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Optimization of touch DNA collection from fired cartridge casings using the M-VAC cell collection system

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Thesis

**OPTIMIZATION OF TOUCH DNA COLLECTION FROM FIRED
CARTRIDGE CASINGS USING THE M-VAC CELL COLLECTION SYSTEM**

by

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ABSTRACT

Brass and nickel-plated fired cartridge casings (FCCs) are among the most common type of evidence encountered by crime agencies and are commonly collected for touch DNA. While the collection of touch DNA on both of these FCCs has been shown to have low success rates, brass FCCs appear to pose more difficulties due to the presence of copper ions that are known to have degradative and inhibitory effects on downstream DNA analysis.

The microbial vacuum (M-VAC®) cell collection system is a sterile wet vacuum device that uses buffer to agitate a sample in order to more effectively loosen the cells that were deposited on the surface of an item. A new method known as the Bardole method™, which utilizes the vacuum filtration feature of the M-VAC®, has been proposed as a collection method for touch DNA on FCCs.

The purpose of this research is to optimize method collection of touch DNA from FCCs using the M-VAC®. Four methods, including the Bardole method™, that incorporate the use of the vacuum filtration are compared to assess the relative successes in collecting touch DNA from brass and nickel-plated FCCs. Chelating-agents that to bind copper ions and reduce the degradative and inhibitory effects on DNA analysis were also assessed. The three remaining methods incorporate one of the following chelating agents each: Chelex®

Beads, Glycine-Glycine-Histidine (GGH) protein, and Ethylenediaminetetraacetic acid (EDTA).

Results indicated that neither the EDTA nor Chelex® methods were effective in collecting touch DNA from nickel-plated and brass FCCs. Both the Bardole method™ and GGH method demonstrated comparable results when being used to collect saliva from FCCs and touch DNA from fired and unfired cartridge casings. One touch DNA sample collected by the Bardole method™ rendered a full and complete profile from one nickel-plated FCC. A further modification of the GGH method demonstrated increased success and resulted in the generation of one extensive partial profile for nickel-plated FCCs.

The Bardole method™ yielded the best overall results when performing DNA collection of touch DNA on FCCs. However, comparisons of DNA concentrations, degradation and the relative DNA recovery efficiency values indicated that the GGH method demonstrated more potential to successfully collect touch DNA from brass FCCs. Thus, further research is required to determine whether the incorporation of a chelating agent, namely the GGH protein, should be incorporated into the collection method for FCCs to maximize success.

TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	iv
ABSTRACT.....	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xi
LIST OF FIGURES.....	xii
LIST OF ABBREVIATIONS.....	xiv
1. INTRODUCTION	1
1.1 Collection and Analysis of Touch Deoxyribonucleic Acid (DNA)	1
1.2 Microbial Vacuum.....	4
1.3 Collection and Analysis of Touch DNA on Fired Cartridge Casings	5
1.3.1 Relevance in Forensic Casework.....	5
1.3.2 Current Collection Methods for Touch DNA on FCCs.....	5
1.3.3 Effect of the Firing Process on Touch DNA Recovery.....	9
1.3.4 Challenges in the DNA Analysis of Touch DNA for FCCs.....	10
1.4 Incorporation of Chelating agents.....	11
1.5 Purpose of Study.....	13
2. MATERIALS AND METHODS	15
2.1 Sample Preparation and DNA Deposition.....	15
2.1.1 Pre-DNA Deposition.....	15
2.1.2 Deposition of Known Concentrations of DNA: Dilute Saliva.....	15

2.1.3 Deposition of Unknown Concentrations of DNA: Touch DNA.....	16
2.1.4 Firing and Collection of Brass and Nickel-plated Cartridge Casings....	16
2.2 DNA Collection.....	17
2.2.1 Bardole Method™.....	18
2.2.2 GGH Method.....	19
2.2.3 EDTA Method.....	20
2.2.4 Chelex Method.....	22
2.3 Post-DNA Collection.....	23
2.4 DNA Extraction.....	23
2.4.1 Reference Samples.....	23
2.4.2 Experimental Samples.....	24
2.5 DNA Quantification.....	24
2.6 DNA Amplification and Capillary Electrophoresis.....	25
2.7 Profile Analysis.....	25
2.7.1 Calculation of Extraction Efficiency.....	26
2.8 Statistical Analysis.....	26
3. RESULTS	27
3.1 Experiment 1: Comparison of the DNA Collection Methods in Recovering DNA from Known Concentrations Across Brass and Nickel-plated FCCs.....	27
3.1.1 Assessment of Contamination.....	27
3.1.2 Average DNA Concentration.....	27
3.1.3 Degradation Index (DI).....	29

3.1.4 DNA Recovery Efficiency.....	30
3.1.5 Average RFUs.....	31
3.1.6 Forensic Application.....	33
3.2 Experiment 2: Comparison of the DNAs Collection Method in Recovering Touch DNA Across Brass and Nickel-plated FCCs.....	35
3.2.1 DNA Concentration.....	35
3.2.2 Assessment of Contamination.....	37
3.2.3 Forensic Application.....	38
3.2.4 Degradation Index.....	41
3.3 Experiment 3: Comparison of the DNA Collection Methods in Recovering Touch DNA For Brass and Nickel-plated FCCs and UCCs.....	42
3.3.1 Average DNA Concentration.....	42
3.3.2 Degradation Index	44
3.3.3 Assessment of Contamination	45
3.3.4 Comparison of Average RFUs by Donor 1.....	46
3.3.5 Forensic Application.....	47
3.4 Experiment 4: Modifications to Improve DNA Collection Efficiency of the Methods.....	49
3.4.1 Incorporation of the Cone Method.....	49
3.4.2 Incorporation of the Consecutive Vortex for the GGH Method.....	51
4. DISCUSSION.....	54
4.1 Presence of Contamination.....	54

4.2 Relative Success of the DNA Collection Methods for FCCs.....	56
4.3 Effect of the Firing Process.....	62
4.4 Forensic Relevance.....	66
4.5 Assessment of the Modifications to the Bardole™ and GGH Methods.....	68
5. CONCLUSIONS.....	70
5.1 Evaluation of the Bardole™, GGH, EDTA, and Chelex® DNA Collection Methods.....	70
5.2 Future Considerations.....	72
APPENDIX.....	74
Appendix A. Amount of DNA amplified (ng) for all FCC and UCC samples.....	74
LIST OF JOURNAL ABBREVIATIONS.....	76
BIBLIOGRAPHY.....	77
CURRICULUM VITAE.....	82

LIST OF TABLES

Table 1. Summary of DI values Per Replicate for Saliva Recovered by Each DNA Collection Method.....	29
Table 2. Comparison of DNA Recovery Efficiency for the Four DNA Collection Methods.....	30
Table 3. DNA-Database Eligibility for Each Profile Generated by Each Collection Method.....	34
Table 4. Presence of a Non-Donor Allele in the DNA Profiles Generated from Touch DNA by Collection Method.....	38
Table 5. DNA-Database Eligibility for DNA Profiles from Touch DNA Samples on FCCs Collected by Each Collection Method.....	38
Table 6. Summary of DI Values Per Replicate Collected by Each DNA Collection Method for FCCs.....	41
Table 7. Summary of DI Values Per Replicate Collected by Each DNA Collection Method for UCCs.....	44
Table 8. Presence of a Non-Donor Allele in the DNA Profiles Generated from Touch DNA by Collection Method	45
Table 9. DNA-Database Eligibility for DNA Profiles from Touch DNA Samples on UCCs Collected by Each Collection Method.....	48

LIST OF FIGURES

Figure 1. Schematic of the Bardole Method™.....	9
Figure 2. Average DNA Concentration of Saliva Recovered by Each DNA Collection Method.....	28
Figure 3. Individual and Average RFUs For Saliva by Each DNA Collection Method.....	32
Figure 4. DNA Concentrations Obtained from Touch DNA Samples by Each DNA Collection Method.....	36
Figure 5a. Blue and Green Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using the Bardole Method™.....	39
Figure 5b. Yellow, Red, and Purple Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using the Bardole Method™.....	40
Figure 6. Average DNA Concentration of Touch DNA Recovered by Each DNA Collection Method from FCCs and UCCs.....	42
Figure 7. Average RFU Levels from Touch DNA Samples from Donor 1 By Each Method.....	46
Figure 8. Comparison of the Effect of the Cone Method on The Performance of the Bardole™ and GGH Methods.....	50
Figure 9a. Blue and Green Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using GGH Method with	

Consecutive Vortex..... 52

Figure 9b. Yellow, Red, and Purple Dye Channels from the DNA Profile for
Sample Collected from Nickel-plated Casings Using GGH Method
with Consecutive Vortex..... 53

LIST OF ABBREVIATIONS

BSA	Bovine Serum Albumin
CODIS	Combined DNA Index System
DNA	Deoxyribonucleic Acid
DI	Degradation Index
EDTA	Ethylenediaminetetraacetic Acid
FCC	Fired Cartridge Casing
GGH	Glycine-Glycine-Histidine (Gly-Gly-His)
GSR	Gunshot Residue
mg/mL	Milligrams/Milliliter
mg/ μ L	Milligrams/Microliter
mL	Milliliter
μ L	Microliter
M-VAC	Microbial Vacuum
ng	Nanograms
ng/ μ L	Nanograms/Microliter
PHR	Peak Height Ratio
RFU	Relative Fluorescent Unit
SRS	Surface Rinse Solution
UCC	Unfired Cartridge Casing

1. INTRODUCTION

1.1 Collection and Analysis of Touch Deoxyribonucleic Acid (DNA)

The discovery that each individual possesses a unique DNA fingerprint prompted the use of DNA profiling in forensic casework back in the late 1980s (1). A DNA fingerprint includes a series of loci for which DNA sequences tend to be highly variable from individual to individual (2). DNA profiles showcase the genotypes at these loci. Forensic experts use DNA profiles to make identifications and establish linkages that are able to place persons of interest at a crime scene (3). As a result, it is critical that forensic specialists are continuously utilizing the most reliable and accurate methodologies to collect, preserve, and analyze DNA evidence effectively and efficiently.

The majority of human DNA resides in the cell nucleus (4). Some of the most commonly encountered sources of nuclear DNA found at a crime scene are biological fluids such as blood, semen and saliva (5). Stains comprised of these fluids are often heavily populated with cells, meaning they have sufficient concentration of DNA to generate complete and interpretable profiles (2).

Over the last 40 years, new developments in instrumentation and techniques have allowed DNA analysis to be performed with increased sensitivity (6). Crime laboratories are able to conduct DNA analysis on items of evidence that may contain minute levels of DNA, including samples known as touch DNA or transfer DNA. This source of nuclear DNA is deposited when an individual makes physical contact with an object, resulting in the shedding of skin cells onto the object's surface. The capability to analyze touch DNA

has proven to be extremely valuable, particularly at crime scenes where there may be a lack of biological fluid evidence. High levels of degradation complicate the collection and analysis of touch DNA (7). This combined with a lack of visibility of skin cells with the naked eye means that the amount of DNA recovered from touch DNA is oftentimes significantly lower than that recovered from biological fluids due to a smaller population of viable cells contained within this source (8,9).

In many cases, only partial profiles can be generated from touch DNA samples due to the lower concentration of DNA being available in this source. Nonetheless, if a sufficient quantity of skin cells is collected from a surface, it is still possible to obtain a complete and interpretable DNA profile (2). There is a need for optimization of the collection methods that are currently being employed in forensic examination for touch DNA. These methods need to be made more efficient and effective in maximizing the recovery of skin cells in order to increase the likelihood of obtaining a full and accurate DNA profile.

In forensic investigations where there are no known persons of interest or reference profiles to compare to the evidence profile, it can be very useful to be able to upload the profile to a DNA database such as CODIS (Combined DNA Index System). However, profiles have to meet certain conditions in order to be admissible to such a database. The CODIS database comprises of local, state and national DNA databases and a profile will originate at the local level and move up towards the national level if certain criteria are satisfied (9). In most cases, profiles are admissible to local and state level databases if they are classified as interpretable, with typically at least twelve or more autosomal alleles

detected (10). To move up to the national level, which allows for the most extensive search, the profile must have allele calls at the thirteen core loci outlined by the FBI (9).

An individual's shedder status refers to how well they are able to slough off skin cells and deposit genetic material upon making contact with a surface (11). One study showed that there is both intra-individual and inter-individual variability and that one's shedder status is subject to changing depending on conditions (12). Another study demonstrated that there is a degree of reproducibility that can be associated with an individual's shedder status (13). Therefore, the shedder status of an individual may vary and can lie on a spectrum ranging from 'good' to 'intermediate' to 'poor' (12-14).

Secondary transfer of DNA may occur if an individual has traces of other sources of DNA on their hand when they touch a surface (11). Goray et al. reported the high frequency with which the genetic material of the non-depositor was detected as a minor contributor in the depositor's DNA profile. This indicates that a mixed profile with an unrelated individual may result during the analysis of touch DNA sample from a crime scene. It has also been demonstrated that in cases where the depositor is a poor shedder, the non-depositors profile could be detected as a major contributor (15,16). In either case, this may serve to complicate crime scene investigations.

The type of collection method that may be utilized when collecting touch DNA is dependent on the type of surface on which the DNA has been deposited. Swabbing (using a wet or dry swab), cutting and tape lifting are three of the commonly performed collection techniques that crime scene technicians employ when collecting touch DNA (7,17). While these techniques have proven to be successful in collecting large amounts of DNA from

smooth and non-porous surfaces, DNA recovery efficiency is much lower for substrates that are rough and porous (18). Thus, alternative methods are needed in order to maximize the collection of touch DNA from rough and porous substrates.

1.2 Microbial Vacuum

The Microbial Vacuum (M-VAC®) cell collection system is an instrument that is being used as an alternative DNA collection method in forensic casework. The M-VAC® is a sterile wet vacuum device that first physically agitates a sample in order to loosen cells that were deposited on the surface of the item before collecting them (19). This allows for improved efficiency in collecting cells from rough and porous surfaces and allows for the collection of cells that may have been too difficult to reach and collect via swabbing (20,21). The M-VAC® has been shown to outperform wet swabbing on porous surfaces with an average DNA yield that was approximately twelve times as high (22). Similarly, another study demonstrated that the M-VAC® showed a 75% increase in DNA yield in comparison to swabbing on rough surfaces such as bricks (23).

The M-VAC® utilizes a phosphate-based collection buffer known as surface rinse solution (SRS) buffer that is sprayed onto the item through the attached nozzle. While the item is being sprayed, the M-VAC® simultaneously vacuums this buffer into a sterile collection container. The collection buffer that is being vacuumed should consist of the loosened cells that were once deposited on the item. This buffer is then poured through a concentration filter, isolating the cells on its surface and allowing the collection buffer to permeate through into a waste collection bottle (24). This system can also be adapted with an optional pre-filter, which serves to prevent additional contaminants exceeding 0.40

microns, that may have also been loosened and vacuumed from the item, from being collected onto the filter (25).

1.3 Collection and Analysis of Touch DNA on Fired Cartridge Casings

1.3.1 Relevance in Forensic Casework

In 2018, there were approximately 14,000 homicides committed using a firearm and approximately 470,800 recorded cases where the use of a firearm resulted in a non-fatal injury of a person aged 12 or older across the United States of America. In the majority of these cases, a handgun was used to commit the acts of violence (26). Fired cartridge casings (FCCs) and unfired cartridge casings (UCCs) are often recovered at crime scenes involving a shooting and are therefore among the most common types of evidence encountered by crime agencies (27). Cartridge casings that hold projectiles are likely to have been handled by the shooter, particularly when loading the projectiles into a magazine. As a result, there may be touch DNA that could be recovered from FCCs and used to help establish linkages between a potential shooter and the crime scene (17).

1.3.2 Current Collection Methods for Touch DNA on FCCs

There are several methods that have had limited success when being used to collect touch DNA from FCCs. A study by Horsman-Hall et al. using the standard double-swabbing technique to collect touch DNA from FCCs showed up to 22% of alleles were detected in the resulting DNA profiles. In this method, one swab was moistened with ultrapure water and rubbed against the exterior surface of the FCC and a subsequent dry swab was then used to collect any residual DNA remaining on the exterior surface. Since only known ‘good shedders’ were handling the FCCs during the experiment, the low

recovery indicates that there is likely a significant portion of DNA that is not being collected (28). While FCCs appear smooth, in actuality their surface consists of microscopic markings that may trap deposited cells. This furrowed surface makes it difficult to employ the swabbing technique as the swabs are unable to reach into these grooves to pick up the cells (29). Moreover, there is published data showing that touch DNA has a propensity to be trapped in the complex matrix of the swab, resulting in a reduced DNA recovery efficiency (30,31). Despite the fact that there are known limitations when utilizing swabs to collect trace DNA, swabbing is still the most commonly utilized method to collect touch DNA evidence. Factors such as low cost, ease of use, minimal required training and the existence of several validated DNA extraction methods that can process swabs all make the use of these collection devices more appealing to law enforcement agencies (32). Thus, there is a need for increased research into alternative collection methods for recovering touch DNA from FCCs.

