Quantification of Metal Ions Recovered During DNA Analysis of Brass Ammunition Using ICP-OES

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INTRODUCTION

It is often challenging to obtain Short Tandem Repeat (STR) profiles from DNA recovered from the surface of "touched items" such as ammunition, especially those comprising copper alloys. This problem is not only due to the low amount of DNA deposited, but also metal ions causing degradation and damage by interacting with the DNA, and/or inhibition during the polymerase chain reaction (PCR). Previous research has demonstrated that copper ions can form strong ionic bonds between DNA, forming metal ion-DNA complexes and causing oxidative damage to the DNA template[1].

Numerous studies have examined collection and extraction methods aimed at increasing genotyping success from these challenging samples[2, 3]. However, results have been inconsistent. Although there is consensus that metal ions, especially copper, can intercalate with DNA or cause PCR inhibition[3-5], to date, no study has quantified the amount of metal ions actually recovered during the collection of touch DNA on brass ammunition, and that may persist after DNA purification.

This study examines the presence and quantity of copper and zinc ions during various DNA collection and purification methods and their possible effects on genotyping success. The effectiveness of various DNA collection and purification methods commonly used by forensic laboratories to process brass ammunition for DNA evidence was investigated. The amount of copper and zinc, and other metals co-recovered from fired and unfired brass casings during DNA collection (using numerous soaking, swabbing, and direct PCR protocols) was quantified via Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES). This same panel of metals was subsequently quantified after DNA lysis and purification. This research can help inform the forensic community on how best to collect and process trace amounts of biological material from brass ammunition and similar evidence.

MATERIALS AND METHODS

Samples

Ninety 9mm rounds of brass ammunition (Sumbro X-Force 9mm Luger 124 grain Full Metal Jacket) were UV sterilized for 60 min in a UV Crosslinker. Each cartridge was spotted with the equivalent of 10ng of DNA (approximately 510 cells/µL) from a buccal cell suspension in a marked location. Cartridges were left to dry completely. Half the cartridges were reserved for firing, while the other 45 rounds were used as unfired samples. Firing was completed using a 9mm Glock 19 by a law enforcement officer wearing gloves at an outdoor range whilst avoiding the area spotted with buccal cells. A clean tarp was placed on the ground to collect the spent casings. Fired samples were immediately transported to the laboratory and analyzed alongside the unfired samples.

Recovery Methods

Five different recovery methods were evaluated: 1) water-moistened nylon swabs, 2) water-moistened cotton double swabs, 3) EDTA-moistened cotton double swabs, 4) soaking, and 5) microFLOQ® direct swabs (COPAN Diagnostics Inc., Murrietta, CA). Prior to DNA collection, all swabs were moistened with 30 µL of sterilized water or EDTA, except for the microFLOQ direct swabs, which were moistened with 1 µL of water. The microFLOQ samples continued directly to amplification.

DNA Extraction

Two different automated extraction methods were used in this study: PrepFiler Express BTA™ Forensic DNA Extraction Kit (ThermoFisher Scientific, Waltham, MA) on the AutoMate Express™ Forensic DNA Extraction System (ThermoFisher Scientific) and the EZ1 DNA Investigator Kit (QIAGEN, Venlo, the Netherlands) on the EZ1 Advanced XL (QIAGEN). Each set of fired and unfired samples were equally distributed so that each recovery method was purified with each of the two extraction methods. Lysis of the soaked samples was performed as per Montpetit et al., with the exception of increasing the volumes (1062.5 µL lysis buffer and 42.5 µL proteinase K) to cover the entire casing. Extraction on the EZ1 was completed using the Large Volume Protocol with a final elution volume of 40 µL. For the PrepFiler BTA lysis protocol, double the volume was used to submerge the nylon swabs. Furthermore, the soaked samples were split into two sample tubes due to volume constraints on the Automate and were pooled after purification. A standard elution volume of 50 µL was used.

RESULTS AND DISCUSSION

Table 1. Average concentrations of copper and zinc ions for each recovery method post collection and purification.

Recovery Method	Metal Ion Concentrations Before Extraction		PrepFiler BTA Express		EZ1 DNA Investigator	
	Avg Cu (mM)	Avg Zn (mM)	Avg Cu (mM)	Avg Zn (mM)	Avg Cu (mM)	Avg Zn (mM)
Water-Moistened Cotton Double Swab- **Unfired**	375.3	21.53	.153	.076	.252	.076
Water-Moistened Cotton Double Swab- **Fired**	460.3	41.68	.150	.076	.153	.076
EDTA-Moistened Cotton Double Swab- **Unfired**	337.94	122.74	.122	.076	.142	.076
EDTA-Moistened Cotton Double Swab- **Fired**	1, 141	354.5	.146	.076	.146	.076
Water-Moistened Nylon Swab- **Unfired**	45.24	.076	.177	.076	.150	.076
Water-Moistened Nylon Swab- **Fired**	40.13	.076	.157	.076	.153	.076
Soaked- **Unfired**	.3	.11	.069	.038	.142	.076
Soaked- **Fired**	.42	.18	.072	.038	.142	.076
MicroFLOQ- **Unfired**	.022	.07	*Direct PCR*			
MicroFLOQ- **Fired**	.05	.012				

