additional five GSR-positive DNA samples for each extraction method were retained for DNA profiling.

Results obtained for DNA extracts without GSR were subtracted from those containing GSR for each extraction type to eliminate those results attributable to the extraction process and buffers used rather than to GSR constituents. Several elements of interest, including antimony and lead, appeared to co-elute at higher concentrations in GSR-containing samples regardless of the extraction method employed. Along with barium, these elements are the most common constituents of cartridge primers (**Figure 9**).

While the exact composition of GSR varies among manufacturers, no brand-specific differences were detected with regard to DNA yield or profile quality in this study (**Figure 10**). To assess the applicability of the GSR metals analysis data to casework samples, fired cartridges were processed using the soak and sonicate method and extracted using each of five extraction methods. Subsequent metals analysis revealed that the co-eluting species identified in fired cartridges were consistent with those observed using GSR on filter paper. The only substantive difference was the additional presence of copper and zinc. This was not unexpected as these are constituents of brass cartridges (**Figure 11**).

There did not appear to be a correlation between the concentration of co-eluting metals and a reduction in DNA yield or profile quality. In an effort to confirm these findings, dilutions of copper, zinc, lead and antimony salts, were added to positive DNA amplification controls at a range of concentrations in an effort to determine if there was a discernable impact on profile quality. No differences in profile quality were observed for any metal salt dilutions assayed (**Figure 12**).

An experiment was also performed to determine whether the addition of metal chelating agents during the extraction process would reduce the amount of co-eluting metallic species in DNA extracts. Two

modified extraction methods involving the addition of Chelex resin as a chelating agent for divalent cations were evaluated. Based on subsequent ICP-MS metals analyses, no substantive reduction was seen in the co-elution of divalent or other metal ions. Similarly, no improvement was seen in the quantitative yield or resulting profile quality of the recovered DNA (**Figure 13**). This suggests that the use of chelation for the removal of co-eluting metallic species from GSR during extraction may not be of any benefit for the concentrations of co-eluting metals seen in casework-type samples. Taken together, studies of co-eluting metals from either GSR or the cartridge case and primer itself failed to indicate a significant correlation between the identified metallic species and a negative effect on downstream genetic profiling. While it may be possible that some of the organic constituents of GSR may have a negative impact on genetic profiling quality, an investigation of this was beyond the scope of this grant.

DNA Transfer: During firing, the outside of the cartridge case makes direct contact with the inside of the firearm as the round passes from the magazine to the firing chamber and then again as the projectile passes through the barrel. In addition, collected cartridge cases are in physical contact with the evidence packaging prior to testing. Given that published studies have shown that DNA can be indirectly/secondarily transferred between substrates[2,3], an additional set of studies were added to the current project to quantify the potential impact of such transfer events during firing and within the evidence packaging.

Thirty brass 9 mm rounds and thirty 45 ACP rounds were handled and fired on three separate days. Prior to firing, the firearm underwent an extensive internal and external decontamination with 70% ethanol and 20% bleach to remove trace DNA. Baseline wet-dry double swabbing of the firing chamber and the barrel were taken and retained as controls. Expelled cartridge casings were collected in a single coin envelope. After firing, the firing chamber and barrel were separately swabbed. This process was repeated after the second and third days of firing. All swabs from the inside of the firearm were extracted using an

[2] Meakin, G. and Allan Jamieson, A. (2013) DNA transfer: Review and implications for casework. Forensic Science International: Genetics 7, 434–443.

[3] Goray, M. *et al.* (2012) DNA transfer within forensic exhibit packaging: Potential for DNA loss and relocation. Forensic Science International: Genetics 6, 158–166.

organic extraction. The collected casings were also processed for DNA analysis using the soak and sonicate recovery method paired with an organic extraction. In addition, the interior of the coin envelope in which the fired casings had been collected and stored was swabbed to assess potential loss of genetic material from the cartridge case due to transfer to the evidence packaging.

Quantitative and qualitative analysis of samples collected from the firearm and coin envelopes provide support to the hypothesis that secondary transfer of genetic material from the cartridge cases to the firearm and the evidence packaging is occurring. While generally low quantities of genetic material were observed from the samplings of the coin envelopes and barrel, significant levels of DNA were consistently recovered from the firing chamber which showed an average gain of 609.68 pg of DNA. The observed post-firing gain in DNA recovered from the firing chamber is substantial when considering that the average quantitative yield from the 9 mm casings was only 56.71 pg (**Figure 14**).

Assessment of MPS Platforms - Replicate sets of both unfired and fired brass samples were prepared for analysis on both an Illumina® MiSeq FGx™ Forensic Genomics System and an Ion Torrent™ S5™ System massively parallel sequencing (MPS) platforms. Data generated on MPS platforms were compared to data from paired samples (*i.e.*, equivalent in concentration) generated on the ABI 3500 Genetic Analyzer. The use of MPS chemistries allowed the analysis of additional informative markers (including SNPs) and the detection of isoalleles. While this aided in contributor identification, no overall gain in sensitivity was noted. An additional set of brass unfired 9 mm Luger samples (n=30) were split volumetrically and analyzed by both the ABI 3500 and the MiSeq FGx. Using DNA Primer Mix B (DPMB), a direct comparison of Universal Analysis Software (UAS)-developed STR results to capillary electrophoresis fragment length results showed 100% genotype concordant results. Allelic dropout was noted with both systems, with similar rates of occurrence; however, an added 37 STR loci and 172 SNP markers were generated using MPS, which could be used to increase discriminatory individualization of samples and generate investigative leads (**Figure 15**). When comparing results between MPS platforms, equivalent average number of donor STR alleles are

recovered (**Figure 16**).

Applicability of the Optimized Workflow to Caseworking Laboratories - To demonstrate the applicability and reliability of the optimized workflow for cartridge cases (*i.e.,* the combination of soak and sonicate recovery; organic extraction and pre-amplification concentration by lyophilization), a range of commonly encountered brass cartridges including 22LR, .380 ACP, 9 mm Luger, .40 S&W, .45 ACP, 5.56 x 45 mm NATO and .308 caliber rounds (10 of each) were fired and assessed. Successful profiling (defined as detecting ≥65% of donor alleles) was achieved for 90% of .22LR rounds and 100% of 9 mm Luger, .40 S&W, 45 ACP and .380 ACP cartridges (**Figure 17**). Experiments with these cartridges were conducted with personal firearms. For the rifle rounds, 5.56 x 45 mm NATO and .308 caliber, rental firearms and magazines from a commercial shooting range were used. While substantial efforts were made to clean the magazines of these firearms prior to use, all samples in these data sets produced complex mixtures of ≥4 individuals. Over the 7 calibers of ammunition, an average of 89% of samples were eligible into one of the 3 forensic CODIS indices.

It is recognized that a number of casework laboratories have moved away from manual extraction in favor of automated extraction platforms. To assess the relative efficacy of such platforms within the optimized workflow, a replicate set of samples was processed substituting the PrepFiler® extraction kit run on an AutoMate *Express*™ Forensic DNA Extraction System robotic platform in place of manual organic extraction. A decrease of 20-70% in overall profiling success rates (≥65% donor alleles detected) was observed with this substitution.

While not planned as part of the original set of core objectives for this project, an additional set of samples were added to assess the potential impact of intra-individual variability in epithelial cell shedding on DNA profiling success with cartridge cases. A total of 100 samples of the most common calibers (brass 9 mm and 45 ACP) were generated using the optimized workflow. A total of 10 participants were used in the generation of this sample set. The average quantity of DNA recovered from 9 mm and 45 ACP brass fired rounds was 174.13 pg (range: 7.21-895.09 pg) and 575.22 pg

(range: 6.96-7,959.76 pg), respectively. Likelihood ratio calculations from these samples were calculated using BulletProof, a fully continuous probabilistic genotypic tool (**Figures 18-20**). The results obtained from this data set further illustrates the utility of the optimized workflow while illustrating the variable nature of touch-DNA samples.

Effect of Loading Order **–** It has been hypothesized that cartridge loading order may correlate with the likelihood of obtaining sufficient DNA to obtain an interpretable profile. This is based on the proposition that the primary transfer of DNA may be enhanced as progressively more force is required to load each cartridge into a magazine. It has also been postulated, however, that as multiple cartridges are sequentially loaded into a magazine, repeated contact with the same skin area will progressively reduce the amount of DNA available for primary transfer. To assess these two hypotheses, the order in which cartridges were loaded in all studies was tracked and recorded. Every 10-round magazine, was independently analyzed using the Pearson product-moment correlation coefficient to assess the correlation (if any) between loading order and DNA yield (**Figure 21**). No correlation was detected between the order in which a cartridge was loaded into a magazine and the DNA yield from the cartridge case or subsequent profile quality. This finding held for fired as well as unfired cartridges.

Summary - Processing of cartridge case evidence has long been especially challenging. The current studies assessed DNA yields and profile quality as a function of the metal substrate, the presence/absence of co-eluting metal species from GSR and the cartridge case itself, and the mechanical transfer of DNA to adjoining surfaces during the firing process. The results obtained suggest that low quantities of genetic material on fired cartridges is not a factor of small quantities of DNA initially deposited nor the presence of potentially reactive metals but may be attributable to effects related to firing and/or transfer of genetic material to the internal mechanisms of the firearm and evidence packaging materials used. However, these results also demonstrate that these factors are not insurmountable barriers to successful DNA profiling. *In toto*, the studies conducted under award 2015-DN-BX-K059 provide support for the proposition that optimized pairing and simple

modifications of existing DNA recovery, extraction and profiling methodologies can substantially improve an analyst's ability to generate informative genetic profiles.

Acknowledgements
The authors would like to acknowledge the valuable contributions of the following individuals and organizations: Yih Ling Saw (CFSRE), Riley Murphy (NMS Labs), Christian Westring, Todd Bille and the ATFE, Daniele Podini (GW University), Thermo Fisher Scientific, Verogen, The University of Copenhagen and staff at the Fredric Rieders Family Foundation. This work was supported by award 2015-DN-BX-K059 from the National Institute of Justice.

EXECUTIVE SUMMARY APPENDIX A: FIGURES AND TABLES

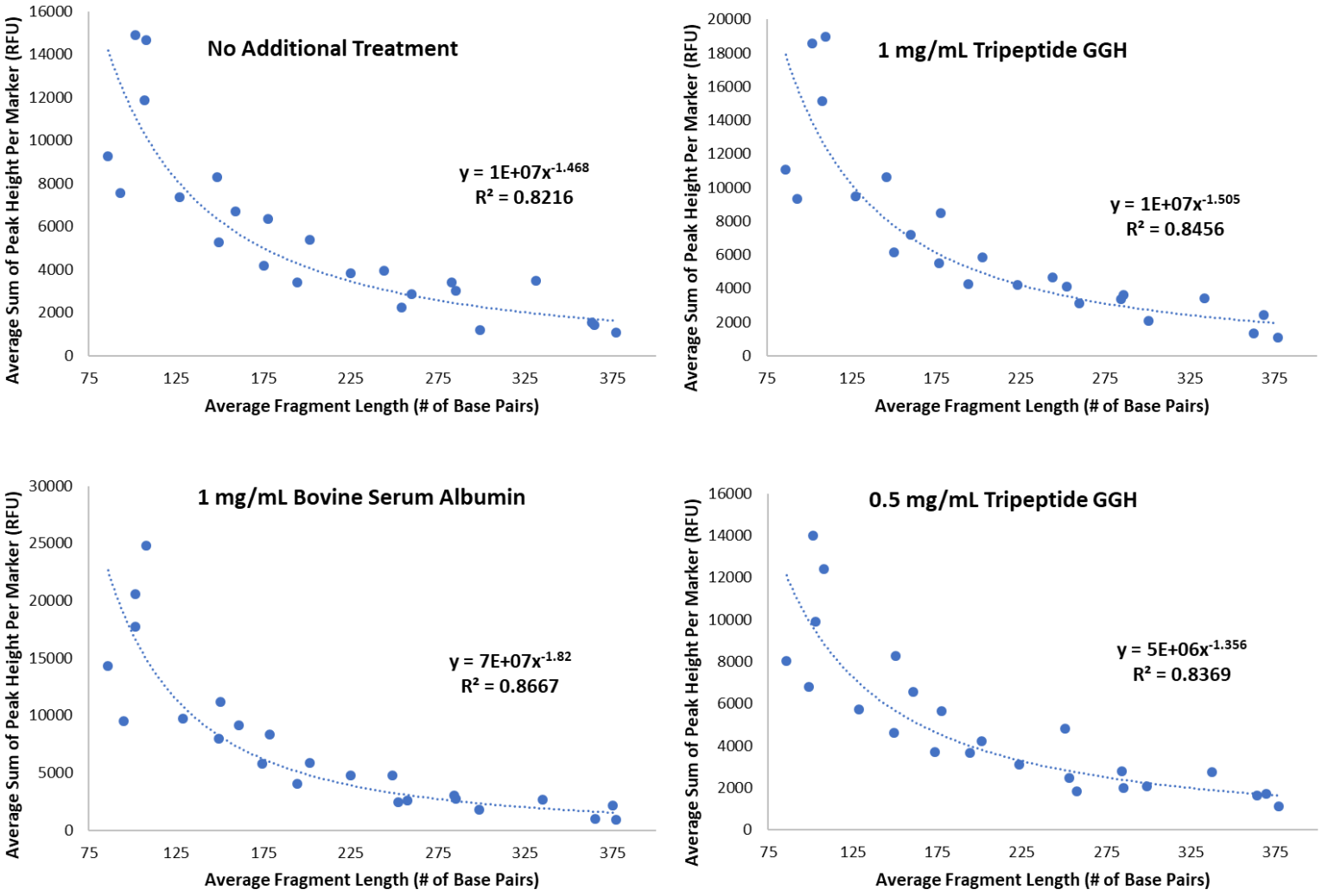

Figure 1: Electropherogram degradation plots for unfired brass 9mm samples of approximately equivalent total DNA input ($\bar{x}$ = 716.82 pg) for each category of solubilization buffer tested (neat solubilization buffer with no additional treatment, solubilization buffer with 1 mg/mL BSA added, solubilization buffer with 1 mg/mL BSA and 0.5 mg/mL GGH added, and solubilization buffer with 1 mg/mL BSA and 1 mg/mL GGH added) showing a decrease in peak heights as the molecular weight of the markers increases. For paired samples with approximately equivalent total DNA input, no statistically significant differences in calculated quantitative degradation indices, average peak heights, average peak height ratios, or qualitative degradation curve slopes were noted between the different compositions of solubilization buffer tested. With the initial concentrations of BSA and GGH assessed, this small-scale study has thus far not seen any significant qualitative benefits to promote the incorporation of BSA or GGH to the solubilization buffer.

