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Comparison of three DNA extraction methods for three different types of fired and unfired ammunition

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ABSTRACT

When handling ammunition for gun loading, epithelial cells from the hands can become adhered to the metal surface, and this trace is a potential source of DNA. This work aimed to compare the efficiency of three DNA extraction methods from fired cartridge cases from three different types of firearms: a 12-gauge shotgun, a point 40 S&W pistol, and a 7.62 mm rifle. Nine volunteers were involved in this study handling 42 pieces of ammunition overall. The unfired ammunition was handled by a known good donor, and we used this data for comparison. DNA profiling was carried out with EZ1 DNA Investigator Kit for EZ1 Advanced XL automated DNA extraction, QIAmp DNA Investigator kit for a non-automated silica-based membrane column method, and direct lysis protocol for a non-automated in-house one. Samples were collected with 0.5 × 0.5 cm pieces of FTA filter paper moistened with distilled water. Quantiplex Pro RGQ kit and Fusion Powerplex 6C were used for genotyping samples. QIAmp DNA Investigator method resulted in the best number of alleles recovered for both conditions tested, both unfired and fired ammunitions: 77 % vs. 19.3 %, followed by the automated extraction (28.6 % vs. 4.3 %) and lysis protocol (0 % vs. 3.9 %). Degradation data from fired cartridge cases were 27 % for column method, 50 % for lysis protocol, and 87 % for EZ1 kit. Kruskal-Wallis test for mean DNA concentration from these samples returned $p < 0.05$, and Dunn's multiple comparison test indicated a significant difference between calibers 0.40 S&W and 12-gauge shotgun from lyses protocol method. We did not detect any other significant differences on the test. The 12-gauge shotgun cartridge cases resulted in a high number of alleles overall (56.8 %). The numerous steps for DNA extraction and purification in the column method may explain its better performance. Although the results obtained indicate that all methods be used for DNA extraction from this type of evidence, the silica-based membrane column method appears to be more efficient.

1. Introduction

In the last decade, the average number of offenses involving firearms was around 43,000 per year in our country. According to the latest national survey on violence (2019), over 30,000 homicides by firearms were registered only in that year. Fired cartridge cases are common materials recovered for forensic analysis. Even though cartridge cases may sustain fingerprints and genetic material that are transferred during handling time before the firing event, the most common analyzes in our country are still regarding forensic ballistics that does not provide necessarily a direct identification of a person of interest in that crime. In 2010, the national DNA database was implemented and since then over 100,000 genetic profiles from objects recovered at crime scenes, missing persons' relatives, human remains,and prisoners imprisoned for heinous crimes were included in the database. Understanding the efficiency of DNA recovery methods across different types of ammunition is a great addition to crime-solving approaches.

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2. Materials and methods

2.1. Sample preparation

Nine volunteers, military-trained and authorized personnel to handle the type of firearms utilized, participated in this study. The ammunitions were cleaned before handling with sodium hypochlorite 2.5 % followed by alcohol 70 %. Firearms were not cleaned before this experiment. The volunteers were instructed to not rub their fingers across the face or other body parts and no extra handling or holding of the ammunition was performed as we aimed for the closest realistic scenario possible regarding this process. The average handling time was 45 s. The firing of each type of ammunition was performed separately. All volunteers fired a 7.62 mm rifle first, followed by a 12-gauge shotgun, and a point 40 S&W pistol. The average time between the firing of the different types of firearms was 30 min. The experiment was carried out in an open training field covered by soil and grass. One of the researchers was safely positioned behind the shooter to track where the cartridge cases were landing. After each shot, the cartridge case was collected with plastic tweezers and stored individually in paper envelopes. The samples were processed up to seven days after the firing.

2.2. DNA collection

To collect DNA from fired cartridges, one small piece (0.5 cm × 0.5 cm) of FTA filter paper moistened with 20 µl of distilled water was used. The collection time was established in 2 min for each cartridge case. The filter paper was transferred to 1.5 ml sterile plastic tubes for DNA extraction.

