Evaluation of soaking to recover trace DNA from fired cartridge cases

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The recovery of trace DNA from cartridge cases is of common interest across many jurisdictions. Soaking offers improved profiling success rates over traditional methods. We evaluated the effects of firing, calibre, and metal composition on controlled and handled DNA samples utilising a soaking method. Our results show that firing decreases the quantities of DNA recoverable from cartridge cases and higher quantities of DNA are recoverable from nickel ammunition compared to brass. In spiked samples, calibre of ammunition had no significant effect on DNA recovery. Despite slight to moderate DNA degradation and variable profiling success rates, spiked unfired and fired nickel cartridges resulted in more usable profiles than brass cartridges. These findings can aid in triaging the types of ammunition subjected to DNA testing.

Keywords: Trace DNA, cartridge case, ammunition, soaking

Introduction

Cartridge cases are common evidence items found at crime scenes involving shootings. As ammunition is handled during the process of loading firearms, the transfer of skin cells can leave behind DNA from the handler of the ammunition prior to a shooting. Due to the lack of success in developing latent prints from cartridge cases [1, 2], DNA evidence is highly sought after. However, unless specifically requested, DNA testing of fired cartridge cases is not a routine method within many jurisdictions due to a perceived lack of success in obtaining usable profiles (data not shown).

Several studies have investigated the recovery of STR profiles from fired cartridge cases [2-5] with varying rates of success. Traditional swabbing methods have resulted in low success rates possibly due to low quantities of deposited DNA as well as factors including high temperatures, pressures and gasses during firing [2, 3]. Other limiting factors also include metal ion inhibition from gunshot residue and lubricants [3, 6].

Soaking cartridge cases has been proposed as an alternative to the traditional swabbing methods. A novel soaking method proposed by Dieltjes et al[4] yielded 'reliable and reportable' STR profiles from 6.9% of 4085 individual items and 26.5% of 616 cases. This method involved soaking non-penetrating bullets, cartridges or cases in a lysis buffer followed by a spin column extraction of DNA from the lysate [4]. As soaking has been found to have no detrimental impact on striation details of cartridges cases [7], this method shows potential for resolving the long-standing issue of obtaining STR profiles from fired cartridge cases. An optimized procedure has also been proposed [5] however as the effects of this method on the striation detail of cartridge cases are unknown, it was not explored in this study.

The aim of this study was to evaluate the soaking method proposed by Dieltjes et al[4] to determine DNA recovery rates, the quality of recovered DNA and STR profile success rates on a set of controlled and handled cartridges and fired cases.

Materials and methods

Experiment setup

Preparation of saliva for spiking

Human Research Ethics Committee (ETH17-1430) approval was obtained for the collection of DNA from volunteers. A 10 mL aliquot of saliva was collected from a single volunteer over the course of an hour. The volunteer was advised to rinse their mouth with water 30 minutes prior to providing the saliva samples in order to obtain a clean sample. The sample was stored at 4°C until extraction and quantification.

To determine the concentration of DNA in the collected 'stock' saliva sample, three 1 mL aliquots were taken from the stock saliva sample and extracted using Chelex® 100

Resin (Bio-Rad) according to the method defined by Yakovchyts[8]. Following DNA extraction, the aliquots were quantitated using the Quantifiler® Trio DNA Quantification Kit (ThermoFisher Scientific, USA) according to the manufactures protocol[9]. The average concentration of the three aliquots was used to determine the concentration of DNA in the collected stock sample.

Experiment 1: Effects of firing, calibre and metal composition

Four calibres of brass and nickel ammunition were chosen for this experiment. These were CCI® .22Long Rifle (.22LR), Federal Premium and Geco 9mm Parabellum (9mmP), PMC and Federal Premium .45Automatic Colt Pistol (.45ACP), and Winchester and American Eagle .357 Magnum (.357MAG) calibre cartridges respectively. These are representative of the common calibres of ammunition encountered in casework within our jurisdiction. Six cartridges for each calibre and metal composition were taken directly from the box and spiked with 2.2 µL (equating to 10 ng) of saliva diluted in water. Spiking was achieved by dotting the saliva randomly across the outer walls of the cartridge before air-drying at room temperature for approximately 1 hour. Half of the spiked cartridges were fired by gloved ballistics experts. The other half were kept unfired.