Bille et al. developed a 'rinse-and-swab' method to recover touch DNA from fired cartridge casings (33). This method consists of initially rinsing the exterior of the FCC with a very small volume of solution followed by swabbing it to collect any residual liquid and cells that may still be on the surface. Both the collected rinse solution as well as the swabs are then combined and undergo DNA extraction and analysis. A comparison between the double-swabbing method and rinse-and-swab method indicated that there was improved DNA recovery efficiency with the rinse-and-swab method though there was a still a significant percentage of the handler's DNA that was not detected (33).

Directly soaking the FCC in a lysis buffer followed by a subsequent dry swabbing of the FCC has also been explored as a potential method to collect touch DNA from FCCs. Dieltjes et al. proposed this method explaining that soaking the FCC in Buffer ATL, which is a tissue lysing buffer, would help access and dislodge a larger number of cells as well as lyse them to release the DNA from the cells into the solution. The results of that study demonstrated a 7.1% success rate in obtaining interpretable profiles from FCCs (34).

A comparison study evaluating the soaking method to swabbing methods in the recovery of touch DNA from cartridges demonstrated that both types of methods performed equally well. Elwick et al. noted the presence of oxidation when the casings were soaked in the lysis buffer due to copper ions from the casing surface being released into the buffer and suggested this would have destructive and degradative effects on downstream DNA analysis (35). To resolve this issue in another study researchers created their own lysis buffer, which did not include Buffer ATL. They demonstrated increased success rates with the soaking method as compared to the double-swabbing method, and were able to generate interpretable profiles for 26.1% of the samples (36). However, there are limitations associated with this method that have to be addressed. Firstly, there is the possibility of metal ions and surface debris from the FCC contaminating the solution, which may have degradative and inhibitory effects on downstream DNA analysis (29). Furthermore, additional forensic examinations such as ballistic and fingerprint analysis may no longer be possible since soaking the FCC in a lysis buffer may erode surface markings as well as wash off any lipid and sebaceous secretions.

One of the most recently developed methodologies to collect touch DNA from FCCs utilizes the vacuum filtration feature of the M-VAC®. This method was developed by West Jordan Utah Police Department's crime scene analyst Francine Bardole in collaboration with M-VAC® Systems Inc and is known as the Bardole method™ (29,37). A schematic of the Bardole method™ is shown in Figure 1. Instead of simultaneously spraying and vacuuming the FCC using the instrument's nozzle, this method involves first adding the FCC to a sterile container with enough SRS collection buffer to completely submerge the casing. The container is then vortexed to agitate and loosen the cells from the casing into the collection buffer, which is then poured through the concentration filter cup while the vacuum is turned on. While pouring, the FCC should not fall onto the concentration filter since this could compromise the filter structure. The vacuum pressure causes the collection buffer to seep through the filter while trapping any cells onto the filter. The eluted buffer is then poured back into the sterile container with the FCC and another vortex and subsequent vacuum step is performed to maximize the amount of touch DNA being recovered. A third vacuum step with only the eluted buffer from the second vacuum step and without the FCC is then conducted to collect any residual cells that may not have been successfully trapped by the filter or that were left behind in the sterile container. The concentration filter is then left to dry in a sterile environment for at least 24 hours before it is cut out of the filter cup and DNA extraction can ensue (37).

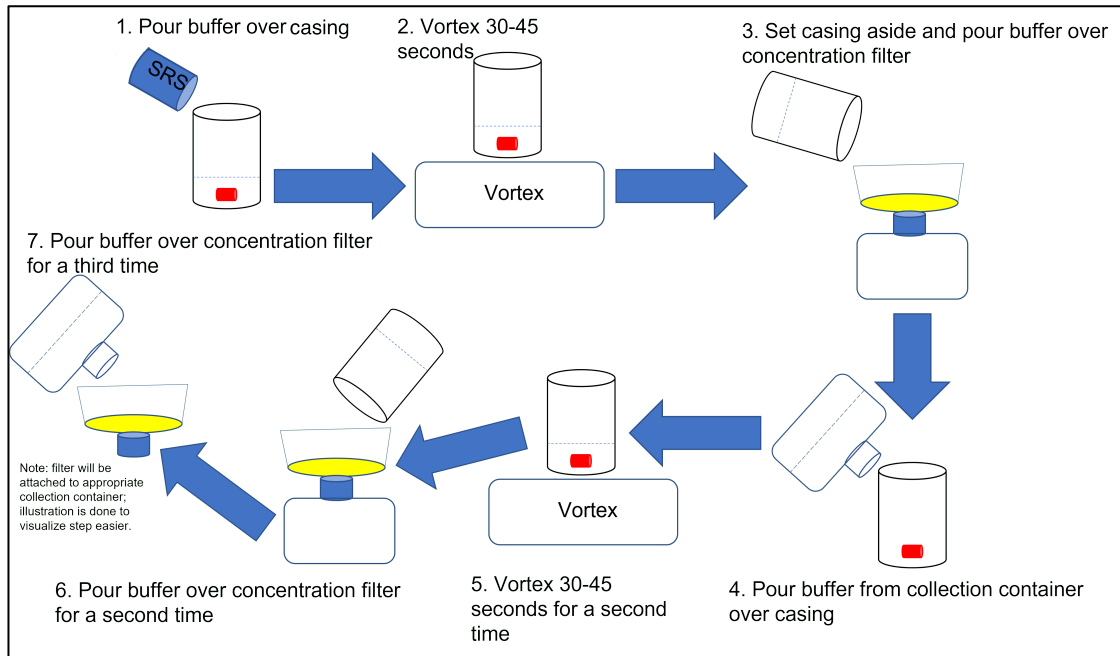


Figure 1. Schematic of the Bardole Method™

It is thought that the high pressure and use of agitation steps in this method help to loosen and collect even those cells that may be embedded deeper within the surface markings of the FCC. Additionally, while this method is more labor-intensive and requires formal training, it does not result in as significant an erosion of the ballistically relevant markings or leaching of metallic ions off the FCC (29).

1.3.3 Effect of the Firing Process on Touch DNA Recovery

Cartridges experience large amounts of force and heat during the gun firing process, which are factors that can denature and degrade DNA. In addition, the firing process causes the surface of the cartridge casing to be covered in gunshot residue (GSR) consisting of organic species such as soot, nitrates, nitrites, as well as metallic species such as lead, barium and antimony found in primer gunshot residue (pGSR) (38). While one study demonstrated that the presence of metallic species did not lead to lower quantity or quality

of DNA yield on fired versus unfired cartridge casings, it is still unknown whether the organic components of GSR have a degradative effect on recovery of touch DNA on cartridge casings (39). Several studies have been conducted to compare the DNA recovery efficiency of UCCs to those of FCCs and have demonstrated inconsistent results. One study found that FCCs generated a 33% lower DNA yield than UCCs (35). In contrast, another study asserted that the temperatures reached during the firing process were not adequate to cause damage to the touch DNA, concluding insignificant differences in the DNA yield between UCCs and FCCs (40).

1.3.4 Challenges in the DNA Analysis of Touch DNA for FCCs

Brass cartridge casings and nickel-plated cartridge casings are two types of cartridge casings commonly encountered in forensic casework (33). Historically, there has been a low success rate in obtaining clear and interpretable DNA profiles from touch DNA on recovered FCCs, which has discouraged many agencies from performing DNA analysis on such items (33,35). It has been hypothesized that this can be largely attributed to the DNA-binding affinity of copper ions, which when bound to DNA result in disruption of the double helix structure and generate reactive oxygen species. The resulting oxidative damage generated may result in degradative effects to DNA as well as inhibitory effects during downstream DNA analysis, particularly in the polymerase chain reaction (PCR) amplification stage (29,33). Since brass is an alloy of copper and zinc, a large population of copper ions is present in brass cartridges (29). In comparison, nickel-plated cartridges have an exterior coating made of nickel. Both nickel and copper have a high binding affinity to DNA, however copper also demonstrates additional inhibitory effects on PCR

(33,41). In the study by Elwick et al., DNA recovery efficiency of touch DNA on brass cartridge casings was more than five times lower than that of nickel-plated cartridge casings (35). Thus, there is much more difficulty in obtaining interpretable profiles from touch DNA on brass versus nickel-plated cartridge casings.

1.4 Incorporation of Chelating Agents

There have been several attempts to optimize the collection of touch DNA on FCCs through the addition of a chelating agent. The purpose of a chelating-agent is to help mitigate and minimize the degradative and inhibitory effects of heavy metals on DNA recovery by binding to the metal ions (42). This bond will help to block the metal ions from being able to bind to DNA. As a result, the metal ions will be filtered out during DNA collection, preventing any degradative downstream effects on DNA analysis (27,35).

There are several studies demonstrating an improved DNA yield when employing the use of chelating agents to collect touch DNA from FCCs. The rinsing solution used in one study used to perform a rinse-and-swab DNA collection method on FCCs incorporated the use of a mixture called 'BTmix'. This consisted of the tripeptide protein Glycine-Glycine-Histidine (GGH) as well as Bovine Serum Albumin (BSA). In a prior study, the addition of BSA during DNA extraction was shown to promote an increased amount of DNA recovery (33). This was attributed to the chemical composition of BSA, which contains a GGH molecule. GGH is a chelating agent that is able to bind and form complexes with copper ions, potentially diminishing the degradative effects of oxidative stress to DNA. Thus, Bille et al. formulated a rinsing solution that includes BTmix in order to reduce the damaging effects caused by copper ions on touch DNA deposited on FCCs,

particularly brass cartridge casings. Results of this study demonstrated that when BTmix was incorporated in the rinsing solution, there was an increased amount of DNA recovery.

In another study, researchers utilized the double-swabbing method and used an ethylenediaminetetraacetic acid (EDTA) solution to moisten the wet swab before rubbing it against the exterior surface of the FCC (27). EDTA has chelating abilities that allow it to bind to heavy metal ions, such as those in copper, and form stable complexes. Through this mechanism, EDTA is able to reduce the degradative impact to touch DNA on brass cartridges caused by the presence of copper ions and promote increased DNA yield during DNA collection.

Lastly, the Broward County Sherriff's Office developed a vacuum filtration method for collecting touch DNA from FCCs by including a step to initially wash the FCCs with Chelex® 100 Resin (43). Chelex® beads have an increased affinity to bind to transition metal ions such as that of magnesium or copper. This chelating property of the Chelex® beads was first exploited to develop an efficient DNA extraction method since the beads were able to chelate magnesium ions effectively, helping to lessen DNA degradation in downstream DNA analysis (44). As a result, researchers at Broward County incorporated the use of Chelex® beads to chelate to the copper ions in brass cartridge casings and reduce the deleterious effects of these ions on downstream DNA analysis. Results indicated an increased DNA yield using this method when collecting touch DNA from brass cartridge casings (44).

1.5 Purpose of Study

Many agencies, including the Worcester Police Department Crime Scene Unit (WPD CSU), are exploring the use of the M-VAC® as an alternative method of collecting touch DNA from FCCs. The current standard operating procedures (SOPs) of the WPD CSU instruct their examiners to use the swabbing method to collect touch DNA on FCCs that were previously processed for latent prints. When latent print processing has not been performed, the WPD CSU uses the Bardole method™ for the recovery of touch DNA from FCCs, incorporating the use of the M-VAC®. Presently, the WPD CSU only attempts to collect touch DNA from FCCs if these were recovered from a crime scene classified as a homicide or as a serious assault and battery with a dangerous weapon. Thus, the purpose of this study is to optimize the method of collecting touch DNA from FCCs using the M-VAC® cell collection system with the goal of improving DNA recovery efficiency, possibly enabling touch DNA analysis on FCCs to expand to less serious crimes.

In this study, the Bardole method™ was compared to three other vacuum filtration methods that were developed and adapted using the general framework of the Bardole method™. Each of these three alternative methods employ the use of a different chelating agent as well as a few procedural differences. One goal of this study was to determine whether the addition of a chelating agent is a modification that should be made to optimize the DNA collection process of FCCs using the M-VAC®. The DNA recovery efficiencies of these methods were initially evaluated using dilute saliva with known levels of DNA concentrations to assess general trends in DNA recovery on FCCs. The success rates of the methods were then compared using touch DNA in order to simulate real forensic casework.

Each method was performed on both brass and nickel-plated cartridge casings to determine the effect of copper ions on the DNA recovery efficiency.

Another goal of this study was to explore the effect of the firing process on the DNA yield generated by each of the four methods. This was accomplished by performing each DNA collection method on both fired and unfired brass and nickel-plated cartridge casings.

Lastly, an additional aim of this study was to optimize DNA collection on FCCs by incorporating or modifying different procedural elements depending on the success rates of each of the methods being evaluated. Factors such as feasibility in the laboratory, cost, time and the ease of performing the method were considered when assessing how to optimize the methods.

2. MATERIALS AND METHODS

2.1 Sample Preparation and DNA Deposition

2.1.1 Pre-DNA Deposition

Unfired 9-millimeter luger projectiles with brass or nickel-plated cartridge casings (Winchester®, East Alton, Illinois) were exposed to ultraviolet radiation for approximately 20 minutes while keeping them in the trays from their original packaging. Next, the cartridges were flipped onto their headstamp and exposed to another 20 minutes of ultraviolet radiation. The trays of cartridges were then placed and packaged into paper evidence bags. The bags were tape-sealed to prevent contamination prior to DNA deposition.

2.1.2 Deposition of Known Concentrations of DNA: Dilute Saliva

All biological samples used in this study were provided by volunteers in accordance with an approved Institutional Review Board protocol.

For *Experiment 1*, human saliva containing a known amount of DNA was deposited onto 40 rounds each of the brass and nickel-plated cartridges. A male donor (Donor 3) provided neat liquid saliva in a 2 milliliter (mL) microcentrifuge tube. A 1:10 dilution of the saliva was made, and 100 microliters (μL) was deposited onto the headstamp of each of the 80 rounds, which were then placed in a laminar flow hood and allowed to dry for 24-48 hours. A total of approximately 34 ng of DNA was deposited on each headstamp using this method. Ten rounds of brass cartridge casings and ten rounds of nickel-plated cartridge casings were subjected to each of the four DNA collection methods being compared. There were two replicates per casing type per DNA collection method and each

replicate consisted of five (of the ten) rounds of either the brass or the nickel-plated cartridge casings. As a result, each replicate in *Experiment 1* had a deposition of approximately 170 ng of DNA.

2.1.3 Deposition of Unknown Concentrations of DNA: Touch DNA

For *Experiments 2, 3* and *4*, success of the four different DNA collection methods was compared using unknown quantities of DNA deposited through touch DNA. Three male donors (D1, D2, D3) were asked to handle 40 rounds of each of the two cartridge types (i.e. each donor handled a total of 80 rounds). Three donors were asked to participate in order to account for variability in shedder status. To prevent secondary transfer events, donors were instructed to wash their hands prior to beginning the handling and not touch any other item until they were finished handling all of the rounds. Each donor handled two or three rounds of the same type of casings in each hand for 15 seconds by rotating them around in the palm of their hand and then placed these rounds back into the projectile trays. Donors were instructed to wait for a period of 15 minutes prior to handling each new set of rounds.

2.1.4 Firing and Collection of Brass and Nickel-plated Cartridge Casings

The third step of sample preparation that was conducted for FCC samples consisted of loading the projectiles into magazines. Magazines were disinfected with a germicidal cloth prior to being loaded. Each magazine held ten rounds of the same type of projectiles. For the rounds containing saliva deposits, loaders were asked to wear two sets of gloves and masks when loading the projectiles in order to prevent contamination. Loaders were also instructed to avoid touching the headstamp of the projectiles so as not to accidentally

remove the cells that had been deposited. For the rounds containing touch DNA deposits, donors were asked to load the cartridges they had handled into the magazines themselves. Loaded magazines were packaged in paper evidence bags and tape-sealed.

An outdoor shooting range was set up to shoot and collect the FCCs. All cartridges were shot using military and police-grade 2.0 chamber .45 automatic handguns (Smith & Wesson, Maryville, TN) issued to officers at WPD. A white tarp was set down to the right of the shooter and covered the area where the FCCs were projected to land. Prior to shooting, the tarp was wiped down with a germicidal cloth. After each magazine (containing ten rounds) had been completely fired, the FCCs were collected in manila evidence envelopes. Collectors adorned double-gloves and facial masks to prevent any contamination. The FCCs were collected in groups of five since each replicate consisted of five of the same type of FCC. The envelopes were brought back to the laboratory where one of the four DNA collection methods being compared was used to perform DNA collection.