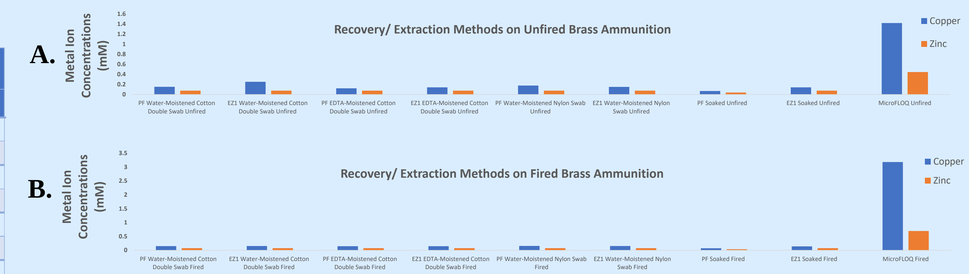

Figure 1. Comparison of metal ion concentrations after DNA extraction of samples collected with the five different recovery methods. Comparison of copper and zinc ion concentrations after purification of **A)** unfired samples, and **B)** fired samples. MicroFLOQ direct swabs were not subjected to any purification, but instead proceeded directly to PCR.

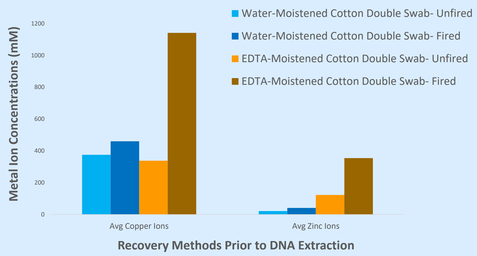

Figure 2. Comparison of metal ions obtained from different recovery methods from fired and unfired copper ammunition. The two methods with the highest concentration of metal ions recovered are shown. A general trend was observed where firing increases the amount of both copper and zinc ions recovered during DNA collection.

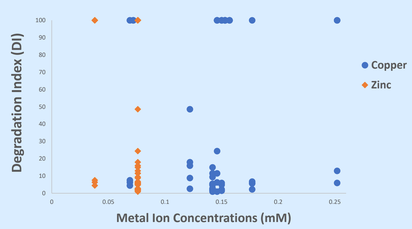

Figure 3. Impact of final metal ion concentrations on the Degradation Index. No relationship was demonstrated between either copper or zinc ion concentration and the degradation index (DI). All samples exhibited some level of degradation, with DI values ranging from 1 to 97.9 for those samples where both targets were amplified. Samples where the large autosomal target was not amplified were assigned values of 100 as an upper limit.

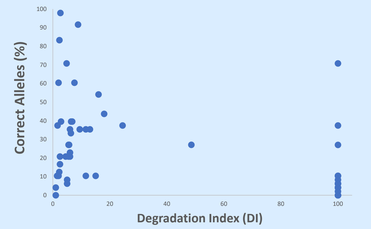

Figure 4. Comparison of DNA degradation and percentage of concordant alleles. No relationship was observed between the DI and percentage of correct alleles recovered. Samples where the large autosomal target was not amplified were assigned values of 100 as an upper limit.

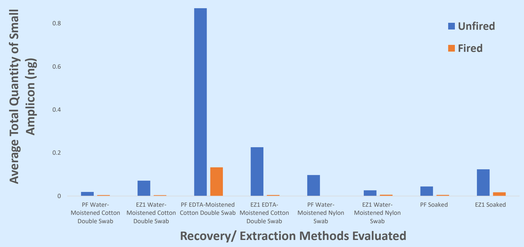

Figure 5. Impact of recovery method and sample type on DNA recovery. As expected, firing substantially decreases the amount of amplifiable DNA recovered from ammunition.

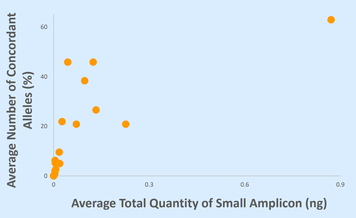

Figure 6. Quantity of DNA recovered and the average genotyping success. The percentage of concordant alleles genotyped was related to the amount of DNA recovered.

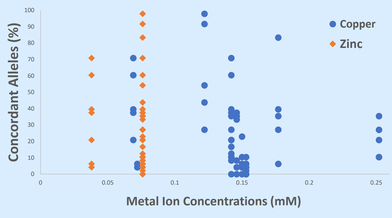

Figure 7. Relationship between metal ion concentrations and percentage of concordant alleles generated. No relationship was observed between the presence of copper or zinc ions after purification and the percentage of correct alleles genotyped.