Experiment 2: Touch DNA

Brass Winchester .22LR and 9mmP calibre ammunition were used for this experiment. For both calibres, three volunteers of unknown shedder status held two cartridges (one in each hand) taken directly from the box for 2 seconds, 15 seconds and 30 seconds each in order to mimic the variations in handling time encountered in casework scenarios. Following handling, one of the two handled cartridges for each hold time were fired by a gloved ballistics expert and the other one kept unfired.

DNA extraction method

DNA extractions were performed in a dedicated pre-PCR facility. DNA collection and extractions were adapted from the soaking method of Dieltjes et al [4] with slight adjustments to reagent volumes to account for reagent efficiency. Collection and extractions were performed utilising the QIAamp® DNA Mini kit (QIAGEN, Germany).

Each unfired or fired cartridge was placed in a single 10 mL tube and submerged in 380 µL of ATL buffer for 20 minutes with occasional rotation to ensure optimal coverage. Following soaking, the unfired and fired cartridges were taken out of the buffer and swabbed with a sterile rayon swab (Copan Innovations). The ATL buffer and swab were transferred to a sterile 2 mL microfuge tube and incubated on a VorTemp™ 56 Incubator/Shaker (Labnet International) at 85°C for 10 minutes while shaking at 800 rpm. Next, 20 µL of Protease K (20 mg/mL: QIAGEN)) was added to each tube and incubated at 56°C for 1 hour while shaking at 800 rpm. Following this, 200 µL of AL buffer was added to each tube which was vortexed for 20 seconds, and then incubated at 70°C for 10 minutes at 800 rpm. Next, 400 µL of absolute ethanol was added to each tube which was briefly vortexed.

Extraction solutions from each tube were transferred into silica spin columns in two aliquots and these were centrifuged at 8000 rpm for 1 minute to allow the DNA to bind to the spin column membrane. The flow through was discarded at each step. 500 µL of AW1 wash buffer was added to each column which was centrifuged at 8000 rpm for 1 minute. Next, 500 µL of AW2 wash buffer was added to each column which was centrifuged again at 12,000 rpm for 3 minutes. Following the washing steps, the silica columns were transferred into clean collection tubes. DNA was eluted by adding 50-100 µL (50 µL experiment 1 and 100 µL experiment 2 respectively) of AE buffer into each

column followed by centrifugation at 8000 rpm for 1 minute. Eluted DNA was stored at -20°C until further analysis.

DNA Quantitation

Plates for experiment 1 were set up on the Tecan Freedom EVO 150 (Tecan Life Sciences, Switzerland). Experiment 2 plates were set up manually. DNA extracts were quantified using the Quantifiler® Trio DNA Quantification Kit (ThermoFisher Scientific, USA) on the either the 7500 Real-Time PCR instrument or the QuantStudio™ 5 Flex for experiment 1 and QuantStudio™ 6 Flex Real-Time PCR instrument (ThermoFisher Scientific, USA) for experiment 2, according to the manufacturers standard protocol[9]. Full volume reactions (20µL) were used for experiment 1 while half volume reactions (10µL) were used for experiment 2. Standards consisting of a dilution series in the range 0.005 – 50 ng/µL, negative controls and positive controls were run with each plate.

PCR Amplification

STR amplification was performed using the PowerPlex®21 Systems (Promega, USA) according to the manufacturers recommended protocol for amplification of extracted DNA[10].

Experiment 1 plates were again set up on the Tecan Freedom EVO 150 with an input quantity of 0.7ng. Amplification was achieved on the GeneAmp PCR System 9700 thermal cycler (ThermoFisher Scientific, USA).

Experiment 2 plates were set up manually with an input amount of 0.5 ng and amplified on the Veriti 96 well thermal cycler (ThermoFisher Scientific, USA). The maximum input volume of 15 µL was added for DNA extracts with less than 33 pg/µL.