2.2 DNA Collection

This study compared the DNA recovery efficiency of four different DNA collection methods that all utilize or were adapted to utilize the M-VAC® (M-VAC® Systems Inc, Sandy, UT). All M-VAC® equipment including the SRS buffer, sterile collection container, the pre-filter cone, the pre-filter shield, the vacuum hose and tubing, the concentration cup (filter) and the collection bottle were purchased from M-VAC® Systems Inc. (37). For each method, two replicates of DNA collection were performed (five FCCs/replicate) per cartridge casing type (brass or nickel-plate) per donor (three donors).

New M-VAC® equipment was used for each new replicate type. Touch DNA from five FCCs was collected onto each filter in order to account for the historically low success rate in obtaining sufficient DNA yields to obtain interpretable profiles (33,35). This was done to increase chances of DNA recovery so that the methods could be evaluated for comparison purposes.

2.2.1 Bardole Method™

Approximately 40 mL of SRS buffer was measured in a graduated cylinder. This was poured into the M-VAC® sterile collection container and an FCC from each replicate was placed into the container using sterile tweezers. The lid of the container was screwed on and then the contents of the container were vortexed using the Vortex-Genie 2 (Scientific Industries, Bohemia, NY) for approximately 45 seconds with the dial turned to 5. The concentration filter was attached to the vacuum hose, and the vacuum was turned on. The SRS buffer in the sterile collection container was poured onto the filter, using sterile tweezers to prevent the FCC from landing on the filter. The vacuum stayed on until all the liquid eluted into the collection bottle. The collection bottle was unscrewed from the collection cup and the eluted liquid was poured back into the sterile collection container with the FCC. The container was vortexed using the same parameters and the liquid was poured over the concentration filter for the second time. This step was added to collect any cells that may not have been released from the FCC in the first round of vortex agitation. Next, the FCC was placed aside, and the collection bottle was unscrewed and used to pour the eluted liquid into an empty sterile container. This was poured over the concentration filter for a third time to capture any cells that were not retained on the filter after the first

two pours. This marked the end of the DNA collection from the first FCC in each replicate. The total volume of buffer in the collection bottle was then discarded. All steps were repeated for each of the subsequent four FCCs using the same M-VAC® equipment. At the end of the collection of the fifth FCC in the group, a small volume of SRS buffer was sprayed (using the nozzle) into the sterile collection bottle and the cap in order to collect any residual cells that may have been left on these surfaces. This small volume was then poured onto the filter while the vacuum was turned on.

2.2.2 GGH Method

The GGH method was adapted from a rinse-and-swab method developed by the Bille et al. and the Bureau of Alcohol, Tobacco, Firearms and Explosives to collect touch DNA from FCCs (33). The collection solution for this method consists of a tissue lysis buffer (Buffer ATL) (QIAGEN, Hilden, Germany) and BTmix, which is a mix of 2 milligrams/microliter (mg/μL) UltraPure™ bovine serum albumin (BSA) (Thermo Fisher Scientific, Waltham, MA) and 62.5 mg/μL GGH tripeptide (Sigma-Aldrich, Burlington, MA). To make approximately 1.6 mL of BTmix, a 50 milligrams/milliliter (mg/mL) BSA solution was combined with a 125 mg/mL GGH solution. For the BSA solution, 64 μL of BSA was added to 736 μL of nuclease-free water. For the GGH solution, 100 milligrams of solid GGH tripeptide was dissolved in 743 μL of nuclease-free water. For each replicate, 50 mL Falcon® conical tubes (Corning Inc., Corning, NY) were prepared containing 2 mL Buffer ATL and 30 μL of BTmix.

An FCC from each replicate was placed into the 50 mL conical tube using sterile tweezers and vortexed using the Vortex-Genie 2 for 45 seconds with the dial at 5. The pre-

filter device, which consists of a pre-filter tube attached to the pre-filter shield, was attached to the vacuum hose. With the vacuum on, the collection buffer was poured onto the pre-filter device, using sterile tweezers to try to prevent the casing from falling onto the pre-filter. This step was intended to filter out any contaminants including the GGH-metal ion compounds. The vacuum hose was then attached to the concentration cup and the eluted liquid in the pre-filter tube was poured onto the concentration cup filter with the vacuum turned on. The eluted liquid was poured back into the tube of the pre-filter device. An additional 1 mL Buffer ATL was added to the conical tube with the FCC and vortexed using the same parameters in order to collect any loose cells that may not have been collected in the first round. This buffer was then poured through the pre-filter device while the vacuum was turned on. The vacuum hose was then attached to the concentration cup filter and the total collection buffer in the pre-filter tube was poured onto the concentration cup filter while the vacuum was turned on. The total liquid in the collection bottle was then poured back through concentration cup filter for a third time, taking care to switch the vacuum on only when the collection bottle was screwed to the bottom of the concentration cup. This was to retain any cells within the collection buffer that were not retained onto the filter in the first two vacuum steps. All steps were repeated for each of the subsequent four FCCs using the same M-VAC® equipment. A new initial conical tube with the BTmix was used for each new FCC.

2.2.3 EDTA Method

For the EDTA method, a 0.5 molar EDTA solution at pH 8.0 collection buffer was prepared. A volume of 1000 mL of EDTA solution was prepared by adding 186.12 grams

of EDTA powder (Sigma-Aldrich) to 800 mL of deionized water. Approximately 5 grams of sodium hydroxide (Sigma-Aldrich) was added and the solution was placed on a heated magnetic stir plate (Thermo Fisher Scientific) to dissolve the EDTA completely. A digital pH meter (Denver Instrument, Inc., Göttingen, Germany) was used to measure the pH level of the solution and Sodium Hydroxide was added until pH reached 8.0 ± 0.4 and the EDTA powder was completely dissolved. The solution was poured into a graduated cylinder and deionized water was added to reach the desired volume of 1000 mL.

As in the Bardole method™, approximately 40 mL of EDTA collection solution was poured into the sterile collection container with an FCC from each replicate and then the container was vortexed. As in the GGH method, the pre-filter device was assembled and the EDTA collection solution was poured onto the device to filter out any contaminants including the EDTA-heavy metal compounds. The eluted liquid in the collection bottle was poured back into the sterile collection container with the FCC and contrary to the GGH method, no additional collection solution was added. The container was vortexed using the same parameters as previously done and the liquid was poured over the pre-filter device for the second and third time as in the GGH method. For the third time, the FCC was placed aside and the eluted liquid in the collection bottle was poured into the empty sterile container. This marked the end of the DNA collection from the first FCC in each replicate. The total volume in the collection bottle was then discarded. All steps were repeated for each of the subsequent four FCCs using the same M-VAC® equipment. As in the Bardole method™, at the end of the collection of the fifth FCC in the group, a small volume of EDTA

was poured into the sterile collection bottle and the cap and this small volume was then poured through the filter.

2.2.4 Chelex® Method

The Chelex® method used was adapted from the protocol developed by the Broward County Sherriff's Office (43). The Chelex® 100 Resin (Bio-Rad Laboratories, Hercules, CA) collection buffer was prepared per replicate by dissolving 2 grams of Chelex® 100 Resin in 25 mL of SRS buffer in a 50 mL Falcon® conical tube.

All five FCCs in each replicate were consecutively vortexed (one after the other) in the same conical tube with collection buffer, using sterile tweezers to add and remove the FCCs. As in the Bardole™, GGH and EDTA methods, the same parameters were used to vortex. After the fifth FCC was set aside, as in the GGH and EDTA methods, the collection buffer was poured onto the pre-filter device to filter out any contaminants including the Chelex-heavy metal compounds. Unlike in the GGH and EDTA methods, the eluted liquid in the pre-filter tube was poured onto the concentration cup filter through a sterile glass funnel with the stem of the funnel approximately centered on the filter. The diameter of the opening at the stem of the funnel was approximately 1.1 centimeters. The funnel was pressed down firmly, creating a tight seal and the purpose of introducing the buffer with the funnel was to concentrate the DNA on a smaller surface area of the filter. The vacuum was left on until the solution drained completely through the funnel. Similar to the GGH and EDTA methods, the eluted collection solution was then poured back through the pre-filter device for a second and third time, though it was conducted by incorporating the sterile glass funnel method. The third vacuum step was added as a modification to the

original method to maintain consistency of three pour-throughs across the four methods. Holding the funnel in place, a small volume of SRS buffer was sprayed around the funnel in order to dislodge any cells that may have been stuck to the sides of the funnel. The total volume in the collection bottle was discarded.

2.3 Post-DNA Collection

For each of the four methods, the filters were allowed to dry for 24-48 hours by placing the collection cups under the laminar flow hood. A sterile scalpel was used to cut the filter out of the concentration cup and the filter was cut into four strips. The strips were placed into two 2 mL microcentrifuge tubes; each tube contained two strips as per the Massachusetts State Police Crime Laboratory (MSPCL) protocol (45). The tubes were transported to the DNA Laboratory at the Boston University Biomedical Forensic Sciences Department for DNA typing to be performed.

2.4 DNA Extraction

2.4.1 Reference Samples

Reference samples from each of the three male donors (Donor 1, Donor 2, Donor 3) were extracted using forensicGEM® DNA Extraction kit (MicroGEM Inc., Charlottesville, VA). Liquid saliva samples from each donor were diluted 1:10 and 10 µL of these samples, 34 µL of deionized water, 5 µL of Blue Buffer 10X and 1 µL of Zygem (EA1 Protease) were added to a 2 mL tube. The samples underwent an incubation at 75°C for 15 mins followed by an incubation at 95°C for 5 mins, to activate and deactivate EA1 protease activity. Samples were stored at -20°C immediately following extraction.

2.4.2 Experimental Samples

All samples were extracted on the EZ1 Advanced Robot (QIAGEN) using the EZ1 1&2® DNA Investigator® Kit (QIAGEN) by following the provided protocol (46). Due to the large size of the filters, prior to extraction on the EZ1, the Investigator® Lyse & Spin Basket kit (QIAGEN) and protocol was followed (47). All four filter strips (across the 2 tubes) were tightly combined in the spin basket. The spin basket was placed in the collection tube and 475 µL of G2 buffer and 10 µL of Proteinase K were added to the spin basket. The sample was vortexed for 10 seconds and then incubated for 1 hour at 56°C and 900 rotations per minute. The samples were centrifuged for 2 mins at 14,000 rotations per minute, forcing the liquid to elute through the spin basket and into the collection tube. The spin basket with the dried filter was placed in a new 2 mL tube and stored at -20°C. The eluted liquid (approximately 485 µL) was transferred to an EZ1 sample tube, and 1 microgram of carrier RNA was added to each sample. Following this, 400 µL of MTL buffer (QIAGEN) was added to each sample. The EZ1 Robot was set-up using the user manual and the samples were extracted using the 'Large-Volume' purification protocol and eluted in 50 µL of Tris-EDTA buffer. Samples were stored at -20°C immediately following extraction.

2.5 DNA Quantification

The Quantifiler® Trio DNA Quantification kit (Thermo Fisher Scientific) and the ABI 7500 Real-Time PCR system (Thermo Fisher Scientific) were used to perform DNA quantification on all samples. The manufacturer's protocol was followed and analysis was performed using the concentration values at the small autosomal target (48).

2.6 DNA Amplification and Capillary Electrophoresis

The Globalfiler™ Amplification kit (Thermo Fisher Scientific) and Veriti Thermal Cycler (Thermo Fisher Scientific) were used to perform DNA amplification. The manufacturer's protocol was followed and experimental and reference samples with small autosomal target concentrations of greater than or equal to 0.001 nanograms/microliter (ng/μL) were amplified (49). Due to low levels of DNA across the entirety of the experimental samples, in cases where the ideal DNA amount of 0.75 ng could not be achieved, the maximum volume of undiluted DNA extract of 15 μL was added to the amplification reaction. The SeqStudio™ Genetic Analyzer v2 (Thermo Fisher Scientific) and an injection time of 10 seconds was used to perform capillary electrophoresis. This kit amplifies DNA at 24 loci and 22 of these are autosomal loci.

2.7 Profile Analysis

GeneMapper™ ID-X v1.6 software was used to analyze the data obtained from capillary electrophoresis and generate DNA profiles. The analysis method used was developed by Bregu et al. (50). It applies a dye-specific analytical threshold: 28 relative fluorescence units (RFU) for the loci in the blue panel, 42 RFU for the green panel, 30 RFU for the yellow panel, 77 RFU for the red panel, 89 RFU for the purple panel, and 20 RFU for the orange (size standard) panel.

Each experimental sample DNA profile was compared to the corresponding reference sample and the 22 autosomal loci were analyzed to determine the number of complete and accurate loci, partially-accurate loci, inaccurate loci, and number of loci with no allelic calls. A locus was categorized as “complete and accurate” if all donor alleles

were detected and if the peak height ratio (PHR) between the RFU of non-donor alleles and the RFU of the lowest donor allele was less than 50%. The PHR was not calculated between donor alleles. If the PHR between non-donor alleles and the lowest donor allele exceeded 50%, this was considered an “inaccurate” locus. If only one allele was called for a heterozygous locus, it was designated a “partially-accurate” locus. The average RFU across the 22 autosomal loci was calculated for each experimental DNA profile.

2.7.1 Calculation of Extraction Efficiency

For samples in *Experiment 1*, the DNA recovery efficiency was calculated using Equation 1. For purposes of this study, two reference samples were used to calculate the total amount of DNA deposited. This was calculated to be approximately 170 ng. The total amount of DNA recovered was calculated using the DNA concentration obtained for a 50 μ L volume (EZ1 extraction elution volume).

$$\text{DNA recovery efficiency (\%)} = \frac{\text{Amount of DNA recovered (ng)}}{\text{Total amount of DNA deposited (ng)}} \times 100 \quad (\text{Equation 1})$$

Degradation index values were analyzed according to Quantifiler™ HP and Trio DNA Quantification Kits User Guide by Thermo Fisher Scientific.

2.8 Statistical Analysis

Student’s t-tests were conducted to make comparisons across two variables. The One-Way ANOVA combined with the Honesty Significant Difference Post-Hoc test was conducted in order to make comparisons across three or more variables. A p-value of 0.05 was used to determine significance.

3. RESULTS

3.1 *Experiment 1: Comparison of the DNA Collection Methods in Recovering DNA from Known Concentrations Across Brass and Nickel-plated FCCs*

3.1.1 Assessment of Contamination

For the Bardole Method™ one of the brass replicates displayed an inaccurate profile that demonstrated a mixture, suggesting there may have been contamination during the sample preparation. There were 27 non-donor alleles that were detected in this profile. All other replicates across the four different methods displayed single-source profiles with only the donor's alleles.

A comparison of the RFU levels of the non-donor alleles compared to the donor alleles for sample B-BC1 indicated that those of the non-donor were a major contributor while the donor was a minor contributor. This implies that prior to or during sample preparation or during DNA collection, the casings in this replicate underwent a significant exposure to an outside DNA source.

3.1.2 Average DNA Concentration

Average DNA concentrations recovered from the FCCs indicate that the Bardole method™, the GGH method and the Chelex® method all demonstrated increased success in recovering saliva from the nickel-plated FCCs in comparison to the brass FCCs (Figure 2). Due to contamination detected in one set of the brass FCCs, only the second set of brass FCCs could be considered when evaluating the Bardole method™. The average DNA concentration of the DNA recovered from both nickel-plated replicates using the Bardole method™ was approximately 8.6x higher than that of the brass replicate. This trend was

consistent for both the Chelex® method for which the average DNA concentration was approximately 9x higher and for the GGH method for which the average DNA concentration obtained was approximately 2x higher. The average DNA concentration obtained from brass casings using the GGH method was approximately 3x and 2x higher than that obtained from the Bardole™ and Chelex® methods, respectively.

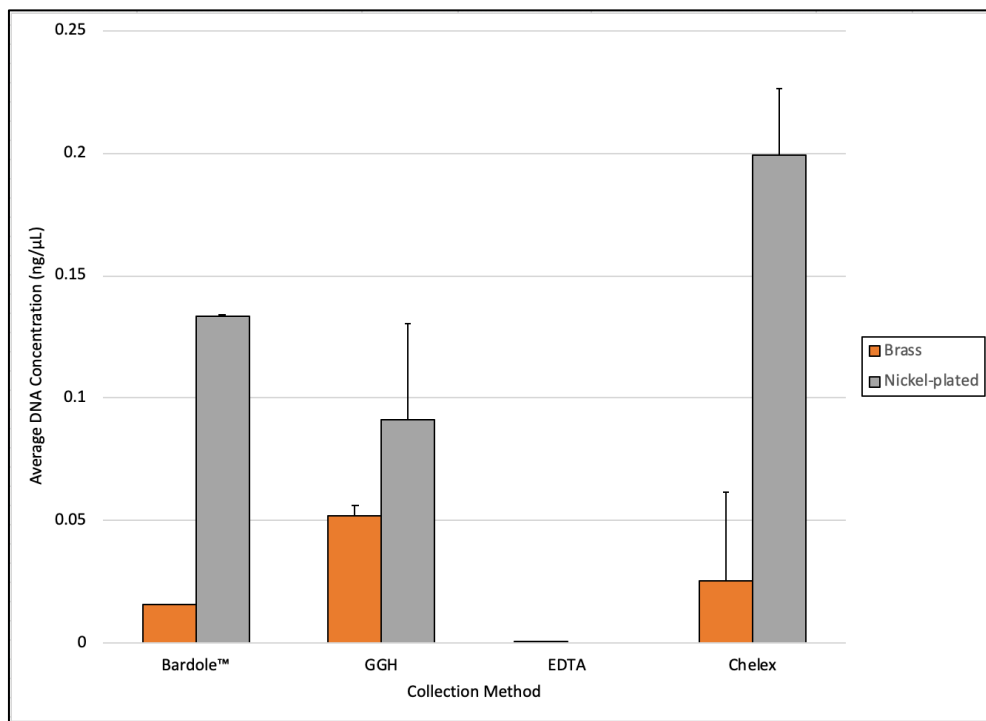


Figure 2. Average DNA Concentration of Saliva Recovered by Each DNA Collection Method. Comparison of average DNA concentrations detected by using Bardole™, GGH, EDTA and Chelex® methods to recover known concentrations of saliva on brass and nickel-plated cartridge casings. Error bars denote one standard deviation.