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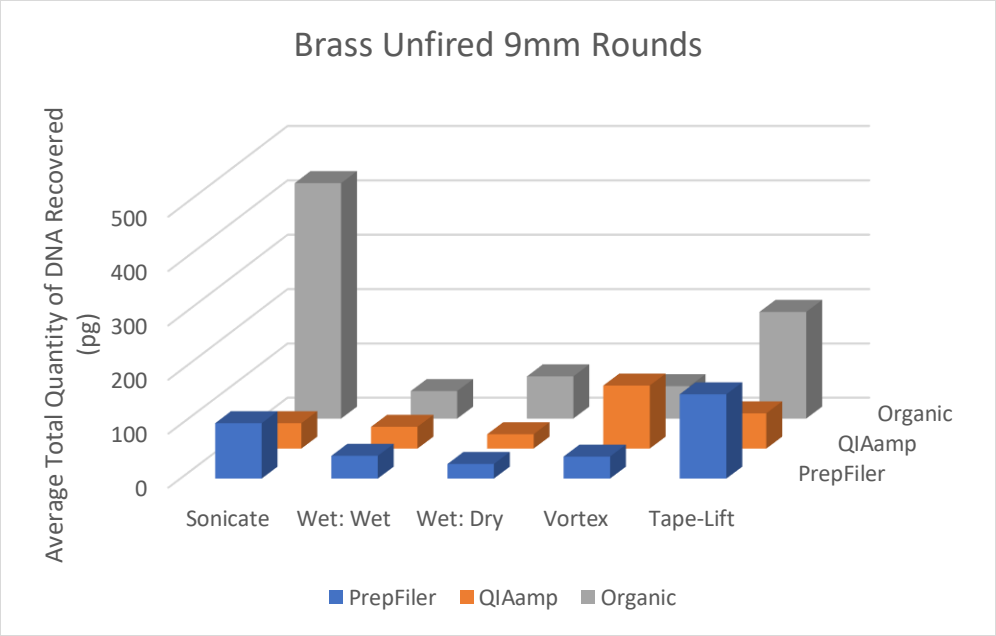

Figure 2: Total DNA yield from 9 mm unfired brass cartridge cases for all pair-wise combinations of DNA recovery and DNA extraction methods. The soak and sonicate DNA recovery method paired with organic extraction generated the highest DNA yield.

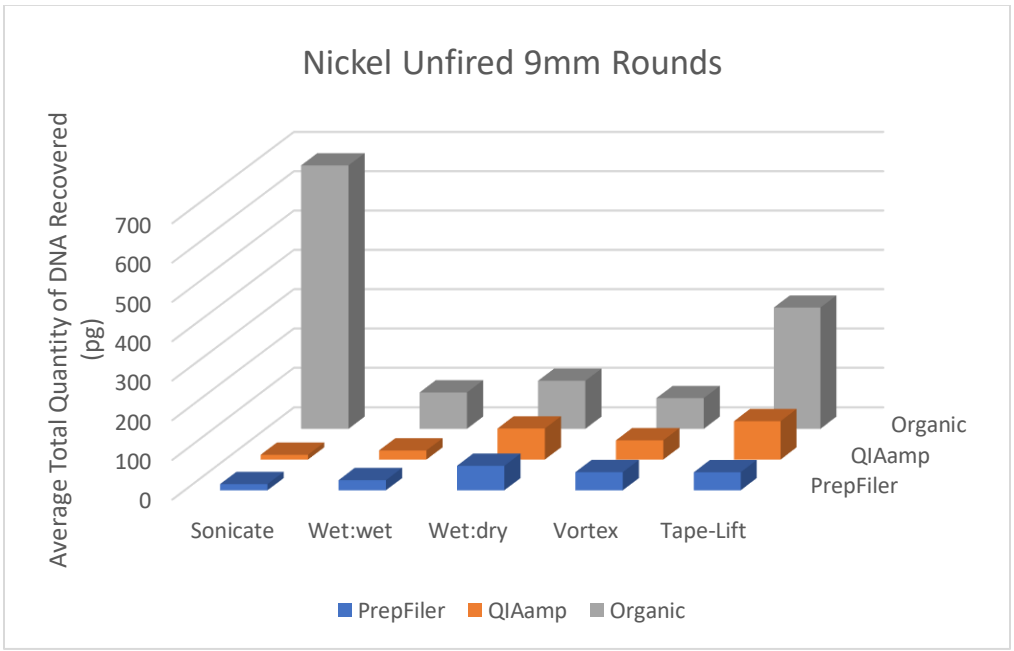

Figure 3: Total DNA yield from 9 mm unfired nickel cartridge cases for all pair-wise combinations of DNA recovery and DNA extraction methods. The soak and sonicate DNA recovery method paired with organic extraction generated the highest DNA yield.

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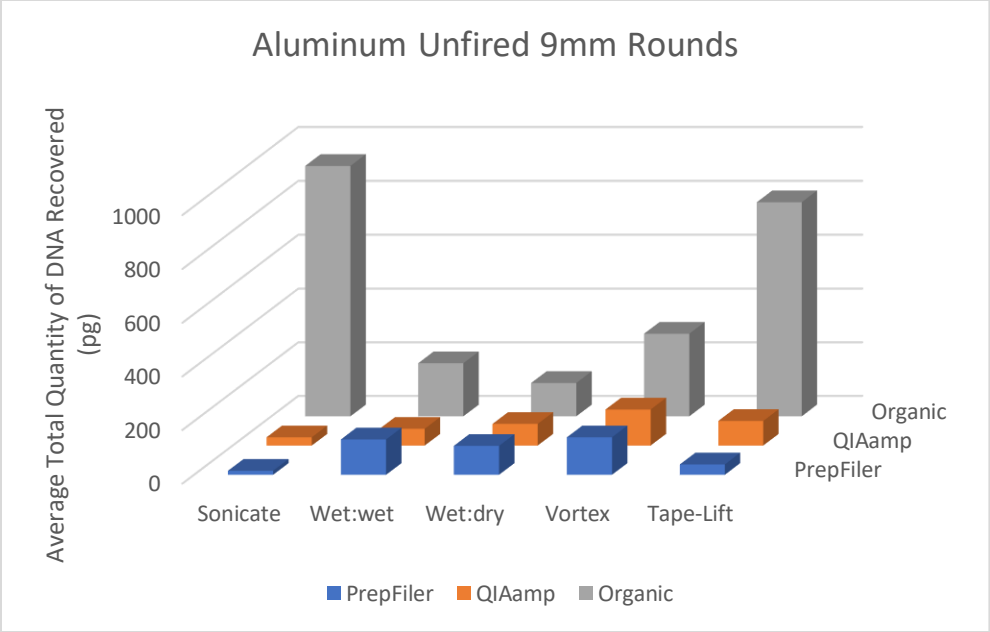

Figure 4: Total DNA yield from 9 mm unfired aluminum cartridge cases for all pair-wise combinations of DNA recovery and DNA extraction methods. The soak and sonicate DNA recovery method paired with organic extraction generated the highest DNA yields.

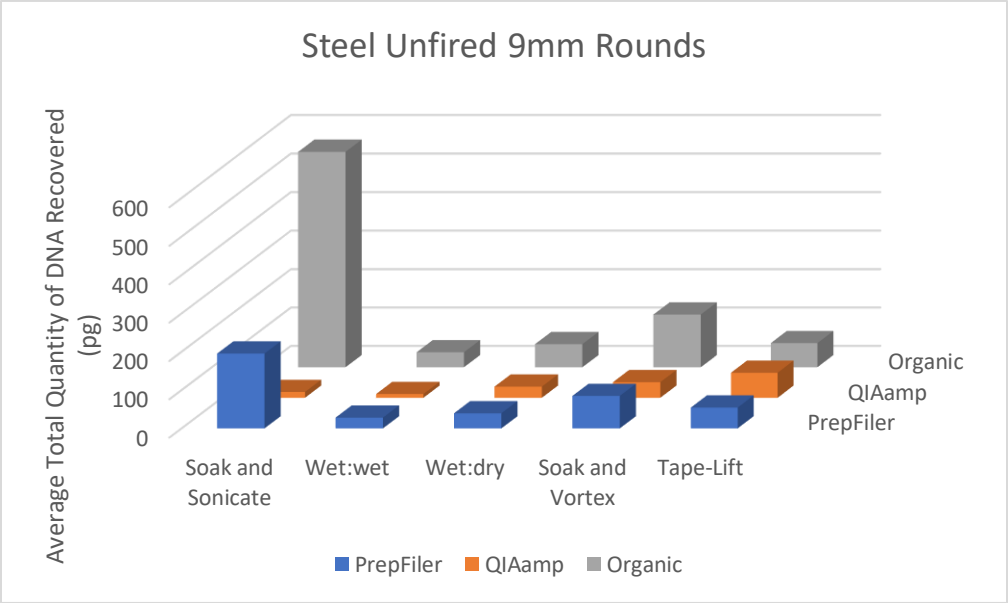

Figure 5: Total DNA yield from 9 mm unfired steel cartridge cases for all pair-wise combinations of DNA recovery and DNA extraction methods. The soak and sonicate DNA recovery method paired with organic extraction generated the highest DNA yields.

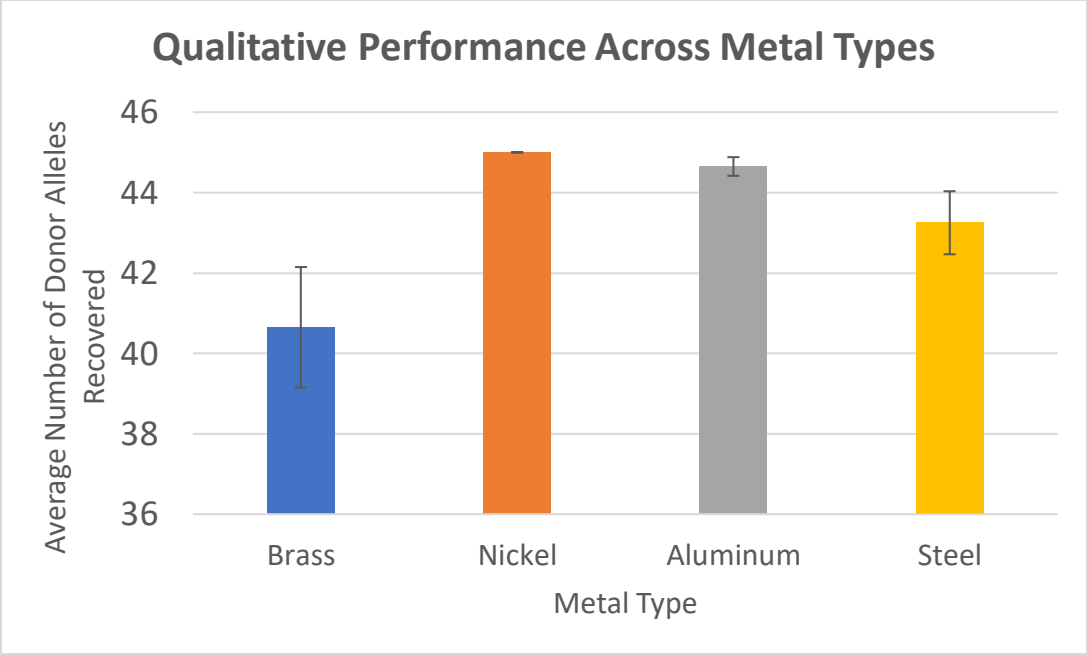

Figure 6: DNA profiling success for 9 mm unfired cartridges as a function of metal type. Success is expressed as a function of the number of donor alleles (out of a total of 45 detected possible alleles) above analytical threshold ± standard error of the mean result. No statistically significant differences in profiling success were observed.

