A COMPARATIVE STUDY OF TOUCH DNA RECOVERY FROM METALS

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A COMPARATIVE STUDY OF TOUCH DNA

RECOVERY FROM METALS

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Abstract: Touch DNA requires a special collection approach to recover the minute amounts of DNA left behind in skin cells. Touch DNA has been successfully recovered from surfaces such as fabric, plastic and wood. However, a consistent effective method to recover sufficient DNA for profiling from metals has yet to be developed. This study investigated in depth the collection of low amounts of DNA deposited on six different types of metal surfaces using five different types of collection tools in combination with four different types of buffer solutions to determine the method that yields the greatest and most consistent recovery of DNA. For all collection procedures, the DNA IQ DNA extraction kit was used to extract and recover DNA collected with the difference swab/solvent combinations. Statistical analysis was completed using ANOVA with Tukey's multiple comparison test.

Sarkosyl buffer combined with tape lift to recover DNA left behind on brass-plated metals showed the highest mean recovery of DNA spotted and dried onto the metal. However the same combination of Sarkosyl buffer and tape lift did not perform similarly across all six metals. This study showed that not only do buffer solution and collection tool play a role in the recovery of touch DNA, but the type of metal the DNA is deposited on also plays a significant role in the recovery rate of touch DNA left behind on metals.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 5

2.1 Touch DNA 5
2.2 Recovering Touch DNA 6
2.2.1 DNA and Metals 6
2.3 Methods 7
2.3.1 Buffer Solutions 8
2.3.2 Type of Recovery Tool 8
2.3.3 Extraction Kits 9
2.4 Discussion 10

III. METHODOLOGIES 12

3.1 Metals and PCR Kit Collections 13
3.1.1 Preparation of the Metal Samples 14
3.2 Creating the Standard Curve 15
3.3 Quantification Real-Time PCR (RT-qPCR) 17
3.3.1 DNA-IQ System DNA Bead Extraction using TE^{-4} and DTT/Lysis 18
3.3.2 DNA-IQ System DNA Bead Extraction using SDS and Sarkosyl 22
3.3.3 RT-qPCR Set Up and Amplification 24
3.4 Determining Which Method Yields the Most DNA 25
3.4.1 Quantitative Amplification using the 7500 Real-Time PCR System 25
3.4.2 Statistical Analysis 28
3.4.3 DNA Profiling 28

Chapter Page

IV. RESULTS 30

4.1 Real-Time PCR (RT-qPCR) 30
4.1.1 DNA Yield 31
4.2 Statistical Analysis 31
4.3 PCR Inhibition 41
4.4 STR Profiling of Recovered DNA 41

V. DISCUSSION 47

5.1 DNA Yield 47
5.1.1 Statistical Comparison of DNA Yield 49
5.1.2 Complications 52
5.2 Future Research and Recommendations 53
5.3 Conclusion 54

REFERENCES 56

APPENDICES 61

LIST OF TABLES

Table Page

1 Standard Curve Dilution 16
2 Multiple Comparison Two-way ANOVA 32

LIST OF FIGURES

Figure Page
1 Spotted DNA on Metal Surfaces....15
2 Tubes with Silica-coated Beads on the Magnetic Stand....20
3 Amplification Plot Showing the Phases of PCR Amplification....26
4 Standard Curve Using Replicate Reactions of Known DNA....27
5A Significant Differences Multiple Comparison of All Metals and Treatments....33
5B Mean DNA Recovery from Metals per Buffer Solution/Collection Device Combination....33
6 Significant Differences Tin Versus All Other Metals....35
7 Significant Differences Aluminum Versus All Other Metals....36
8 Significant Differences Brass Versus All Other Metals....37
9 Significant Differences Copper Versus All Other Metals....38
10 Significant Differences Steel Versus All Other Metals....39
11 Significant Differences Brass Plated Versus All Other Metals....40
12 Electropherogram Using Cotton Swabs Combined with TE^{-4} on Tin....43
13 Electropherogram Using Dacron Swabs Combined with Lysis on Steel....44
14 Electropherogram Using Cotton Swabs Combined with Lysis on Aluminum....45
15 Electropherogram Using Cotton Swabs Combined with TE^{-4} on Brass Plated....46

CHAPTER I

INTRODUCTION

Television shows such as *CSI* and *Law and Order: SVU* have become extremely popular among the public since the early 2000s. Such shows often portray forensic science as fast and infallible, always producing analysis results that will lead to the perpetrator.[1] On these shows the most popular type of evidence depicted that ties a perpetrator to the crime is DNA, or deoxyribonucleic acid. This molecule containing unique hereditary material is found in body tissues such as hair, blood, saliva, semen, and skin cells.[2] Because the popular television shows produce infallible evidence, jurors in real trials may expect investigators to provide DNA evidence in every case before returning a guilty verdict. Without DNA evidence, juries may find no connection and not convict.[3] A juror's unrealistic expectation of hearing DNA testimony in every case for a conviction to occur has led to scientists studying the amount of DNA left behind in cells that are transferred during the "touch" process in which an item is handled by a perpetrator.

When DNA was first introduced into the forensic lab in the 1980s, investigators needed a blood or semen sample about the size of a quarter for analysis. As DNA typing procedures evolved to greater sensitivity, analysts required less and less sample to produce a genetic profile. Now, analysts do not even need a body fluid such as blood or semen to produce results.

Investigators can utilize as a source of DNA what is referred to as touch DNA, or low copy number DNA, in order to obtain a profile. Touch DNA is just what it sounds like: DNA that is left behind on items that have been touched by an individual.[4] A person may shed up to 1,000,000 skin cells per day.[5] These cells contain either fragmented DNA or cell-free nucleic acids and can contain enough DNA to produce a genetic profile using polymerase chain reaction (PCR) amplification techniques.[6] However, existing methods for collecting trace DNA samples from solid surfaces, especially metals, are less than optimal and therefore more research directed at improving swab based DNA collection methods is needed.[7]

Studies have shown that touch DNA can easily be collected from surfaces such as glass, plastic, and fabric to obtain a genetic profile.[8] However, surfaces such as metal have proven to be more difficult to reliably recover sufficient DNA to produce a profile.

Metals have a range of electron affinities and ionization energies that would affect their interaction with negatively charged compounds such as DNA.[9] The backbone of DNA consists of negatively charged phosphates which confer on DNA properties that allow base pairs to form ionic bonds with metal ions, which may prevent the release of touch DNA once it has bonded with a metal surface.[10] However, because of advances with DNA extraction from certain metals, studies have also shown that small amounts of touch DNA may be obtained from ridged metal surfaces such as guns and bottle tops.[11] So far, the studies that have been conducted on the recovery of touch DNA from metals have been extremely limited, and the best method of recovery has yet to be defined.

Metals may be found in abundance at a crime scene. Perhaps an intruder may leave prints behind on an aluminum window frame or a brass doorknob. A gun may contain touch DNA on the trigger, the slide or the butt. A perpetrator may think to clean all possible traces of DNA from a gun but may never think to wipe down the bullets that he loaded into the gun without wearing

gloves. All of the above mentioned scenarios are only a few examples of where touch DNA may be located on metals at a crime scene. Because metals can be located at a crime scene and can harbor touch DNA, a sample recovery method that elevates the efficiency of isolating and recovering touch DNA from metals would greatly benefit the crime laboratory during the investigation of the crime.

Currently, the primary method of transferring possible touch DNA from a surface for later DNA analysis is to pre-wet a swab of some type and then "scrub and roll" the swab over the surface thought to harbor DNA.[12, 13] Various studies on touch DNA recovery from surfaces other than metals (glass, plastic, fabric, and wood) have shown that swab type and swabbing technique play a role in the amount of touch DNA recovered from surfaces.[14, 15] Such studies evaluated methods to recover touch DNA using tape lift, cotton swabs, flocked swabs, and Polymerase Chain Reaction (PCR) squares, which are small squares of filter paper that may be manipulated with a tool to reach tight areas that a swab may not reach. Results varied among studies as to which method worked best. Studies on recovering touch DNA from surfaces also explored the efficiency of DNA recovery when the swab is pre-wet with various types of buffer solutions. Solutions tested as wetting agents for a swab chemically aid in solubilizing DNA from the tissue structures that contain it may also bind metal cations, while inactivating DNA nucleases and protecting the DNA from degradation.[16] A study by Thomasma and Foran was conducted using various buffer solutions including a "lysis buffer" composed of Sodium Dodecyl Sulfate and Triton X-100, and a TE (Tris-EDTA) buffer to moisten the swab prior to swabbing a surface for touch DNA.[17] Thomasma and Foran determined that buffer solution does have an effect on the amount of touch DNA recovered from a given surface. Since both the swab solution and the recovery method have been shown to contribute to the amount of touch DNA recovered from surfaces other than metals, the purpose of this study was to determine which swab type in

combination with which buffer solution works best to recover the maximum amount of touch DNA left behind on metals including brass, copper, aluminum, steel, tin, and brass plated alloy.

Answering the question "Does swab solution and swab type determine the amount of DNA recovered from metals?" will further improve the likelihood of producing DNA typing results for investigators and prosecutors in developing a more compelling case against a defendant where other probative evidence may be lacking. Jurors continue to expect sophisticated methods that have a high rate or reliability for identifying the perpetrators of a crime. The ability to recover sufficient DNA from touch evidence for the production of a DNA profile, regardless of the substrate the touch DNA is deposited on, enhances the forensic capabilities of law enforcement in general, and opens the door to the application of DNA typing in property crimes, which are the principle type of crimes not being processed due to low success rates, and hence the type of case that forms the bulk of the backlog in the U.S. and around the world.

CHAPTER II

REVIEW OF LITERATURE

Deoxyribonucleic acid (DNA) found in blood, saliva, semen, and tissue evidence has been significantly helpful to forensic scientists since DNA analysis first appeared in forensics in the late 1980s.[18] While traditional techniques such as fingerprinting are still used for individualization, DNA evidence may provide a much stronger link between a perpetrator and a particular crime. DNA is present in relatively high amounts in tissues and blood; however, this evidence may not always be left at a crime scene. Perhaps a spent bullet cartridge is found but there is only a partial print, or copper wire is used to strangle someone. Although there are no body fluids or tissues present, this evidence may still be very useful to forensic scientists as it may contain small amounts of trace DNA, referred to as "touch DNA".

2.1 Touch DNA

Touch DNA is DNA left behind by a person after coming into contact with an item. However minimal this DNA may be, it often can still be used for forensic analysis. Research shows a person may slough off thousands of dead skin cells per day.[19] These cells contain cellfree nucleic acids, or CNAs. Suzanna Ryan, a consultant in Forensic DNA, states that although the cells no

longer contain nuclei, a DNA profile can still be generated from the CNAs, which are composed of free DNA fragments, that may be contained in sweat.[20] She has also noted that forensic DNA investigations are becoming more and more complex due to touch DNA. Cells containing CNAs are extremely limited, and may become degraded much more easily than DNA contained within tissue (such as bloodstain, semen stain or saliva stain). The type of material the DNA is bound to will also predict impediments to recovering sufficient DNA for analysis. Several studies have examined what affects the transfer of CNAs onto particular surfaces. These studies include looking at shedder status (the rate at which skin cells are shed), hygiene practices, and handling time of an item. Studies have determined the longer an item is handled, the more optimal the chance of DNA recovery.[21] However, vigorous hand washing causes a substantial decrease in shedder status, which is extremely significant in obtaining touch DNA from objects.[22]

2.2 Recovering Touch DNA

Recovering touch DNA has been successful in some circumstances, but yet proven difficult in other circumstances for multiple reasons. Studies have shown that the type of surface the DNA is being deposited on matters significantly. The more abrasive the surface, the better the chance more skin cells will be sloughed off during contact.[23] The literature has shown successful recovery of touch DNA from plastic, glass, and wood. However studies have found difficultly in recovering DNA from certain types of metal such as brass and copper.