Results obtained from the EDTA method demonstrated that this method was ineffective at collecting DNA from brass and nickel-plated FCCs. There was no DNA detected for these samples during DNA quantitation.

3.1.3 Degradation Index (DI)

The average DI obtained from brass FCC samples was higher than that obtained from the nickel-plated FCC samples for the Bardole™, GGH and Chelex® methods (Table 1). Particularly for the brass FCC samples, the high DI values exceeding a value of 1 appear to indicate that there was potentially PCR inhibition that was present in these samples.

Table 1. Summary of DI values Per Replicate for Saliva Recovered by Each DNA Collection Method. Comparison of DI values across samples using Bardole™, GGH, EDTA and Chelex® methods to recover saliva on brass (BC) and nickel-plated cartridge casings (NC). (X) denote blank values obtained by the software. Red = samples for which one or more allele call was made.

Cartridge Type	Degradation Index (DI)			
	Bardole™ (B)	GGH (G)	EDTA(E)	Chelex® (C)
BC1	N/A*	2.48	7.31	4.53
BC2	4.74	3.33	X	1.76
NC1	1.55	1.32	X	1.43
NC2	1.28	1.22	X	3.71

The average DIs for obtained from nickel-plated FCCs using both the Bardole™ and the GGH methods were relatively close to the value of 1, which represents minimal levels of DNA degradation. In contrast, the average DI for DNA obtained from nickel-plated FCCs using the Chelex® method was approximately 1.5, indicating a slight increase in degradation. The average DI for brass FCCs observed with the Bardole™ method was approximately 1.5x higher than the average DI for brass FCCs observed with the GGH and the Chelex® methods. For the EDTA method, one brass replicate and both nickel-plated replicates displayed blank values indicating a lack of DNA detected at the small and large autosomal regions for these samples.

3.1.4 DNA Recovery Efficiency

The DNA recovery efficiency values across all four of the collection methods were low. Both the GGH and the Chelex® methods displayed DNA recovery efficiencies that were approximately 3.3x more efficient than the Bardole method™ in recovering DNA from the brass FCCs (Table 2). In contrast, for the nickel-plated FCCs, the Bardole method™ generated a DNA recovery efficiency that was approximately 1.5x than that of the GGH method. In addition, the Bardole method™ demonstrated a DNA recovery efficiency for collecting DNA on nickel-plated FCCs that was statistically higher ($p = 0.0312$) than that of the Chelex® method. This suggests the Bardole method™ may be the method of choice among the methods tested here for DNA recovery from nickel-plated FCCs.

Table 2: Comparison of DNA Recovery Efficiency Across the Four DNA Collection Methods
Results include the amount of DNA recovered from saliva samples by Bardole™, GGH, EDTA and Chelex® methods as a percentage based on the amount of DNA deposited.

Sample Name	Cartridge Casing Type	Collection Method	DNA Recovery Efficiency (%)	
B-BC1	Brass	Bardole™	N/A*	
B-BC2		Bardole™	0.44	
G-BC1		GGH	1.47	
G-BC2		GGH	1.47	
E-BC1		EDTA	0.00	
E-BC2		EDTA	0 [†]	
C-BC1		Chelex®	0.00	
C-BC2		Chelex®	1.47	
B-NC1		Nickel-plated	Bardole™	3.82
B-NC2			Bardole™	3.82
G-NC1	GGH		3.23	
G-NC2	GGH		1.76	
E-NC1	EDTA		0 [†]	
E-NC2	EDTA		0 [†]	
C-NC1	Chelex®		1.17	
C-NC2	Chelex®		0.00	

* Sample was removed and not included in analysis due to evidence of contamination.

[†] DNA for these samples was not detected at the small autosomal target region.

A lack of concentration values in the samples collected by the EDTA method meant the amount of DNA recovered was low and was undetectable for these samples.

3.1.5 Average RFUs

The RFU level of the brass FCC sample (B-BC2) collected by the Bardole method™ was approximately 80% lower than the average RFU levels for brass replicates by the GGH method and 60% lower than that generated by the Chelex® method (Figure 3A). The amount of DNA amplified for both brass replicates of the Bardole method™ was approximately 33-66% less than that amplified for the GGH and Chelex® methods (Appendix A: Table A). Even so, a partial profile at several loci was still detected for B-BC2 indicating that this method was sufficiently effective in collecting DNA from these casings. The average RFU level generated from the nickel-plated samples by the Bardole method™ was comparable to that of the GGH method, suggesting that both of these methods are equally effective collecting DNA from these samples (Figure 3D).

Brass and nickel-plated samples that were collected by the GGH method displayed the highest RFU levels amongst the samples collected by all methods (Figure 3B). In addition, the GGH method demonstrated the highest success rate generating interpretable DNA profiles, with both nickel-plated FCC replicates and one brass FCC replicate generating complete and accurate profiles. An accurate partial profile that was three-quarters complete was generated by the remaining brass FCC sample for this method.

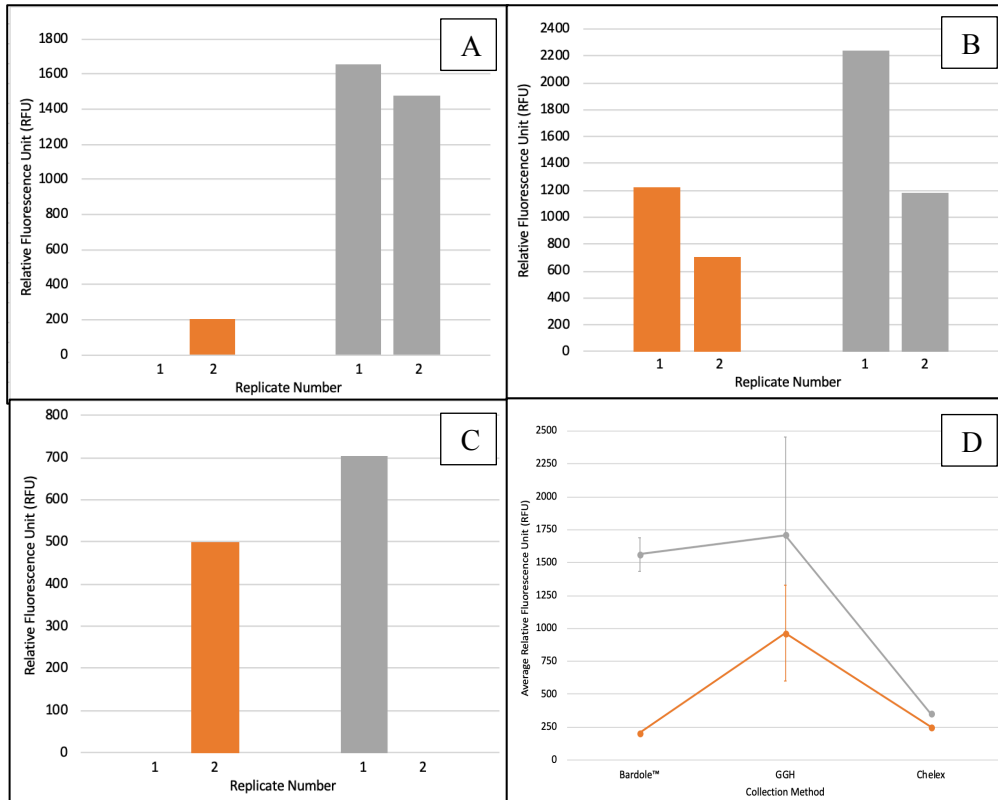


Figure 3. Individual and Average RFUs For Saliva by Each DNA Collection Method.

RFU levels per replicate detected by using A) Bardole Method™; B) GGH Method; C) Chelex® method. D) Average RFU levels across brass and nickel-plated cartridges. Error bars denote one standard deviation. Color: ■ Brass ■ Nickel-plated

Despite having average RFUs that were 20-25% lower than that produced by the GGH method across all samples, the Chelex® method was still successful in collecting sufficient quantity and quality of DNA to render interpretable profiles for one replicate from each of the brass and nickel-plated FCCs (Figure 3C). The DNA profile generated for the brass FCC sample (C-BC1) was complete and accurate while the profile generated for the nickel-plated FCC (C-NC2) was complete and accurate at all but one locus. Moreover, the amount of DNA amplified for sample C-NC2 was also approximately 22% lower than that amplified for the nickel-plated samples for the Bardole™ and GGH methods. The

Chelex® method failed to collect sufficient DNA to generate an interpretable or even a partial profile for the other respective replicates (C-BC1 and C-NC2).

The use of the EDTA method to perform DNA collection did not have any success in generating interpretable profiles for either the brass or nickel-plated FCCs and therefore generated average RFUs equal to baseline values for all samples.

3.1.6 Forensic Application

The primary goal for forensic DNA analysis is to develop interpretable profiles so that they can be used to establish linkages between evidence, persons of interest and the crime scene. As mentioned in section 1.1, an interpretable profile that may be admissible to a local/state DNA database is one that is considered to have at least 12 allele calls (10). Moreover, for a DNA profile to be submitted to CODIS, allele calls must be made at the FBI's 13 core STR loci (9). Thus, all of the profiles that were generated in *Experiment 1* of this study were evaluated against this criterion to determine how effective DNA collection methods were in rendering interpretable and CODIS-eligible DNA profiles (Table 3).

Table 3. DNA-Database Eligibility for Each Profile Generated by Each Collection Method. Results include proportion of donor's alleles that were detected (n = 44). Red = Uninterpretable, Yellow = Eligible for local/state level DNA databases, Green = CODIS-eligible.

Cartridge Casing Type	Collection Method	Number of Donor's Autosomal Alleles Detected (n=44)
Brass	Bardole™	N/A*
	Bardole™	23
	GGH	44
	GGH	34
	EDTA	0
	EDTA	0
	Chelex®	0
	Chelex®	44
Nickel-plated	Bardole™	44
	Bardole™	44
	GGH	44
	GGH	44
	EDTA	0
	EDTA	0
	Chelex®	43
	Chelex®	0

* Sample was removed and not included in analysis due to evidence of contamination.

It is evident that there was increased success in developing complete and accurate profiles for the nickel-plated FCCs across the different methods in comparison to the brass FCCs. Five of the eight nickel-plated samples displayed complete and accurate profiles at all autosomal loci rendering them all CODIS-eligible. In contrast, two of the eight brass FCC samples had complete and accurate profiles at all autosomal loci, indicating the success rate of CODIS-eligible profiles differs by more than 50% across the two different FCC types.

The Bardole™ and GGH methods demonstrated the most success in generating interpretable and CODIS-eligible profiles across both brass and nickel-plated FCCs. For the nickel-plated FCCs, both methods were equally successful, generating two complete

and accurate profiles at all autosomal loci. The GGH method was able to render one CODIS-eligible DNA profile for one brass replicate that had 100% detection of the donor's alleles as well as one interpretable profile that had 75% detection of the donor's alleles for the other brass replicate. In comparison, for the Bardole method™, due to the extensive contamination in one brass replicate that had significant evidence of a mixture, this would not be eligible to be added to any DNA database. However, the other brass replicate for the Bardole method™ was considered to be interpretable as it had slightly more than 50% detection of the donor's alleles. This profile was not CODIS-eligible due to the presence of allelic dropout at 8 of the 13 core loci outlined by the FBI. The Chelex® method yielded complete and accurate profiles 50% of the time across brass and nickel-plated samples.

3.2 Experiment 2: Comparison of the Performance of the DNAs Collection Method in Recovering Touch DNA Across Brass and Nickel-plated FCCs.

3.2.1 DNA Concentration

The DNA concentrations observed across the collected touch DNA samples on brass and nickel-plated FCCs displayed a large amount of variability across different methods and different donors (Figure 4). The concept of variability in shedding status amongst different individuals and by the same individual was evident in the DNA concentrations from samples obtained by both of these methods (Figure 4A).

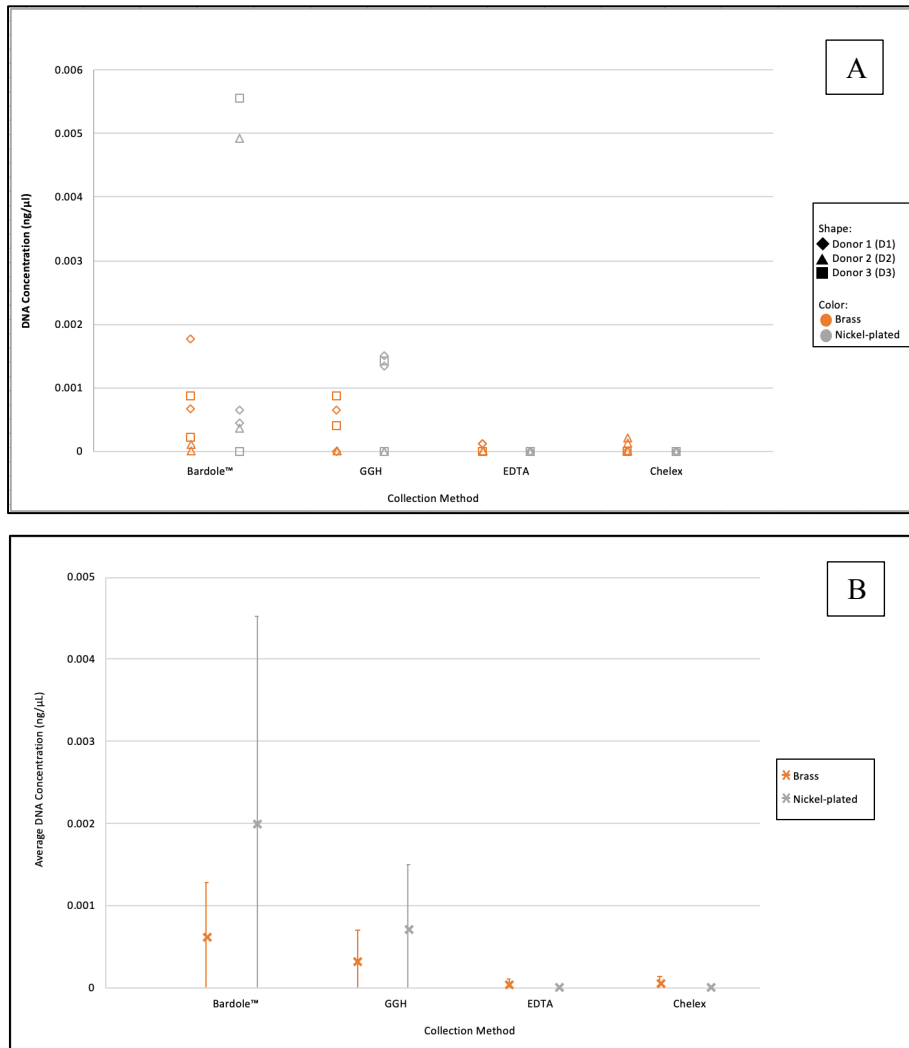


Figure 4. DNA Concentrations Obtained from Touch DNA Samples by Each DNA Collection Method. A) Distribution of individual DNA concentrations across three Donors; B) Comparison of average DNA concentrations detected by using Bardole™, GGH, EDTA and Chelex® methods to recover touch DNA on brass and nickel-plated cartridge casings. Error bars denote one standard deviation.

For the brass FCC replicates using the Bardole method™, it seemed that Donor 1 was the best shedder. This was not consistent across the nickel-plated FCC replicates, where the lowest and highest DNA concentrations were obtained from samples from the same individual (Donor 3) and differed by approximately 0.0055 ng/μL. Considering that

a total of 0.75 ng of DNA is recommended for DNA amplification in order to obtain interpretable profiles and that DNA was eluted in 50 μL in this study, a difference of 0.0055 ng/ μL would be 0.25 ng in solution. This is one-third of the required amount of DNA for amplification, which is a significant amount. As a consequence, average DNA concentrations will be compared as opposed to individual DNA concentrations of each donor in order to evaluate the success of the method against the average donor.

