Qualification of Metallic Ions Recovered from Swabbing Unfired Brass Bullet Casings.

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Abstract:

The presence of metallic ions has been established to inhibit DNA Taq Polymerase, a key component for the Polymerase Chain Reaction (PCR), making amplification challenging when trying to generate DNA profile of bullet handler transferred DNA, commonly called touch DNA from the unfired bullet casing surface. The goal of this research is to determine the amount of various bivalent cations such as Copper (Cu+2) and Zinc (Zn+2), which are suspected PCR amplification reaction inhibitors. A known concentration (0.1ng/µL) of liquid standardized human DNA, the total amount (1 ng, 3 ng, and 5 ng) was deposited at outlined locations onto the unfired brass ammunition, and the samples were air dried in the clean hood. The deposited DNA was lifted by sterile cotton double swabbing technique, using two (2) different solutions such as Rapid Stain Identification (RSID) kit universal buffer, TE dilution buffer or deionized water. To determine the quantity (percentage) of various metallic ions recovered from swabbing, Electron Micro Probe Analyzer (EPMA) technique was utilized to quantify (percentage) various bivalent cations collected from the swabbed samples. Each of the swab samples recovered more Cu+2 ions than Zn+2 ions, though the ratios were variable between solutions. Deionized water was shown to recover more bivalent cations than any other tested solution. Deionized water recovered 6 times more bivalent cations than RSID buffer and 1.6 times more than TE dilution buffer.

Introduction:

Every touch leaves a trace, and every trace tells a story. Welcome to the intricate world of forensic science, where even the smallest detail can hold the key to unraveling a mystery.

Ballistics evidence is important in forensics, because roughly 85% of homicides were committed using a firearm in the US, in 2021.[5] This percentage has been steadily increasing since 2014 [5], with handguns being the primary type of firearm used in these cases. The ammunition used in handguns is usually made of brass [5], which is a mixture of copper and zinc. By itself, copper would be far too delicate to tolerate everyday handling, let alone the high pressures found in contemporary cartridges.[2] This is why zinc is added to give the alloy more hardness.[2]

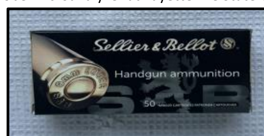
Metal ions can directly crosslink DNA, preventing Polymerase Chain Reaction (PCR) products from accessing the DNA template. One example of a metal-DNA interaction is copper, which binds to DNA bases with a high affinity.[4] The presence of metallic ions has been established to inhibit DNA Taq Polymerase, a key component for the PCR making amplification challenging when trying to generate a DNA profile of bullet handler transferred DNA, commonly called "touch DNA" from the unfired bullet casing surface.[4]

In forensic science, 'Locard's Exchange Principle' holds that "the perpetrator of a crime will bring something to the crime scene and will leave with something from it"; this comes from a French criminologist named Dr. Edmond Locard.[6] In layman's terms, this means everything touched by a person will leave a trace, and that trace is what would be used in a criminal investigation.[6] This trace can be referred to as "Touch DNA". Touch DNA is any DNA from any source that is present in trace amounts and is typically recovered speculatively without a visible bodily fluid or stain being visible. This might involve "touch deposits," which are materials left behind after a substrate has been handled or touched.[1]

Considering the prevalence that PCR inhibitors have in recovering DNA from brass bullets, the question at hand is, can copper (Cu+2) and zinc (Zn+2) ions be qualified and quantified? With that, this research aims to determine the amount of various bivalent cations such as Copper (Cu+2) and Zinc (Zn+2), using different solvents, which are suspected PCR amplification reaction inhibitors.

Materials:

The primary focus of this research revolves around the examination of bullets used in firearm-related crimes. A total of 42 unfired brass bullets of the nine-millimeter Luger caliber, sourced from the 'Sellier & Bellot' brand, were utilized as the main component of this study. Applied Biosystems AmpFiSTR control DNA was deposited on and recollected from the casings using Fisher brand cotton tipped swabs with three different solutions as the independent variable in this series of experiments. The recovery solutions used included RSID Universal Buffer, dilution buffer (provided with the AmpFiSTR kit), and deionized water, which is analogous to the solution used in local forensics swabbing. Analysis was conducted using the Joel JXA-8530F Field Emission Electron Probe Microanalyzer at Fayetteville State University's Microprobe Laboratory.



Methods:

Sterilization of the bullets was conducted using a meticulous procedure to ensure the removal of any potential contaminants and prepare the surfaces for DNA deposition. Initially, each bullet underwent manual washing with ethanol, followed by air drying to eliminate any residual impurities. Subsequently, a thorough wash with deionized water was performed, followed by another round of air drying. To complete the sterilization process, the bullets were exposed to UV radiation in a designated cabinet, with sterilization carried out in increments of 1/3 turns for a duration of one hour each. This methodical approach ensured comprehensive sterilization, with each set of bullets undergoing the process for a minimum of 24 hours.

