Evaluation of Metal Ion and DNA Recovery from the Surface of Brass Ammunition to Improve STR Profiling

Natalia Czado, MS; Rachel Houston, PhD; Sheree Hughes, PhD
Department of Forensic Science, Sam Houston State University, Huntsville, Texas, USA

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

WASHINGTON, DC ISFG 2022 INTERNATIONAL SOCIETY FOR FORENSIC GENETICS

INTRODUCTION

Given the large amount of violent gun crimes committed globally, firearms and ammunition are routinely submitted as evidence to crime laboratories. During the handling and loading of a gun, DNA can be deposited and used to generate STR profiles. However, generating profiles has traditionally been difficult due to the low amounts of DNA deposited, the presence of possible mixtures, and the DNA degradation and inhibition caused by the metal ions. Inhibition is often more pronounced for samples with a copper alloy composition.

Studies have shown that the presence of copper ions can negatively affect DNA recovery and genotyping for several reasons. First, the copper ions can form strong ionic bonds with the DNA[1,2]. These metal/ion complexes not only affect the collection of DNA, but also cause inhibition of the DNA polymerase during PCR. Furthermore, oxidative damage to the DNA can result in amplification issues. Lastly, the presence of metal ions during capillary electrophoresis can lead to mobility issues[2]. Although there is consensus that metal ions play a detrimental role in DNA analysis, to date, no study has quantified the amount of metal ions co-recovered during the collection of touch DNA on brass ammunition, and that may persist after DNA purification.

This study aims to evaluate several different collection methods to determine which recover the most amplifiable DNA and generate the most complete STR profiles. Furthermore, this study will examine the identity and quantity of various metal ions co-recovered during DNA collection from the surface of brass ammunition. 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 metallic evidence.

MATERIALS & METHODS

Samples

- 110 Sumbro X-Force 9mm Luger 124 grain full metal jacket rounds of UV sterilized 9 mm brass cartridges
- Spotted with ≈ 510 cells/µL of buccal cell suspension (≈ 10 ng DNA) in designated area (Fig. 1)
- Fired by a law enforcement officer using a 9mm Glock 19

Figure 1. Buccal cell suspension was deposited in the area designated by the circle.

Recovery Methods

Six different recovery methods were evaluated:

1) Water-moistened nylon swabs
2) Water-moistened cotton double swabs
3) EDTA-moistened cotton double swab
4) Soaking[3]
5) BTMix rinse/swab[4]
6) microFLOQ® direct swabs (COPAN Diagnostics Inc., Murrietta, CA)

DNA Extraction

Two different automated extraction methods were used:

1) PrepFiler Express BTA™ Forensic DNA Extraction Kit (ThermoFisher Scientific, Waltham, MA) on the AutoMate Express™ Forensic DNA Extraction System (ThermoFisher Scientific) using a standard elution volume of 50 µL.
2) EZ1 DNA Investigator Kit (QIAGEN, Venlo, the Netherlands) on the EZ1 Advanced XL (QIAGEN) using the Large Volume Protocol with a final elution volume of 40 µL.

Modifications

Lysis of the soaked samples was performed as per Montpetit et al[3], with the exception of increased volumes (to 1062.5 µL lysis buffer and 42.5 µL proteinase K) to cover the entire casing. 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.

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 when the R^2 value was ≥ 0.99.

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). STR profiles were analyzed using GeneMapper® ID-X Software v1.4 (ThermoFisher Scientific). An analytical threshold of 100 RFUs was applied.

RESULTS & DISCUSSION

Table 1. Metal ion concentrations for all expected metals recovered using representative methods. Copper and zinc were recovered in the highest concentrations.