2.2.1 DNA and Metals

The inability to recover touch DNA from metal may be due to the affinity DNA exhibits towards metal. DNA is associated with metal cations in the phosphate backbone structure: the

principal structural support and contributor to the structure of the double helix. Metal cations are also integral to the control of gene expression with chromosomal DNA,[24] and studies have shown that the nucleotide base pairs in DNA are able to form an ionic bond with certain metal ions.[25] The known interaction of DNA base pairs with metal cations offer insight into why it may be difficult to release DNA from a metal surface.

Metals are found in weapons and ammunition, explosives, door handles, window frames and screens, home furnishings, as well as other evidence common to crime scenes. The literature has cited very few examples of the successful recovery f trace amounts of DNA from metals sufficient to produce a DNA profile. The few studies that do cite recovery from metals have limited metal samples and do not include copper as a surface being used during the study. One particular study focused on designing an extraction method sensitive enough to recover DNA left behind on cartridges and bullets.[26] This method showed that soaking the sample in a lysis buffer to release the DNA worked better than just swabbing the surface of the sample. However, soaking a piece of evidence may not always be feasible.

Another possible reason metals like brass and copper have proven particularly challenging for the recovery of touch DNA may relate to copper acting as a catalyst promoting or enhancing the degradation of DNA on the metal surface. Copper exhibits antimicrobial effects, known to be due to the detrimental effect of the metal on microbial DNA. And, because brass is an alloy of copper, the same problems exist.[27] Due to the difficulty of recovering touch DNA left on particular types of metal, and the substantial amount of metal that may be found at a crime scene, there is a pressing need to research the best recovery method of touch DNA left behind on metal surfaces.

2.3 Methods

Although current research is discouraging, studies have provided valid extraction methods of recovering DNA from some metals, such as steel.[28] Other surfaces such as plastic and glass have shown success with particular extraction methods, which could be tested with metal surfaces.[29]

2.3.1 Buffer Solutions

Studies have shown that the buffer solution type used in a touch DNA recovery method plays an important role due to the binding ability of the DNA to the metal. Buffers aid in lysing the cells and releasing the DNA while maintaining the integrity of the DNA. A particular pH is necessary during this step.[30] Research has been completed comparing multiple buffer solutions used to recover DNA. During one study, touch DNA samples were prepared from volunteers and analyzed using detergents, commercial solutions, and water. Using solutions containing a detergent such as SDS or Triton X-100 rather than using only water worked best for obtaining a higher recovery of touch DNA.[31] Other studies have shown that buffers containing a phosphate buffered saline worked very well at obtaining a profile from trace amounts of DNA.[32]

2.3.2 Type of Recovery Tool

In addition to the effect of the buffer solution used for DNA recovery, the collection device may also play an important role in the recovery of touch DNA. One particular swabbing technique or device may be better than another for the recovery of DNA from surfaces. Flocked swabs have shown success with direct amplification procedures.[33] Swabs such as cotton, Dacron, and nylon have all been used to recover touch DNA from samples. Research has shown even a method other than swabbing, such as tape lift or Polymerase Chain Reaction (PCR) squares,

which are small squares of filter paper, may be used.[34] PCR squares have been used in order to obtain access to small areas possibly containing DNA. The small squares can be manipulated easier than a traditional swab. However, studies on ridged surfaces have shown that tape-lift methods work best to recover touch DNA.[35]

2.3.3 Extraction Kits

Kits containing everything needed for efficient extraction and amplification of DNA are available commercially for DNA recovery. Comparative studies have been completed on commercial kits to determine the efficiency and sensitivity using whole-blood samples. The results showed that bead extraction kits like the DNA IQ system from Promega Corp. (Madison, WI) yielded lower DNA concentrations than desired, while other kits such as the Millipore Montage were difficult to use.[36] Research has also shown the difficulty in obtaining a DNA profile from metals often used in the manufacture of firearms because commercial STR typing kits available do not amplify STR alleles sufficiently for the low DNA levels left behind on metals. One study focused on using an STR MiniFiler kit, which is designed for low level DNA. During the study, the MiniFiler kit amplified a higher number of alleles when compared with kits such as the PowerPlex 16 Bio kit.[37] Another study comparing kits and direct PCR analysis showed that direct PCR rather than extraction maintains the quality of DNA with lower allele dropout and higher peak RFU values.[38] Scientists have also developed a highly sensitive procedure for detecting low amounts of DNA on firearms, and yet this process yielded only a 26% success rate.[39] This method uses a DNA mini kit but makes specific changes that include soaking the casing in a buffer solution for a given amount of time. Although the success rate was low, the details for this method provides information as to what may work to obtain a DNA profile from metals, and shows how the buffer used may determine the success rate of recovery.

2.4 Discussion

All of the research performed to date on producing DNA profiles from trace amounts of DNA has shown that a single optimal DNA extraction and recovery procedure has not yet been developed. Although some studies have been performed on firearms and cartridges, these results have had very low success rates. Because metal is ubiquitous, there is a high likelihood of encountering metal in a forensic investigation. Based on the body of previous research, this study was designed to investigate combinations of recovery buffers and collection devices to identify an improved trace DNA collection procedure. This study included various collection devices including Dacron swabs, cotton swabs, glass-fiber swabs, PCR squares, and tape lift; which were pre-wetted before DNA collection with buffers including Sarkosyl/Proteinase-K buffer, Sodium Dodecyl Sulfate/Proteinase-K buffer, Tris-EDTA buffer and a lysis buffer containing a concentrated chaotropic salt (guanidine isothiocyanate) supplied with a commercial DNA extraction and recovery kit (Promega DNA-IQ System). A study of this type had not yet knowingly been performed on copper, brass and aluminum.

Previous research in our laboratory described the development of a novel glass-fiber swab,[40] and was included in the current study as one of the investigated methods for recovering DNA from metals. Because DNA is attracted to silica, the rationale for the collection method was that DNA should naturally bind to the glass-fiber filter material used to make the glass-fiber swab when it is pre-wetted with a concentrated chaotropic salt solution (i.e. lysis buffer). The glass-fiber swab study was completed concurrently with the current study on touch DNA recovery from metals. Other research that was conducted concurrently involved the recovery of touch DNA from fabrics. Collection techniques in the fabric study were similar to those in the current study, except that actual cuttings from the samples were taken rather than the use of PCR squares.

It is known that a single cell contains about 6 pg of DNA.[41] Depending on the surface of the item touched and how long the item is handled, a very minute amount of touch DNA may be left behind. In this study, a total of 10 ng of extracted and purified DNA was spotted onto the different metal surfaces to represent low copy number DNA left behind on objects.

In summary, the lack of substantial literature on recovering touch DNA from metals underscores the need for this research. Other research has been completed on various surface types such as plastics, glass, and wood. But, because metal evidence is ubiquitous at crime scenes, the ability to recover maximal amounts of trace DNA to yield a genetic profile would aid crime laboratories in producing probative DNA profiles for law enforcement and the courts in prosecuting offenders. This study will help close a gap in the literature on techniques for obtaining DNA profiles from touch DNA left behind on metal.

CHAPTER III

METHODOLOGIES

DNA, a valuable source of evidence for criminal cases, can sometimes be left behind in fingerprints or on items touched by perpetrators.[42] Touch DNA, or low copy number DNA, samples may contain less than 300 pg of genomic DNA template for STR analysis, resulting in a loss in sensitivity and completeness of STR profiles produced from evidence.[43] Studies have shown that touch DNA is deposited more easily onto rough surfaces such as wood as opposed to smooth surfaces such as metal.[44] However, metal is one of many types of evidence that is found in abundance at crime scenes. Metal evidence may include pure metals such as steel or aluminum, or plated metals such as brass plated fixtures. Because so little DNA may be on the evidence found at a crime scene, it is crucial to maximize the amount of DNA recovered from touched objects.

In addition to the surface type of the substrate affecting DNA recovery, the amount of DNA recovered is also dependent upon the method of collection. Studies have shown that the type of swab and the buffer solution used to pre-wet the swab play a crucial role in the recovery of touch DNA.[45] The purpose of this study was to determine which swab and buffer combination works best to recover touch DNA left behind on various types of metals to achieve maximum

yield amounts for quantitation. The DNA used in this study was a purchased stock DNA. Since no human participants were involved during this research, IRB approval was not required.

3.1 Metal and PCR Kit Collections

For this study, five different types of pure metals and one brass plated metal were purchased at a local hardware store. These included a brass pipe, a copper pipe, a brass plated kick plate, and three sheet metals consisting of tin, steel, and aluminum. The metals were purchased new to avoid corrosion and oxidation. The metals were cut into smaller pieces approximately 5x5-in, except for the pipes, which were cut into lengths of 5 in. The five different collection techniques included the use of Puritan Sterile cotton tipped applicators, Pur-Wraps sterile Polyester tipped applicators (Dacron swabs), Scotch tape, Polymerase Chain Reaction (PCR) squares (small squares of Whatman Chromatography filter paper #1, approximately 0.6x0.6-in), and glass-fiber swabs (produced by hand in the OSU-CHS lab from sheets of Whatman GF/C filter paper glued to a plastic applicator with superglue). Dacron and cotton swabs were considered sterile when obtained from an unopened package. PCR squares, the wooden sticks used to manipulate the squares, and the glass fiber swabs were placed into an ultraviolet (UV) irradiator at an energy density of 3000 mJ/cm^2 to ensure no DNA contamination was present. Tape was considered sterile if it was on the roll.

Four different extraction buffers were used with each collection device on each metal: lysis buffer (~8M guanidine isothiocyanate pH 8.0), which is supplied with the DNA IQ DNA extraction kit, containing 10 mM dithiothreitol (DTT); TE^{-4} solution containing 10 mM Tris-Cl, pH 8.0 with 0.1 mM EDTA (TE^{-4}); a 2% sodium dodecyl sulfate (SDS) solution containing 955 µL TNE (10mM Tris-Cl, pH 8.0; 0.2 M NaCl; 0.1 mM EDTA), 25 µL 20% SDS, and 20 µL 20 mg/ml Proteinase K (Pro-K); and a 2% Sarkosyl buffer solution was created using 855.2 µL TNE

(10 mM Tris-Cl, pH 8, 0.2 M NaCl, 1 mM EDTA) added to 20 µL 20% Sarkosyl, 125 µL of 20 mg/ml Proteinase K, and 0.8 µL DTT (1M). Buffer solutions use protease enzymes to lyse open the cells to allow for DNA binding, which is essential in touch DNA recovery. A series of experiments using each specific collection method was conducted with each buffer.

Once DNA has been recovered from the solid substrate, the DNA was recovered from the swab/filter square/tape lift using the DNA IQ extraction kit (Promega Corp., Madison WI). This system is specifically designed for extracting and recovering genomic DNA for forensic and paternity applications.[46] The kit contains silica-coated magnetic beads that bind DNA in the presence of a high salt environment such as that provided by the lysis solution supplied with the DNA IQ kit. In order to extract DNA from a biological sample, a lysis buffer containing 6-8 M guanidinium isothiocyanate is added to a sample and allowed to solubilize any biological materials for 30 minutes. Magnetic silica-coated beads are then added to the extract which binds the DNA. Samples are washed repeatedly and then purified DNA may then be eluted from the silica beads using TE^{-4} pre-warmed to 65°C. The DNA eluted from the beads is generally free of PCR inhibitors and is ready for PCR amplification.