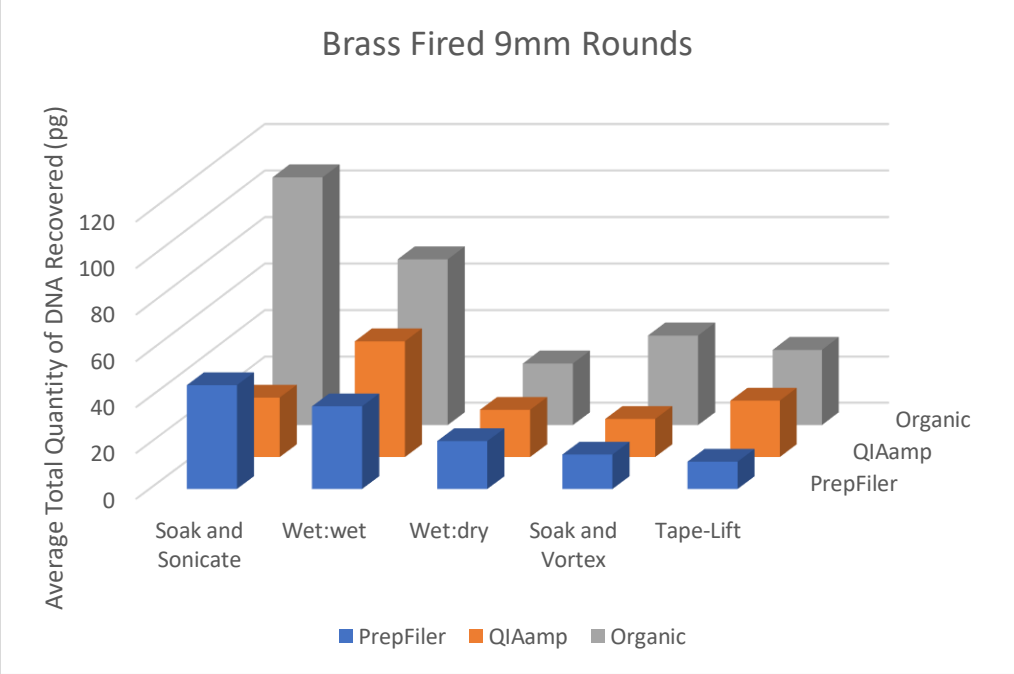

Figure 7: Total DNA yield from 9 mm fired brass cartridge cases for all pair-wise combinations of DNA recovery and DNA extraction methods. The soak and sonicate DNA recovery method paired with organic extraction generated the highest DNA yield.

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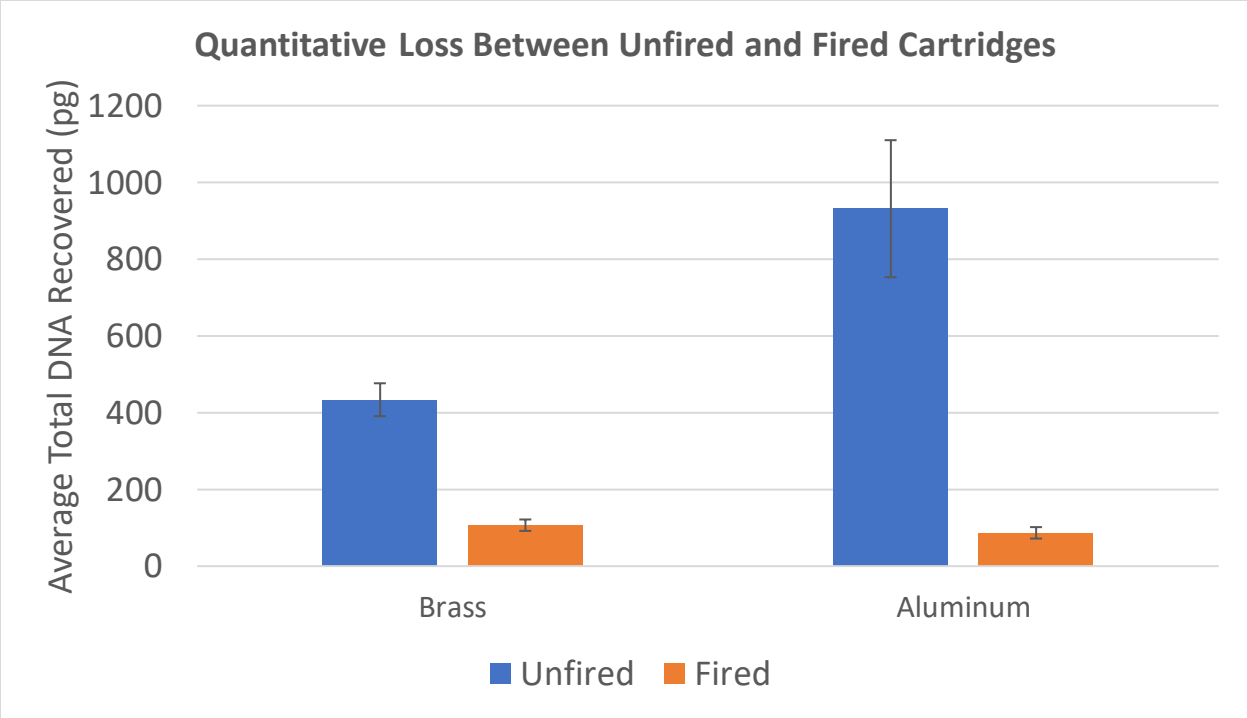

Figure 8: Quantitative comparison of average DNA yields (± standard error of the mean) for 9 mm fired versus unfired brass and aluminum cartridges. There was a decrease in DNA yield of 67.85% for fired relative to unfired brass cartridges. For fired relative to aluminum cartridges, the decrease in DNA yield was 90.68%.

[GSR+DNA]-[DNA-BUFFER]

LOQ	0.55	10	1.1	5.5	4	0.55	5.5	1.1	0.55	2.75	2.75	0.275
Method	55 Mn	56 Fe	60 Ni	65 Cu	66 Zn	71 Ga	88 Sr	118 Sn	121 Sb	137 Ba	208 Pb	209 Bi
Chelex				13.00					29.84	2.50	45.04	
Organic				29.60					97.35	153.43	800.08	2.01
PrepFiler		447.86			6.00				36.37		550.32	16.05
QIAamp									90.67	439.29	27.64	
EZ1								0.44	60.09		13.91	

Figure 9: Heat map of ICP-MS elemental metal analysis as a function of the DNA extraction method for epithelial cell samples mixed with gunshot residue from fired Winchester cartridges. Green boxes indicate either the non-detection of metal or a ≤0 quantity after subtracting out any metals detected in the reagent blank control. Red boxes indicate the presence of quantities of metal species in excess of any detected in the reagent blank control.

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[GSR+DNA]-[DNA-BUFFER]

LOQ	0.55	0.55	10	1.1	5.5	4	0.55	5.5	1.1	0.55	2.75	2.75	0.275
Method	52 Cr	55 Mn	56 Fe	60 Ni	65 Cu	66 Zn	71 Ga	88 Sr	118 Sn	121 Sb	137 Ba	208 Pb	209 Bi
5% Chelex					11.06					10.41		18.07	
Organic							1.32			35.86	110.55	51.40	
PrepFiler			1,082.86							22.56		229.64	
QIAamp			15.57		17.32				1.26	71.10	59.29	5.84	
EZ1									1.95	19.73		4.85	

Figure 10: Heat map of the results of ICP-MS elemental metal analysis as a function of the DNA extraction method for epithelial cell samples mixed with gunshot residue from fired Federal cartridges. Green boxes indicate either the non-detection of metal or a ≤0 quantity after subtracting out any metals detected in the reagent blank control. Red boxes indicate the presence of quantities of metal species in excess of any detected in the reagent blank control.

[CASING]-[DNA-BUFFER]

LOQ	0.55	0.55	10	1.1	5.5	4	0.55	5.5	1.1	0.55	2.75	2.75	0.275
Method	52 Cr	55 Mn	56 Fe	60 Ni	65 Cu	66 Zn	71 Ga	88 Sr	118 Sn	121 Sb	137 Ba	208 Pb	209 Bi
5% Chelex	5.45		93.50	13.90	1,390.20	877.20	0.75			473.25	444.25	495.20	
Organic				40.75	4,642.35	2,011.80	4.95			486.35	751.35	722.25	
PrepFiler	2.90	1.15	2,835.50	22.75	436.90	25.20			5.10	28,562.05		90.45	
QIAamp				1.80	98.70					23.60		8.80	
EZ1							0.70			183.50			

Figure 11: Heat map of ICP-MS elemental metal analysis as a function of the DNA extraction method for manually loaded and fired Winchester cartridges. DNA was recovered using the soak and sonication method. Green boxes indicate either the non-detection of metal or a ≤0 quantity after subtracting out any metals detected in the reagent blank control. Red boxes indicate the presence of quantities of metal species in excess of any detected in the reagent blank control.

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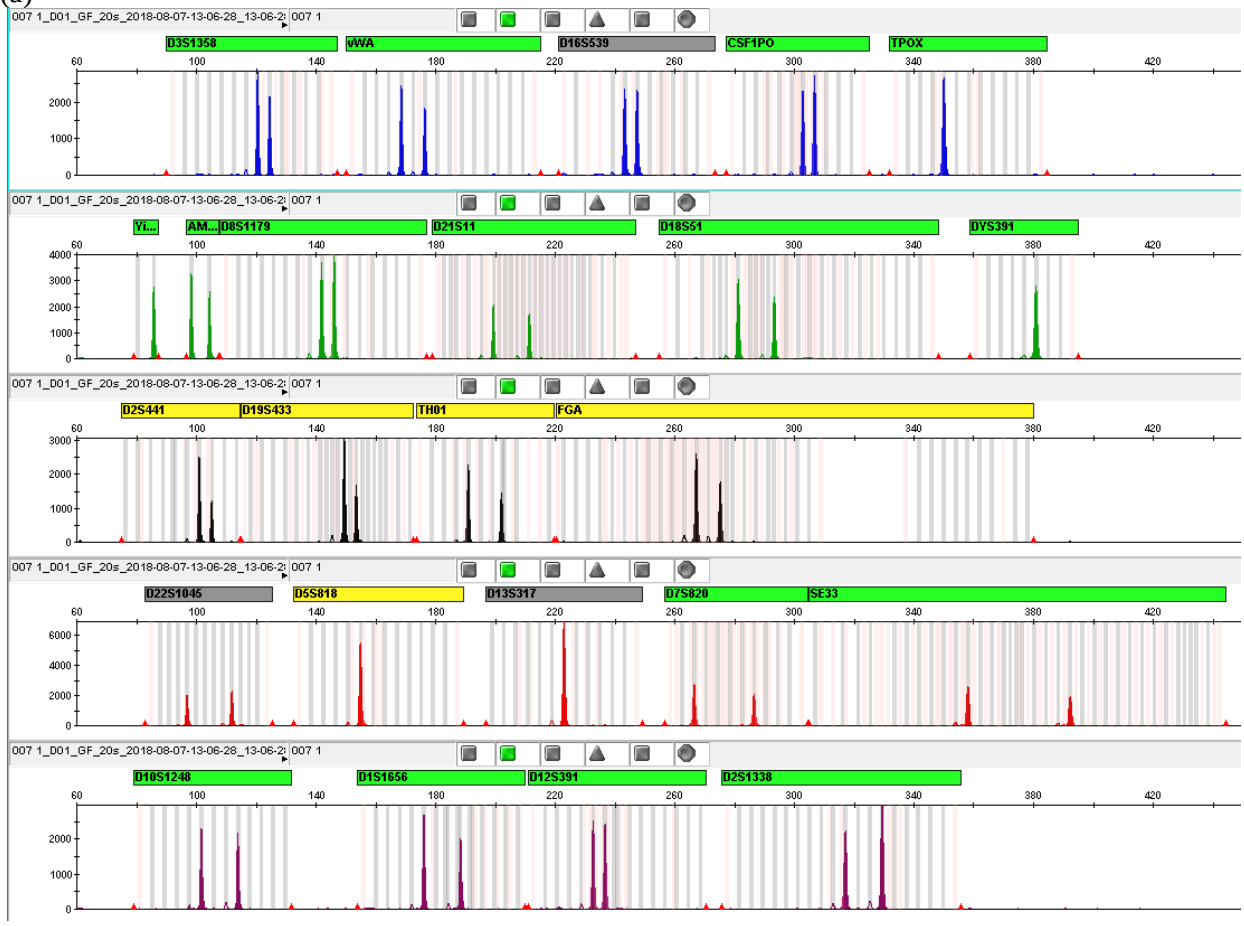
(a)