Metal	Fired Casings (mg/kg)	Soaked Fired Casings (mg/kg)	Soaked Unfired Casings (mg/L)	EDTA-Moistened Cotton Double Swab- Fired Casings (mg/kg)	EDTA-Moistened Cotton Double Swab- Unfired Casings (mg/kg)
Copper	411	26.4	19.84	58	17.18
Zinc	198	11.74	5.63	18.54	6.42
Barium	206	3.21	0.087	5.26	1.89
Lead	184	6.38	0.49	7.34	0.22
Iron	120	0.06	0.077	3.00	4.00
Antimony	71.5	2.53	0.025	0.112	0.29
Aluminum	52.9	0.014	0.014	1.25	0.54
Nickel	4.11	0.033	0.004	0.015	0.004

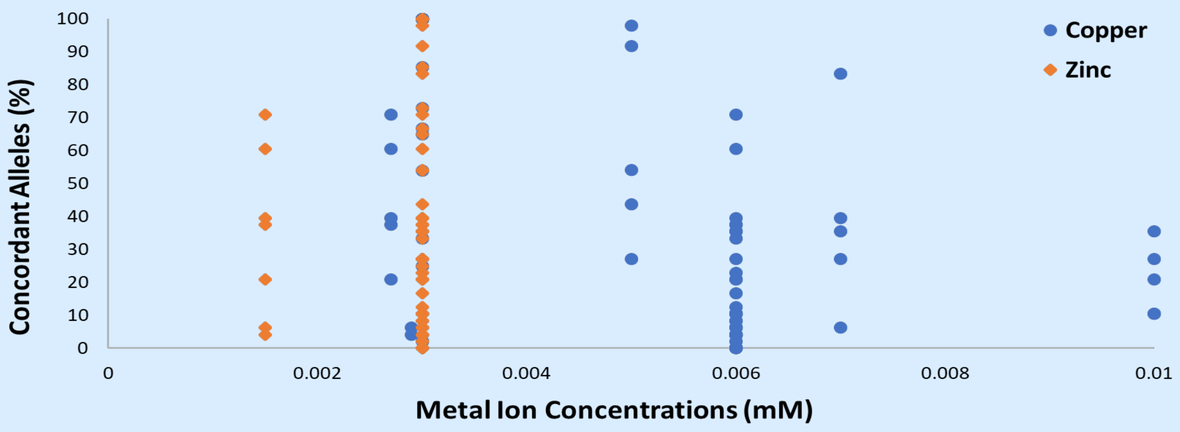

Figure 2. Comparison of ion concentration to genotyping success. No relationship was observed between the presence of copper or zinc ions after purification and the percentage of correct alleles genotyped.

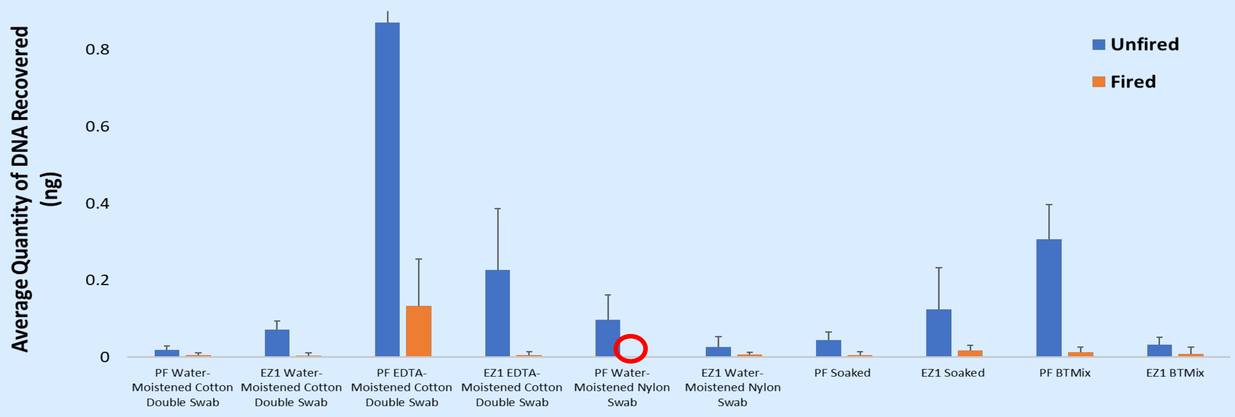

Fig. 3. Comparison of DNA quantity recovered with each collection method. Samples swabbed from fired casings with water-moistened nylon swabs and extracted with PrepFiler BTA chemistry failed to yield any DNA. The difference between unfired and fired was not statistically significant (p-value=0.936 ; α=.05). The difference observed between PrepFiler and EZ1 was not statistically significant (p-value= 0.769416; α=.05).