3.1.1 Preparation of the Metal Samples

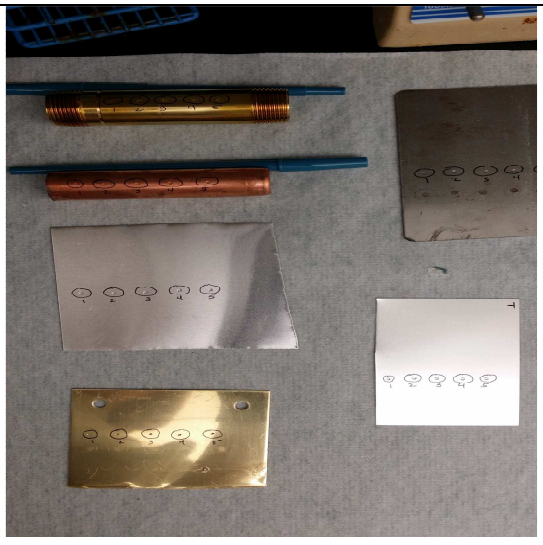
Each metal was cleansed with a 10% bleach solution, rinsed with deionized water, and wiped with ethanol and a Kimwipe. This cleansing procedure was repeated twice. The metals were allowed to dry before being placed into a UV irradiator and processed at energy density of 3000 mJ/cm^2 to ensure any DNA contamination was eliminated. The metals were then placed onto a clean workbench where samples of a known DNA concentration were spotted onto them and allowed to air dry. DNA was diluted for spotting to 5 ng/µL using TE^{-4} (10 mM Tris-Cl, pH 8.0, 0.1 mM EDTA). Diluted DNA in the amount of 2 µL was then pipetted onto each metal in

five marked locations resulting in five samples for each of the metals and a total of thirty samples (Figure 1). The DNA spots were allowed to dry overnight. This process was repeated prior to each experiment to create the samples for extraction. The samples were labeled sample 1 through sample 5 for each respective metal (i.e. Copper 1, Copper 2…Copper 5).

3.2 Creating the Standard Curve

Recovered DNA was quantified using the Quantifiler real time PCR kit from Applied Biosystems (Foster City, CA). To quantify DNA recovered using the different methods, a standard curve must be created. A standard curve aids in determining the concentration of DNA in an unknown sample. If any mistakes are made when creating the standard curve, the results of the unknown samples will be inaccurate.

Figure 1. Spotted DNA onto Metal Surfaces



Five marked locations on each metal show where to spike known DNA samples. Each marked location contains 2 µl of 5 ng DNA for a total of 10 ng concentrated DNA per location.

The standard curves used in this study consisted of 8 concentrations of human genomic DNA diluted from 50 ng/µL down to 23 pg/µL as described in the instructions accompanying the Quantifiler kit (Table 1). To create a master mix for the reactions, a ratio of 4:5 µL of Quantifiler Human primer mix to Quantifiler PCR reaction mix was mixed thoroughly in a 1.5 mL reaction tube. Enough master mix was created for ten reactions to allow for the eight standard reactions plus two additional reactions to account for pipetting error.

Table 1. Standard curve dilutions

Standard	Concentration (ng/ µL)	Amounts
Std. 1	50.000	5 µL [200 ng/ µL stock] + 15 µL TE^{-4} buffer
Std. 2	16.700	10 µL [Std. 1] + 20 µL TE^{-4} buffer
Std. 3	5.560	10 µL [Std. 2] + 20 µL TE^{-4} buffer
Std. 4	1.850	10 µL [Std. 3] + 20 µL TE^{-4} buffer
Std. 5	0.620	10 µL [Std. 4] + 20 µL TE^{-4} buffer
Std. 6	0.210	10 µL [Std. 5] + 20 µL TE^{-4} buffer
Std. 7	0.068	10 µL [Std. 6] + 20 µL TE^{-4} buffer
Std. 8	0.023	10 µL [Std. 7] + 20 µL TE^{-4} buffer

Standard curves were produced using a 96-well PCR reaction plate. Master mix was pipetted into wells A1 through A8 in the amount of 9 µL. Next, 1 µL of DNA standard 1 was pipetted into well A1, 1 µL of DNA standard 2 into well A2, and repeated for each well with each respective DNA standard. The standards were performed in replicates of six. A1, B1, C1…F1 representing standard 1 (50 ng/µL). A2, B2, C2…F2 representing standard 2 (16.7 ng/µL). A3, B3, C3…F3 representing standard 3 (5.56 ng/µL). This was repeated until each standard had six replicates. The reaction plate was then sealed with optical adhesive tape. The plate was

centrifuged at 3000 Xg for approximately 20 seconds to remove any bubbles in the wells. The plate was then ready for amplification on the Applied Biosystems 7500 real time thermal cycler in accordance with the manufacturer's instructions.

This process was repeated twice with the results from 12 standard curves pooled and averaged for comparison of our unknown samples.

3.3 Quantitative Real-Time PCR (RT-qPCR)

Quantitative real-time PCR (RT-qPCR) assay is a quantitation method for DNA samples that have been extracted and purified. DNA is amplified and copied in order for quantitative measurement using fluorescent probes. Primers and probes specific for a target sequence are added to a PCR mixture. The probe is labeled with a reporter fluorescent dye and a quencher dye that will reduce fluorescence when near the reporter. During the extension phase of the PCR reaction, Taq polymerase degrades the probe, which allows the reporter to fluoresce, as it no longer associated with the quencher. The amount of the fluorescence increases with each round of amplification, and is directly proportional to the amount of product being produced, which is proportional to the amount of input DNA template. Real-Time PCR analyzes and measures the change in florescence during PCR and with each round of amplification. The assay can also detect PCR inhibition and is sensitive to the threshold of 0.0001 pg/μL.[47] Because of sensitivity, the RT-qPCR assay is a good method to quantify DNA.

The samples for this study were quantified using the Applied Biosystems Quantifiler Human DNA Quantification kit. The kit utilizes the TaqMan technology described above to label PCR product with fluorescent probes, so the fluorescence may be measured to determine the amount of product in a given sample. This kit is used to produce reliable results from samples containing DNA concentrations of 0.023 ng/μL to more than 50 ng/μL. The kit contains all of

the necessary reagents required for amplification, including a PCR mix, a primer mix, and a human DNA standard. The PCR mix contains buffers, AmpliTaq Gold DNA polymerase and a reference standard. The primer mix contains primers and probes that are formulated to amplify the Human Telomerase Reverse Transcriptase gene. The human DNA standard is simultaneously amplified with forensic samples to determine inhibition or samples that do not contain human DNA. Quantifiler kits have been optimized to be used with the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Inc, Foster City, CA).[48]

3.3.1 DNA-IQ System DNA Bead Extraction using TE^{-4} and DTT/Lysis

Experiments were conducted utilizing each collection device with each type of buffer. The experiments were grouped into series, completed when each collection device had been utilized to swab each metal with the different buffer solutions. A typical experiment consisted of pipetting 2 μL of diluted DNA onto five locations on each type of metal substrate. The spotted DNA was allowed to dry overnight, and the collection method to be tested was used to recover DNA from the spotted DNA location on each metal. The metals were cleansed and prepared by spotting diluted DNA onto new locations prior to each experiment. This basic process was repeated with all metals until all five collection techniques were used with the first type of buffer solution to complete a single series of experiments. The process was repeated using a different type of buffer solution for each series, for a total of four series.

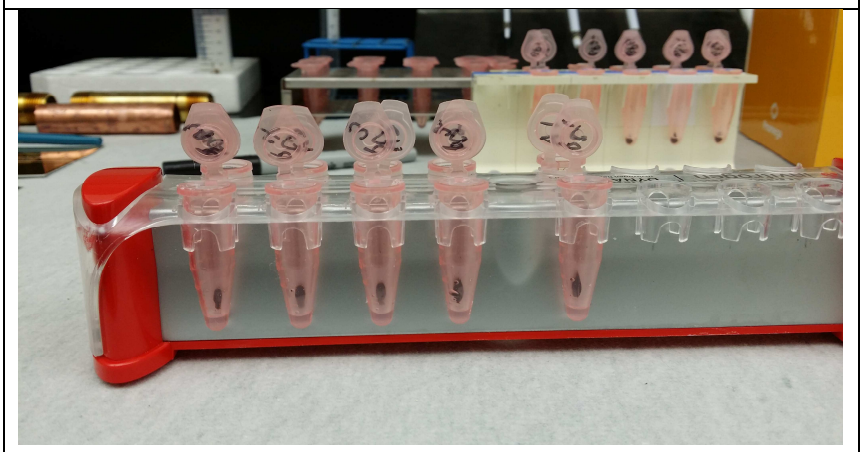
For the first series of experiments, TE^{-4} (10 mM Tris-Cl, pH 8.0, 0.1 mM EDTA) was used. Thirty-five 0.5 mL Eppendorf tubes were needed. The tubes were labeled with each metal and a corresponding sample number (ex. Copper 1, Copper 2…Copper 5, Brass 1, Brass 2…Brass 5) until 30 tubes were labeled for each of the individual samples. There were a total of five samples per metal for a total of 30 samples. The last five tubes were labeled 'positive control 1'

through ‘positive control 5’. A cotton swab was moistened with 70 µL of TE^{-4}, and then the DNA sample on the copper metal spot 1 was swabbed in a back and forth motion while rotating the swab to avoid redeposit of the DNA onto the metal. The swab was placed into the 0.5 mL tube labeled ‘Copper 1’, and then 130 µL of DTT/Lysis buffer (dithiothreitol mixed with Lysis 100:10 µL/mL ratio) was pipetted into the tube. The stick of the swab was cut so only the tip of the swab containing the sample with the solution was left in the tube, and the tube could be closed. A hole was punched in the bottom of the tube using a sterilized tack, and the entire tube was then placed into a larger 1.5 mL tube. This process was repeated with a sterile cotton swab for each of the samples on each of the metals.

The positive control tubes had 2 µL of diluted DNA (5 ng/ µL), 130 µL of DTT/Lysis buffer and 70 µL of TE^{-4} pipetted into each tube prior to punching a hole in the bottom of the tube. No swab was used for the positive control tubes. The double tubes were then centrifuged at 6000 Xg for three minutes. The smaller 0.5 mL tubes were then discarded since the liquid containing DNA had been spun down into the larger 1.5 mL tube. Magnetic silica-coated beads were pipetted into each of the sample tubes in the amount of 7 µL, the amount capable of binding 100 ng of genomic DNA (DNA IQ protocol), and the tubes were vortexed for approximately 5 seconds. The tubes were allowed to sit for 2-3 minutes before being placed on a magnetic microfuge tube stand. The liquid phase was removed from each tube via pipette, leaving the beads behind in the tube. The liquid contained the contaminants and extracellular material that is not needed for amplification. The beads were then washed with 50 µL of 2X wash buffer (included with the DNA IQ kit and consisting of TE^{-4} buffer containing 35% ethanol and 35% isopropanol) and the wash removed via pipette for each sample. This process was repeated leaving only the beads in the tube after the final removal of the wash buffer. The tubes with the beads still in them were allowed to sit on the magnetic stand to air dry for five minutes. The DNA was then eluted with 30 µL heated TE^{-4} (65°C). The tubes were then vortexed for

approximately 10 seconds and were incubated at 65°C for approximately five minutes. The samples were placed back onto the magnetic microfuge tube stand and allowed to sit for one minute so the beads could be drawn to the back of the tube (Figure 2). The liquid which contained the DNA was pipetted into a clean 1.5 mL tube labeled with the date, the type of metal the sample was spotted onto, the type of buffer used and the type of collection method used. The eluted DNA was refrigerated until DNA quantitation was performed. This process was repeated using a different collection method each time.

Figure 2. Tubes with Silica-coated Beads on the Magnetic Stand



Magnetic silica-coated beads are pulled to the back of the tube when using the magnetic stand. This allows for fluids to be drawn out while the DNA remains bonded to the beads.

The second collection method evaluated utilized collection onto a Dacron swab. The third collection method evaluated utilized a glass fiber swab, composed of glass fiber filter material used to form a swab head on a plastic applicator stick. The glass fiber swab (GF) was developed at OSU-CHS. The fourth collection method evaluated utilized collection onto a PCR square, which is a small square of Whatman Chromatography filter paper #1, approximately

0.6x0.6-in that may be manipulated using a wooden stick. The PCR square was pre-wet with only 35 µL of TE^{-4} to avoid oversaturation. To compensate for the difference in liquid, 165 µL of DTT/Lysis was added to the tubes after the PCR square was placed into the tube. To 'swab' with the PCR square a wooden stick was used to maneuver the square over the sample area in a circular motion. The sticks had previously been placed into a UV irradiator and processed at energy density of 3000 mJ/cm^2 to ensure DNA contamination was not present. The fifth collection method explored was tape lift. The DNA was dry-lifted and the tape cutting placed into the 0.5 mL tube with 70 µL of TE^{-4} and 130 µL of DTT/Lysis.