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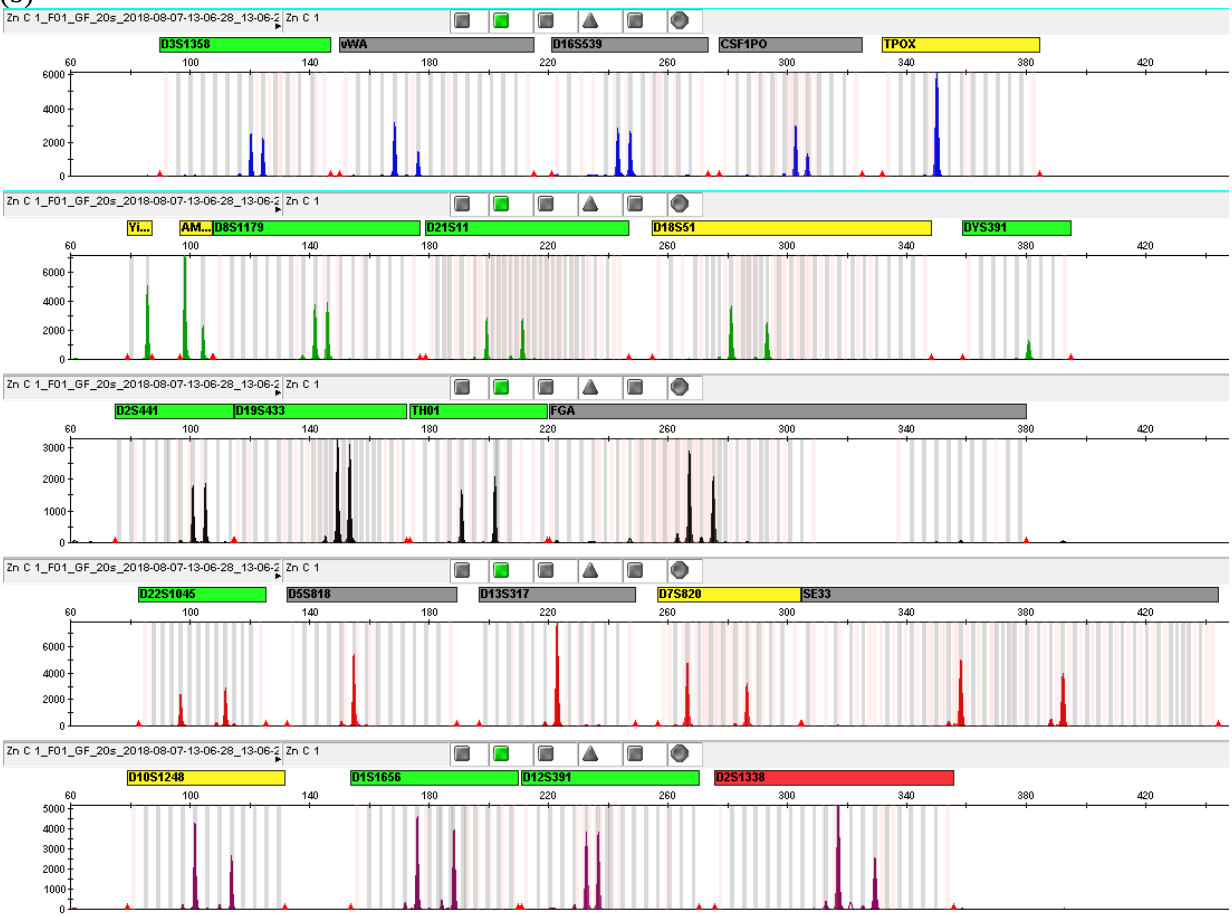

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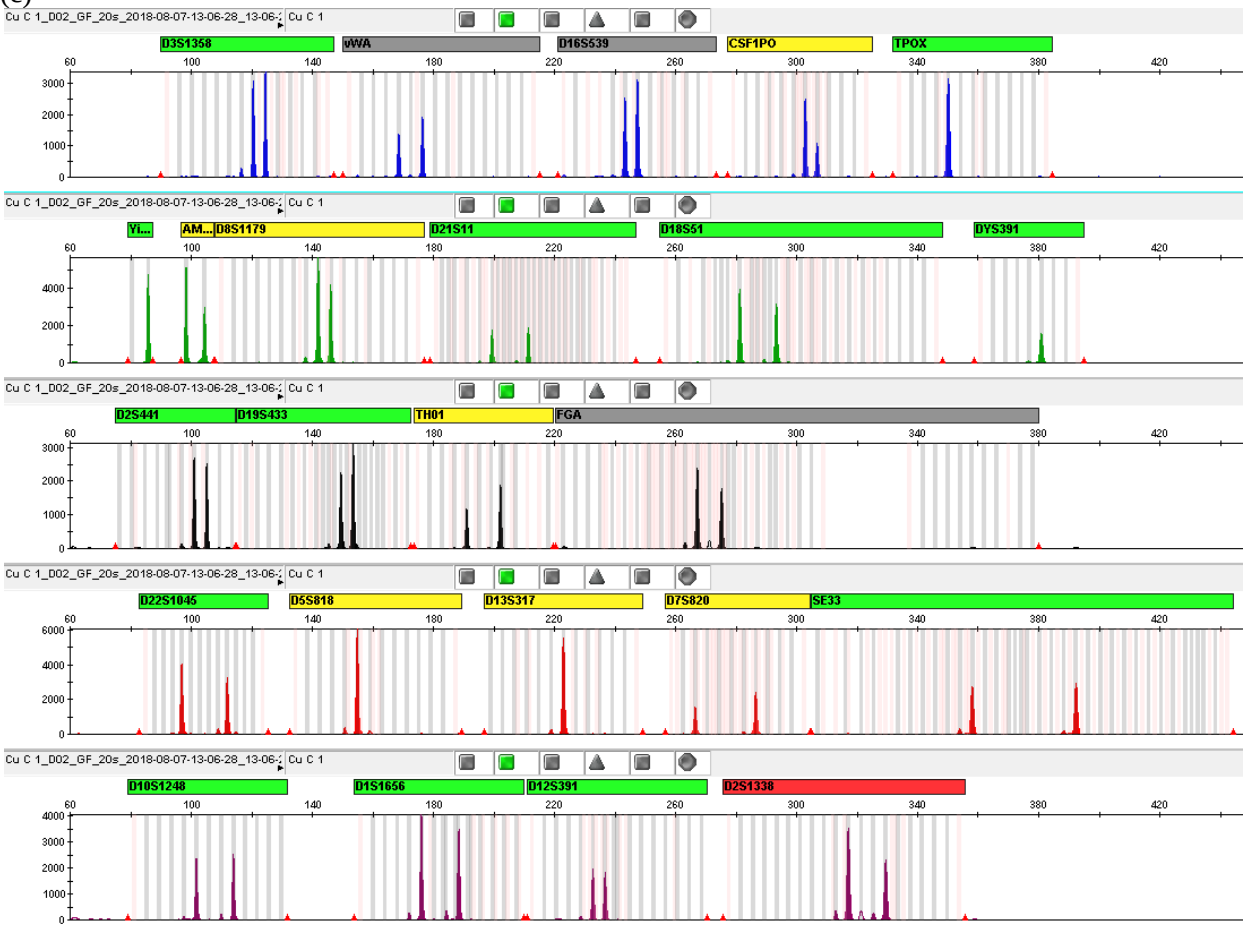
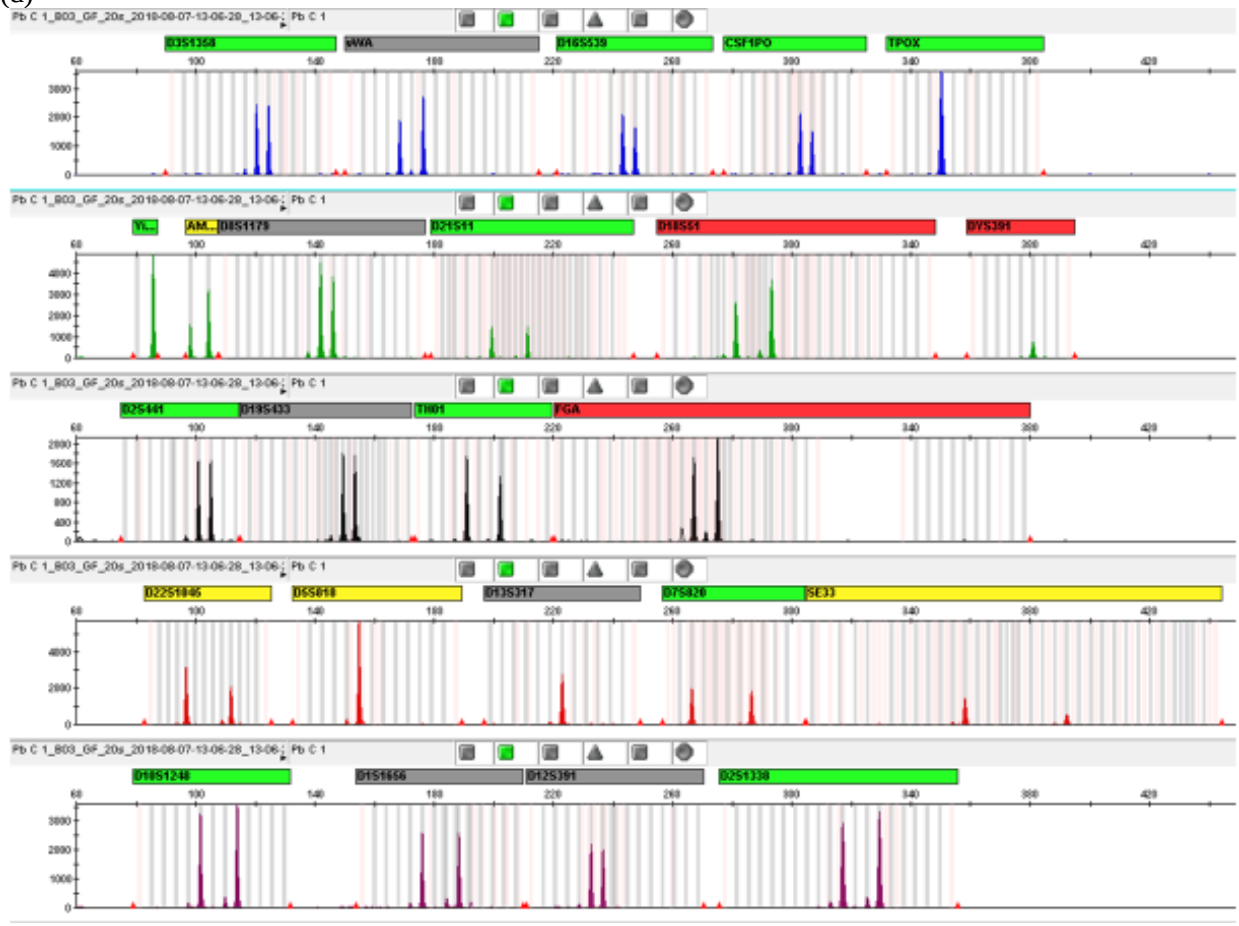


(d)



(e)

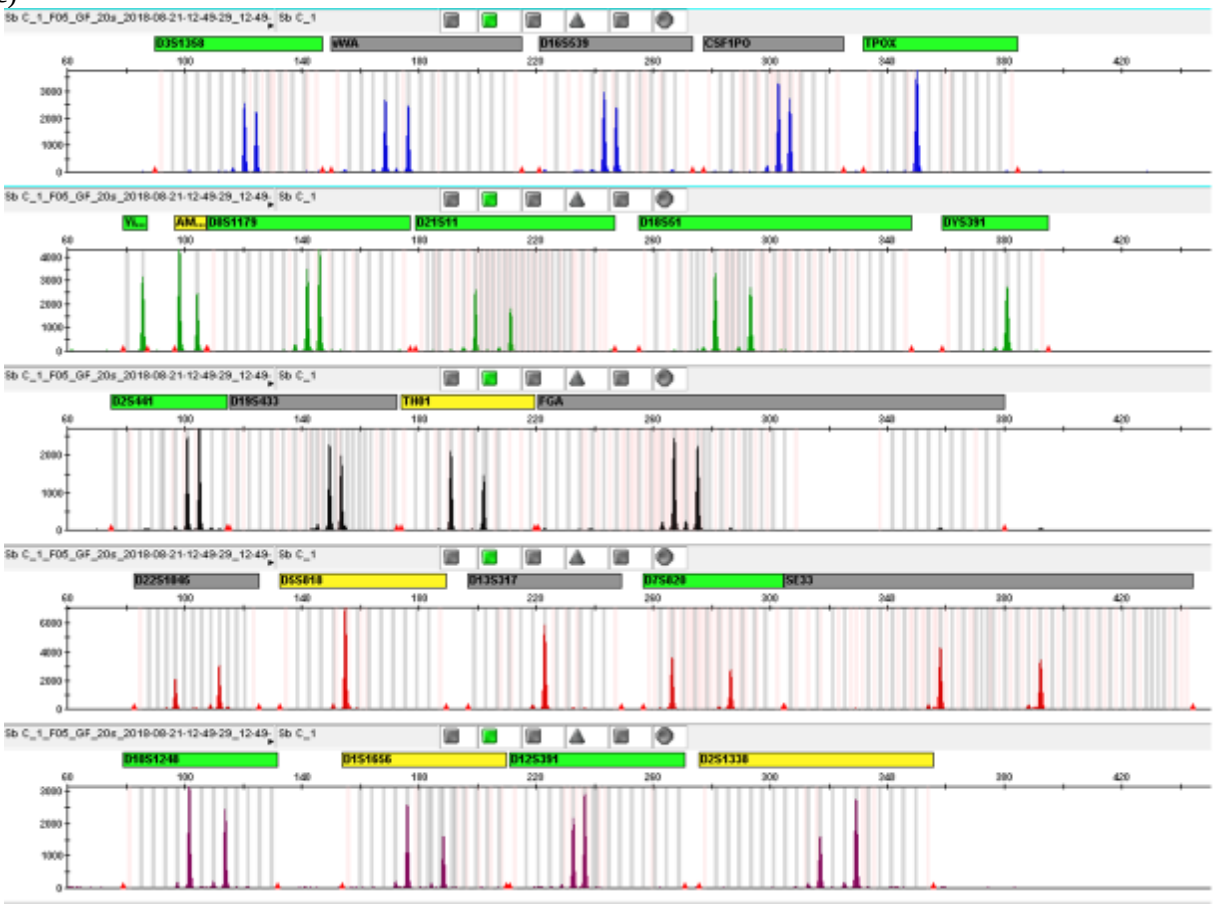


Figure 12: Representative electropherograms generated from DNA extracts generated as part of the metal titration testing. The highest concentration tested for each metal is represented here. No substantive differences in electropherogram quality were noted for any of the co-eluting metallic species. Profile quality was assessed on the basis of the number and signal intensity of the alleles detected. (Figure Panels: (a) 007 control DNA, (b) zinc at 20 ppm, (c) copper at 50 ppm, (d) lead at 20 ppm and (e) antimony at 30 ppm)

[GSR+DNA]-[DNA-BUFFER]

LOQ	0.55	10	1.1	5.5	4	0.55	5.5	1.1	0.55	2.75	2.75	0.275
Method	55 Mn	56 Fe	60 Ni	65 Cu	66 Zn	71 Ga	88 Sr	118 Sn	121 Sb	137 Ba	208 Pb	209 Bi
5% Chelex				6.26					7.80		9.39	
10% Chelex				14.55					10.88	3.63	6.94	
Organic	0.75			15.02	4.02				58.81	223.07	78.72	
Organic with 5% Chelex	1.02			17.82	13.81				144.25	426.41	217.27	

Figure 13: Heat map of elemental analysis for fired Winchester cartridge casings by extraction method. Buccal cell slurry was added to filter paper with and without gunshot residue, extracted and analyzed via ICP-MS. Green boxes indicate negative values before or after subtraction from blank buffer controls. Red boxes indicate the presence of a metallic species after blank subtraction. Addition/increase of chelating agent did not affect amount of co-eluting divalent metallic species and subsequently had no beneficial impact on electropherogram quality.