The Bardole™ and the GGH methods were the best performing methods for both brass and nickel-plated FCCs and across all three donors (Figure 4B). The use of the Bardole method™ resulted in higher average DNA obtained across all FCCs across the three donors. None of the samples across the three donors produced sufficient DNA quantities to generate a DNA profile when DNA collection was performed using the EDTA or Chelex® methods.

3.2.2 Assessment of Contamination

Out of the forty-eight samples for which touch DNA collection was performed for this experiment, only four samples yielded DNA profiles with allele calls (Table 4). Of these four samples, the DNA profiles of the two replicates collected using the GGH method (D1 G-BC1 and D3 G-NC2) each indicated the presence of one non-donor allele.

Table 4. Presence of a Non-Donor Allele in the DNA Profiles Generated from Touch DNA by Collection Method.

Sample name	Cartridge Casing Type	Collection Method	Number of Non-Donor Alleles
D1 G-BC1	Brass	GGH	1
D3 B-NC1	Nickel-plated	Bardole™	0
D1 B-NC1		Bardole™	0
D3 G-NC2		GGH	1

3.2.3 Forensic Application

Of the four DNA profiles that were generated in *Experiment 2*, three of these were classified as uninterpretable since only one allele was detected in each profile (Table 5). The Bardole method™ demonstrated some success in collecting touch DNA from nickel-plated FCCs (D3 B-NC1). Not only was the resulting profile classified as interpretable, but it was also classified as CODIS-eligible. Thus, this profile would be very valuable if obtained in forensic casework. The only DNA profile generated for brass FCCs was collected using the GGH method, however only 1 allele call was made which would not have any value in forensic casework.

Table 5. DNA-Database Eligibility for DNA Profiles from Touch DNA Samples on FCCs Collected by Each Collection Method. Results include proportion of donor's alleles that were detected (n = 44). Red = Uninterpretable, Yellow = Eligible for local/state level DNA databases, Green = CODIS-eligible.

Sample name	Cartridge Casing Type	Collection Method	Number of Donor's Alleles Detected (n=44)
D1 G-BC1	Brass	GGH	0
D3 B-NC1	Nickel-plated	Bardole™	42
D1 B-NC1		Bardole™	1
D3 G-NC2		GGH	0

Sample D3 B-NC1 had 20 complete and accurate autosomal loci and 2 partial and accurate autosomal loci (Figures 5a and 5b). The DNA concentration for this sample was

approximately 0.0055 ng/μL and thus, only 0.08 ng of DNA could be amplified for this sample (Appendix A: Table B). This is approximately one-tenth of that amplified from the saliva on the nickel-plated FCCs collected by the Bardole™ method in *Experiment 1*. The average RFU of this profile was 585.18, which is approximately one-third of that seen in *Experiment 1* for the nickel-plated FCCs for the Bardole method™. In addition, the DI for this sample was approximately 1.05, indicating small amounts of degradation.

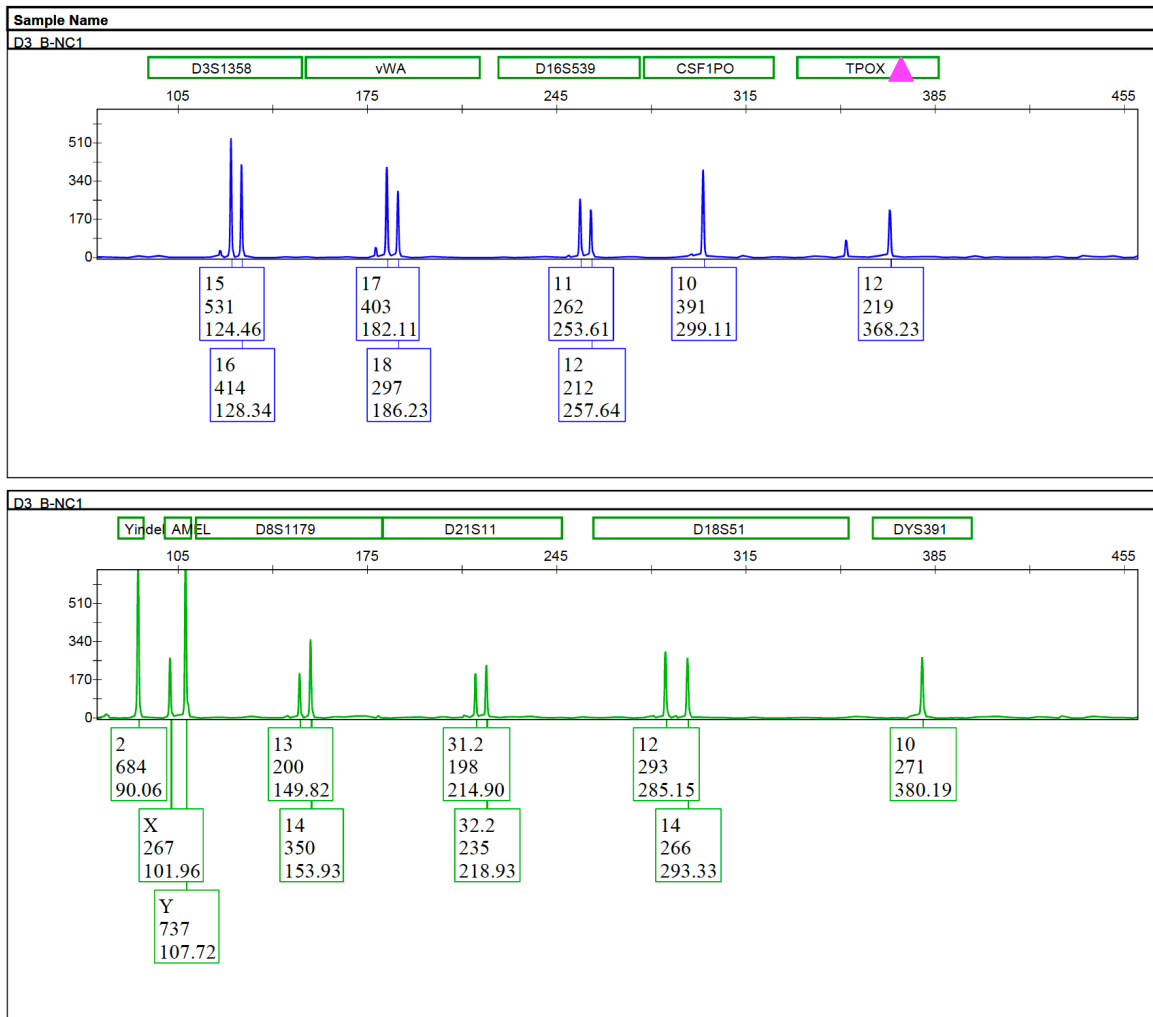


Figure 5a. Blue and Green Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using the Bardole Method™. All alleles and loci are complete and accurate given the following symbols: ▲ Partial locus

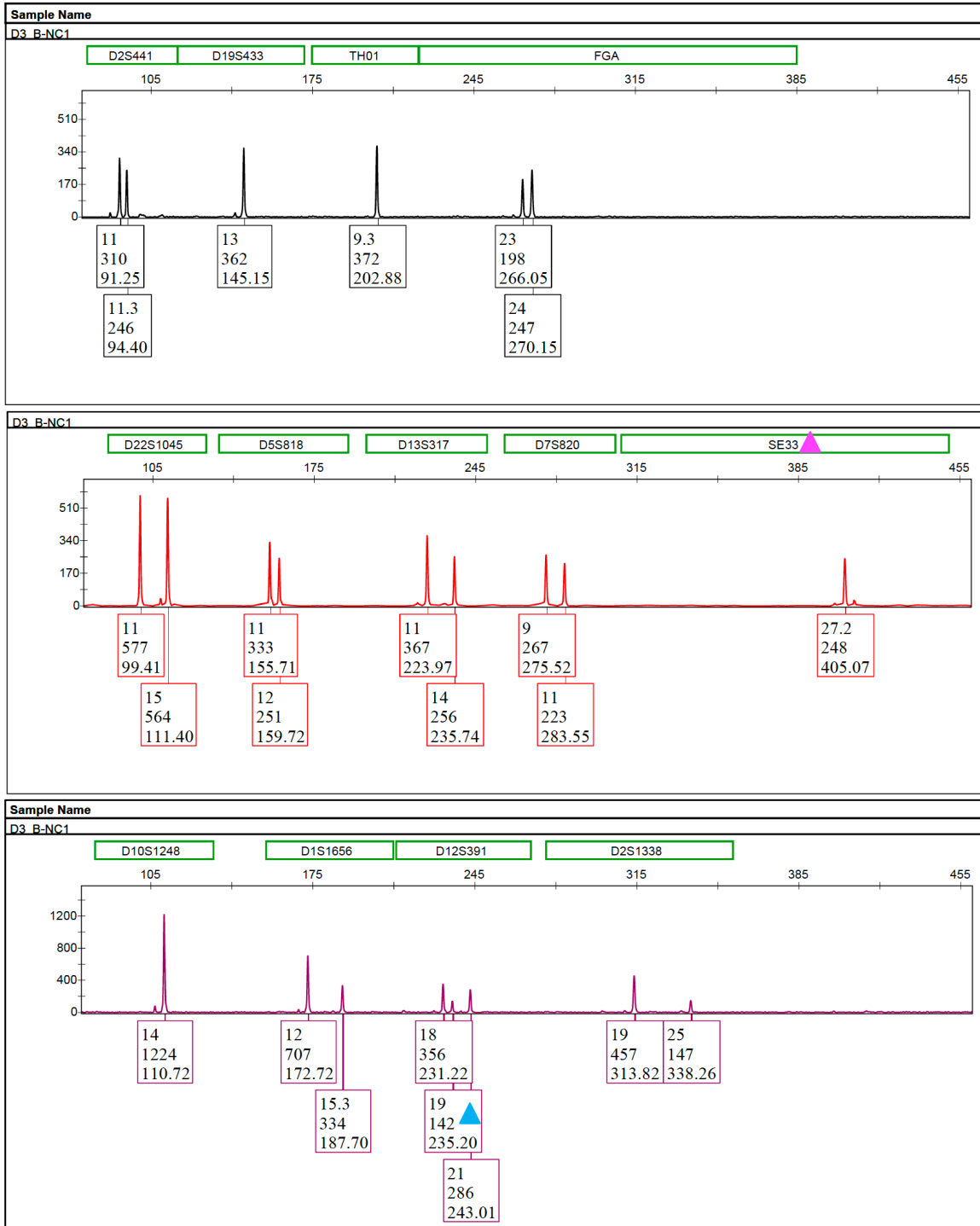


Figure 5b. Yellow, Red, and Purple Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using Bardole Method™. All alleles and loci are complete and accurate given the following symbols: ▲ Non-donor allele; ▲ Partial locus.

3.2.4 Degradation Index

The vast majority of all the brass and nickel-plated FCC samples collected by the EDTA and Chelex® method were accompanied by blank DI values indicating the lack of DNA detected in these samples (Table 6). This indicates that neither of these methods is well-suited to collecting touch DNA from cartridge casing samples and could also be explained by the presence of high levels of DNA degradation. The simple detection of the DI value in many more samples collected by either the Bardole™ or the GGH methods suggests that these methods are better adapted to collecting touch DNA samples from FCCs. The DI values across these two methods were quite comparable regardless of the type of FCC as well as the method.

Table 6. Summary of DI Values Per Replicate Collected by Each DNA Collection Method for FCCs.

Comparison of DI values across samples using Bardole™, GGH, EDTA and Chelex® methods to recover touch DNA on brass (BC) and nickel-plated cartridge casings (NC). (X) denote blank values obtained by the software. Red = samples for which one or more allele call was made.

Donor and Cartridge Type	Degradation Index (DI)			
	Bardole™(B)	GGH (G)	EDTA(E)	Chelex® (C)
D1 BC1	3.10	2.49	X	X
D1 BC2	X	N/A*	X	X
D2 BC1	X	X	X	X
D2 BC2	X	X	X	X
D3 BC1	2.48	0.90	X	X
D3 BC2	X	3.17	1.74	X
D1 NC1	2.53	1.02	X	1.35
D1 NC2	1.69	0.95	X	X
D2 NC1	59.1	X	X	X
D2 NC2	3.01	X	X	X
D3 NC1	1.05	2.62	X	X
D3 NC2	X	2.59	X	X

*Sample was removed from dataset due to error during extraction process.

3.3 Experiment 3: Comparison of the DNA Collection Methods in Recovering Touch DNA For Brass and Nickel-plated FCCs and UCCs

3.3.1 Average DNA Concentration

In the third part of this study, DNA collections were performed for touch DNA on UCCs using the four different methods and across the three donors. The relative success of the methods in being able to collect touch DNA from the UCCs was then compared to that for the FCCs in *Experiment 2*. Due to limitations in materials, each donor only handled one set of five brass or nickel-plated UCCs so there were three replicates of UCCs for each method (one per donor).

In general, it was observed that for each method the UCCs obtained higher average DNA concentrations than the FCCs across all methods (Figure 6).

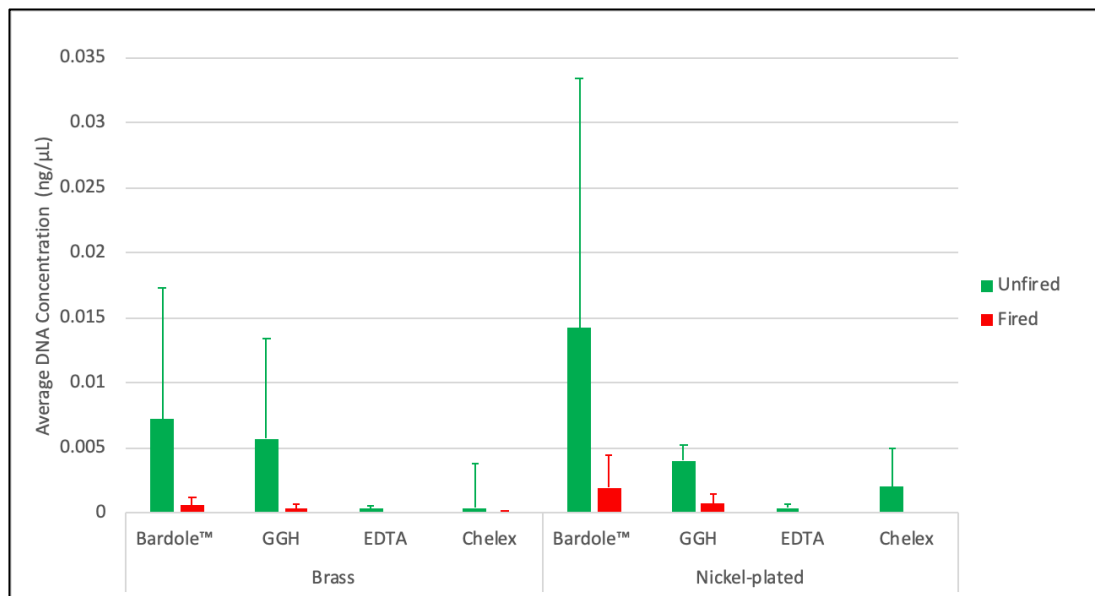


Figure 6. Average DNA Concentration of Touch DNA Recovered by Each DNA Collection Method from FCCs and UCCs. Comparison of average DNA concentrations detected by using Bardole™, GGH, EDTA and Chelex® methods to recover of touch DNA from brass and nickel-plated cartridge casings. Error bars denote one standard deviation.

Though there were increases in average DNA concentrations observed for the EDTA method for the UCCs in comparison to the FCCs, this increase was very slight. The overall average DNA concentrations were still extremely low for both brass and nickel-plated UCCs in comparison to the other methods.

UCC samples collected by the Chelex® method demonstrated increased average DNA concentrations in comparison to the FCC samples. This was true particularly for the nickel-plated samples. There was an approximately 2.5-fold increase in average DNA concentrations from the nickel-plated versus the brass UCC samples collected using this method.

Both the Bardole method™ and the GGH method displayed the highest average DNA concentrations obtained from the UCCs in comparison to the FCCs. For the nickel-plated UCCs, average DNA concentrations were approximately 7x and 4x higher than those obtained from nickel-plated FCCs for the Bardole™ and GGH methods, respectively. Similarly, for the brass UCCs, the average DNA concentrations were approximately 7x and 5x higher than those obtained from brass FCCs for the Bardole and GGH methods, respectively. Additionally, the Bardole method™ was able to render samples with average DNA concentrations that were approximately 2x higher from nickel-plated UCCs than from brass UCCs. In addition, the average DNA concentrations for samples collected from nickel-plated UCCs using the Bardole method™ were also approximately 3x those collected using the GGH method. The average DNA concentrations obtained from samples collected

by the GGH method were comparable across both nickel-plated and brass samples, with only a slight noticeable increase for the brass UCCs.