Capillary electrophoresis and analysis

Capillary electrophoresis was performed on a 3500 Genetic Analyzer (Applied Biosystems®) according to the parameters defined in the PowerPlex® 21 Systems technical manual[10]. Data generated from runs were analysed using the GeneMapper IDX software (Applied Biosystems). All profiles were analysed using an analytical threshold of 80 RFU and a stochastic homozygote of 700 RFU.

Data analysis

General data analysis was performed using Microsoft Excel. Kruskal Wallis and Mann-Whitney U tests were performed using SPSS statistics 25 (IBM).

In order to evaluate the soaking technique, four parameters for each experiment were documented. These were the total quantity of DNA recovered, DNA quality as indicated by the internal positive control (IPC) cycle threshold (Ct) values and degradation index, and the number of alleles (derived from the known donors) detected.

Degradation index is calculated as the concentration of a small autosomal DNA target divided by the concentration of a large autosomal DNA target, both amplified in the Quantifiler® Trio DNA Quantification kit. In accordance with the manufacturer's guidelines[9] for assessing degradation, a degradation index of < 1 represents no degradation or inhibition, 1-10 represents slight to moderate degradation, and > 10 significant degradation and possible inhibition. No amplification of large autosomal targets indicates significant degradation, therefore samples exhibiting undetermined large autosomal target concentrations were designated 'DI >10',

Alleles were counted as a maximum of two alleles (regardless of any additional alleles) per locus, according to the PATs defined above. The maximum possible number of

alleles for any profile was 42. Profiles deemed to be uploadable consisted of 12 or more alleles in addition to both amelogenin alleles, as required by the database requirements in our jurisdictions.

Results

Experiment 1: Effects of firing, calibre and metal composition

Figure 1 shows the total DNA recovered from unfired and fired brass and nickel cartridge cases, with a total of twelve replicates for each group (three replicates for each of the four calibres). As there were no statistically significant differences ($p < 0.05$) found among calibres, these were pooled for comparisons between firing status and metal composition.

The mean values in Figure 1 show greater total DNA recovery from unfired cartridges compared to fired cases which was found to be statistically significant ($p = 0.000$). Similarly, significant differences were also found between the total DNA recovered from nickel cartridges compared to brass cartridges ($p = 0.000$).

Figure 2 shows the degradation indices for the DNA recovered from unfired and fired brass and nickel cartridges. Analysis of cycle threshold (Ct) values showed no evidence of inhibition in any samples, therefore degradation indices were used to assess sample degradation. Both metals showed varying DNA degradation. A large percentage (67-100%) of the DNA recovered from unfired and fired brass and nickel cartridges were slightly to moderately degraded. DNA recovered from unfired nickel samples revealed the least amount of degradation whilst DNA from only fired brass samples displayed severe degradation.

Figure 3 shows the number of donor alleles detected between unfired and fired brass and nickel cartridges. From the three replicates for each calibre, the DNA

concentration closest to the average was chosen for amplification. Therefore, for each sample category only 4 of 12 DNA samples were amplified.

A slight decrease was observed in the number of alleles detected from fired cartridge cases compared to unfired cartridge cases. Amplification of DNA from unfired and fired brass samples show that 3 of 4 (75%) and 1 of 4 (25%), respectively, resulted in profiles usable for upload onto our jurisdiction's database. The remaining samples had less useful profiles. Amplification of DNA from unfired nickel cartridges resulted in 4 of 4 (100%) complete profiles, while 4 of 4 (100%) fired samples resulted in partial but uploadable profiles.

Experiment 2: Touch DNA

Figure 4 shows the total amount of DNA recovered from handled unfired and fired cartridges. There was no statistical significance found amongst the three handling times (2 sec, 15 sec, 30 sec) of unfired and fired .22LR and 9mmP cartridges, therefore all three handling times were pooled together for further analysis.

Significantly less DNA was recovered from handled 9mmP ($p = 0.001$) fired cartridge cases than unfired cartridges. No significant differences were found between unfired .22LR cartridges and fired cases ($p = 0.200$).