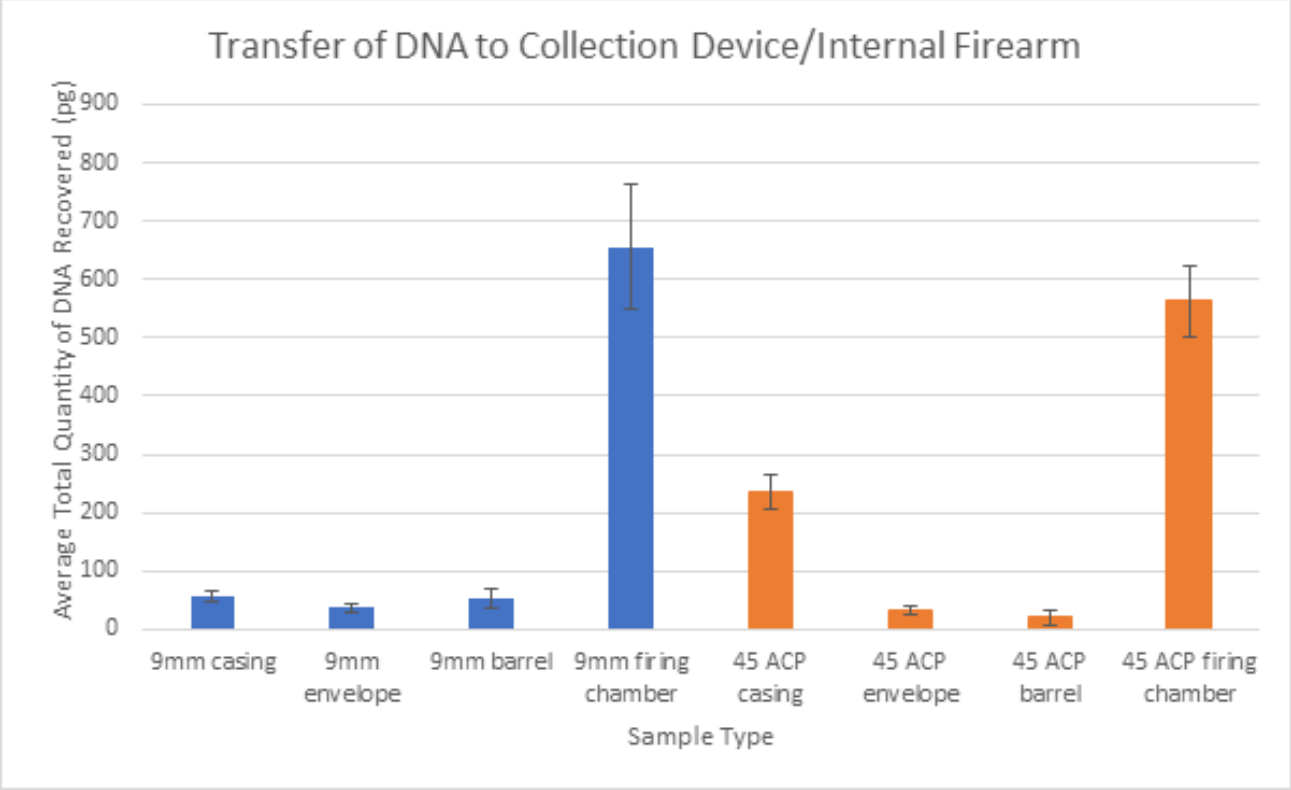


Figure 14: Transfer of touch-type/trace DNA from fired cartridges to internal components of a firearm (*i.e.*, firing chamber and barrel) as well as the interior surface of the evidentiary packaging used to hold the cartridge cases after firing. Results displayed show the average net total quantity of DNA recovered (± standard error of the mean) due to firing.

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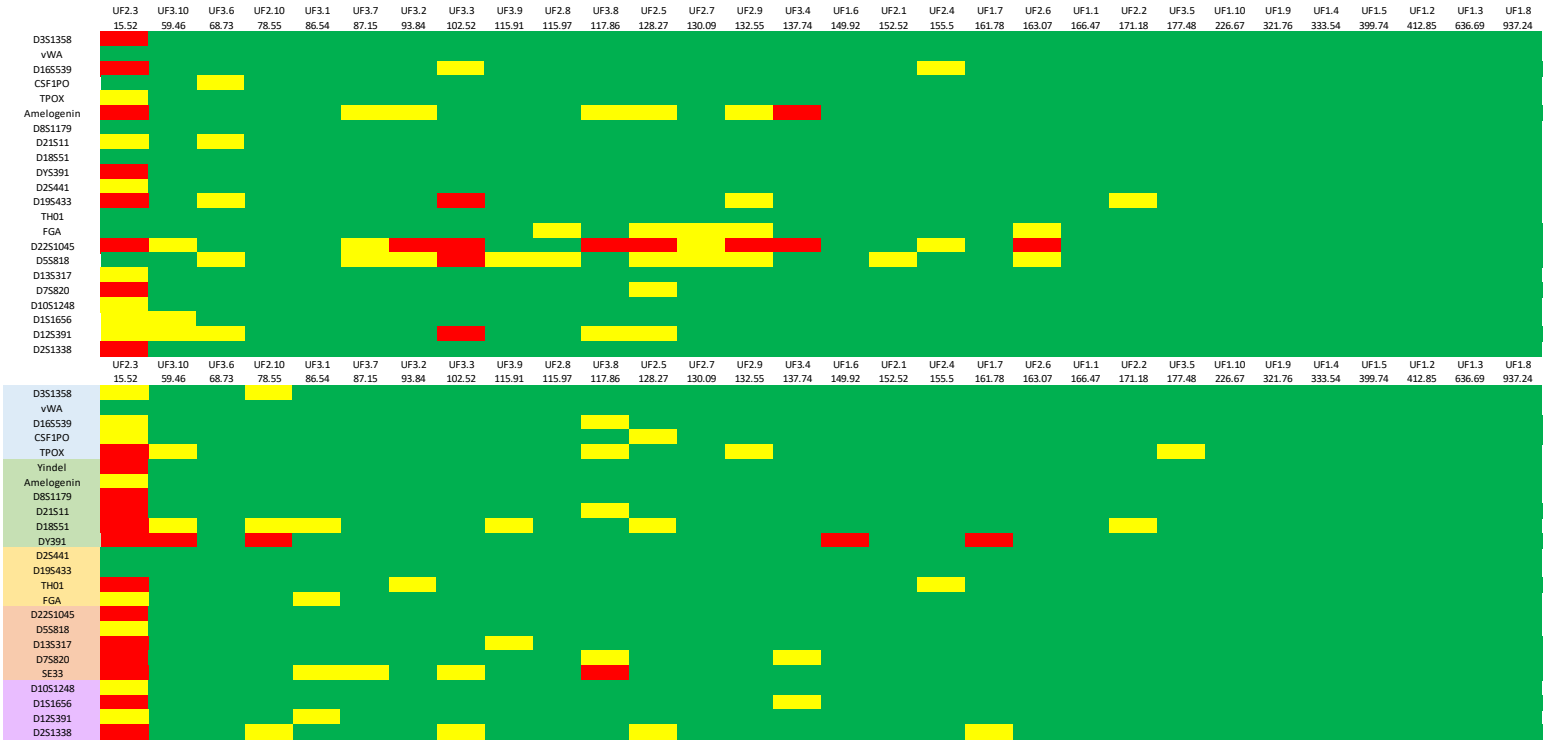

Figure 15: Concordance map displaying matched loci across both amplification kits (ForenSeq DNA Signature Prep Kit and GlobalFiler™ PCR amplification kit) and genetic analysis technologies (next-generation sequencing using the MiSeq FGX and capillary electrophoresis using the ABI 3500). Samples are arranged horizontally by increasing concentration. (Green cells: 100% of donor alleles detected; yellow cells: 50% of donor alleles detected; red cells: 0% of donor detected).

	ForenSeq (DPMB) Workflow	Ion Torrent Workflow
Average Total DNA Input Quantity (pg)		57.09
Average Number of Autosomal STR Alleles Detected	25.70 / 53	37.94 / 59
Average Number of X STR Alleles Detected	2.41 / 7	N/A
Average Number of Y STR Alleles Detected	8.67 / 26	1.94 / 3
Average Number of Identity Informative SNP Calls Detected	31 / 137	N/A

Figure 16: Average quantitative inputs and number of donor-STR alleles and SNP calls detected from fired brass 9 mm cartridge casings processed using the optimized workflow and analyzed using the ForenSeq and Ion Torrent workflows. Displayed detection data indicates the average number of alleles or calls detected out of the total number of known donor alleles. Both workflows were able to detect approximately equivalent numbers of donor-STR alleles (ForenSeq = 36.78, Ion Torrent = 39.88), with the ForenSeq workflow being able to generate additional informative information due to the additional SNP markers present within the kit.

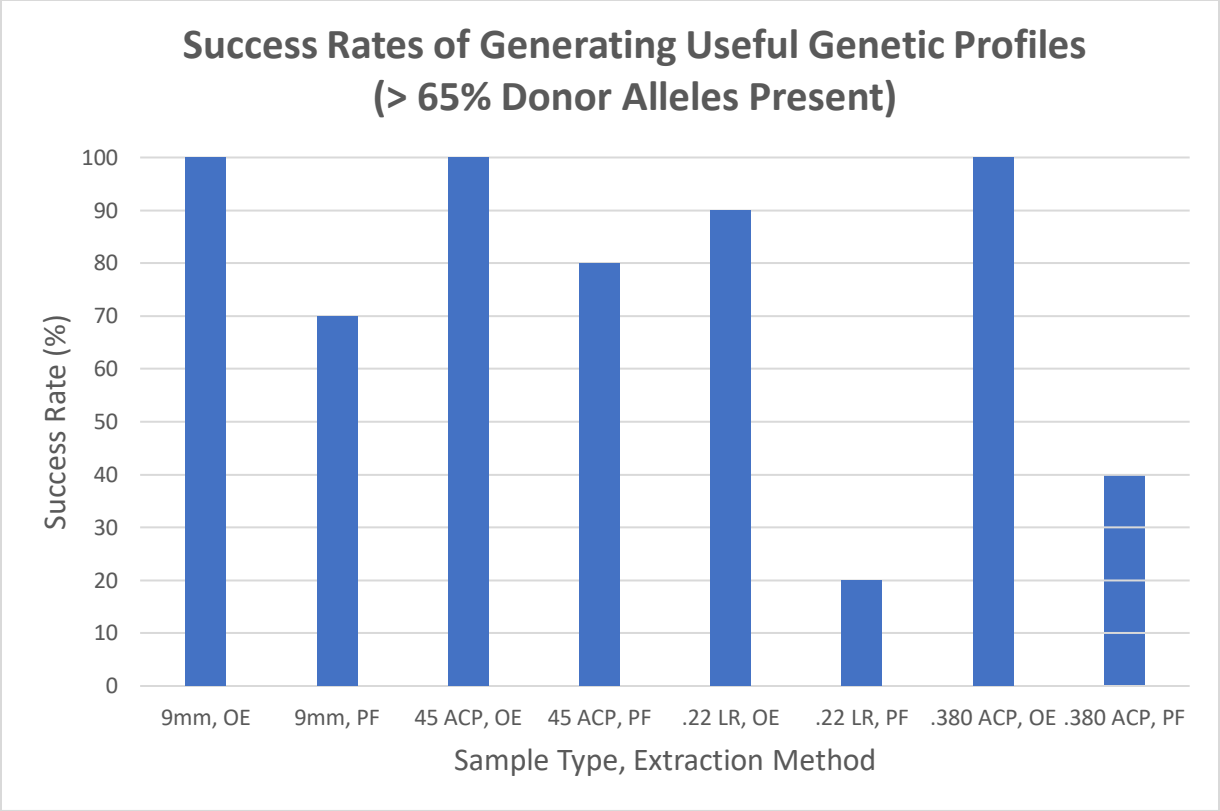

Figure 17: Comparison of DNA profiling success rates (success = ≥65% of donor alleles detected) as a function of cartridge type and workflow. Workflows included both manual and automated extraction protocols. A decrease of 20-70% in overall profiling success rates was observed when an automated workflow was used in place of manual organic extraction. (OE: Organic Extraction; PF: PrepFiler).