To ensure no contamination was present on each collection method, the glass fiber swabs, the PCR squares and the wooden sticks used to maneuver the squares were placed into a UV irradiator and processed at energy density of 3000 mJ/cm^2. The Dacron and cotton swabs were considered sterile if retrieved from an unopened package. The tape was considered sterile if it came directly from the roll.

The second series of experiments used the same bead extraction process to recover DNA from the collection device, however the TE^{-4} was replaced with Lysis/DTT to pre-wet the collection device in each set of experiments. Each collection device was pre-wet with 70 µL of DTT/Lysis solution (dithiothreitol mixed with Lysis 100 µL:10 mL ratio), except for the PCR squares which were pre-wet with 35 µL of buffer solution. The lysis was obtained from the Promega DNA IQ extraction kit. Each sample spotted onto each metal was swabbed and then the collection devices were placed into individually marked Eppendorf tubes. DTT/Lysis solution was added to each tube in the amount of 130 µL for each collection device except for the PCR square and tape lift methods. The PCR squares had 165 µL DTT/Lysis solution added to the tube. The tape lift method utilized dry-lifting the DNA from each sample and placing the tape cutting into the Eppendorf tube and then adding 200 µL of DTT/Lysis buffer solution. Five positive controls were created by pipetting 2 µL of diluted DNA directly into 200 µL DTT/Lysis

solution contained within 0.5 mL Eppendorf tubes labeled 'Positive 1, Positive 2…Positive 5. From this point, the experiments were completed following the same procedures as the first series, utilizing magnetic silica-coated beads and the 2X wash from the Promega DNA IQ extraction kit, resulting in 35 samples (5 from each metal and 5 positive controls) of eluted DNA being refrigerated until DNA quantitation was performed.

3.3.2 DNA-IQ System DNA Bead Extraction using SDS and Sarkosyl

The third and fourth series of experiments varied during the extraction process due to the use of a 2% SDS buffer solution (20% Sodium Dodecyl Sulfate in dH_2O) for one experimental series and a 2% Sarkosyl buffer solution for the final experimental series. For the each series of experiments the metals were prepared in the same manner as before. Sample locations were marked on the metals as described above and 2 µL of DNA were spotted on to the metals and allowed to dry overnight. The collection device was then pre-wet with the prepared buffer and each sample was swabbed with the collection device. The swab head/filter square/tape lift was then placed into a 0.5 mL tubes. Additional buffer solution was added to each tube. In order for digestion to occur, the samples in the tubes were allowed to incubate at 56° C overnight.

For the third series of experiments, a 2% SDS extraction buffer was created using 955 µL TNE (10 mM Tris-Cl, pH 8, 0.2 M NaCl, 1 mM EDTA) added to 20 µL of 20 mg/ml Proteinase K and 25 µL 20% SDS (Sodium dodecyl sulfate). A sterile cotton swab was pre-moistened with 70 µL SDS buffer and used to swab each sample in a back and forth motion while rotating the swab to avoid redeposit of the DNA sample. The entire swab head was cut and collected in a 0.5 mL tube, and 130 µL SDS extraction buffer was added to each sample. The samples were allowed to incubate overnight at 56°C for digestion to occur. After incubation, a hole was punched in the bottom of the tube using a sterilized tack, and the entire tube was placed into a

larger 1.5 mL tube. The samples were centrifuged at 6000 Xg for 3 minutes. The 0.5 mL tubes were thrown away once the liquid had been spun into the larger tube. Once the liquid phase was collected in the 1.5 mL tubes 400 µL DTT/Lysis was added to each tube in addition to 7 µL of magnetic silica-coated beads, the amount capable of binding 100 ng of genomic DNA (DNA IQ protocol), from the bead extraction kit. The samples were vortexed for 30 seconds and then allowed to incubate at room temperature for five minutes. The samples were then vortexed again for 30 seconds and placed onto a magnetic stand allowing the beads to pull to the back of the tube. The liquid containing the contaminants was removed and disposed of leaving behind the beads containing DNA. The beads were then washed with 100 µL of Lysis/DTT followed by two washes of the alcohol containing wash buffer provided with the DNA IQ extraction kit. The DNA was eluted from the beads using 30 µL hot TE^{-4} to release the DNA as described above. The samples were refrigerated until quantitation was performed using qPCR. Positive controls were created by adding 2 µL of 5 ng DNA to the tube with 200 µL SDS solution for overnight incubation along with the samples. The remainder of the extraction process was completed alongside the samples in the same manner.

To complete the third series of experiments, the process above was repeated with each collection device using the SDS buffer solution. When the glass fiber swab was used, the swab was pre-moistened with 70 µL of Lysis/DTT and then used to swab the DNA sample from the metal. The swab head was cut into a 0.5 mL Eppendorf tube prior to adding 200 µL of the SDS buffer solution to the tube and allowing overnight incubation to occur. The sample was then spun through using the same technique described above. Once the liquid was spun through, only 330 µL of Lysis/DTT was added to the samples to compensate for the 70 µL Lysis/DTT used on the swab. When the PCR squares were used, 20 µL of the SDS buffer solution was used to pre-wet the squares prior to swabbing the samples and placing the squares into a 0.5 mL tube. Then, 180 µL SDS was added to the tube prior to overnight incubation. The remainder of the procedure was

the same as described above. When the tape lift method was performed, the DNA samples were dry-lifted and then the tape cuttings were placed into a 0.5 µL tube prior to 200 µL SDS buffer solution being added to each tube and then allowing overnight incubation. The remaining of the procedure followed was the same as described above.

The final series of experiments followed the same methods as the third series except that the buffer solution used was a 2% Sarkosyl buffer solution in place of the 2% SDS buffer solution. The 2% Sarkosyl buffer solution was created using 855.2 µL TNE (10 mM Tris-Cl, pH 8, 0.2 M NaCl, 1 mM EDTA) added to 125 µL of 20 mg/ml Proteinase K, 0.8 µL DTT (1M) and 20 µL 20% Sarkosyl. A low Lysis/DTT solution (75 µM) was used for the Sarkosyl series of experiments to account for the DTT already present in Sarkosyl. The collection devices were pre-moistened in the same manner as described above, substituting Sarkosyl for the SDS buffer, and then following the same overnight incubation and extraction protocol as described above. Each series of experiments resulted in 35 samples, accounting for five samples for each metal and five positive controls that were refrigerated until DNA quantitation could be completed.

To ensure no contamination was present on each collection method, the glass fiber swabs and the PCR squares were placed into a UV irradiator and processed at energy density of 3000 mJ/cm^2. The Dacron and cotton swabs were considered sterile if retrieved from an unopened package. The tape was considered sterile if it came directly from the roll.

3.3.3 RT-qPCR Set Up and Amplification

Similar to setting up for the standard curve, master mix was created using a ratio of 4:5 µL of Quantifiler Human Primer Mix to Quantifiler PCR Reaction Mix. This kit is used to quantify total human DNA.[49] Enough master mix was made for the appropriate number of reactions being run plus two to ensure enough mix was available for all wells, and to account for

possible pipetting mistakes. All of the reactions were run in duplicate. Master mix was added in the amount of 9 µL to the number of wells needed in a 96-well PCR reaction plate for each sample to be quantified in duplicate. 1 µL of each sample was then added to the appropriate well. A standard created with a known quantity of DNA was included in the plate. This included a repeat of the standard curve for three of the known concentrations: 50 ng/µL, 16.7 ng/µL, and 5.56 ng/µL. The standard curve was used to ensure accuracy and sensitivity thresholds were met. The plate was sealed with optical adhesive cover and centrifuged at 3000 Xg for about 20 seconds to eliminate bubbles in the reaction wells. The plate was then placed on the Quantifiler 7500 real-time thermal cycler and analyzed according to the manufacturer's instructions.

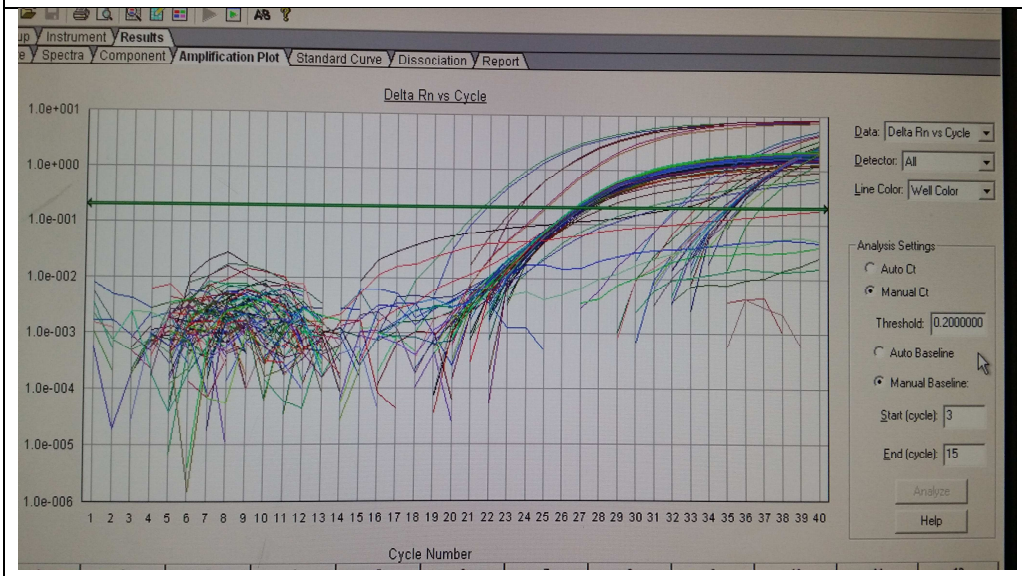
3.4 Determining Which Method Yields the Most DNA

3.4.1 Quantitative Amplification using the 7500 Real-Time PCR System

Real-time PCR employing the ABI 7500 real time instrument and the Quantifiler DNA quantitation kit (both from Applied Biosystems, Inc, Foster City, CA) was used for this study to quantitate the DNA in each sample. Real Time PCR follows the accumulation of fluorescence as PCR product accumulates during thermal cycling. During the amplification process, a fluorescent reporter (on one end of an added, sequence specific probe) is in close association with a quencher at the other end of the probe. During the elongation phase of amplification, the Taq polymerase complex encounters the hybridized probe and displaces and degrades the probe separating the Fluor from the quencher and fluorescence is produced. The fluorescence of the reporter increases as the products accumulate with each round of amplification. The Applied Biosystems 7500 Real-Time PCR system uses this process with several dyes as a reference to distinguish fluorescence of target DNA to be amplified. SDS software provided with the ABI 7500 analyzes the cycle-cycle change in the fluorescent signal during amplification to determine the relative

quantity of target DNA.[50] An amplification plot is generated to show the changes in the reporter signal for each cycle (Figure 3). The point where the reporter signal crosses a threshold defined as the point during amplification at which fluorescence is increasing logarithmically is referred to as the cycle threshold or C_T.[51] The C_T when plotted against known input DNA amounts will allow a standard curve to be produced against which DNA content in unknowns can be estimated.