Figure 5 displays the degradation of DNA recovered from handled cartridges and cases. Again, Ct values indicated no evidence of inhibition and a greater number of samples show slight to moderate degradation with no apparent trends across the data.

Figure 6 shows the average number of alleles detected in the profiles generated from unfired and fired handled cartridges. From the .22LR cartridges, 8 of 9 (88.89%) unfired and 7 of 9 (77.79%) fired cartridges produced usable profiles. The remaining samples did not produce profiles. From the 9mmP cartridge cases, 8 of 9 (88.89%)

unfired cartridges produced usable profiles whilst 9 of 9 (100%) fired cases produced less useful profiles.

Discussion

To determine relative DNA recovery, the cartridges used in this study were each spiked with a known quantity of DNA. This enabled a direct comparison between samples by minimising any variation that could be introduced when using touch DNA samples. Saliva was chosen as the DNA source material for spiking as the buccal epithelial cells would best mimic epithelial cells transferred from touch. Touch DNA samples handled by three volunteers of unknown shedder status were also analysed as they would best replicate the samples encountered in routine casework.

DNA recovery rates showed interesting trends among fired and unfired cartridges, metal composition, calibre and handling times. Firstly, firing was shown to decrease total DNA recovery (Figure 1, Figure 4) except for .22LR handled cartridges where there was no significant difference. The influence of firing was expected as high pressures and temperatures during the firing process are likely to damage DNA. Although Gashi et al[11] have shown that internal temperatures during firing are not sufficient to degrade DNA present on ammunition, our results support those of Thanakiatkrai & Rerkamnuaychoke[12] and Monpetite et al[5] who have also shown firing to significantly reduce the amounts of DNA recovered from fired cartridges using direct PCR and soaked extracts, respectively.

Furthermore, although there was no statistically significant difference, DNA recovery from unfired .22LR cartridges (Figure 4) appeared greater than for fired cartridges, due to a potential outlier for unfired cartridges, demonstrating the variability in DNA deposition that is typically expected from touch samples.

Secondly, significant differences were also found between brass and nickel cartridges and cases (Figure 1). While soaking the cartridges, it was observed that brass ammunition caused the lysis buffer solution to turn blue, indicating the presence of copper (II) ions. This reaction was not present with the nickel cartridges. Copper ions are known to have an effect on DNA by destabilising hydrogen bonds between base pairs[13]. The presence of this reaction may potentially explain the discrepancies in DNA recovery between brass and nickel cartridges.

Finally, no differences were found between calibres and between handling times suggesting that surface area and length of contact have little influence on DNA recovery. The lack of difference found between contact times is also consistent with the general observations of van Oorschot and Jones[14].

Degradation indices revealed that the metallic composition of cartridges and firing status had no significant effect on DNA degradation (Figure 2). DNA degradation between unfired and fired samples was shown to be highly variable. Firing was found to severely degrade DNA in some fired brass cartridges from experiment 1 (Figure 2) and 9mmP samples from experiment 2 (Figure 6). Again, moderate DNA degradation was expected from both fired and unfired cartridge cases due to the high temperatures and pressures associated with firing and the exposure of the negatively charged backbone of DNA to metal ions and metallic surfaces, respectively[15]. As there are no studies to our knowledge that have investigated DNA degradation between unfired and fired cartridge cases, comparisons with other studies could not be made.

Firing of brass cartridge cases was shown to lower the number of alleles detected when compared to nickel cartridges which all produced usable profiles (Figure 3). However, as the number of samples ($n = 4$) was limited we could not make an authoritative assessment of whether this trend is reflective of all brass and nickel

cartridges and fired cases. The same trend was also observed amongst touch samples where the number of alleles recovered from fired 9mmP cartridges decreased in comparison to unfired samples. As touch DNA is highly variable between persons and objects [16], once again we could not accurately assess whether firing definitively decreases the number of alleles. Nonetheless our results again support previous research[5] that firing reduces successful DNA typing.