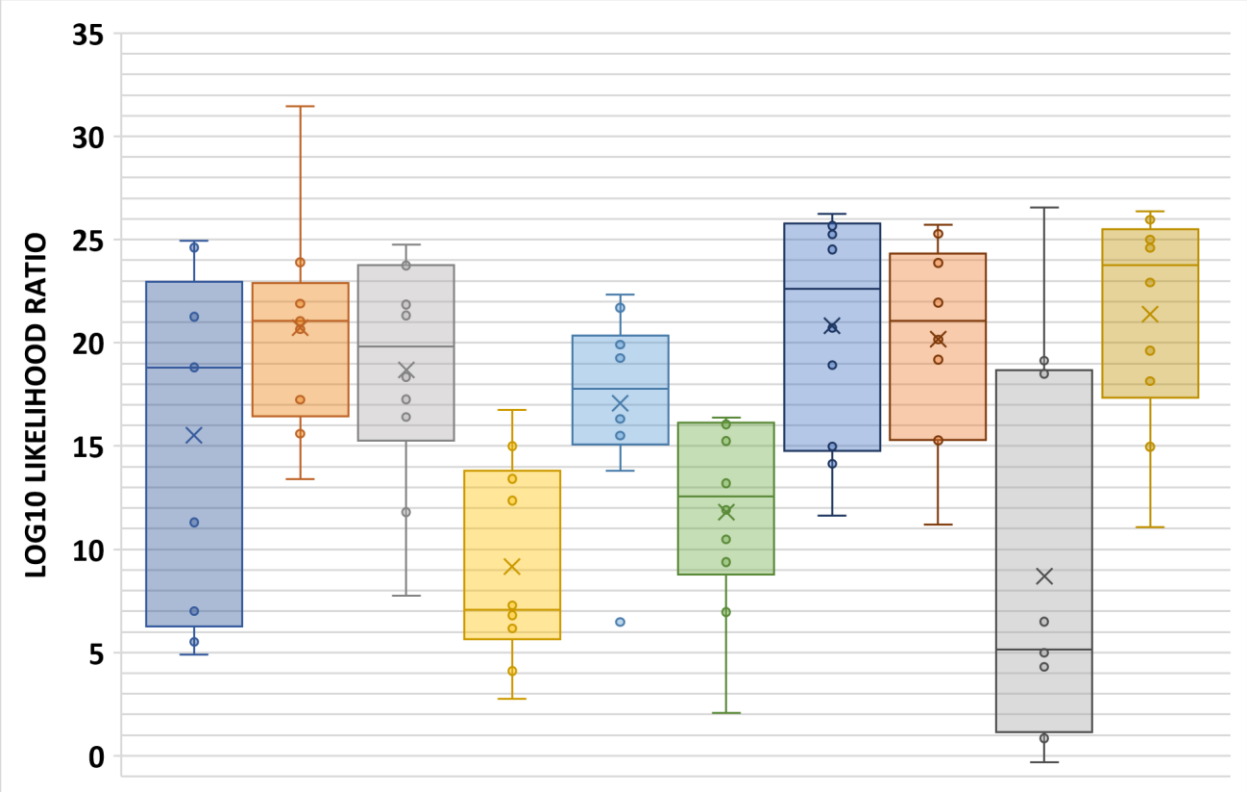

Figure 18: Box and whisker plots of calculated likelihood ratios (displayed in logarithmic form on the Y-axis) from 9mm fired cartridge casings processed using the optimized workflow. Each plot corresponds to one of the ten unique male participants who handled the rounds of ammunition prior to firing. Default parameters were used, in addition to the inclusion of degradation modelling, to calculate the maximum likelihood ratio using the FBI Caucasian population frequency database for each sample. Propositions were set based on the assumed number of contributors for each sample; most samples showed evidence of either 2 or 3 contributors. As such, the H_p was set to the known contributor and 1 or 2 additional unknown contributors while the H_d was set to 2 or 3 total unknown contributors

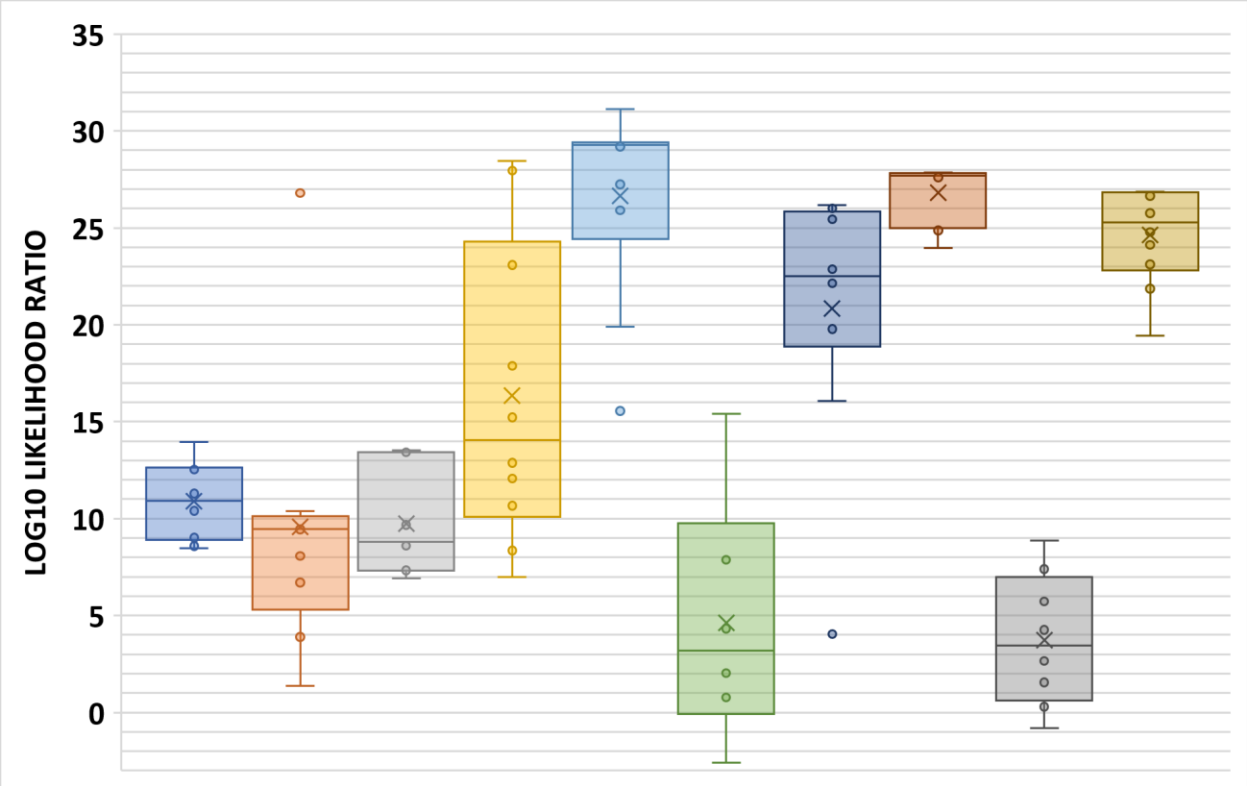

Figure 19: Box and whisker plots of calculated likelihood ratios (displayed in logarithmic form on the Y-axis) from 45 ACP fired cartridge casings processed using the optimized workflow. Each plot corresponds to one of the ten unique male participants who handled the rounds of ammunition prior to firing. Default parameters were used, in addition to the inclusion of degradation modelling, to calculate the maximum likelihood ratio using the FBI Caucasian population frequency database for each sample. Propositions were set based on the assumed number of contributors for each sample; most samples showed evidence of either 2 or 3 contributors. As such, the H_p was set to the known contributor and 1 or 2 additional unknown contributors while the H_d was set to 2 or 3 total unknown contributors

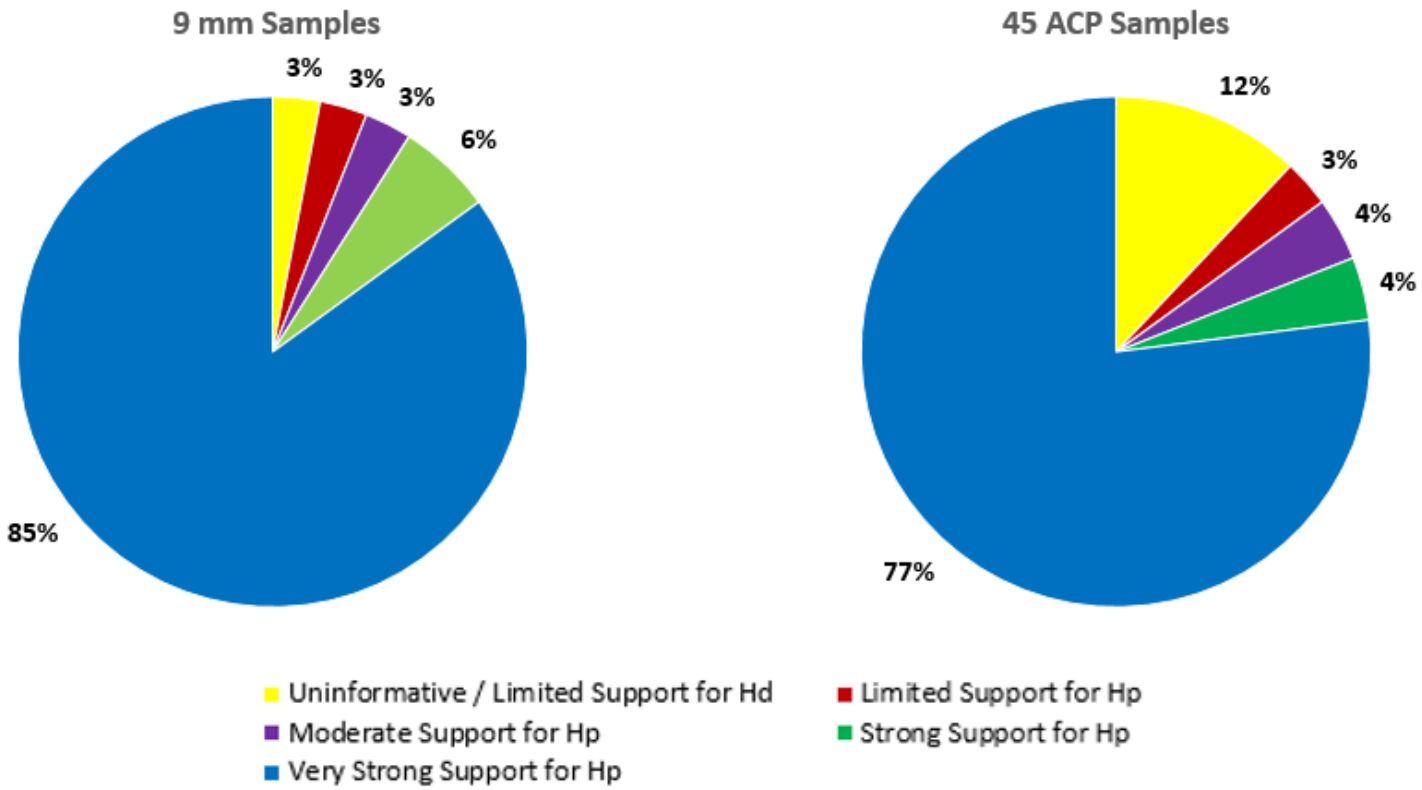

Figure 20: Visual breakdown of verbal qualifiers of likelihood ratios (as recommend by SWGDAM) for fired cartridge casings (both 9mm and 45 ACP handled by 10 unique male participants) processed using the optimized workflow. Despite the majority of the samples displaying degradation, allelic and locus dropout, and the presence of additional contributors, the optimized workflow was able to generate powerfully discriminatory data. Analysis of 9 mm samples revealed that 97% of the samples processed using the optimized workflow resulted in data which provides some degree of support for the prosecution's hypothesis (the known contributor plus 1 or 2 additional unknown contributors), with 85% of samples providing very strong support. Analysis of 45 ACP samples revealed that 88% of the samples processed using the optimized workflow resulted in data which provides some degree of support for the prosecution's hypothesis, with 77% of samples providing very strong support. These results confirm that utilization of the optimized workflow can lead to the generation of interpretable and highly discriminatory STR profiles from fired cartridge casings (FCCs) handled by individuals with variable shedding propensities.