Figure 3. Amplification Plot Showing the Phases of PCR Amplification

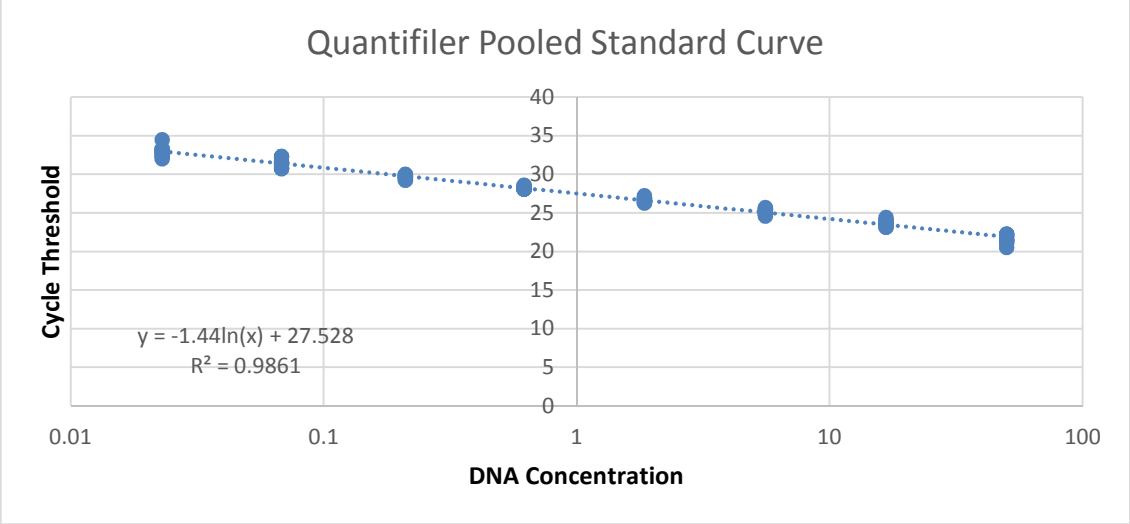


Change in Rn represents the reporter signal. Geometric phase is below the threshold line (shown in green); linear phase is above the threshold line. Point at which the reporter signal crosses the threshold is measured as the cycle threshold.

The number of cycles it takes to amplify the unknown DNA was plotted against the DNA concentration, which was then compared to the standard curves. A standard curve plot of C_T versus DNA concentration for the known standards was created, which was amplified at the same time as the unknown samples. The results were statistically analyzed to determine which method yielded the most DNA quantity.

The linear regression analysis generates an equation that was used to quantify the unknown amounts of DNA of the samples. The trend line equation used for analysis was the trend line generated from the pooled standard curve: $y = -1.44\ln(x) + 27.528$ with an $R^2 = 0.98614$ (Figure 4), which represents an acceptable level of accuracy from the pooled standard curve. Because the R^2 value does not equal 1.0 the C_T for each sample must be entered into the trend line equation to estimate DNA concentration in each sample. The C_T value for each sample was inserted for y and the equation was solved for x to determine the DNA quantity per µL. Since the samples were eluted in 30 µL, each sample was multiplied by 30 to account for the total DNA recovered. Each sample was calculated to pg/µL to prepare for statistical analysis. DNA yield was determined by extrapolating the data of each experimental run from a linear regression of analysis of the standard curve.

Figure 4. Standard Curve Using Replicate Reactions of Known DNA.



Cycle threshold plotted against DNA concentration for standard curve.

3.4.2 Statistical Analysis

Prism statistical software was used to analyze DNA recovery results for significance. The mean recovery of DNA (expressed as pg/μL) was calculated for the replicate DNA recoveries for each experimental condition. A 5x4x6 factorial was a comparison of each collection method against each buffer solution per metal. Means were then compared using analysis of variance (ANOVA) to determine if there was a significant difference between the experimental groups. A p-value greater than 0.05 indicated there is no significant difference between the series of experiments, while a p-value less than 0.05 indicated there is significant difference between the series. P-values less than 0.01 indicated a highly significant difference. The experiments were analyzed using a two-way ANOVA for a grouped analysis to determine if there were significant differences in the total experiment for each metal. Tukey's multiple comparison test was used for the grouped analysis.

3.4.3 DNA profiling.

Attempts were made to produce STR profiles from selected DNA extracts produced from the different metals under the different recovery methods to confirm that profiles could be produced in the different conditions. The Identifiler Plus™ (Applied Biosystems Inc., Foster City, CA) kit was used for short tandem repeat (STR) analysis. The Identifiler Plus STR kit contains all reagents needed to produce STR profiles from amounts of genomic DNA ranging from 200-300 picograms and up to 1000 picograms of genomic DNA template. In our attempts to produce STR profiles, 3.5 μL of the sample DNA was added to each PCR reaction, which was then cycled per the manufacturer's recommendations. STR profiles were produced using a 3130XL Genetic Analyzer (Applied Biosytems, Foster City, CA).

For the ID-Plus kit, a HiDi formamide + Liz 500 mixture was created by mixing 1.0 mL HiDi formamide with 7.0 µL Liz 500 size standard. The amplified samples were prepared for electrophoresis on the 3130 Genetic Analyzer by adding a 1.0 µL aliquot of the sample to a mixture 20 µL of the HiDi formamide-Liz 500 mixture and pipetted into a 96-well plate. An ID-plus ladder (Applied Biosystems) was included in the 96-well plate for allele comparisons. A rubber septum was used to cover the plate prior to the plate being loaded into the 16-capillary genetic analyzer and allowed to electrophorese. For this study, not all samples were genetically analyzed. Rather, a somewhat random selection of DNA extracts from the different experimental conditions was profiled. All STR profiles were analyzed using GeneMapper ID software (ver. 3.2) provided by Applied Biosystems Inc. (Foster City, CA).

CHAPTER IV

RESULTS

The ability to successfully recover DNA from any touched surface is of great value to any criminal investigations lab. As stated earlier, swab type and swabbing technique may play a role in how much DNA is recovered from a surface. Determining which type of swab and wetting solution combination works best to recover optimal amounts of DNA for processing will allow laboratory analysts to provide essential information to law enforcement. The results of this study explore if there is significant difference between swab type and wetting solution combinations among various metals. Each mean of each experiment was compared to every other experiment. Significant difference of 0.05 or less requires additional exploration into the experiment exhibiting the difference to determine whether or not the difference is attributable to sample error.

4.1 Real-time PCR (RT-qPCR)

DNA was quantified for each swab recovery method (swab matrix and wetting agent) using RT-qPCR as described in the Methods section. Every sample, including positive control DNAs used for the standard curve was amplified in duplicate. Average yield of DNA recovered from each metal and recovery method was determined and the raw data is attached as Appendices A-F.

4.1.1 DNA Yield

Each sample yielded a highly variable amount of recovered DNA; some samples not showing any recovery at all. For copper, seventy-one of the samples (35.5%) returned results. Recovery of DNA spotted onto brass were lower with only fifty samples (25%) returning results. Steel had better recovery rates with 107 samples out of 200 (53.5%) producing results, and recoveries from aluminum were similar to steel with 106 samples (53%) producing results. Brass plated steel also yielded similar results with 109 samples (54.5%) producing results. The highest recovery rate was observed for tin with 60.5% of 121 samples returning DNA results.

Recovery of DNA from copper using Glass Fiber and Sarkosyl were based upon an average recovery of 632.56 pg/µL in one sample while recovering between 0.00 and 5.75 pg/µL in the other four samples. The recovery rate among brass plated metal samples recovered using tape lift and Sarkosyl was consistent across the five samples recovering 135.34 pg/µL (4.06 ng total genomic DNA) in average. The recovery across the five samples of steel using the Sarkosyl and PCR square treatment was also consistent with a mean recovery of 126.33 pg/µL (3.8 ng total genomic DNA) across the samples (Appendix C).

Although there was nearly 50% recovery rate of DNA from each metal, many samples yielded a very miniscule amount, some resulting in only mere picograms per microliter. The range of DNA recovery was quite variable across all experiments, with a range of 0 to 632 pg/µL.

4.2 Statistical Analysis

The sample replicates were averaged creating a total of five values per metal for each swab/buffer recovery combination. A 5x4x6 factorial was designed to compare the collection types with the buffer solutions across the metals. A two-way ANOVA compared the means of recovery to determine if there were statistical differences among the means (Table 2). A p-value less than 0.05 indicated significant

differences among the means. These values were calculated and results plotted using GraphPad Prism software version 6.05. Data was plotted using the mean and the range. Tukey's multiple comparison analysis was completed considering data from the entire study, showing a significant difference within the mean comparisons of $P < 0.0001$, which is indicates there is a highly significant difference among the mean DNA recoveries within the groups of experiments. This indicates the need to analyze each experiment individually to determine which particular recovery method was better when compared to another.

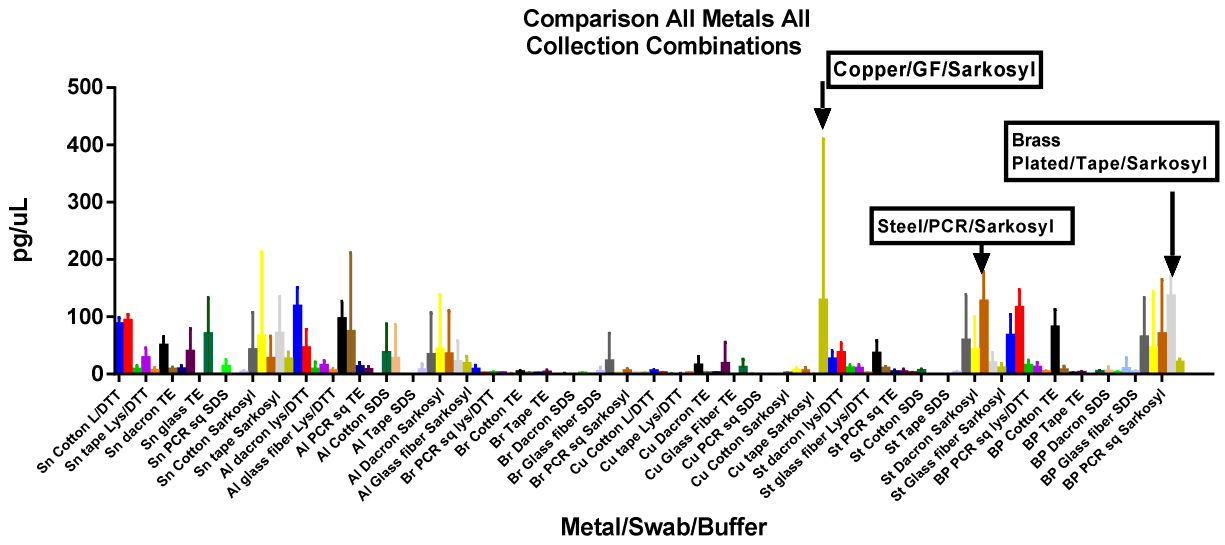
Table 2. Multiple Comparison Two-way ANOVA.

Source of Variation	% of total variation	P value	P value summary	Significant?	
Row Factor	0.4222	0.4909	ns	No	
Column Factor	40.82	< 0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	6214	4	1553	F (4, 476) = 0.8551	P = 0.4909
Column Factor	600691	119	5048	F (119, 476) = 2.778	P < 0.0001
Residual	864789	476	1817		

Two-way ANOVA comparing all metals using all collection tools and all buffers. Alpha =0.05

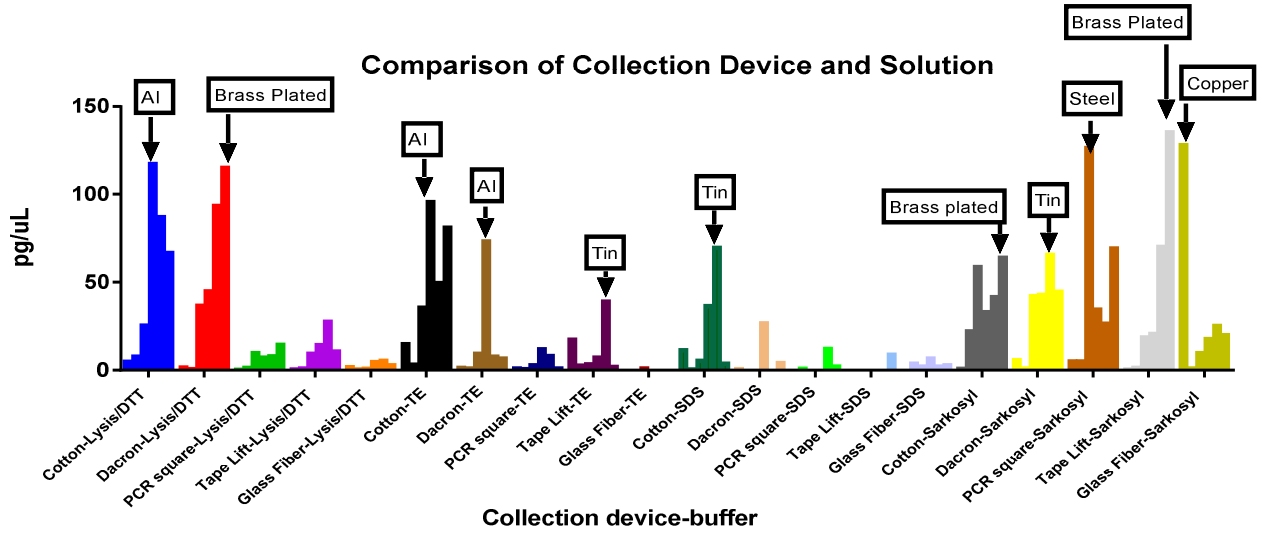
As seen in Figure 5 below, the multiple comparison test compares every mean recovery of each experiment with each other experiment. The data has been grouped both by metal (Figure 5A) and also by collection matrix (Figure 5B) to facilitate the comparisons.