2.3. DNA extraction

2.3.1. EZ1 DNA Investigator Kit

DNA extraction was carried out with EZ1 DNA Investigator Kit for EZ1 Advanced XL automated DNA extraction. Preliminary lysis according to the manufacturer's protocol for FTA® cards was performed. After incubation, the samples were loaded into EZ1 Advanced XL equipment following the manufacturer's recommendations. The Tip Dance protocol recommended for samples containing solid particles was set. The final DNA extract volume was 40 µl eluted in TE buffer. No additional purification steps were performed.

2.3.2. QIAmp DNA Investigator Kit

For a non-automated silica-based membrane column method, QIAmp DNA Investigator Kit was used according to the manufacturer's protocol for FTA® cards. The final DNA extract volume was 35 µl eluted in TE buffer. No additional purification steps were performed.

2.3.3. Direct enzymatic lysis protocol

For a non-automated in-house method, direct enzymatic lysis was performed by mixing 7.2 µl of Proteinase K with 198.2 µl of SDS 0.05 % detergent for each sample followed by incubation at 56 °C for 30 min in a thermomixer at 1400 RPM, 100 °C for 10 min (no agitation) and 4 °C for 5 min. DNA extract was purified and concentrated to 19 µl final volume through centrifugation in Microcon® Centrifugal Filters 0.5 ml 100 K (Merck Millipore) for 15 min.

2.4. DNA quantitation and STR amplification

DNA extracts from fired cartridge cases were quantified using Investigator® Quantiplex® Pro RGQ kit (QIAGEN) according to the manufacturer's recommendations. Positive and negative controls were included in each run. For STR amplification PowerPlex® Fusion 6C kit (Promega Corporation) was used following the manufacturer's protocol. An analytical threshold for peak calling was set at 75 RFU.

3. Results

For the unfired cartridges, no profiles were obtained for the direct lysis method; the average number of donor alleles recovered was 77 % for the QIAmp DNA Investigator methodology and 28.6 % for the EZ1 DNA Investigator Kit. For the fired cartridges, 12-gauge shotgun and 7.62 mm cartridges yielded no profiles and partial profiles were obtained for all point 40 S&W cartridges samples, meaning 11 % of alleles recovered (Direct Lysis method). Considering the membrane column method, only partial profiles were obtained: 46.9 % of alleles recovered for the 12-gauge shotgun cartridges, 9.2 % for the 7.62 mm cartridges, and 1.8 % for the point 40 S&W cartridges. For the automated DNA extraction method,only partial profiles were obtained: 9.9 % of alleles recovered for the the12-gauge shotgun cartridges, 2.5 % for the 7.62 mm cartridges, and 0.6 % for the point 40 S&W cartridges (0.6 %).

4. Discussion

Despite the DNA extraction technique used, the STR results from both unfired and fired ammunition indicated DNA loss and degradation. Detection of alleles, in general, was poor, however, better results were obtained for plastic cartridge cases (12-gauge shotgun) overall. Copper ions present in metal surfaces may interfere with the hydrogen bonding of the nucleic acid, thus changing the DNA molecule structure [1,2] and this may explain the challenges in obtaining interpretable DNA profiles from metal cartridge cases. Also, as Wood [3] indicates epithelial cells from the hands may not become easily adhered to the metal surface as they would in texturized plastic surfaces such as the 12-gauge shotgun surface. The membrane column method yielded a higher percentage of alleles recovered across all the volunteers and calibers tested. Similar results were found by Stephen [4]. The numerous steps for DNA extraction and purification in the column method contribute to washing PCR inhibitor(s) produced during the firing event and may explain the QIAmp DNA Investigator kit's better performance. This observation may also be supported as poor results were obtained by Martin [5] in a recent direct PCR approach for shotgun cartridge cases research.

5. Conclusion

Our results showed that several factors may influence DNA profiling from fired cartridge cases, but the type of surface appears to play a major role in these analyzes. Regarding the DNA extraction methodology, even though partial profiles were obtained from the three methods tested, the silica-based membrane column method showed the best results and therefore may be a preferred method for these types of samples.

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Conflict of interest

None.

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