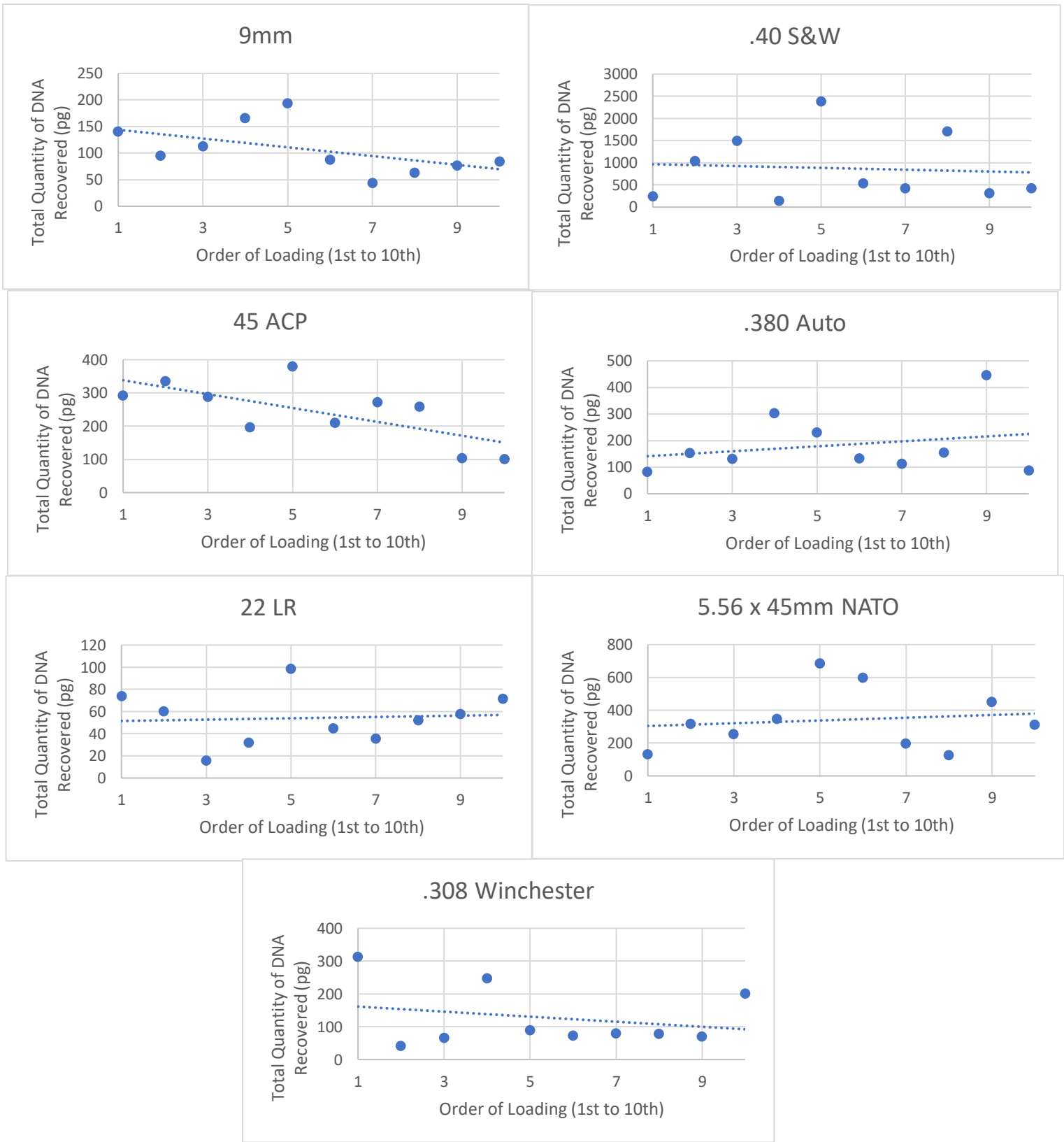

Figure 21: Pearson product-moment correlation coefficient to assess the degree of correlation between magazine loading order and the quantity of DNA recovered from a cartridge case. No correlation was detected between the order in which a cartridge is loaded into a magazine and DNA yield.

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EXECUTIVE SUMMARY APPENDIX B: DISSEMINATION OF RESEARCH

Appendix B: Dissemination of Research Findings

Presentations

- *Jim Dawson.* "Who Loaded the Gun? Recovering DNA from Bullet Casings." *Forensic Magazine,* September 27, 2016. https://www.forensicmag.com/article/2016/09/who-loaded-gun-recovering-dna-bullet-casings.

- *Advances in Forensic DNA Technology,* Phillip Danielson*, Founders Forum, University of Denver, Denver, CO, 2016

- *Touch Type and Trace DNA Evidence,* Phillip Danielson*, Colorado Public Defender Annual Conference, Snowmass, CO, 2017

- *Touch Type and Trace DNA Evidence,* Phillip Danielson*, Office of the Public Defender and Office of the Alternate Defense Counsel, Washoe County, NV, 2017

- *The Evaluation and Optimization of DNA Recovery and Amplification from Bullet Cartridge Cases.* Heather Milnthorp*, Heather McKiernan, Phillip Danielson. 70th Annual Meeting of the American Academy of Forensic Sciences, Platform Presentation, February 2018.

- *Evaluation and Optimization of DNA Recovery from Bullet Cartridge Cases.* Heather Milnthorp, Heather McKiernan*, Phillip Danielson. 19th Annual Meeting of the Mid-Atlantic Cold Case Homicide Investigators Association, Platform Presentation, August 2018.

- *The Evaluation and Optimization of DNA Recovery and Amplification from Bullet Cartridge Cases.* Heather Milnthorp, Heather McKiernan*, Phillip Danielson. 6th Annual Meeting of the Encontro Nacional de Quimica Forense (ENQFor), Platform Presentation, November 2018.

- *Evaluation and Optimization of DNA Recovery from Bullet Cartridge Cases.* Heather McKiernan* Department of Forensic Medicine, University of Copenhagen

- *Evaluation and Optimization of DNA Recovery from Bullet Cartridge Cases.* Heather McKiernan* Baltimore County Police Department, Division of Homicide and Missing Persons Units

- *An Evaluation of Performance and STR Concordance Between Massively Parallel Sequencing (MPS) and Capillary Electrophoresis (CE) Platforms for the Forensic Analysis of Challenging Sample Types.* Heather Milnthorp*, Yih Ling Saw, Heather McKiernan, Phillip Danielson. 12th Annual Meeting of the Green Mountain DNA Conference, Poster Presentation, July 2019.

- *An Evaluation of Performance and STR Concordance Between Massively Parallel Sequencing (MPS) and Capillary Electrophoresis (CE) Platforms for the Forensic Analysis of Challenging Sample Types.* Heather Milnthorp, Yih Ling Saw, Heather McKiernan*, Phillip Danielson. 28th International Congress of the International Society for Forensic Genetics (ISFG), Poster Presentation (pending), September 2019.

Manuscripts In Preparation

- Validation of an Optimized Workflow for the Processing of Cartridge Case Evidence
- Evaluation of DNA Transfer and Persistence within Firearms: Implications for DNA Analysis and Interpretation
- An Evaluation of Performance and STR Concordance Between Massively Parallel Sequencing (MPS) and Capillary Electrophoresis (CE) Platforms for the Forensic Analysis of Challenging Sample Types Bullets-
- Improving the Analysis of DNA from Fired Cartridge Cases: Recommendations for Forensic Practitioners

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SOP: **SOAK AND SONICATE COLLECTION TECHNIQUE FOR THE RECOVERY OF TOUCH DNA FROM SMALL CALIBER CARTRIDGE CASE EVIDENCE**

PURPOSE: To ensure proper sample processing and recovery of DNA from the outside of unfired and/or fired cartridge casings.

SCOPE: This SOP pertains to the recovery of DNA from the outside of unfired and/or fired cartridge casings utilizing a soak and sonicate collection technique. Employees who perform sample processing and recovery of genetic information for downstream DNA analysis shall be familiar with this SOP.

A. Materials:

1. Bench paper
2. 14 mL Falcon™ round-bottom polypropylene tubes
3. 2.0 mL microcentrifuge tubes
4. Vortex
5. Microcentrifuge or pulse spinner
6. Rubber-tipped forceps
7. Test tube rack
8. Microcentrifuge tube rack
9. Pipettes
10. Pipette tips
11. Permanent marker/pen
12. Lint-free lab tissue
13. VWR Symphony Ultrasonic Cleaner
14. Eppendorf™ Vacufuge® plus speed vacuum concentrator

B. Reagents:

1. Sodium hypochlorite solution, 20% (bleach)
2. Deionized water (diH_2O)
3. Salt-Free Solubilization Buffer
   a. 426.5 mL deionized water
   b. 5 mL 1M Tris-HCl
   c. 10 mL 0.5M EDTA
   d. 50 mL 20% sodium dodecyl sulphate (SDS)
   e. 8.5 mL proteinase K, 20 mg/mL, room-temperature stable

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C. Controls:

1. Reagent Control (RC) – an empty 14 mL Falcon™ or 2 mL microcentrifuge tube that undergoes identical processing as the samples being collected to test the reagents used for contamination. The RC should be placed as the last sample in the batch.

D. Equipment Parameters:

1. Prior to processing of samples, ensure that the VWR Symphony Ultrasonic Cleaner is turned on.

    a. The temperature setting should be set for 56°C so the water bath can heat appropriately before being used.

    b. The timer duration for sonication should be set for 30 minutes.

    c. Ensure that an appropriate volume of water is contained within the unit. If more volume is needed, add deionized water until the fill line is reached.

2. Prior to processing of samples, ensure that the Eppendorf™ Vacufuge® plus speed vacuum concentrator is turned on.

    a. The temperature setting should be set for 60°C so the unit can heat appropriately before being used.

    b. The timer duration for lyophilization should be set for at least 2 hours.

    c. Ensure that the brake is set to OFF and the vent mode selected displays V-AQ.

    d. The rotor as well as the inside of the vacuum concentrator should be cleaned with 20% bleach followed by deionized water prior to use.

E. **Initial Preparations:**

1. Prepare the work area. Bench surfaces must be cleaned with 20% bleach followed by deionized water prior to opening an item of evidence as well as between each case.

2. The work area must be covered with clean bench paper to prevent the loss of sample material and to minimize the cross-transfer of materials from one item of evidence to another. Clean bench paper will be put down between each piece of ballistic evidence examined. The necessary tools and reagents for the recovery of DNA from these items should be conveniently placed.

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3. Sterilize all utensils (rubber-tipped forceps) needed for sample collection.

4. Depending on the caliber of ammunition being processed, label an appropriately sized soaking vessel (2 mL microcentrifuge tube for smaller calibers such as .22 LR or 14 mL Falcon™ round-bottom polypropylene tubes for other handgun calibers to include 9 mm, .45 ACP, .40 S&W, and .380 Auto) for each sample with the corresponding sample identifier using a permanent marker. Make sure to include one soaking vessel to serve as the reagent control (RC).

5. For any samples being processed in a 14 mL Falcon™ round-bottom polypropylene tube, additionally prepare a 2.0 mL microcentrifuge tube and label accordingly with the sample identifier.

6. Check that all reagents have not expired.

F. **Recovery Protocol:**

1. Carefully open the evidence packaging. Using sterile rubber-tipped forceps, remove the intact cartridge or casing and place primer side down into the appropriately labelled soaking vessel.

2. Depending on the caliber of ammunition being processed and the size of the soaking vessel being used, pipette an appropriate volume of salt-free solubilization buffer down the side of the soaking vessel such that the liquid immerses approximately 80% of the outside of the casing (400 µL for .22LR caliber and 800 µL for most other calibers)

   NOTE: If processing a spent casing, it is imperative that the liquid does not go inside the cartridge case to minimize the amount of overall solubilization buffer necessary to immerse the casing.

3. Cap the tubes and place samples into an appropriately sized test tube rack.

4. Place the test tube rack into the VWR Symphony Ultrasonic Cleaner and sonicate at 56°C for 30 minutes.

5. Once the sonication is complete, remove the test tube rack and samples from the ultrasonic bath.

6. Uncap the tubes and carefully remove the cartridge or casing from the tube using sterile rubber-tipped forceps and dispose.

7. If samples were processed in a 14 mL tube, carefully transfer the lysate into the appropriately labelled 2.0 mL microcentrifuge tube.

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8. Pulse spin the 2.0 mL tubes in a microcentrifuge for 5 seconds to force any condensate to the bottom of the tube.

9. Uncap the sample tubes and place into the rotor of the vacuum concentrator, ensuring adequate spacing to balance the rotor.

10. Securely close the lid of the vacuum concentrator and dry samples at 60°C for 2 hours (or until all buffer has evaporated completely).

11. Remove tubes from the vacuum concentrator rotor and cap tubes.

12. Proceed directly with extraction digest; or store the samples at 4°C (short term) or in the freezer (long term > 24 hours).

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SOP: **TAPE-LIFT COLLECTION TECHNIQUE FOR THE RECOVERY OF DNA FROM CARTRIDGE CASE EVIDENCE (RIFLE ROUNDS)**

PURPOSE: To ensure proper sample processing and recovery of DNA from the outside of unfired and/or fired rifle cartridge casings

SCOPE: This SOP pertains to the recovery of DNA from the outside of unfired and/or fired large caliber cartridge casings utilizing a modified tape-lift collection technique. Employees who perform sample processing and recovery of genetic information for downstream DNA analysis shall be familiar with this SOP.

A. Materials:

1. Bench paper
2. 2.0 mL microcentrifuge tubes
3. Sterile cotton-tipped swabs
4. Scalpel
5. Tape Dots (Scotch® Restickable Mounting Dots, 7/8 in x 7/8 in, Clear)
6. Microscope slides
7. Vortex
8. Microcentrifuge or pulse spinner
9. Rubber-tipped forceps
10. Forceps
11. Microcentrifuge tube rack
12. Pipettes
13. Pipette tips
14. Permanent marker/pen
15. Lint-free lab tissue
16. UV lamp or PCR hood with UV capabilities

B. Reagents:

1. Sodium hypochlorite solution, 20% (bleach)
2. Deionized water (diH_2O)
3. 2% sodium dodecyl sulfate (SDS) solution

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C. Controls:

1. Reagent Control (RC) – a blank tape dot that undergoes identical processing as the samples being collected to test the reagents used for contamination. The RC should be placed as the last sample in the batch.