To facilitate the deposition of DNA on the sterilized bullets, a systematic approach was employed. Among the 42 sterilized bullets, 20 were designated with a 'deposition zone,' delineated using a blue Sharpie marker for precise targeting and deposit mass was labeled on the projectile of the bullet. The DNA used for deposition consisted of Amp FISTR control (female) DNA, with a concentration of 0.1 ng/µL. Various volumes of DNA were deposited on each bullet, with the aim of achieving targeted concentrations. Specifically, 10 µL of DNA was deposited in incremental steps, corresponding to different deposit amounts: bullets intended to receive 1 ng of DNA received a single 10 µL drop, those aimed for 3 ng received three 10 µL drops, and those designated for 5 ng received five 10 µL drops. Following each deposition, the bullets were allowed to air dry completely for approximately 45 minutes within a sterile hood environment to ensure adherence of the DNA to the surface before subsequent deposits were made.

Table 1: Bullet Groups

Bullet Deposition Groups	GROUP A (RSID Universal Buffer)	GROUP B (RSID Universal Buffer)	GROUP C (Sterile Deionized Water)	GROUP D (Dilution Buffer)
SET 1 (1 ng)	1A	1B	1C	1D
SET 3 (3 ng)	3A	3B	3C	3D
SET 5 (5 ng)	5A	5B	5C	5D
NEGATIVE CONTROL	No DNA Deposited			

To collect DNA samples from the bullets, a 'Double-Swab' Technique was employed, recognized for its efficacy in maximizing DNA recovery.[3] This technique involves the use of two swabs sequentially to ensure comprehensive sample collection. Initially, 50 µL of solvent was applied to one swab, saturating it for optimal DNA extraction. Subsequently, a dry swab was utilized to complement the solvent-saturated swab, facilitating the absorption of any residual DNA present on the bullet surface.

To prepare for Electron Probe Microanalyzer (EPMA) analysis, three Fisher brand regular glass slides were utilized, each equipped with three evenly spaced pieces of carbon tape. Each piece of tape was labeled accordingly: '5B,' '5C,' '5D'; '1B,' '1C,' '1D'; and 'B,' 'C,' and 'D.' Subsequently, 8 µL of sample was deposited onto each slide, with deposition carried out in 2 µL increments. Following each deposition, the slides were allowed to air dry for approximately 45 minutes to ensure the formation of a visible residue. For the blank samples ('B,' 'C,' and 'D'), specific solvents were applied: 'B' received universal buffer, 'C' received deionized sterile water, and 'D' received dilution buffer. Notably, Group A was excluded from testing due to spatial constraints on the EPMA slide and its utilization of the same solvent as Group B. Finally, the samples were subjected to analysis using the EPMA to characterize their elemental composition and distribution.

EPMA Results:

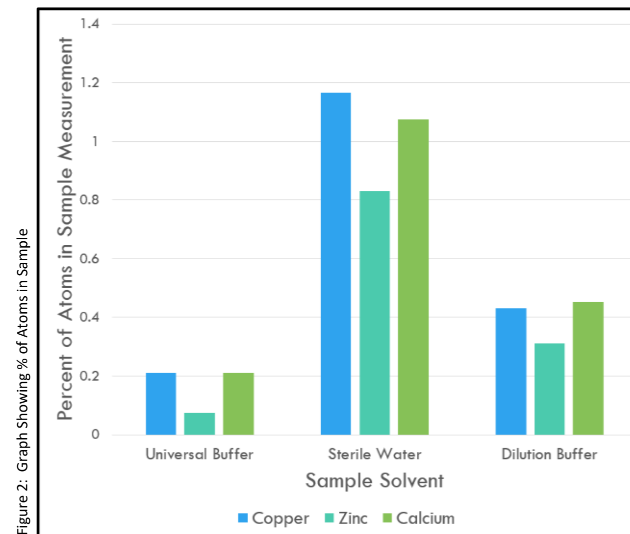


Figure 2: Graph Showing % of Atoms in Sample

This graph visually represents the percentages of atoms recovered in samples, providing a comprehensive illustration of the distribution and efficacy of the recovery process across various experimental conditions and solvents.

Discussion:

In conclusion, the analysis revealed that each sample consistently recovered a higher concentration of Cu^2+ ions compared to Zn^2+ ions, albeit with varying ratios observed among the solutions tested. Notably, deionized recovered six times more bivalent cations than RSID buffer and 1.6 times more than TE dilution buffer. These findings underscore the importance of solvent selection in optimizing the recovery of bivalent cations. Since sterile water is typically used in swabbing techniques employed by local forensics units, the reconsideration of swabbing solution may reduce recovery of bivalent cations and increase the viability of DNA evidence in such firearms cases.

Future Research:

Currently, replicate studies are underway to validate and expand upon the preliminary findings presented herein. It's noteworthy that a transition occurred from utilizing female control DNA to male-specific DNA, driven by emerging research highlighting the disproportionate involvement of males in firearm-related crimes. This adjustment reflects a commitment to aligning the research with contemporary insights into criminal demographics. Moving forward, the next phase of the research will involve conducting polymerase chain reaction (PCR) assays, coupled with strategies for mitigating PCR inhibitors. Subsequently, the collected DNA samples will undergo analysis to generate comprehensive DNA profiles, ultimately contributing to a deeper understanding of the forensic implications surrounding firearm-related incidents.

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