3.3.2 Degradation Index

In comparison to the DI value results in *Experiment 2*, there was an increased consistency in the DI values as the blank values were observed in a much smaller proportion of the samples (Table 7). All of the UCC samples for the Chelex® method from this experiment had actual numerical DI values, unlike in *Experiment 2* where all the FCC samples collected by this method rendered blank DI values.

Table 7. Summary of DI Values Per Replicate Collected by Each DNA Collection Method for UCCs. Comparison of DI values across samples using Bardole™, GGH, EDTA and Chelex® methods to recover touch DNA on brass (BU) and nickel-plated cartridge casings (NU) for three donors (D1, D2, D3). (X) denote blank values obtained by the software. Red = samples for which one or more allele call was made.

Donor and Cartridge Type	Degradation Index (DI)			
	Bardole™(B)	GGH (G)	EDTA(E)	Chelex® (C)
D1 BU	0.76	1.40	0.52	0.32
D2 BU	7.65	1.26	X	3.46
D3 BU	X	0.72	X	2.27
D1 NU	0.71	0.55	8.57	1.08
D2 NU	X	1.11	X	2.21
D3 NU	2.51	0.85	0.63	2.49

Across all four methods, it appeared that the samples collected by the GGH method had the lowest DI values and that these samples had of minimal levels of degradation and in some cases no degradation at all. The DI values for the samples collected from UCCs were approximately 2-fold lower than those collected from FCCs for this method,

indicating that the performance of this collection method may have been impacted by the firing process. This was true across both brass and nickel-plated cartridge casings.

The range of degradation values obtained by samples collected using the Bardole method™ displayed highest variability across both brass and nickel-plated UCCs and amongst the four methods. Sample D2 B-BU appeared to be an anomaly since there were allele calls generated despite having a moderate level of degradation.

3.3.3 Assessment of Contamination

Half of the samples that were collected on UCCs generated sufficient DNA concentrations to be amplified and analyzed by capillary electrophoresis (Table 8). Of the resulting DNA profiles, three of the nickel-plated replicates (D1 B-NU, D3 B-NU, D2 G-NU) showed the presence of non-donor alleles. Two of these replicates were collected using the Bardole method™ and one with the GGH method.

Table 8. Presence of a Non-Donor Allele in the DNA Profiles Generated from Touch DNA on UCCs by Collection Method.

Sample name	Cartridge Casing Type	Collection Method	Number of Non-Donor Alleles
D1 B-BU	Brass	Bardole™	0
D2 B-BU		Bardole™	0
D1 G-BU		GGH	0
D1 C-BU		Chelex®	0
D3 C-BU		Chelex®	0
D1 B-NU	Nickel-plated	Bardole™	1
D2 B-NU		Bardole™	0
D3 B-NU		Bardole™	1
D1 G-NU		GGH	0
D2 G-NU		GGH	1
D3 G-NU		GGH	0
D1 C-NU		Chelex®	0

3.3.4 Comparison of average RFUs by Donor 1

A comparison of the average RFUs generated by Donor 1 across samples collected on brass and nickel-plated UCCs seemed to mirror trends that were seen in *Experiment 1* for the Bardole™, GGH and Chelex® methods (Figure 7). The amount of DNA amplified for the nickel-plated samples for the Bardole method™ was approximately 7x that amplified for both the GGH and the Chelex® methods (Appendix A: Table C). The highest average RFU was that observed in the nickel-plated sample that was collected by the Bardole method™. This was approximately 7.5x and 5.3x higher than that generated by the Chelex® method and the GGH method, respectively. It was also approximately 3x higher than the sample collected from brass UCCs using the Bardole method™.

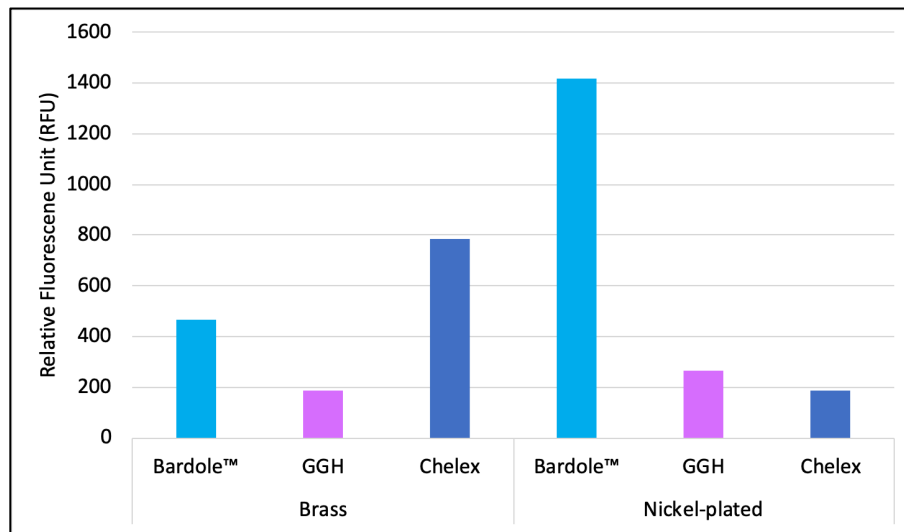


Figure 7. Average RFU Levels from Touch DNA Samples from Donor 1 By Each Method. Comparison of average RFU levels from donor 1 samples across brass and nickel-plated cartridges collected by the using Bardole™, GGH, and Chelex® methods.

The average RFUs of touch DNA samples collected from brass and nickel-plated UCCs using the GGH method observed were comparable even though only approximately

one-third of the amount of DNA was amplified for the nickel-plated UCCs in comparison to the brass UCCs for this method.

For the brass UCCs, the amount of DNA amplified for the samples collected using the Bardole™ and the GGH methods was approximately twice that for the Chelex® method. Even so, the average RFU generated from the touch DNA sample on the brass UCCs collected using the Chelex® method was the highest amongst the three methods and was approximately 2x and a 4x higher than that for the Bardole method™ and GGH method, respectively. This appears to suggest that the Chelex® method demonstrated the best performance when collecting touch DNA on brass casings, which does not correlate to the results for this method observed in *Experiments 1* and *2*. In addition, the RFUs from touch DNA collected on brass UCCs by the Chelex® method was also approximately 4x higher than that from the nickel-plated UCCs.

Similar to *Experiments 1* and *2*, the samples collected using the EDTA method did not result in the generation of any interpretable DNA profiles.

3.3.5 Forensic Application

While the Chelex® method was unable to provide interpretable profiles for touch DNA on both brass and nickel-plated FCCs, profiles were produced with both brass and nickel-plated UCCs (Table 9). The use of this method to perform DNA collections resulted in two CODIS-eligible DNA profiles (D1 C-BU, D1 C-NU). The Bardole method™ was also able to generate DNA profiles that were CODIS-eligible, generating one for brass UCCs and one for nickel-plated UCCs (D1 B-BU, D1 B-NU). Each of these four CODIS-eligible profiles had more than 91% of alleles detected (at least 40 out of 44 alleles),

indicating that these profiles would be valuable in a forensic investigation. As a result, both the Bardole method™ and the Chelex® method demonstrated equally high ability in being able to collect touch DNA samples across both brass and nickel-plated UCCs. The four CODIS-eligible profiles generated from samples collected by the Chelex® and Bardole™ methods all came from Donor 1 samples, indicating this donor was also likely a good shedder in comparison to the other two donors.

Table 9. DNA-Database Eligibility for DNA Profiles from Touch DNA Samples on UCCs Collected by Each Collection Method. Results include proportion of donor's alleles that were detected (n = 44) for all samples that contained sufficient DNA concentration for amplification to occur (> 0.001 µg). Red = Uninterpretable, Yellow = Eligible for local/state level DNA databases, Green = CODIS-eligible.

Sample name	Cartridge Casing Type	Collection Method	Proportion of Donor's Alleles Detected (n=44)
D1 B-BU	Brass	Bardole™	42
D2 B-BU		Bardole™	4
D1 G-BU		GGH	32
D1 C-BU		Chelex®	44
D3 C-BU		Chelex®	0
D1 B-NU		Nickel-plated	Bardole™
D2 B-NU	Bardole™		4
D3 B-NU	Bardole™		18
D1 G-NU	GGH		38
D2 G-NU	GGH		4
D3 G-NU	GGH		29
D1 C-NU	Chelex®		40

While the use of the GGH method did not result in collecting samples with CODIS-eligible DNA profiles, it was able to render DNA profiles for two nickel-plated UCC samples and one brass UCC sample that were interpretable (D1 G-BU, D1 G-NU, D3 G-NU). These profiles demonstrated a 66% coverage of Donor 3's alleles for one nickel-plate

UCC sample and a 73% and 86% coverage of Donor 1's alleles for samples of brass and nickel-plated UCCs respectively. Furthermore, the use of the Bardole method™ was also able to render an interpretable profile from one nickel-plated UCC sample with 41% coverage of Donor 3's alleles. Thus, the use of the GGH method was able to deliver a higher overall average coverage of alleles across all donors in comparison to the Chelex® and Bardole™ methods. Regardless, the development of all of these profiles would also likely serve to help further forensic investigations.

Three out of the four methods, excluding the EDTA method, were able to generate more interpretable profiles when being used to collect touch DNA from UCCs as opposed to FCCs.

3.4 *Experiment 4: Modifications to Improve DNA Collection Efficiency of Methods*

3.4.1 Incorporation of the Cone Method

The vacuum filtration step took an increased amount of time with the cone method due to the collection solution being eluted through a smaller area of the filter as determined by the circumference of the stem of the cone. Thus, the cone method was done in conjunction with the consecutive vortex of all five cartridge casings in the set in order to optimize the amount of time it would take to perform the method. Without the consecutive vortex, collection of each replicate would involve a total of fifteen vacuum filtration steps since there would be three per cartridge casing. With the incorporation of the consecutive vortex, only three vacuum filtration steps in total were performed per replicate.

With the addition of the cone method and the consecutive vortex element, the GGH method demonstrated an increased success in recovering of touch DNA from brass FCCs

as shown by the approximate 3-fold increase in average DNA concentrations (Figure 8). This can also be seen by the approximate 2-fold reduction in average DI levels for these samples. While the Bardole method™ also displayed an increase in average DNA concentrations for the brass FCCs, there was a slight increase in the average DI values with the use on the cone for these samples.

For the nickel-plated FCCs, the lack of success of incorporating the cone method with the Bardole method™ could be seen through drop in average concentrations of the DNA. There appeared to be almost no difference in the performance of the GGH method in collecting DNA from nickel-plated cartridges with and without the cone method as seen by the comparable average DNA concentrations and DI values.

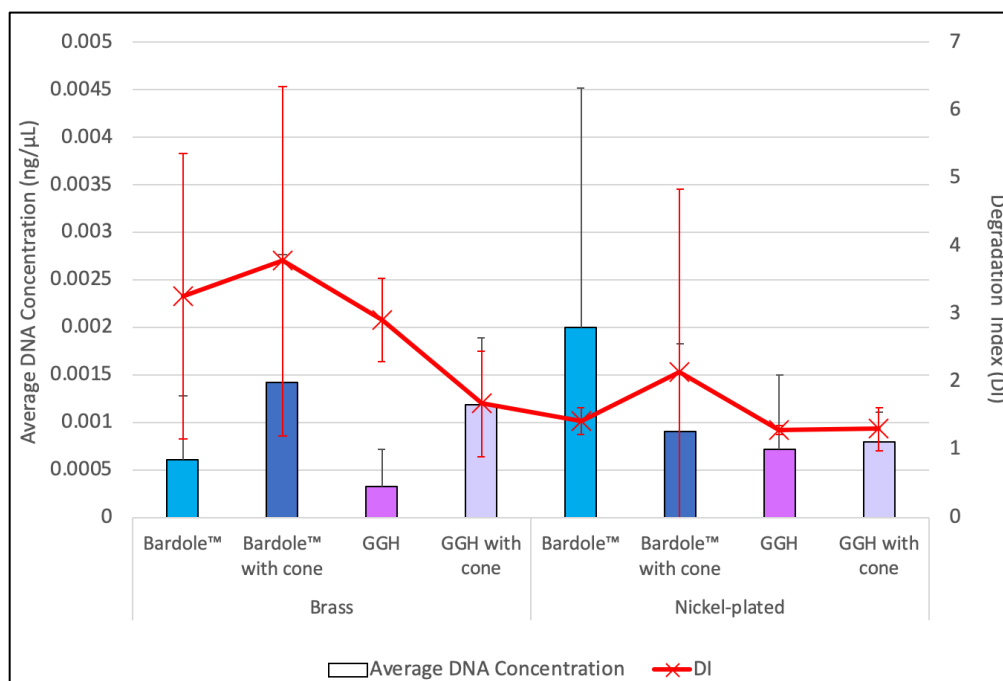


Figure 8. Comparison of the Effect of the Cone Method on the Performance of the Bardole and GGH Methods. Comparison of average DNA concentrations and DI values for touch DNA across brass and nickel-plated cartridges collected by the using 4 methods: Bardole™ method, GGH method, Bardole™ with cone method, GGH with cone method. Error bars denote 1 standard deviation.

Ten FCC samples collected using either the Bardole™ with cone or GGH with cone methods advanced to the PCR amplification stage and an average of approximately 0.03 ng of DNA was amplified for these samples (Appendix A: Table D). There were no alleles detected for any of these samples. This suggests that the cone method is not an effective modification to make to either of these methods since they displayed more success in generating interpretable profiles across the FCCs and UCCs without the use of the cone method as seen from *Experiments 1* and *2*.

3.4.2 Incorporation of Consecutive Vortex for the GGH Method

An additional experiment was conducted in which only the consecutive vortex element, without the cone method, was incorporated to the GGH method to see if this modification could help increase the DNA recovery efficiency. The use of this method was successful in generating a partial profile for nickel-plated FCCs (Figures 9a and 9b).

This profile contained 16 complete and accurate loci, 4 loci with no alleles detected and 2 partial loci. While this profile may not be CODIS-eligible, it is interpretable and would be considered forensically relevant for casework. The DI value for this sample was 1.50 indicating this sample did not have significant degradation; however, the ski-slope effect evident across the panels in the DNA profile appears to suggest otherwise. Lastly, this sample had a high DNA concentration of 0.025 ng/μL and thus, 0.37 ng of DNA was amplified (Appendix A: Table D).



Figure 9a. Blue and Green Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using GGH Method with Consecutive Vortex. All loci are complete and accurate except those marked with the following symbols: ▲ Partial locus; ▲ Locus with no allele detection. CV denotes consecutive vortex.

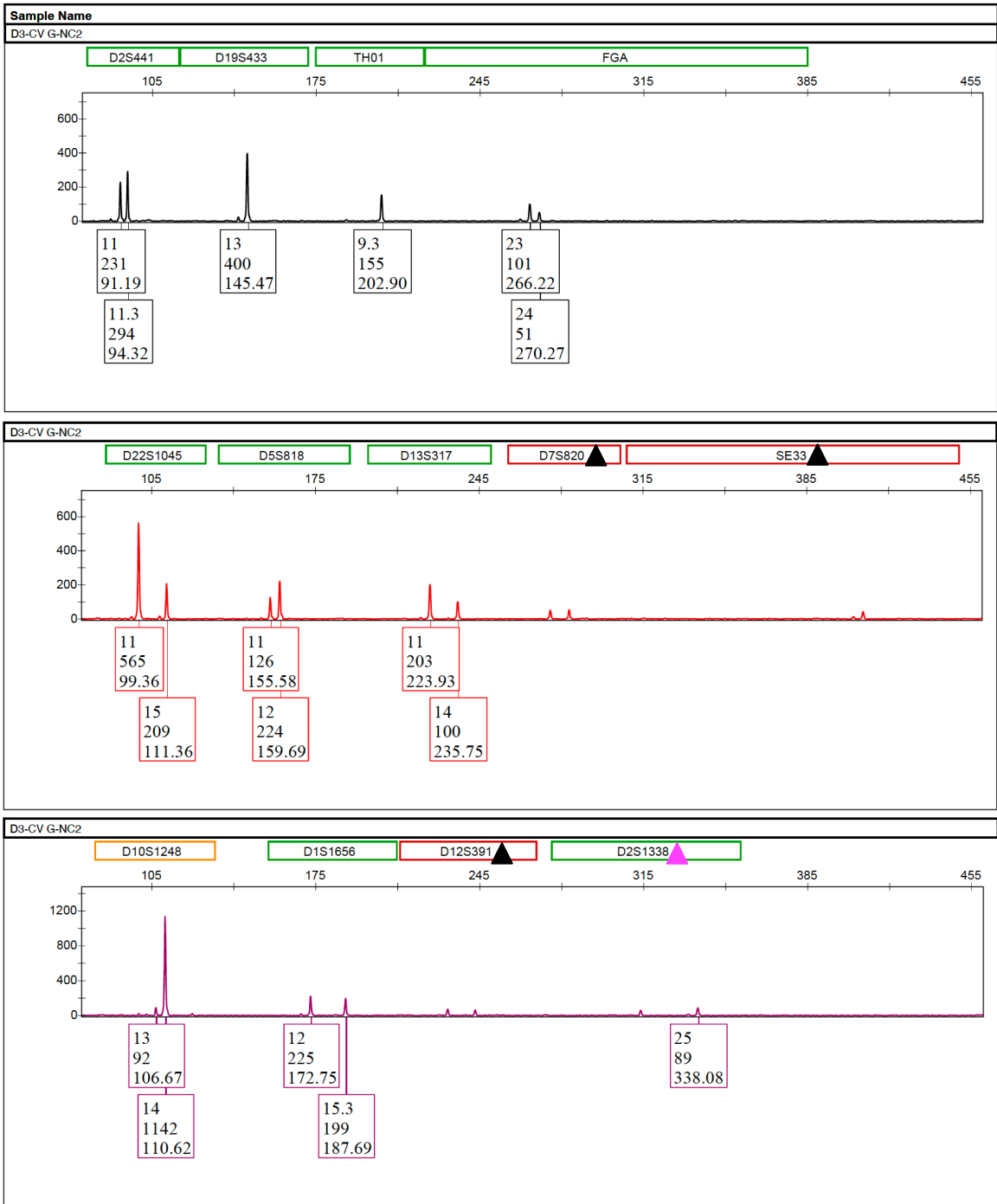


Figure 9b. Yellow, Red, and Purple Dye Channels from the DNA Profile for Sample Collected from Nickel-plated Casings Using GGH Method with Consecutive Vortex. All loci are complete and accurate except those marked with the following symbols: ▲ Partial locus; ▲ Locus with no allele detection. (CV) denotes consecutive vortex.