Table 2. Average concentrations of copper and zinc ions for each recovery method post collection and purification. As expected, the highest concentration of metal ions were detected after collection and before DNA extraction. PCR inhibitors present after DNA collection were removed during DNA purification.

Recovery Method	Metal Ion Concentrations *Before* Extraction		Metal Ion Concentrations *After* Extraction							
			PrepFiler BTA Express				EZ1 DNA Investigator			
	Avg Cu (mM)	Avg Zn (mM)	Avg Cu (mM)	Cu removed (%)	Avg Zn (mM)	Zn removed (%)	Avg Cu (mM)	Cu removed (%)	Avg Zn (mM)	Zn removed (%)
Water-Moistened Cotton Double Swab- **Unfired**	0.30	0.017	0.006	***98***	0.003	***82.35***	0.01	***96.67***	0.003	***82.35***
Water-Moistened Cotton Double Swab- **Fired**	0.37	0.033	0.006	***98.37***	0.003	***90.91***	0.006	***98.37***	0.003	***90.91***
EDTA-Moistened Cotton Double Swab- **Unfired**	0.27	0.098	0.005	***99.87***	0.003	***96.94***	0.006	***97.78***	0.003	***96.94***
EDTA-Moistened Cotton Double Swab- **Fired**	0.91	0.28	0.006	***99.34***	0.003	***98.93***	0.006	***99.34***	0.003	***98.93***
Water-Moistened Nylon Swab- **Unfired**	0.04	0.005	0.007	***80.56***	0.003	***36.17***	0.006	***83.33***	0.003	***36.17***
Water-Moistened Nylon Swab- **Fired**	0.032	0.015	0.0062	***80.63***	0.003	***80***	0.006	***81.25***	0.003	***80***
Soaked- **Unfired**	0.30	0.09	0.0027	***99.1***	0.0015	***98.26***	0.006	***98***	0.003	***96.51***
Soaked- **Fired**	0.42	0.18	0.0029	***99.31***	0.0015	***99.17***	0.006	***98.57***	0.003	***98.33***
BTMix- **Unfired**	0.06	0.006	0.003	***95***	0.003	***50***	0.003	***95***	0.003	***50***
BTMix- **Fired**	0.07	0.005	0.003	***95.83***	0.003	***66.67***	0.003	***95.83***	0.003	***66.67***
MicroFLOQ- **Unfired**	0.022	0.07	*Direct PCR*							
MicroFLOQ- **Fired**	0.05	0.012								