Further to the above, we also investigated the effect of time between DNA deposition and collection (immediately vs. 48 days) through a series of spiked fired and unfired cartridges (Supplementary Figure 1). Time before collection was not shown to affect the quantities of DNA or the number of alleles detected, indicating that this is not a constraint when utilising the soaking method as a means of DNA recovery from cartridge cases.

Additionally, cartridges taken directly from the box and not spiked with any DNA were also tested (Supplementary Figure 1). The amount of recovered DNA and number of alleles detected were highly variable, indicating the presence of background DNA. Our findings concur with the general likelihood of DNA being deposited onto handled objects [17]. Hence, caution must be exercised when interpreting profiles derived from cartridge cases.

Conclusion

The quantities of DNA recovered from ammunition by soaking in lysis buffer is shown to be dependent on factors including firing and metal composition. Firing was found to decrease total DNA recovery and brass ammunition yielded less DNA than nickel ammunition.

Slight to moderate degradation was found in most DNA samples recovered. Differences in DNA degradation between unfired and fired cartridges were variable between experiments.

The numbers of alleles detected were also highly variable between samples, with unfired and fired nickel samples showing slightly less variability than brass. Overall, usable profiles to aid investigations could be obtained from both fired and unfired brass and nickel cartridges, indicating soaking to be a useful method for DNA recovery from fired cartridge cases. To conclude, our results could potentially help triage the types of cartridges that can be subjected to DNA extraction by soaking.

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Figure captions

Figure 1. The total DNA (ng) recovered from unfired (n = 24) and fired (n = 24) brass (n = 24) and nickel (n = 24) cartridge cases (12 samples for each of the four individual categories) spiked with 10 ng saliva. Error bars represent standard deviations.

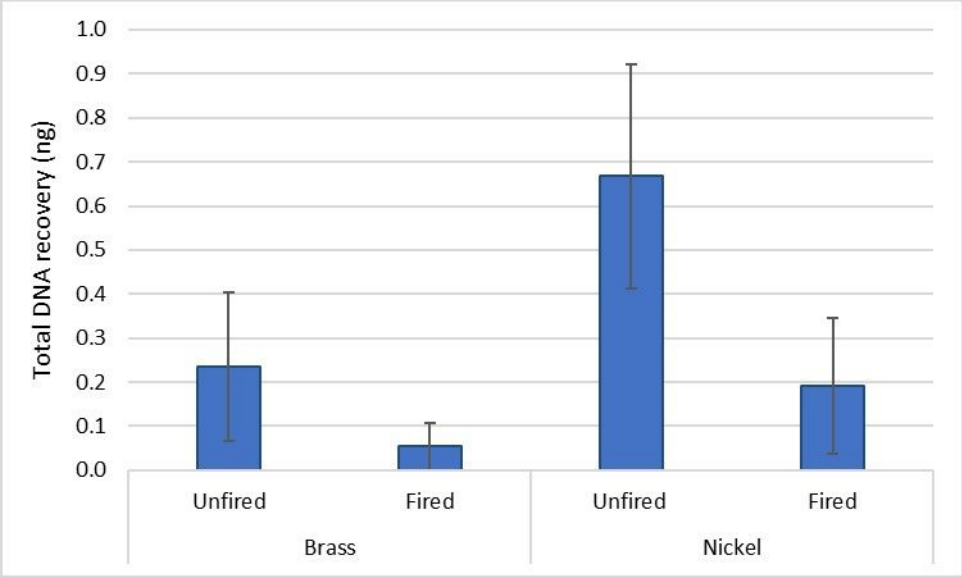
Figure 2. Degradation indices of unfired and fired brass and nickel cartridge cases. DI = degradation index. 2 of 3 fired brass cartridge cases showed no amplification of the large autosomal target.