Figure 5A. Significant Differences Multiple Comparison of all Metals/Treatments



Comparison of each buffer used with each collection tool across the six metals. The 3 methods that had the most significant difference are notated.

Figure 5B. Mean DNA Recovery from Metals per Buffer Solution/Collection Device Combination

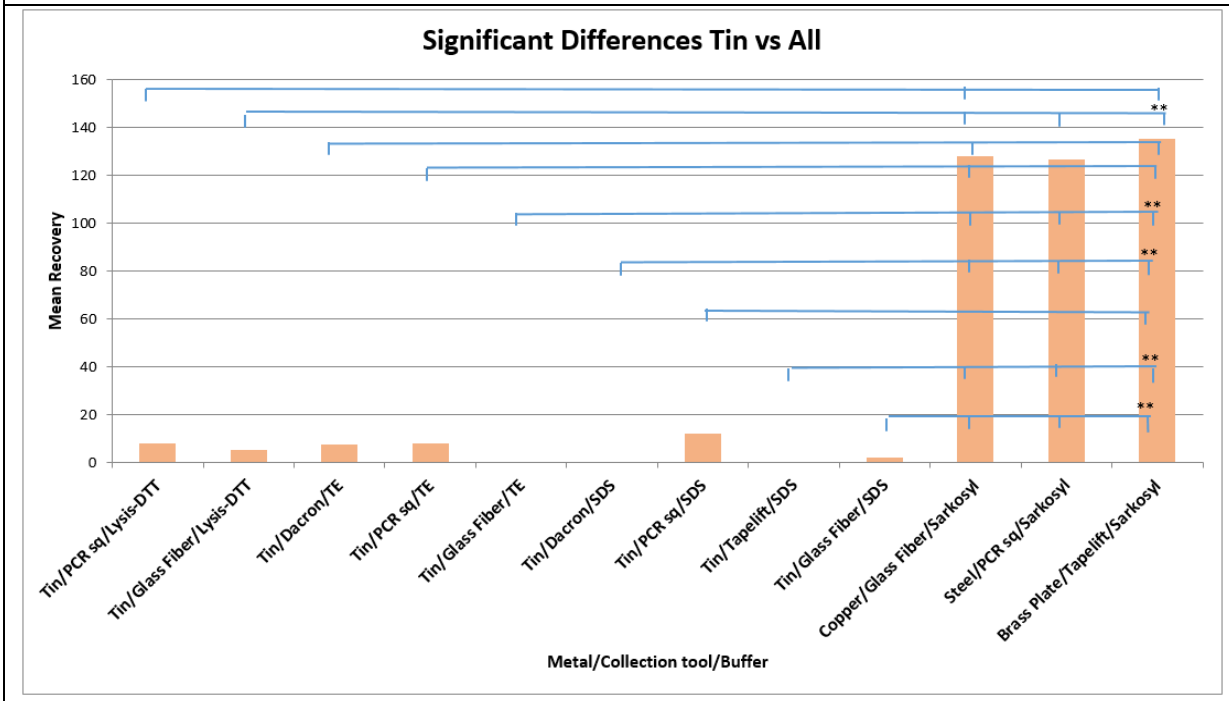


Comparison of each metal (copper, steel, brass, aluminum, tin, brass plate per group) grouped by solution and collection device.

When DNA recovery from all six metals was evaluated, the performance characteristics of three recovery methods stood out more than other combinations. The combinations showing significant elevated recovery compared to the remaining methods were Copper/Glass Fiber/Sarkosyl, Steel/PCR squares/Sarkosyl, and brass plate/tape lift/Sarkosyl. These three combinations showed significantly greater mean DNA recoveries when compared to the other recovery methods as shown in Figures 6 – 10 below. These three conditions are used for comparison of DNA recovery to all other collection methodologies with each of the metals (Figures 6 – 10). Significance for each of the comparisons is denoted with the asterisks. The number of asterisks denotes the degree of significance (0.05 versus 0.01).

Comparing DNA recovery from tin utilizing the various collection tool/buffer combinations to all other metals using the same combinations, Copper had a mean DNA recovery of 127.94 pg/µL when the glass fiber swab was used with Sarkosyl. Using this buffer on Steel had a mean recovery of 126.33 pg/µL when PCR squares were used. Brass plated metal had a mean recovery of 135.34 pg/µL when tapelift was used with Sarkosyl. These three treatments exhibited significant increase in the yield of DNA recoveries when compared to the treatments used with tin (Figure 6). Means not showing significant difference were not displayed in the figure.

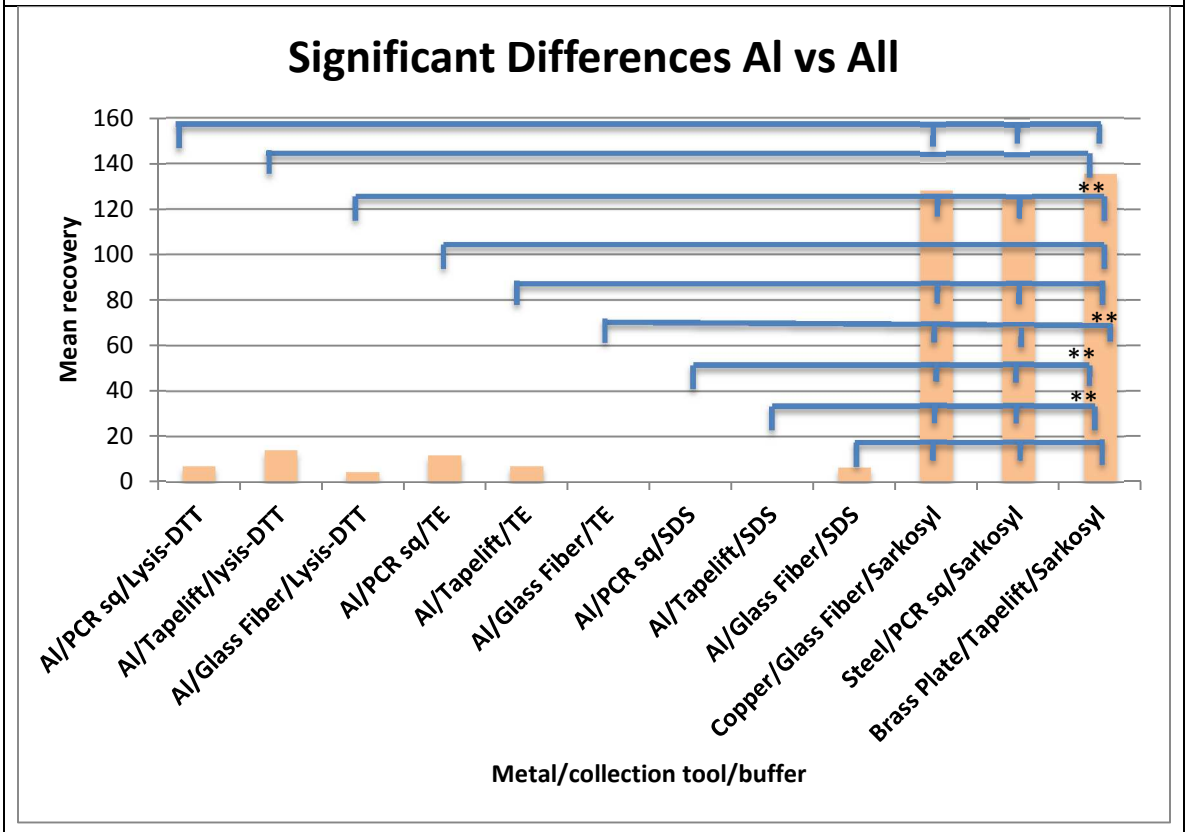
Figure 6. Significant Differences Tin Versus Other Metals



** signifies $P \leq 0.01$; all other significant interactions marked signify $P \leq 0.05$

DNA recovery from Aluminum with the five collection tools used with four wetting agents yielded results comparable to the results obtained from tin (Figure 7). None of the different recovery methods performed as well as Copper/Glass Fiber/Sarkosyl, Steel/PCR Squares/Sarkosyl, and brass plate/tape lift/Sarkosyl. Other significant differences of $P \leq 0.05$ among Aluminum are shown in Figure 7 below.

Figure 7. Significant Differences Aluminum Versus All Other Metals.



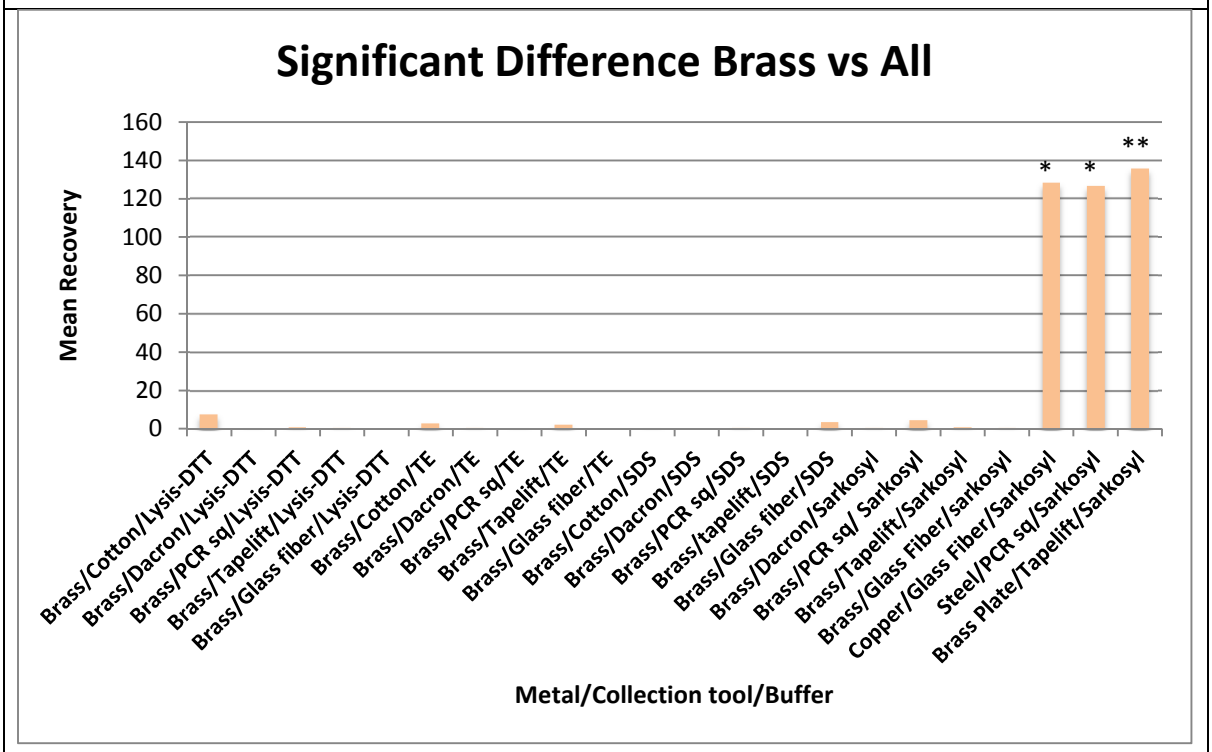
** signifies $P \leq 0.01$; all other significant interactions marked signify $P \leq 0.05$

Copper had a mean DNA recovery of 127.94 pg/µL (3.8 ng total genomic DNA) when the glass fiber swab was used with Sarkosyl, while using this buffer on Steel had a mean recovery of 126.33 pg/µL (3.8 ng total) when PCR squares were used. Brass plated metal had a mean recovery of 135.34 pg/µL (4.06 ng total) when tape lift was used with Sarkosyl. These are significantly different than the means of 0.00 to 14.14 pg/µL recovered from Aluminum when using the other noted treatments.