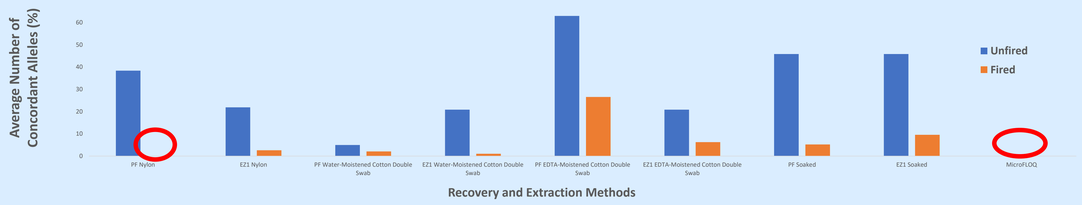

Figure 8. Relationship between genotyping of DNA from fired and unfired brass ammunition after recovery using several common methods.
A greater percentage of concordant alleles were recovered from unfired ammunition. MicroFLOQ swabs were the only recovery method which failed to yield any concordant STR alleles for both fired and unfired brass ammunition. Nylon swabs were unable to recover any amplifiable DNA from fired brass ammunition after extraction using PrepFiler Express BTA chemistry.

MATERIALS AND METHODS

DNA Quantification

DNA extracts were quantified using Quantifiler Trio DNA Quantification Kit (ThermoFisher Scientific) on the 7500 Real-time PCR System (ThermoFisher Scientific). Results were accepted as reliable when an R^2 value of at least .99 was obtained.

Metal Ion Quantification

A portion of each extract was removed and sent to the Texas Research Institute for Environmental Studies (TRIES) Facility at Sam Houston State University for copper and zinc ion quantification using inductively coupled plasma-optical emission spectrometry (ICP-OES).

DNA Amplification

DNA extracts were amplified using the VeriFiler™ Plus PCR Amplification Kit (ThermoFisher Scientific) on the ProFlex PCR System (ThermoFisher Scientific) according to manufacturer's protocol. For direct PCR of the microFLOQ swabs, a 10 µL reaction volume was used.

Separation, Detection, and Analysis

Amplified DNA was separated and detected on the ABI 3500 Series Genetic Analyzer (ThermoFisher Scientific) using a 36 cm capillary array and POP-4 configuration (ThermoFisher Scientific). STR profiles were analyzed using GeneMapper® ID-X Software v1.4 (ThermoFisher Scientific). An analytical threshold of 100 RFUs was applied.

CONCLUSIONS

- The highest concentrations of metal ions results after collection of DNA (*copper > zinc > other metals*) (Table 1; Fig 2), and substantially decrease after DNA purification (Table 1; Fig. 1). Therefore, further purification after automated extraction is unnecessary, and unlikely to yield greater genotyping success.
- Any PCR inhibitors present after DNA collection were removed during DNA purification.
- All samples exhibited some level of DNA degradation regardless of the extraction method (Fig. 3), supporting the hypothesis that DNA is damaged prior to or during recovery, and that amplification is not adversely affected by the presence of inhibitory metal ions. Instead, poor genotyping results are most likely the result of damage and degradation of the DNA template due to the initial interaction with the metal ions.
- Overall, results demonstrate that STR profiles can be successfully obtained from brass ammunition even in the presence of metal ions (Fig. 7). However, firing substantially increases both metal ion concentrations and DNA damage, and decreases genotyping success (Figs. 2, 5 and 8).
- MicroFLOQ swabs were the only recovery method to yield no concordant STR alleles for both fired and unfired brass ammunition (Fig. 8). This is most likely due to the smaller recovery surface and the higher amounts of metal ions recovered using these swabs, and no purification step. For these reasons, our results do not support microFLOQ direct swabs as an appropriate recovery method for touch DNA on brass ammunition.
- 60% of nylon swabs were unable to recover any amplifiable DNA from fired brass ammunition (Fig. 8). This may be due to the nylon swabs becoming easily oversaturated when moistened and causing more damage to the trace amount of degraded DNA after firing.
- The soaking method produced the most consistent genotyping results for unfired ammunition, and EDTA-moistened swabs yielded the most successful results overall (Fig. 8). Due to the additional time needed to complete the soaking protocol, the use of EDTA to moisten a swab may be the best approach for laboratories to adopt.
- Further research into the use of smaller, alternative markers to genotype "touch" and degraded DNA from brass ammunition is warranted.

REFERENCES

1. Moreno LI, McCord BR. Understanding metal inhibition: The effect of copper (Cu2+) on DNA containing samples. Forensic Chemistry. 2017;4:89-95.
2. Montpetit S. Obtaining DNA from ammunition: A review. Wiley Interdisciplinary Reviews: Forensic Science. 2020;2(2):e1352.
3. Bille TW, Fahrig G, Weitz SM, Peiffer GA. An improved process for the collection and DNA analysis of fired cartridge cases. Forensic Science International: Genetics. 2020;46:102238.
4. Combs LG, Warren JE, Huynh V, Castaneda J, Golden TD, Roby RK. The effects of metal ion PCR inhibitors on results obtained with the Quantifiler® Human DNA Quantification Kit. Forensic Science International: Genetics. 2015;19:180-9.
5. Vincent M, Duval RE, Hartemann P, Engels-Deutsch M. Contact killing and antimicrobial properties of copper. Journal of applied microbiology. 2018;124(5):1032-46.

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