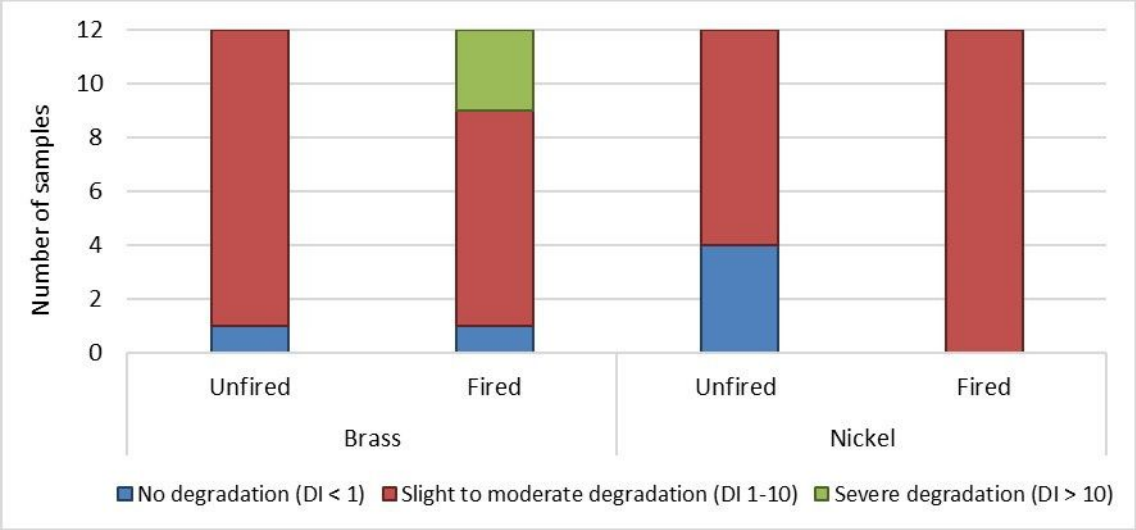
Figure 3. The number of donor alleles from unfired (n = 8) and fired (n = 8) brass (n = 8) and nickel (n = 8) cartridge cases (4 samples for each of the 4 individual categories). The maximum number of expected alleles is 42. Error bars represent standard deviations.

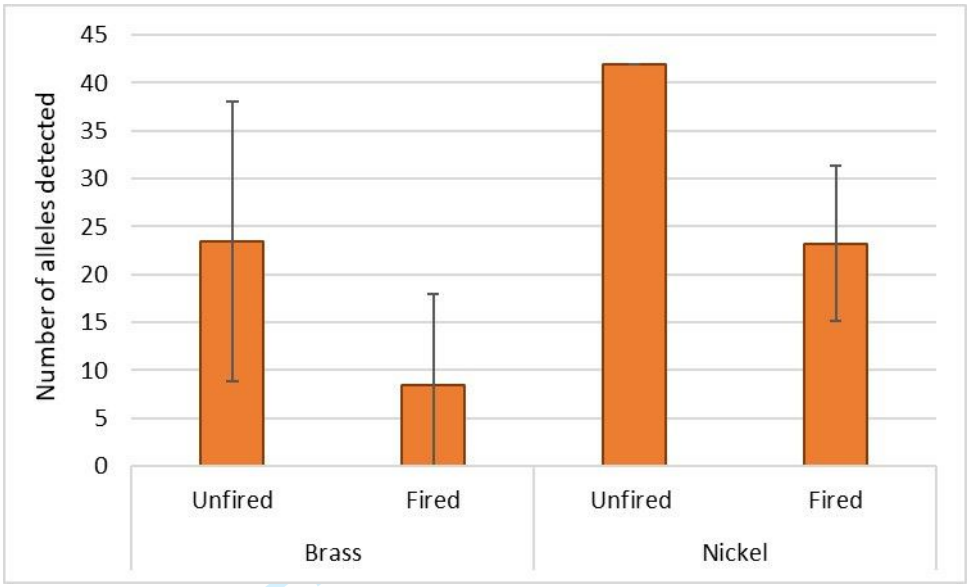
Figure 4. Total DNA recovered from handled cartridges, fired (n = 18) and unfired (n = 18) .22LR (n = 18) and 9mmP (n = 18) calibres (9 samples in each of the four individual categories). Error bars represent standard deviations.