D. **Initial Preparations:**

1. Prepare the work area. Bench surfaces must be cleaned with 20% bleach followed by deionized water prior to opening an item of evidence as well as between each case.

2. The work area must be covered with clean bench paper to prevent the loss of sample material and to minimize the cross-transfer of materials from one item of evidence to another. Clean bench paper will be put down between each piece of ballistic evidence examined. The necessary tools and reagents for the recovery of DNA from these items should be conveniently placed.

3. Sterilize all utensils needed for sample collection and processing.

4. For each cartridge or casing being processed, label a glass microscope slide with the sample identifier using a waterproof marker.

   a. Using forceps, remove the clear bottom liner from two tape dots for each sample. Place the two tape dots, sticky side down, side-by-side onto the labelled slide.

   b. Using forceps, remove the plastic protective cover from the top of the tape dots which are now mounted onto the microscope slide.

   c. Place microscope slide under UV light for 10 minutes to sterilize the tape dot surface.

5. For each sample being processed, prepare and label a 2.0 mL microcentrifuge tube with the appropriate sample identifier.

6. Check that all reagents have not expired.

E. **Recovery Protocol:**

1. Carefully open the evidence packaging. Using sterile rubber-tipped forceps, remove the intact cartridge or casing and place primer side down onto one of the tape dots on the appropriately labelled microscope slide.

   NOTE: When processing fired casings, minimize contact with the outside of the casing by touching only the inside of the cartridge casing with the forceps.

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2. While holding the item with the rubber-tipped forceps, push the primer side down onto the tape dot with firm pressure

    a. Repeat this for a total of three times on separate areas of the tape dots to ensure sufficient transfer of genetic material from the textured primer base of the cartridge to the tape dots.

3. Roll the exterior of the cartridge or casing lengthwise across the two tape dots with firm pressure.

    a. Repeat this step such that the entirety of the exterior of the cartridge or casing has contacted the tape dots to ensure uniform sampling of the item.

4. Perform a wet: wet double swabbing of the surface of the tape dots using sterile cotton-tipped swabs moistened with 1-2 drops of 2% SDS solution.

5. Using a sterile scalpel, remove the head of the cotton swab from the wooden shaft for both collected swabs. Transfer the entire swab head from both swabs into the appropriately labelled 2.0 mL microcentrifuge tube.

6. Proceed directly with extraction digest; or store the samples at 4°C (short term) or in the freezer (long term > 24 hours).

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SOP: **ORGANIC EXTRACTION OF GENETIC MATERIAL RECOVERD FROM CARTRDIGE CASE EVIDENCE**

PURPOSE: To ensure proper chemical separation and purification of DNA from other cellular components, inorganic material, and/or inhibitors for downstream genetic analysis.

SCOPE: This SOP pertains to the extraction of DNA recovered from unfired and/or fired cartridge casings. Employees who perform sample lysis and purification of genetic material for downstream DNA analysis shall be familiar with this SOP.

A. Materials:

1. Bench paper
2. 2.0 mL microcentrifuge tubes (containing collected DNA samples)
3. Spin-X Baskets
4. 1.5 mL microcentrifuge tubes
5. Vivacon®-500 ETO Centrifugal Concentrators
6. Vivacon® microcentrifuge filtrate tubes
7. Thermomixer
8. 15 mL conical tube
9. Vortex
10. Microcentrifuge
11. Pulse spinner
12. Forceps or tweezers
13. Microcentrifuge tube rack
14. Pipettes
15. Pipette tips
16. Permanent marker/pen
17. Lint-free lab tissue

B. Reagents:

1. Sodium hypochlorite solution, 20% (bleach)
2. Deionized water (diH_2O)
3. Organic Extraction Buffer
4. Proteinase K, 20 mg/mL solution
5. Phenol: chloroform: isoamyl alcohol (PC/IA) (25:24:1 v/v)
6. Water-saturated butanol

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7. TE^{-4} Buffer

C. Controls:

1. Reagent Control (RC) – a blank tube that undergoes the same extraction process as the samples to test the reagents being used for contamination. The RC should be placed as the last sample in the batch.

2. Extraction Control (EC) – an extraction control of a known DNA profile (reference blood or saliva) is required for each analytical batch which undergoes the same extraction process as the sample to demonstrate that the procedure is performing as expected. The EC should be placed as the first sample in the batch.

D. Equipment Parameters:

1. Prior to initial preparations, ensure that the thermomixer is set to 56°C and 850 RPM so that it has adequate time to heat up before being used.

E. Initial Preparations:

1. Prepare the work area. Bench surfaces must be cleaned with 20% bleach followed by deionized water and covered with a fresh piece of clean bench paper prior to beginning a new extraction batch. The necessary tools and reagents for the extraction of DNA from samples should be conveniently placed.

2. Sterilize all utensils needed for substrate removal (tweezers or forceps).

3. Check that all reagents have not expired.

4. Prepare sample tubes as follows:

   a. Prepare 2 sets of 1.5 mL microcentrifuge tubes for each sample. Each should be marked with the sample identifier.

   b. Prepare 1 set of filtrate collection tubes with a Vivacon®-500 ETO filter for each sample. Each should be marked with the sample identifier.

   c. Prepare 1 additional set of filtrate collection tubes for the wash step. Each should be marked with the sample identifier.

   d. Prepare 1 additional set of 1.5 mL microcentrifuge tubes for each sample for recovery and storage. Each should be marked with the sample identifier.

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F. Sample Lysis:

1. Prepare one master mix containing the appropriate volumes of reagents for each analytical batch into an appropriately sized conical tube:

   NOTE: A master mix correction factor has been included in the sample count of +2.

   Number of samples (including RCs and ECs) + 2 x 300 µL Extraction Buffer
   Number of samples (including RCs and ECs) + 2 x 5 µL Proteinase K

2. Vortex and/or invert the master mix thoroughly and add 305 µL to each sample tube.

   NOTE: Additional master mix may be needed for absorbent samples containing multiple swab heads to ensure complete submersion of the sample. Adjust the number of samples in the master mix calculation accordingly. The RCs should reflect the largest volume of master mix used.

3. Vortex the tubes for approximately 3 seconds and pulse spin for approximately 5 seconds to force the substrate material into extraction fluid, if necessary.

4. Place the samples into a shaking thermomixer set for 850 RPM at 56° for overnight (12 hours minimum / 18 hours maximum) incubation.

   NOTE: Analysis can be paused at this point by placing the samples in the -20°C freezer. If stored at -20°C, then the samples should be warmed at 56°C for several minutes before starting phenol/chloroform extraction. Once started, the extraction must be carried through to the centrifugal filtration and elution steps.

5. Remove the samples from the thermomixer and pulse spin for approximately 5 seconds to force the condensate into the bottom of the tube.

   a. Proceed with step G.1 if the sample was processed using the soak and sonication collection technique.

   b. If the sample was processed using the tape lift collection technique, the following additional steps are necessary:

      1. Sterilize forceps or tweezers by rinsing in a 20% bleach solution follow by diH_2O. Wipe dry with a clean lint-free tissue.

      2. Gather a Spin-X Basket for each sample. Using clean forceps for each sample, transfer the sample substrate into the Spin-X Basket. Place the Spin-X Basket back into the respective 2.0 mL microcentrifuge tube and cap the tube.

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3. Place the tubes in a microcentrifuge and spin for 5 minutes at 13,000 RPM. Remove and discard the Spin-X Basket with cutting into the biohazard waste container.

G. Sample Purification and Concentration:

1. To each 2.0 mL microcentrifuge tube add 300 µL of PCIA (**bottom layer**). Vortex the mixture for approximately 10 seconds to obtain a milky emulsion.

2. Spin the tubes in a microcentrifuge for 3 minutes at 13,000 RPM.

   a. While the samples are spinning down, add 100 µL TE^{-4} Buffer to the surface of the Vivacon®-500 ETO filter. The TE^{-4} Buffer must be added before the sample to avoid possible loss of membrane integrity from excess butanol and/or PCIA.

3. Transfer the aqueous layer (**top layer**) to a fresh 1.5 mL microcentrifuge tube. Discard the old tube, capped, with the PCIA (bottom layer) in the biohazard waste.

4. Repeat the previous 3 steps (G.1-G.3) once more for a total of **two** PCIA washes.

5. To each sample tube add 300 µL of water saturated butanol.

   NOTE: Do not swirl or mix the water saturated butanol. The water and the butanol should remain separate. Always pull from the **top** layer.

6. Vortex the mixture for approximately 10 seconds to obtain a milky emulsion.

7. Spin the tubes in a microcentrifuge for 3 minutes at 13,000 RPM.

8. Transfer the aqueous layer (**bottom layer**) from the tube to the concentrator.

   NOTE: Avoid pipetting organic solvent (top layer) from the tube into the concentrator. Do not overfill the concentrator filter (add a maximum of 300 µL from the aqueous layer at one time). In the event that excess sample remains in the aqueous layer, add the remainder of the aqueous layer into the original concentrator filter after the initial spin and centrifuge again.

9. Cap the concentrator (gently so that the filter does not crack) and spin in a microcentrifuge for 10 minutes at t 5,900 RPM for 10 minutes, or until all liquid has spun through the concentrator.

10. Carefully remove the concentrator unit from the assembly and discard the filtrate tube into the biohazard waste container.

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11. Return the concentrator to a fresh filtrate tube. Add 200 µL TE-4 Buffer to the concentrator. Replace the cap and spin in a microcentrifuge for 10 minutes at 5,900 RPM, or until all liquid has spun through concentrator.

H. Sample Recovery:

1. Add 50 µL of TE-4 Buffer to the concentrator.

2. Uncap and place the remaining labelled 1.5 mL microcentrifuge tube on top of the concentrator unit. While gently pinching the microcentrifuge tube against and down on the filter unit, pull the concentrator out of the old filtrate tube. Discard the previously used filtrate tube.

3. Flip the 1.5 mL microcentrifuge tube containing the concentrator upright and spin the assembly in a microcentrifuge for 2 minutes at 5,000 RPM.

NOTE: The microcentrifuge tubes with not cap with the inverted filter in them.

4. Remove the samples from the microcentrifuge. Remove and discard the concentrator.

5. Samples can be proceeded directly to quantification or stored at 4°C (short term – same day) or frozen (long term > 1 day). Prior to using stored samples, samples should be thawed, vortexed, and spun down for approximately 5 seconds.

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SOP: **PRE-AMPLIFICATION CONCENTRATION OF EXTRACTED GENETIC MATERIAL FROM CARTRIDGE CASE EVIDENCE**

PURPOSE: To successfully concentrate low-quantity DNA samples which were recovered from cartridge case evidence to ensure the highest quality of genetic information can be obtained in downstream testing.

SCOPE: This SOP pertains to the pre-amplification concentration of low quantity extracted DNA samples recovered from cartridge case evidence. Employees who perform sample processing and downstream genetic testing to include PCR amplification and STR typing shall be familiar with this SOP.

A. Materials:

1. Bench paper
2. Extracted genomic DNA
3. 1.5 mL microcentrifuge tubes
4. Microcentrifuge tube rack
5. Microcentrifuge or pulse spinner
6. Vortex
7. Permanent marker/pen
8. Paper towels
9. Eppendorf™ Vacufuge® plus speed vacuum concentrator

B. Reagents:

1. Sodium hypochlorite solution, 20% (bleach)
2. Deionized water (diH_2O)

C. Controls:

1. Reagent Control Prime (RC') – an empty 1.5 mL microcentrifuge tube that undergoes identical processing as all extracted samples and controls being dried down to ensure that the equipment being used does not introduce any contaminants.

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D. **Equipment Parameters:**

1. Prior to processing of samples, ensure that the Eppendorf™ Vacufuge® plus speed vacuum concentrator is turned on.

    a. The temperature setting should be set for 60°C so the unit can heat appropriately before being used.

    b. The timer duration for lyophilization should be set for at least 1 hour.

    c. Ensure that the brake is set to OFF and the vent mode selected displays V-AQ.

    d. The rotor as well as the inside of the vacuum concentrator should be cleaned with 20% bleach followed by deionized water prior to use.

E. **Initial Preparations:**

1. Prepare the work area. Bench surfaces must be cleaned with 20% bleach followed by deionized water. The work area must be covered with clean bench paper to prevent the loss of extracted sample material and to minimize the cross-transfer of genetic material between samples.

2. Retrieve the necessary samples and controls to be processed from storage and allow to thaw completely at room temperature.

    NOTE: This protocol should only be used for samples which generated quantitative values of less than or equal to (≤) 0.02 ng/µL.

F. **Pre-Amplification Concentration Protocol:**

1. Vortex the sample tubes for approximately 3 seconds and pulse spin for approximately 5 seconds to force all extract to the bottom of the tube.

2. Uncap the microcentrifuge sample tubes (as well as the RC' tube) and place into the rotor of the vacuum concentrator, ensuring adequate spacing to balance the rotor

3. Securely close the lid of the vacuum concentrator and dry samples at 60°C for 1 hour (or until samples have completely dried down).

4. Remove tubes from the vacuum concentrator rotor and cap tubes.

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5. Proceed directly with PCR amplification (resuspend lyophilized samples using ultra pure water and the maximum sample input volume based on the protocol and amplification kit being used); or store the samples at 4°C (short term) or in the freezer (long term > 24 hours).

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