Recovery amounts were very low with Brass (Figure 8), therefore nearly every buffer treatment and collection method used with Brass was significantly different from the three more optimal recovery methods discussed above that yielded the highest amounts of DNA. Sarkosyl used with tape lift on brass plated metal yielded a significant difference of $P \leq 0.01$ (Figure 8). Sarkosyl used with glass fiber on

copper, and Sarkosyl used with PCR squares on Steel are among other significant differences of $P \leq 0.05$ with Brass displayed in Figure 8 below.

Figure 8. Significant Differences Brass Versus All Other Metals.



** signifies $P \leq 0.01$; all other significant interactions are $P \leq 0.05$

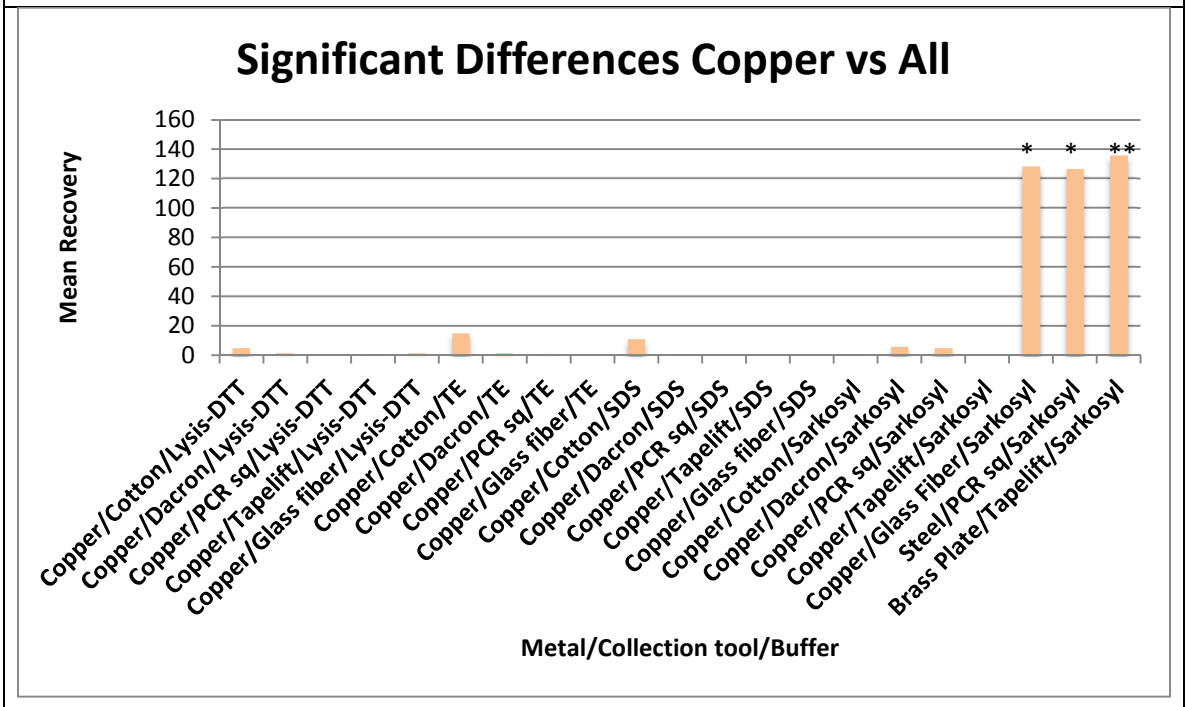
The rate of recovery was much higher among the three significantly different treatments listed. The only recovery method with brass that was not statistically different was Sarkosyl used with Cotton swabs, therefore it is not displayed on the Figure above. The average DNA recovery of 21.99 pg/µL (660 pg total) from brass while using Sarkosyl with cotton swabs may be considered skewed due to recovering an extremely high amount of 109.97 pg/µL from only one of the samples, with the remaining four samples showing no recovery (Appendix B).

Copper, like Brass, also had very low recovery rates, with the exception of the glass fiber swab and Sarkosyl as seen with the previous reports. The mean for glass fiber and Sarkosyl was 127.94 pg/µL

(for a total of 3.8 ng of genomic DNA) when recovered from Copper, however this was based on the reporting of a single sample yielding a high amount of 632.56 pg/µL while the other samples yielded anywhere between 0.00 and 5.75 pg/µL (172.5 pg total) when recovered from Copper using the Sarkosyl and a glass fiber swab. Other average yields from Copper ranged between 0.00 and 17.23 pg/µL (517 pg total), with cotton swabs and TE^{-4} providing the most consistent recovery, recovering measureable DNA from every sample (Appendix A).

The low DNA recovery from copper was very similar to recoveries from brass previously discussed. Sarkosyl used with tape lift on brass-plated metal yielded a significant difference of $P \leq 0.01$ when compared to the other collection methods used on Copper (Figure 9). Sarkosyl used with glass fiber on copper and Sarkosyl used with PCR squares on Steel also exhibited significant differences of $P \leq 0.05$ as shown in Figure 9 below.

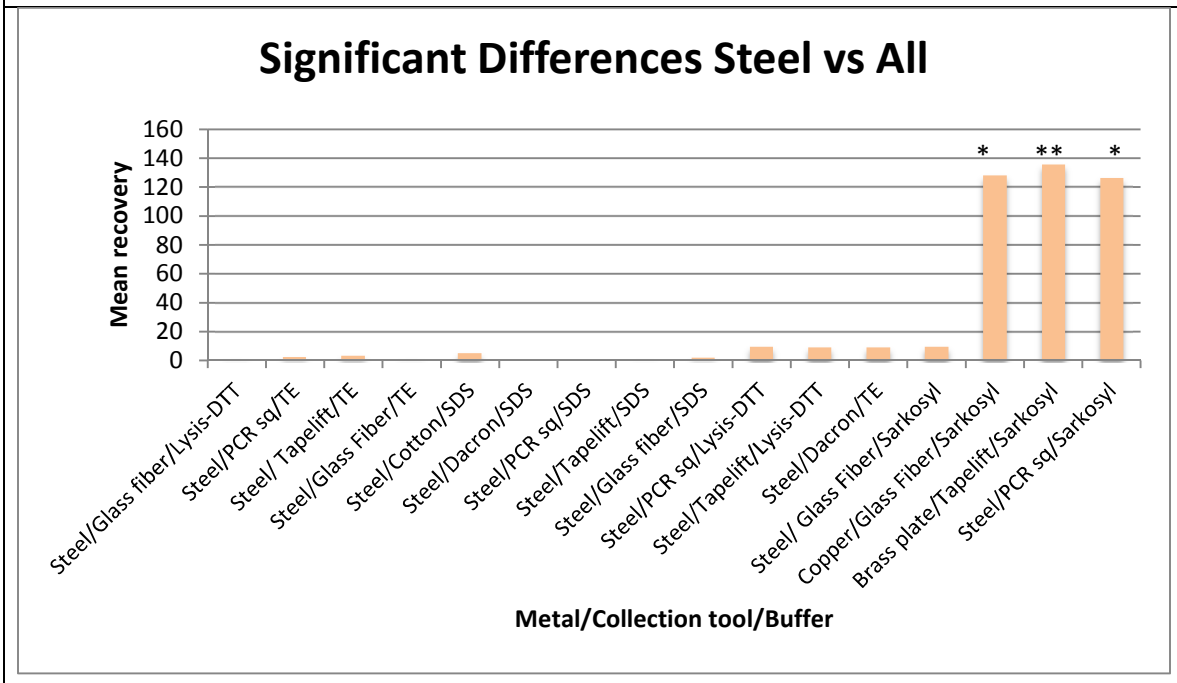
Figure 9. Significant Differences Copper Versus All Other Metals.



** signifies $P \leq 0.01$; all other significant interactions are $P \leq 0.05$

Comparing Steel across all of the treatments and all of the other metals, Sarkosyl used with Glass fiber on Copper, tape lift on brass plated and PCR squares on Steel stood out as significant. Using Sarkosyl with tape lift on brass plated metal had a $P \leq 0.01$ when compared to the other treatments on Steel (Figure 10). Other significant differences of $P \leq 0.05$ among Steel are shown in Figure 10 below.

Figure 10. Significant Differences Steel Versus All Other Metals.

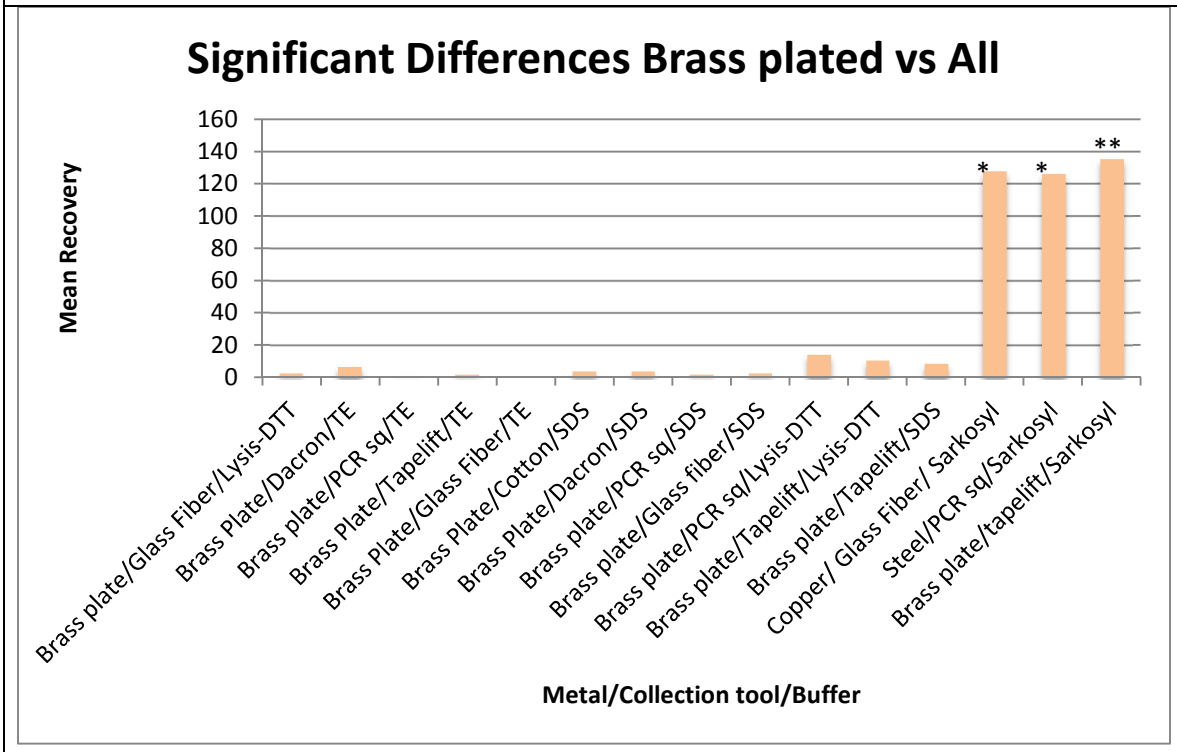


** signifies $P \leq 0.01$; all other significant interactions are $P \leq 0.05$

Brass plated metal showed the most significant difference among the metals for mean recovery. Dacron swabs combined with DTT/Lysis had a mean recovery yield of 115.09 pg/µL and. Glass fiber combined with Sarkosyl/Pro-K showed a more consistent recovery across the samples, however 19.75 pg/µL is still a low mean yield for consideration in profiling. The most significant difference when compared to all of the metals and all of the treatments was Sarkosyl used with tape lift. With a mean recovery of 135.34 pg/µL (4.06 ng total) and consistent recovery across all of the samples, this collection method on brass-plated metal, with a $P \leq 0.01$, stood out above the rest of the methods (Figure 11). Other

collection methods that showed a significant difference of $P \leq 0.05$, including Copper/glass fiber/Sarkosyl and Steel/PCR squares/Sarkosyl are included on Figure 11.