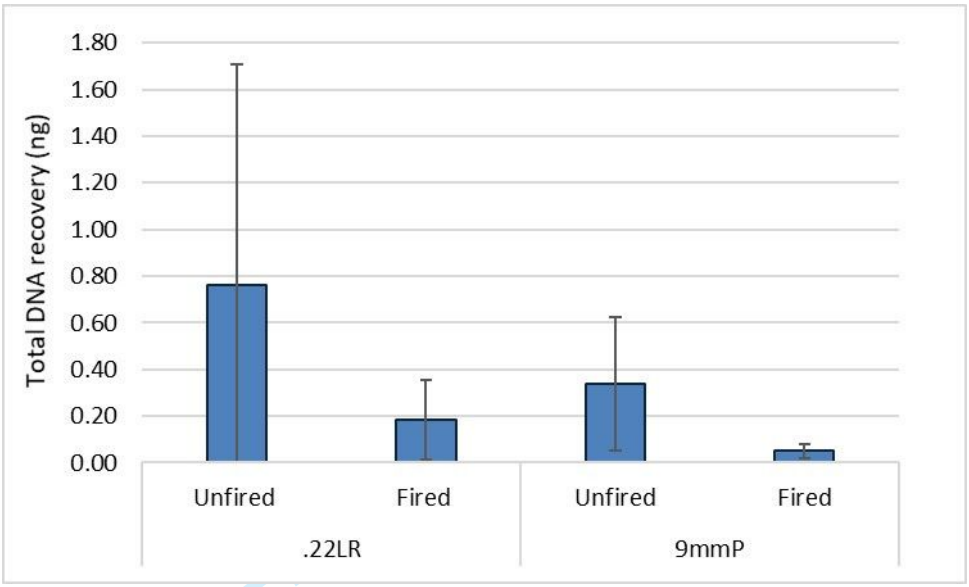
Figure 5. Degradation indices of DNA recovered from fired and unfired .22LR and 9mmP handled cartridge cases. DI = degradation index.

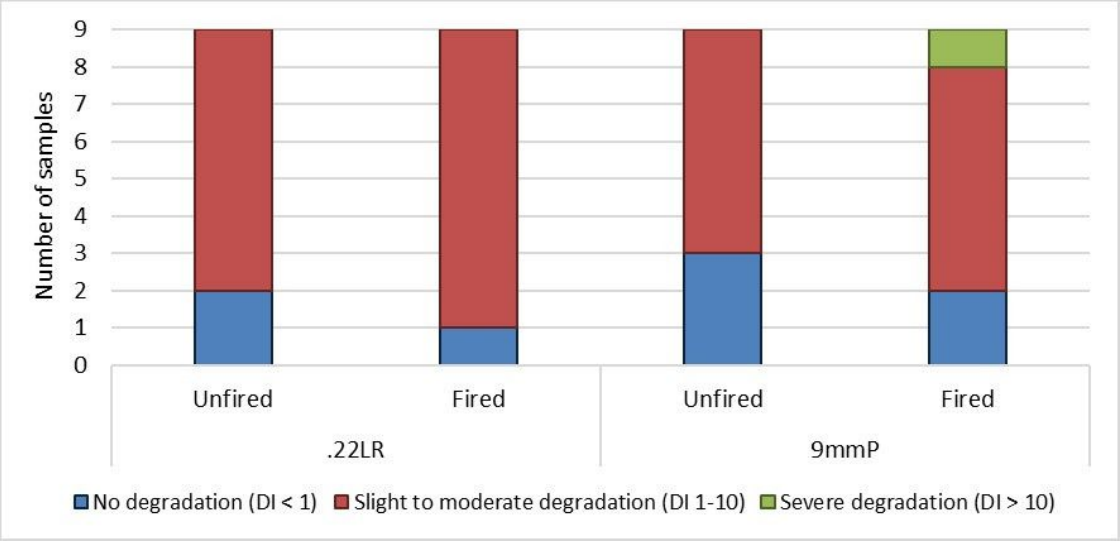
Figure 6. The number of alleles detected from unfired (n = 18) and fired (n = 18) handled .22LR (n = 18) and 9mmP (n = 18) cartridges. The maximum number of expected alleles is 42. Error bars represent standard deviations.