4. DISCUSSION

4.1 Presence of Contamination

There was a comparable amount of low-level contamination present in samples that were collected using the Bardole™ and GGH methods. For *Experiment 1*, whether the contamination occurred during DNA collection or subsequent processing could not be ascertained due to lack of comparison profiles from the analysts that performed DNA collection. Despite wearing the appropriate personal protective equipment, one potential source of contamination could be the transfer of DNA from the analyst when performing the collection method (51). The contamination could also have arisen from the outdoor range where the cartridges were shot since this is a space where WPD officers practice shooting and therefore numerous casings were scattered on the ground. While a tarp was wiped down with disinfectant wipes and set down on the ground to catch and provide a barrier between the sample cartridge casings and extraneous cartridge casings, there may have been some residual sub-surface DNA on the tarp. Moreover, due to lack of control of where the casings might land, there were a few instances where the casings landed in the areas outside the tarp. Though casings were collected promptly, this may have also led to a rise in contamination. Since only one of the tested samples displayed contamination and the remaining samples collected by the Bardole™ and other three methods did not display non-donor alleles, this suggests that using these methods to perform DNA collection on brass and nickel-plated casings is still quite effective. It also suggests that the presence of contamination in casing B-BC1 could be an isolated incident and not characteristic of inadequacies in this method.

Lastly, though the magazines of the handgun being used to fire the projectiles were wiped down with disinfectant wipes, the firearm was the active duty weapon of an officer. As a result, these weapons were not sterile and could have been exposed to a number of contaminating sources. In addition, the exterior surface of the handgun was not wiped down prior to shooting. Thus, there could have been some secondary transfer of cells during the loading and firing process.

Contrary to *Experiment 1* which used known saliva dilutions, the quantity and quality of DNA that was being deposited through physical contact could not be controlled in *Experiments 2, 3, and 4*. For these set of experiments, only the method and time over which the DNA was deposited was controlled. In addition to the aforementioned potential sources of contamination for *Experiment 1*, another source of contamination for *Experiments 2, 3 and 4*, could be due to the occurrence of secondary transfer of DNA cells, which is widely associated with touch DNA (11). Though donors were instructed to wash their hands prior to handling the cartridges and also asked to refrain from touching any other items during the handling process, they were not closely monitored during this process. As a result, if the instructions were misunderstood or if they accidentally made contact with a different surface while they were handling the cartridge casings, the accidental transfer of DNA cells from other sources could have occurred. In addition, if the donor had recently had contact with a very good shedder, it could mean that the cells of this shedder were able to persist on the donor's hands and be transferred onto the surface on the cartridge casings.

The presence of contamination in the UCC samples in *Experiment 3* suggests that exposing the cartridge casings to the outdoor firing range was not the main source of contamination. It stresses the importance of incorporating measures to minimize the possibility of secondary transfer.

4.2 Relative Success of the DNA Collection Methods for FCCs

The results from the DNA recovered from brass and nickel FCCs using known concentrations of saliva DNA in *Experiment 1* were important since they showcased the general trends and comparative success rates when conducting DNA collection on these sample types using each of the four methods. In forensic casework, however, DNA on cartridge casings comes from skin cells as opposed to large quantities of saliva. As a result, the second part of this study focused on comparing the DNA collection of touch DNA from brass and nickel-plated cartridge casings by the four different collection methods.

In *Experiment 1*, all four methods demonstrated decreased average DNA concentrations from the saliva samples collected from brass FCCs in comparison to those from nickel-plated FCCs. This may imply that all of the collection methods are experiencing the deleterious effects on downstream DNA analysis caused by an abundance of copper ions in brass FCCs. Thus, the results highlight the increased difficulty in conducting analysis on brass FCCs in comparison to the nickel-plated FCCs regardless of collection method.

The absence of a chelating agent and the lack of the use of a pre-filter device in the Bardole method™ may explain why both the Chelex® and GGH methods were able to yield higher average DNA concentrations from brass casings in *Experiment 1*. They may be more

effective in removing copper ions from the DNA collected, thereby preventing the ions from having deleterious effects on downstream DNA analysis. Moreover, the difference in average DNA concentrations obtained from brass casings between the GGH method and the Chelex® method could imply that the GGH chelating agent is a more effective chelating agent than the Chelex® beads. Additionally, it has been shown when Chelex® beads carry over from extraction to amplification, an inhibitory effect on PCR is observed (52). Thus, it may be that the Chelex® beads were not effectively removed during DNA collection despite the use of the filter device, indicating a potential limitation to using the Chelex® method.

In *Experiment 1*, the DNA concentrations obtained from saliva samples collected from the Bardole™ and GGH methods demonstrated an equal success rate for nickel-plated FCCs. This trend was not mirrored from the DNA concentrations obtained from the touch samples in *Experiment 2* for which the Bardole method™ demonstrated the higher average DNA concentrations for touch DNA samples on brass FCCs. This difference may be attributed to the difference in the number of cells since the efficiency of the methods may be affected when using a much smaller quantity of cells.

DNA collection of the FCCs using the EDTA method was unsuccessful when the source of DNA was from touch DNA, just as it was when the source of DNA was from saliva, indicating that the EDTA method may not be well-suited to collecting DNA from FCCs. The collection of touch DNA on FCCs performed by the Chelex® method yielded results that were as poor as those performed by the EDTA method. This was not expected since the Chelex® method demonstrated some success, albeit fluctuating in nature, in being

able to recover sufficient DNA from saliva samples in *Experiment 1*. This may indicate that this method is not well suited to collecting samples with significantly lower levels of DNA. In addition, it may suggest that the use of the Chelex® beads as a chelating agent is not the most effective element to incorporate when performing the collection of touch DNA from FCCs. Moreover, the protocol for the Chelex® method developed by the Broward County Sherriff's Office utilizes a different buffer whereas for this study, the phosphate-based SRS buffer was utilized. This difference in collection solution could have also impacted the success of this method in collecting touch DNA from the FCCs.

The overall results of the very low DNA recovery efficiency values obtained using the known saliva samples in this study indicate that more research is required to optimize the use of the vacuum filtration methods in collecting touch DNA from FCCs. A significant amount of DNA is either not being recovered or is being lost when these four methods are being utilized. This is particularly concerning since these DNA recovery efficiency values were obtained for DNA in saliva samples, which are more highly concentrated and robust than touch DNA samples. As a result, the DNA recovery efficiency for touch DNA is likely lower than that for the saliva DNA. In the study by Elwick et al. average DNA recovery efficiency of human epidermal keratinocytes was compared between the GGH rinse-and-swab method and a soaking method using a Proteinase K solution (35). They demonstrated an average recovery rate of 5-6% for both the rinse-and-swab and the soaking methods. These calculations included approximately 150 samples of brass and nickel-plated FCCs and UCCs each per method. In this study, the DNA recovery efficiency values for brass and nickel-plated FCCs for all of the methods excluding the

EDTA method primarily ranged between 1-4%, with the GGH method falling at the higher end of the range. Thus, the DNA recovery efficiencies are comparable across the two studies despite the large difference in sample size. It also suggests that the rinse-and-swab, soaking and vacuum filtration techniques have similar success rates when being used to collect DNA from FCCs.

Based on the DNA recovery efficiency values obtained during this study it would appear that there is a benefit to including chelating agents such as the Chelex® beads and the GGH protein in the collection method when collecting DNA from brass FCCs. This finding correlates to the idea that chelating agents can help prevent the copper ions from having inhibitory and degradative effects on downstream DNA analysis.

When using the GGH and the Bardole™ methods, it was observed that each time the solution was poured through the filter cup, a small volume of collection solution was being lost to the thin rim of plastic surrounding the filter as well as on the interior walls of the filter cup. Since the collection solution was poured through the filter cup thrice per replicate, this could mean there were several instances where DNA yield was being lost. This issue was not observed with the Chelex® method due to the incorporation of the cone method, which funneled the collection solution so that it was directly introduced to the filter and did not make contact with the interior walls of the cup or fall into the lip surrounding the filter. Thus, this could be in part responsible for the increased DNA recovery efficiency observed with the Chelex® method. Though the GGH method did not use the cone method, it used a significantly smaller amount of collection solution; the total volume was approximately 20x less than the Bardole method™. The Chelex® method

utilized 25 mL of collection solution, which is approximately 62.5% of the volume used in the Bardole method™. It has been shown in the literature that concentrating DNA in a smaller volume is an effective technique when trying to maximize yield (53,54). Thus, there may be a benefit in using a smaller collection solution volume when collecting touch DNA from FCCs.

Furthermore, the statistically significant increase in DNA recovery efficiencies between the Bardole™ and the Chelex® methods for the nickel-plated FCCs may suggest that the presence of chelating agents in the absence of copper ions can be disruptive to overall DNA recovery. Chelating agents need to be filtered out prior to DNA extraction since these agents may have inhibitory effects on downstream PCR analysis. As a result, both the Chelex® and GGH methods employ the use of the pre-filter device, which adds another surface to which some DNA could be lost. Since the Bardole method™ does not use this device, it does not experience the potential loss of DNA that the GGH and Chelex® methods may be experiencing.

Elwick et al. reported average keratinocyte DNA concentrations of 0.004 ng/μL for brass UCCs and FCCs, and 0.027ng/μL for nickel-plated UCCs and FCCs, and 0.012ng/μL across all FCCs (35). The average touch DNA concentrations for brass FCCs in this study were comparable to those in the Elwick et al. study. In contrast, the average touch DNA concentrations for nickel-plated FCCs and therefore the overall average for FCCs in this study were both approximately 10-fold lower. While this may be attributed to the large difference in sample size, it could also indicate that the soaking or rinse-and-swab methods are more optimal for nickel-plated FCCs than the vacuum filtration methods.

A consistent increased level in degraded DNA obtained from the saliva samples on brass FCCs in comparison to the nickel-plated FCCs could explain why the average DNA concentrations obtained from the brass FCCs were consistently lower than those of the nickel-plated FCCs across these methods. It also implies that the presence of copper ions is correlated to increased amounts of degradation, which correlates to the known literature that copper ions may have degradative and inhibitory effects on downstream PCR analysis (27,35,42). Moreover, it provides additional support for why the GGH, Bardole™, and Chelex® methods appear to be more successful when collecting DNA from nickel-plated FCCs as opposed to the brass FCCs.

The consistently high RFU levels generated from the saliva samples across both types of FCCs using the GGH method suggests that there were no significant inhibitory effects to PCR generated from using this method. This once again highlights the effectiveness of the GGH chelating agent in chelating the copper ions for the brass samples. It also demonstrates the effectiveness of this method in being able to render a sample that has a sufficient quantity and quality of DNA present to generate a DNA profile. This is consistent with a study by Bille et al. which indicated that the incorporation of the GGH protein in the recovery method yielded profiles with higher RFUs per locus (33). However, Bille et al. reported an average RFU of 200 for touch DNA samples on FCCs collected using the rinse-and-swab method with GGH whereas in this study, there were no interpretable profiles generated for touch DNA samples on FCCs collected using the GGH method (33). This may suggest that if incorporating the GGH protein in the recovery method, a rinse-and-swab method is more effective and successful than a vacuum filtration

method. This appears to stress the importance to utilizing a smaller volume of collection solution when collecting touch DNA from FCCs.

The RFU results from *Experiment 1* also suggest that the Chelex® method may not be as reliable a method in collecting DNA from FCCs. One set of casings for both the brass and the nickel-plated FCCs demonstrated alleles for at least 43 of 44 alleles while the other set of casings failed to detect any alleles. Inefficiencies in the method in filtering out the Chelex® beads using the pre-filter device and the subsequent inhibitory effects in PCR could explain why the two samples did not have any alleles detected. This could also explain why the average RFU level for the nickel-plated FCCs is low and similar to that of the brass FCCs for this method.

4.3 Effect of the Firing Process

The purpose of *Experiment 3* was to determine whether the process of firing affected the success and effectiveness of each method in collecting sufficient amounts of DNA from FCCs to generate interpretable profiles. The large amounts of heat and force that are elements of the firing process may serve to further degrade DNA present on the surface of the FCCs. In addition, the exposure of the FCCs to the components of GSR may also result in degradative and inhibitory effects during downstream DNA analysis (38).

The DNA concentration results for touch DNA on UCCs generated using the EDTA method suggests that the process of firing the casings may not be the primary factor that is interfering with successful DNA analysis using this method. Instead, it appears to point to inefficiencies of the actual EDTA method itself that may be at the root of the cause for why samples being analyzed using this method are not generating interpretable profiles.

However, this would be contrary to what was suggested by Holland et al., whose study showed positive results when using EDTA in their method for collecting touch DNA on unfired cartridge casings (27).

As seen with the FCCs, the GGH and Bardole™ methods also generated the highest DNA concentrations from the touch DNA samples collected from the UCCs. Moreover, the increased DNA concentrations obtained from the UCCs in comparison to the FCCs using these methods indicates that the quantity and quality of DNA that may be obtained using both of these methods is negatively affected by the firing process. This was also demonstrated in the study by Elwick et al. as they reported an average DNA concentration of 0.019 ng/μL across all UCCs compared to 0.012 ng/μL across all FCCs for the rinse-and-swab and soaking methods (35). The average DNA concentrations for a small number of samples collected on UCCs using the Bardole™ and GGH methods were comparable to the values reported by the Elwick et al. study.

The increased success of the Bardole method™ reported in this study in obtaining high DNA concentrations from the nickel-plated UCCs versus from the brass UCCs corresponds to the trends seen for the FCCs in *Experiment 1* and *2*. It suggests that the increased presence of the copper ions was a factor that limited the extent of success of the Bardole method™ in *Experiments 1* and *2* as opposed to the firing process. The average DNA concentration level generated by the GGH method for touch samples on UCCs were comparable across the brass and nickel-plated samples. This may suggest that this method is influenced by the firing process since unlike with the FCCs, the GGH method was able to generate similar average DNA concentrations across all UCCs. In contrast, the high

variability across the DI values for all UCCs for the Bardole method™ may indicate that the exposure to the firing process does not affect the quality of touch DNA samples that are collected using this method. Moreover, these results may also indicate that the GGH protein is so effective at chelating copper ions that it is able to generate comparable DNA concentration results across both cartridge casing types. The Chelex® method generated UCC samples with DI values comparable to those generated by the Bardole™ and the GGH methods in this study. This suggests that the exposure of the cartridge casings to the firing process may be contributing to the lack of success of the Chelex® method.

The GGH method was able to generate complete and accurate profiles for the saliva samples across both types of FCCs and able to generate interpretable profiles for the touch DNA samples across both types of UCCs. In contrast, the GGH method was unable to render interpretable profiles for touch DNA samples on all FCCs. It is difficult to ascertain whether it is the exposure to the firing process or another underlying factor that is affecting the success of the GGH method in collecting touch DNA from FCCs. It is possible that the success in collecting a sufficient quantity and quality of DNA with the saliva samples but not with the touch DNA samples on the FCCs is due to the characteristics of touch DNA with regard to the number and condition of the cells.

The GGH method was able to generate similar results across the two different types of casings, which is contrary to a previous study that indicated that nickel-plated cartridges tend to yield higher concentrations of touch DNA than brass cartridges (35). This could be attributed to the effectiveness of the GGH protein, allowing for touch DNA samples on

brass UCCs to be collected with the same efficiency as those on the nickel-plated UCCs in the absence of the firing process.