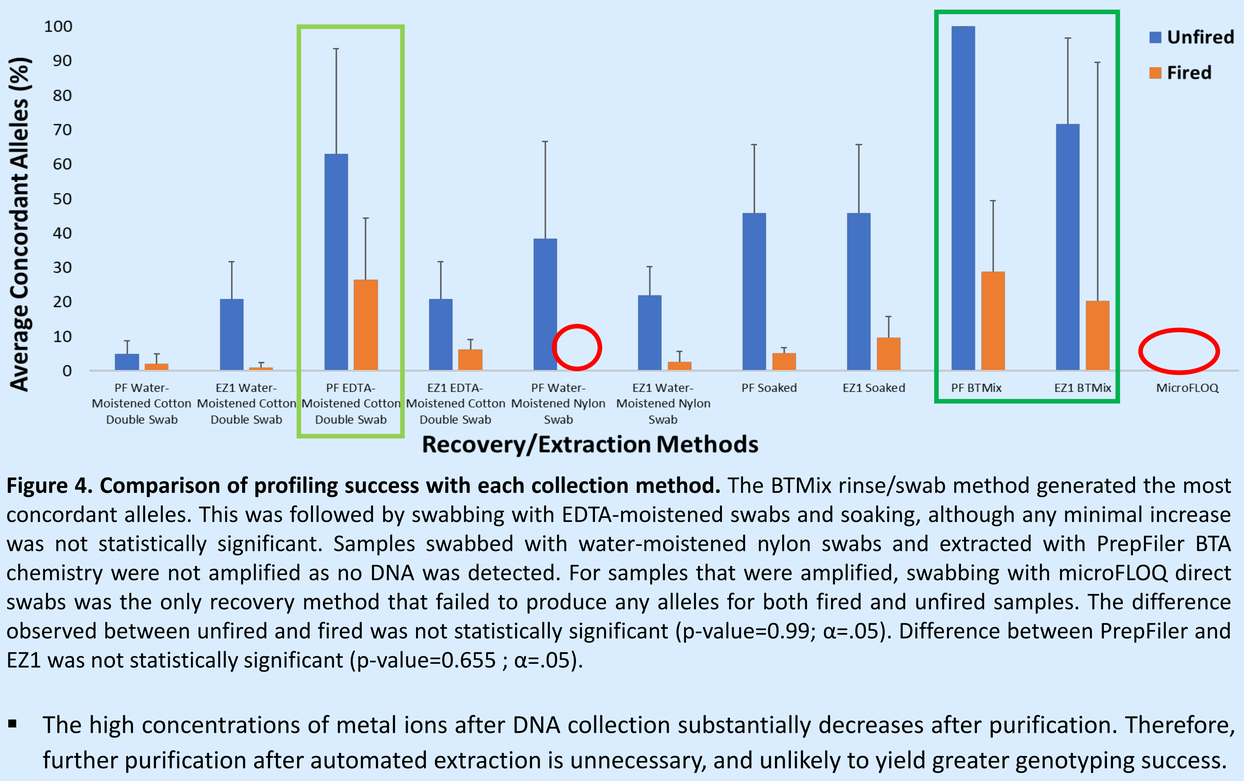

Figure 4. Comparison of profiling success with each collection method. The BTMix rinse/swab method generated the most concordant alleles. This was followed by swabbing with EDTA-moistened swabs and soaking, although any minimal increase was not statistically significant. Samples swabbed with water-moistened nylon swabs and extracted with PrepFiler BTA chemistry were not amplified as no DNA was detected. For samples that were amplified, swabbing with microFLOQ direct swabs was the only recovery method that failed to produce any alleles for both fired and unfired samples. The difference observed between unfired and fired was not statistically significant (p-value=0.99; α=.05). Difference between PrepFiler and EZ1 was not statistically significant (p-value=0.655 ; α=.05).

CONCLUSIONS

- Despite reports of metal ions causing significant PCR inhibition, our results show that metal ions (copper and zinc) present after DNA collection were removed during DNA purification.
- STR profiles can be obtained from brass ammunition with some success. Poor STR results are most likely explained by the very low levels of good quality "trace" DNA recovered.
- Firing increases both metal ion concentrations and DNA damage, thereby, decreasing genotyping success.
- microFLOQ direct swabs were not appropriate for recovery of LT-DNA on brass ammunition in this study.
- Use of chelating agents (EDTA and BTMix) are recommended, followed by soaking to improve DNA recovery.
- The high concentrations of metal ions after DNA collection substantially decreases after purification. Therefore, further purification after automated extraction is unnecessary, and unlikely to yield greater genotyping success.
- Because microFLOQ swabbed samples did not undergo purification, metal ions were directly introduced into the PCR amplification reaction. The lack of purification and less DNA being collected using the smaller recovery surface most likely led to genotyping failure using this swab.
- All samples exhibited some level of DNA degradation, with average degradation index (DI) values ranging from 2.5 to 80.4 in samples where both targets were amplified. There was no indication of PCR inhibition during quantification. These results, along with the DI values, support our hypothesis that the DNA template is most likely damaged and degraded due to the initial interactions with the metal ions prior to recovery, and amplification is not adversely affected by the presence of inhibitory metal ions.

ACKNOWLEDGEMENTS

We would like to thank Officer Jon Keith with the League City Police Department for assisting with the firing of samples. We would also like to thank the Department of Forensic Science and the Graduate School at Sam Houston State University for funding portions of the project, as well as ThermoFisher Scientific for providing some reagents.

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