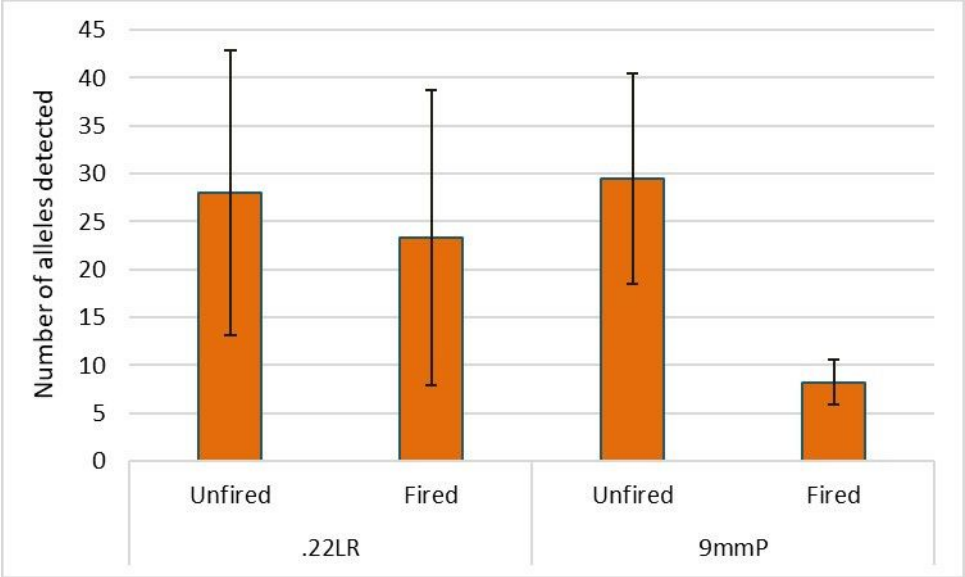


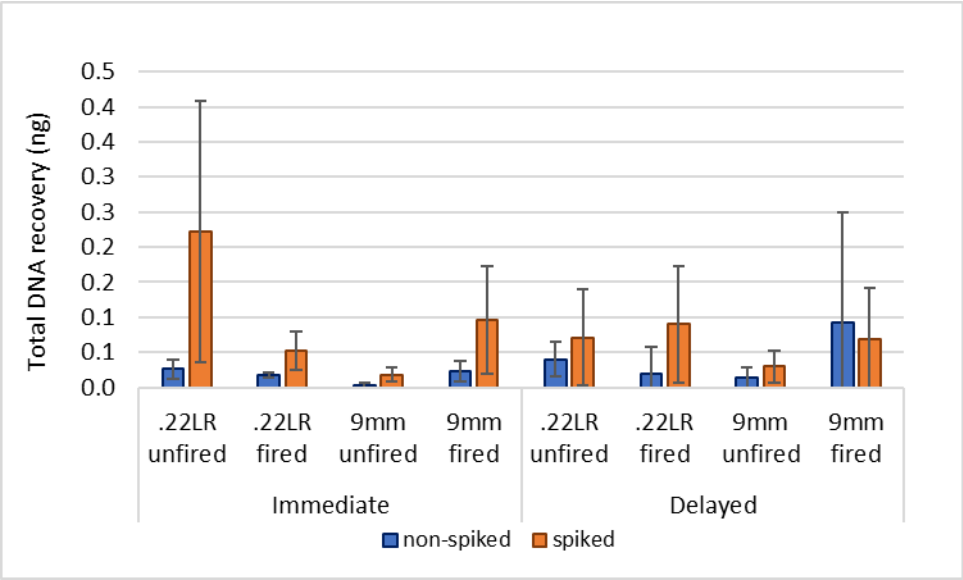












Supplementary Figure 1. Total DNA recovery from spiked and non-spiked cartridges following immediate DNA extraction (n=78) vs. delayed extraction (n=48) after 48 days. Error bars represent standard deviation.