The average RFU generated using the Chelex® method was higher than that of the Bardole™ and GGH methods for touch DNA samples on brass UCCs despite a larger amount of DNA being amplified for the Bardole™ and GGH methods. This seems to indicate once again that the exposure to the firing process is a major factor hindering the success of this method since the Chelex® method demonstrated a complete failure to render any alleles in *Experiment 2*. Moreover, though the Chelex® method generated a higher RFU level for the brass UCCs in comparison to the nickel-plated UCCs, even though a comparable amount of DNA was amplified for both cartridge casing types. This could indicate that the Chelex® method is capable of effectively collecting touch DNA from UCCs irrespective of the type of cartridge casing. This lack of consistency could be attributed to potential inefficiencies in filtering out the Chelex® beads prior to DNA extraction leading to downstream inhibition or due to the effectiveness in the beads as a chelating agent.

In this study, the GGH method demonstrated the best ability to collect DNA samples from donors with varying shedder statuses. This method displayed the highest overall average coverage of alleles amongst the three donors in the profiles generated in *Experiment 3*. It is important to note that only Donor 1's RFU levels and quantity of DNA amplified were compared and considered in *Experiment 3* due to a lack of alleles detected in the samples across the other two donors. Thus, it is unclear whether the results from this data are applicable to the average DNA donor. The high variability in shedding status that

the same individual may experience must be taken into consideration when analyzing the results from just one donor (15). As a consequence, it is important to keep in mind that the results from these RFUs may not be reflective of the average donor. Additional data would be required in order to make more definitive claims surrounding the relative success and performance of these methods. The Bardole method™ was able to perform at a level comparable to that of the Chelex® and the GGH methods for the UCCs, which suggests that the lack of a chelating agent in the Bardole method™ does not serve as a significant disadvantage for this method in being able to render forensically relevant DNA profiles.

The lack of success in using the EDTA was consistent for both the UCCs and the FCCs stressing that this method was not well-adapted to collecting touch DNA from cartridge casings, fired or unfired. This finding does not conform to the existing literature on using EDTA as a chelating agent on UCCs in which sufficient touch DNA was detected to generate DI values though the DI values did indicate high levels of degradation (27). Overall, the results suggest that the firing process appears to affect the success rate of the Chelex® method to a much larger extent than that of the Bardole™ and GGH methods.

4.4 Forensic Relevance

In *Experiment 1*, 100% of the saliva samples collected using the Bardole™ and the GGH method yielded interpretable or CODIS-eligible DNA profiles. In stark contrast, only one interpretable and CODIS-eligible DNA profile was generated across all touch DNA samples in *Experiment 2* and this profile was for a nickel-plated casing using the Bardole method™. This conforms with the widely known concept that there is a relatively low

success rate associated with the DNA recovery from touch DNA on cartridge casings, particularly for brass FCCs.

Despite the low-level success, *Experiment 2* indicates that the Bardole method™ can be considered an effective collection method for touch DNA on FCCs, particularly nickel-plated FCCs. Factors such as the DI values and the DNA concentrations suggest that the GGH method may be preferable only when analyzing brass FCCs. Nonetheless, the lack of interpretable profiles from touch DNA samples collected using the GGH, Chelex®, and EDTA methods seems to indicate that the addition of a chelating agent in the DNA collection method does not lead to more accurate and improved DNA results across FCCs, particularly ones that are nickel-plated. Since the Bardole method™ does not incorporate the use of a chelating agent or the pre-filter device, this means it is more cost and time-efficient in comparison to the other three methods. The lack of reliability demonstrated by the Chelex® and EDTA methods in collecting touch DNA may discourage a laboratory from selecting them as a collection method for FCCs.

Since it is widely known that touch DNA samples often have significant amounts of degradation, it was hypothesized that any DNA profiles that were generated would likely be partial profiles (7). Thus, the generation of a complete and accurate DNA profile (D3 B-NC1) exceeded the expectations for *Experiment 2*. Though this sample had a low DI, the quantity of DNA that could be amplified did not meet the 0.75 ng as recommended by the Globalfiler™ Amplification kit. Thus, the generation of this profile is somewhat of an anomaly. The generation of this DNA profile may indicate that even if samples have a very low DNA concentration, if the corresponding DI indicates low levels of degradation this

may be sufficient to render an interpretable profile. It should not be overlooked that for this study, each sample or replicate consisted of five cartridge casings processed together. Therefore, the generation of a complete and accurate profile may simply be attributed to this procedural element, which is not a common practice in forensic casework.

4.5 Assessment of the Modifications to the Bardole™ and GGH methods

Since the Bardole™ and the GGH methods demonstrated the most success in collecting touch DNA from FCCs, inefficiencies in these methods were targeted for improvement. During *Experiments 1* and *2*, it was observed that both of these methods may have been experiencing losses in DNA during the elution-vacuum filtration steps. There were noticeable volumes of collection solution that accumulated on the interior walls and within the interior lip area surrounding the filter. Additionally, the GGH method produced significant foaming during the vortex steps leaving a considerable amount of residual foam on the sides of the conical tubes and in the concentration cup. These losses in collection solution and DNA were not observed when performing the Chelex® method.

It was hypothesized that the consecutive vortex combined with the cone method in the Chelex® method helped maximize the interaction between the collection solution containing DNA and the concentration filter. As a result, the incorporation of these two elements in the Bardole™ and GGH methods could potentially minimize the loss of DNA to the other surfaces. An additional purpose of the cone method was to concentrate the DNA on a smaller area of the filter to increase extraction efficiency and help maximize DNA recovery. However, the size of filters that were cut and extracted across all samples in *Experiment 4* were comparable to those of the methods without the cone method.

Furthermore, the DNA concentrations and DI values of samples indicated that this modification did not generate improved collection efficiency, particularly for the Bardole™ method.

The incorporation of the consecutive vortex and cone method elements in the GGH method resulted in a significantly reduced amount of foaming observed. Modifying the GGH method with only the consecutive vortex element rendered one interpretable profile of a nickel-plated FCC sample, highlighting the effectiveness of this element. This step involved concentrating the same amount of DNA in a smaller volume providing the same effect as if reducing the elution volume to concentrate DNA (53,54). Though in forensic casework it may not be common practice to collect DNA from multiple cartridge casings on the same filter, the success of this modification indicates that it may be beneficial to reduce the collection solution volume when collecting touch DNA from a single FCC. It is important to be aware that the success of this modification may have been attributable to a donor with a very good shedder status. Additional samples and testing would be needed in order to determine whether the success of this sample was due to the incorporation of the consecutive vortex element or due to shedder status of the donor.

5. CONCLUSIONS

5.1 Evaluation of the Bardole™, GGH, EDTA, and Chelex® DNA Collection

Methods

The Bardole method™ yielded the best overall results when performing DNA collection of touch DNA on FCCs. Despite the absence of a chelating agent, the Bardole method™ generated a larger quantity of high-quality interpretable DNA profiles with an increased frequency in comparison to the Chelex® method. In addition, the Bardole method™ was shown to recover a statistically higher amount of touch DNA from nickel-plated FCCs in comparison to the Chelex® method.

Comparisons of DNA recovery efficiency, average DNA concentrations, DI values and RFUs demonstrated that both the GGH and the Bardole method™ had comparable success rates in collecting touch DNA samples on FCCs that generated forensically relevant profiles. The Bardole method™ appeared to be best suited to collecting touch DNA samples from nickel-plated FCC and was the only method in which the collection of touch DNA from an FCC resulted in a DNA profile that could be considered CODIS-eligible. Though the incorporation of the cone method in the GGH method was able to achieve some success in rendering a sample from a nickel-plated FCC with an interpretable profile, the allelic coverage of this profile was not as extensive as that rendered using the Bardole method™.

The GGH method appeared to achieve better quality and higher quantities of DNA from touch DNA samples collected from brass FCCs compared to the EDTA and Chelex® methods, indicating the effectiveness of the GGH chelating agent. However, in these

samples there was not enough DNA to produce an interpretable DNA profile. In addition, the Chelex® method demonstrated complete lack of success in being able to collect touch DNA from brass or nickel-plated FCCs. Additionally, the complete lack of success of the EDTA method in collecting DNA from FCCs and UCCs throughout this study suggests that this method is incompatible with touch DNA recovery on cartridge casings. The lack of success of both of these methods also suggests that neither the use of Chelex® beads or EDTA chelating agents was well-suited for collection of touch DNA on FCCs in this study.

Despite not rendering any interpretable profiles with the FCCs in *Experiment 2*, the GGH protein appears to be successful in reducing the effects of copper ions as seen from improved DNA concentrations and DI values. Therefore, it suggests that other aspects of the method require optimization.

Lastly, due to the high variability of shedder status that is associated with touch DNA samples, the measured success of these methods may vary depending on the donor of DNA. Both the GGH method and the Bardole method™ generated interpretable DNA profiles across multiple donors. Though the relative success and performances of both the Bardole method™ and the GGH method are comparable, the GGH method required a longer amount of sample preparation time and is more expensive due to the high cost of the GGH protein and BSA. In addition, the GGH method also required the pre-filter device. Taking into consideration these factors and the results from this study, the method recommended for crime laboratories such as WPD CSU to use when collecting touch DNA from FCCs is the Bardole method™. This is particularly recommended for smaller crime laboratories that typically have lower budgets.

5.2 Future Considerations

Despite the higher associated cost and time to perform, it may be beneficial to assess the effectiveness of adding a "pre-filter step" to the Bardole method™ to help eliminate any GSR components, debris or metal ions that are found on the surface of FCCs. Failure to remove such contaminants could result in high levels of degradation and PCR inhibition and be responsible for the limited successes in complete and accurate DNA profiles being generated by this collection method.

A study utilizing a rinse-and-swab touch DNA collection method demonstrated that the DNA collection should be performed as soon as possible once touch DNA is deposited on the casings in order to obtain the best quality DNA profiles (55). Thus, varying the time for which the deposited DNA is left on the FCCs prior to DNA collection is another variable that can be explored since this may indicate when is the optimal time to perform DNA collection using the M-VAC® cell collection system.

Comparing the performance of the Bardole™ and GGH collection methods when using different volumes of collection solution is also recommended. Studies have shown that increasing elution volume during DNA extraction helps promote higher DNA yield but lower concentrations, whereas decreasing the elution volume promotes low total DNA yield but higher concentrations (53,54). Thus, optimizing the volume of collection solution may be critical for developing a method that promotes both sufficient yields and concentrations of touch DNA.

The benefits of the use of carrier ribonucleic acid (RNA) in increasing DNA yield of low-level samples during when conducting DNA extraction have been demonstrated by

several studies. Carrier RNA is added to mimic nucleic acids and prevent compounds from irreversibly binding target DNA, therefore increasing DNA recovery (56). The addition of carrier RNA in the collection solutions of touch DNA collection methods such as the Bardole method™ could serve to reduce the amount of DNA molecules that bind to copper ions. Thus, a future study should incorporate carrier RNA in the collection solution for both the Bardole and GGH methods to evaluate the effect on DNA recovery efficiency of touch DNA on FCCs across the two methods.

Lastly, for this study, all sample replicates consisted of DNA that was collected from five cartridge casings processed together. Though this was done primarily in an effort to increase the surety of generating sufficient levels of DNA for comparison purposes, results of this study demonstrate that this was not always achieved. The use of five cartridge casings per replicate also meant that the total number of sample sets was small for this study, making it difficult to make meaningful comparisons and determine the statistical significance of the results. In addition, using five FCCs per replicate was also not very realistic for forensic casework since consecutive sampling of FCCs on the same filter is likely not a standard practice in casework. To ensure that the results obtained are as reflective of real casework as possible, future studies where the collection of each casing is done individually should be performed.

APPENDIX

Appendix A: Amount of DNA amplified (ng) for all FCC and UCC samples

Table A. Summary of Values for the Amount of Saliva DNA Amplified (ng) Per Replicate for Each DNA Collection Method in Experiment 1. Comparison of DNA quantity values across samples using Bardole™, GGH, EDTA and Chelex® methods to recover saliva DNA on fired brass (BC) and nickel-plated cartridge casings (NC). (N/A) denotes samples for which no DNA was detected.

Cartridge sample	Amount of DNA Amplified (ng)			
	Bardole™(B)	GGH	Chelex®	EDTA
BC1	0.51	0.75	N/A	N/A
BC2	0.23	0.74	0.75	N/A
NC1	0.75	0.75	0.59	N/A
NC2	0.75	0.75	N/A	N/A

Table B. Summary of Values for the Amount of Touch DNA Amplified (ng) Per Replicate for Each DNA Collection Method in Experiment 2. Comparison of DNA quantity values across samples using Bardole™, GGH, EDTA and Chelex® methods to recover touch DNA on fired brass (BC) and nickel-plated cartridge casings (NC). (N/A) denotes samples for which no DNA was detected.

Donor and Cartridge Type	Amount of DNA Amplified (ng)			
	Bardole™(B)	GGH (G)	EDTA(E)	Chelex® (C)
D1 BC1	0.03	0.01	N/A	N/A
D1 BC2	N/A	N/A	N/A	N/A
D2 BC1	N/A	N/A	0.04	N/A
D2 BC2	N/A	N/A	N/A	N/A
D3 BC1	N/A	N/A	N/A	N/A
D3 BC2	N/A	N/A	N/A	N/A
D1 NC1	0.01	N/A	N/A	N/A
D1 NC2	N/A	N/A	N/A	N/A
D2 NC1	N/A	N/A	N/A	N/A
D2 NC2	0.07	N/A	N/A	N/A
D3 NC1	0.08	N/A	N/A	0.01
D3 NC2	N/A	0.02	N/A	N/A

Table C. Summary of Values for the Amount of Touch DNA Amplified (ng) Per Replicate for Each DNA Collection Method in Experiment 3. Comparison of DNA quantity values across samples using Bardole™, GGH, EDTA and Chelex® methods to recover touch DNA on unfired brass (BU) and nickel-plated cartridge casings (NU) for three donors (D1, D2, D3). (N/A) denotes samples for which no DNA was detected.

Donor and Cartridge Type	Amount of DNA Amplified (ng)			
	Bardole™ (B)	GGH (G)	EDTA(E)	Chelex® (C)
D1 BU	0.28	0.22	N/A	0.11
D2 BU	0.03	N/A	N/A	N/A
D3 BU	N/A	N/A	N/A	0.02
D1 NU	0.54	0.07	N/A	0.08
D2 NU	0.02	0.04	N/A	N/A
D3 NU	0.07	0.07	N/A	N/A

Table D. Summary of Values for the Amount of Touch DNA Amplified (ng) Per Replicate for Each DNA Collection Method in Experiment 4. Comparison of DNA quantity values across samples using the Bardole™ and GGH methods to recover touch DNA on fired brass (BC) and nickel-plated cartridge casings (NC) for three donors (D1, D2, D3). (N/A) denotes samples for which no DNA was detected.

Donor and Cartridge Type	Amount of DNA Amplified (ng)		
	Bardole™ (B) – cone method	GGH (G) – cone method	GGH (G) – consecutive vortex
D1 BC1	0.02	0.02	N/A
D1 BC2	0.06	0.02	N/A
D2 BC1	N/A	0.02	N/A
D2 BC2	N/A	0.03	N/A
D3 BC1	N/A	N/A	N/A
D3 BC2	0.02	N/A	N/A
D1 NC1	0.03	N/A	N/A
D1 NC2	N/A	N/A	0.37
D2 NC1	N/A	0.02	N/A
D2 NC2	N/A	N/A	N/A
D3 NC1	0.03	N/A	N/A
D3 NC2	N/A	N/A	N/A

LIST OF JOURNAL ABBREVIATIONS

Clin Chim Acta	International Journal of Clinical Chemistry
Coord Chem Rev	Coordination Chemistry Reviews
Croat Med J	Croatian Medical Journal
Forensic Sci Int	Forensic Science International
Forensic Sci Int Genet	Forensic Science International: Genetics
Forensic Sci Int Genet Suppl Ser	Forensic Sci International. Genetics Supplement Series
Fron Ecol Evol	Frontiers in Ecology and Evolution
Forensic Sci Int	Forensic Science International
Glob Med Gen	Global Medical Genetics
Int Congr Ser	International Congress Series / Excerpta Medica
Int J Environ Res Public	International Journal of Environmental Research and Public Health
Int J Mol Sci	International Journal of Medical Sciences
Int J Legal Med	International Academy of Legal Medicine
J Assoc Crime Scene Reconstr	ACSR
J Clin Microbiol	Journal of Clinical Microbiology
J Forensic Identif	Journal of Forensic Identification
J Forensic Sci	Journal of Forensic Sciences
Sci Justice	Science & Justice
WIREs Forensic Sci	Wiley Interdisciplinary Reviews. Forensic Science

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CURRICULUM VITAE