Figure 11. Significant Differences Brass Plated Versus All Other Metals.



** signifies $P \leq 0.01$; all other significant interactions are * signifying $P \leq 0.05$

The ANOVA analysis and Tukey's multiple comparison was used to analyze all of the treatments across all of the metals. The means of each treatment were compared for statistical difference. There was a significant difference of $P < 0.0001$ among all of the means. Throughout the analysis, using Sarkosyl with the glass fiber swab on Copper and using Sarkosyl with the PCR squares on Steel maintained a statistical difference of $P \leq 0.05$ when compared to all other treatments on all other metals. When

Sarkosyl was used with tape lift on brass plated metal, a statistical difference of $P \leq 0.01$ was most commonly seen when compared to all other treatments and all other metals.

4.3 PCR Inhibition

The Quantifiler Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) contains a pre-formulated internal PCR control (IPC) for each reaction to detect inhibition in each sample. When the IPC is not detected, PCR inhibition has likely occurred. Several samples using SDS and Sarkosyl exhibited complete inhibition. When SDS was used as the wetting solution, all metals exhibited some degree of inhibition in the majority of samples regardless of collection device (greater than 50% inhibition). When Sarkosyl was used as the solution, complete inhibition was also seen in greater than 50% of the following samples: copper using cotton and tape lift collection devices; brass using cotton and Dacron devices; steel using cotton and Dacron devices; and in aluminum, tin and brass plated samples using cotton, Dacron and PCR squares.

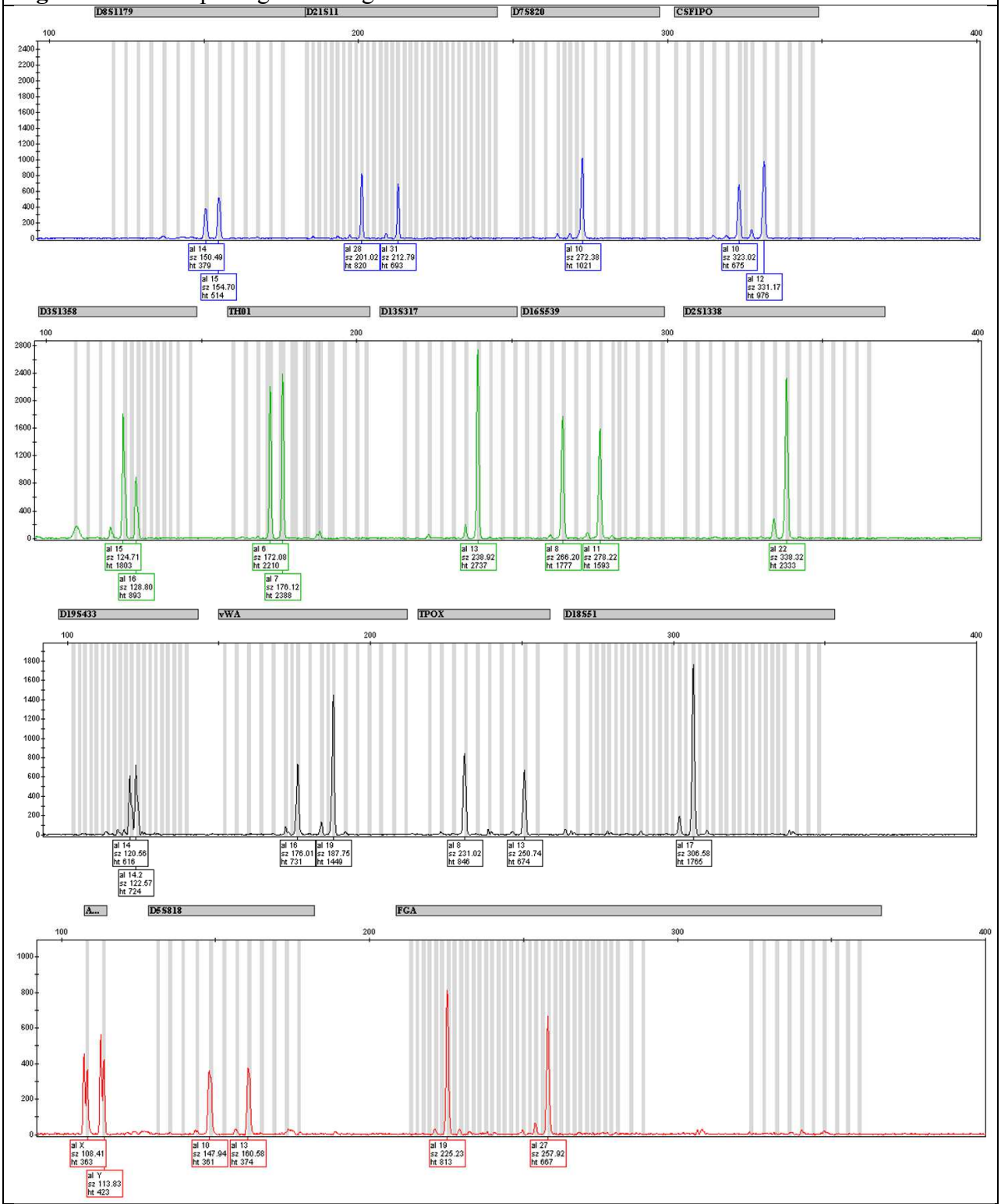
The probability of PCR inhibition in forensic samples is high given the variety of sampling conditions such as having the presence of blood, tissue, fibers or soil. Purification is imperative to remove inhibition. Ensuring the proper polymerase is used and using sound handling and processing techniques is imperative to reduce inhibition. Given the inhibition experienced in our sample set, it is possible that more DNA was recovered from those metals yielding little DNA, but that the quantitation methodology was inaccurate due to PCR inhibition.

4.4 STR Profiling of Recovered DNA

STR typing using the Identifiler Plus multiplex kit was performed on twelve recovered DNA samples ranging in concentration from 25 pg/µL to 135 pg/µL and recovered from different metals using

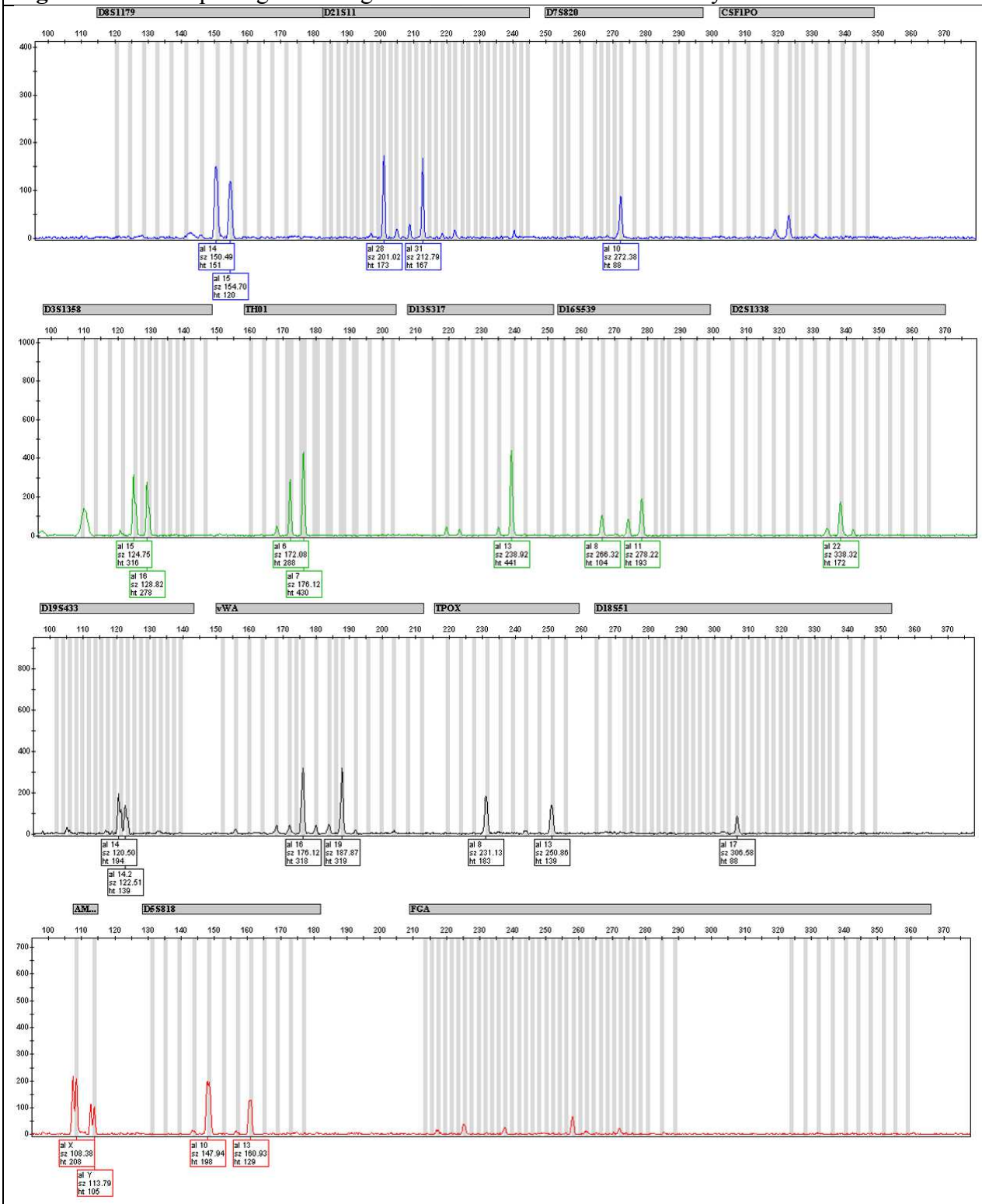
different methods. Of the twelve samples amplified, six generated all or part of an STR profile; two of the profiles were complete 16 locus profiles whereas four of the DNA samples produced only a partial profile (Figures 12 – 15). The 3130xl and GeneMapper software is capable of creating a genetic 'fingerprint' using STR analysis (short tandem repeats). A complete STR profile would produce alleles for 15 autosomal STR loci plus amelogenin to determine sex.[52] All DNA samples spotted onto the metals initially were derived from the same donor, and therefore all profiles produced from the samples would be expected to match. Indeed, the STR profiles produced from those DNA samples were able to produce a profile consistent with originating from a single male donor (Figures 12 – 15). DNA recovered from tin using cotton combined with TE^{-4} produced a full STR profile from 171.5 pg of recovered DNA (Figure 12). DNA recovered from steel using a Dacron swab wetted with DTT/Lysis also generated a nearly full profile (14/16 loci) with the addition of 126 pg of template (Figure 13). Similarly, a cotton swab combined with DTT/Lysis used on aluminum also produced a partial profile (14/16) loci with a greater input of recovered DNA (517 pg total) (Figure 14). DNA recovered from the brass plated steel (81 pg/μL) using a cotton swab pre-wetted with TE^{-4} also generated a full matching profile (Figure 15).

Figure 12. Electropherogram Using Cotton Swabs Combined With TE^{-4} on Tin.



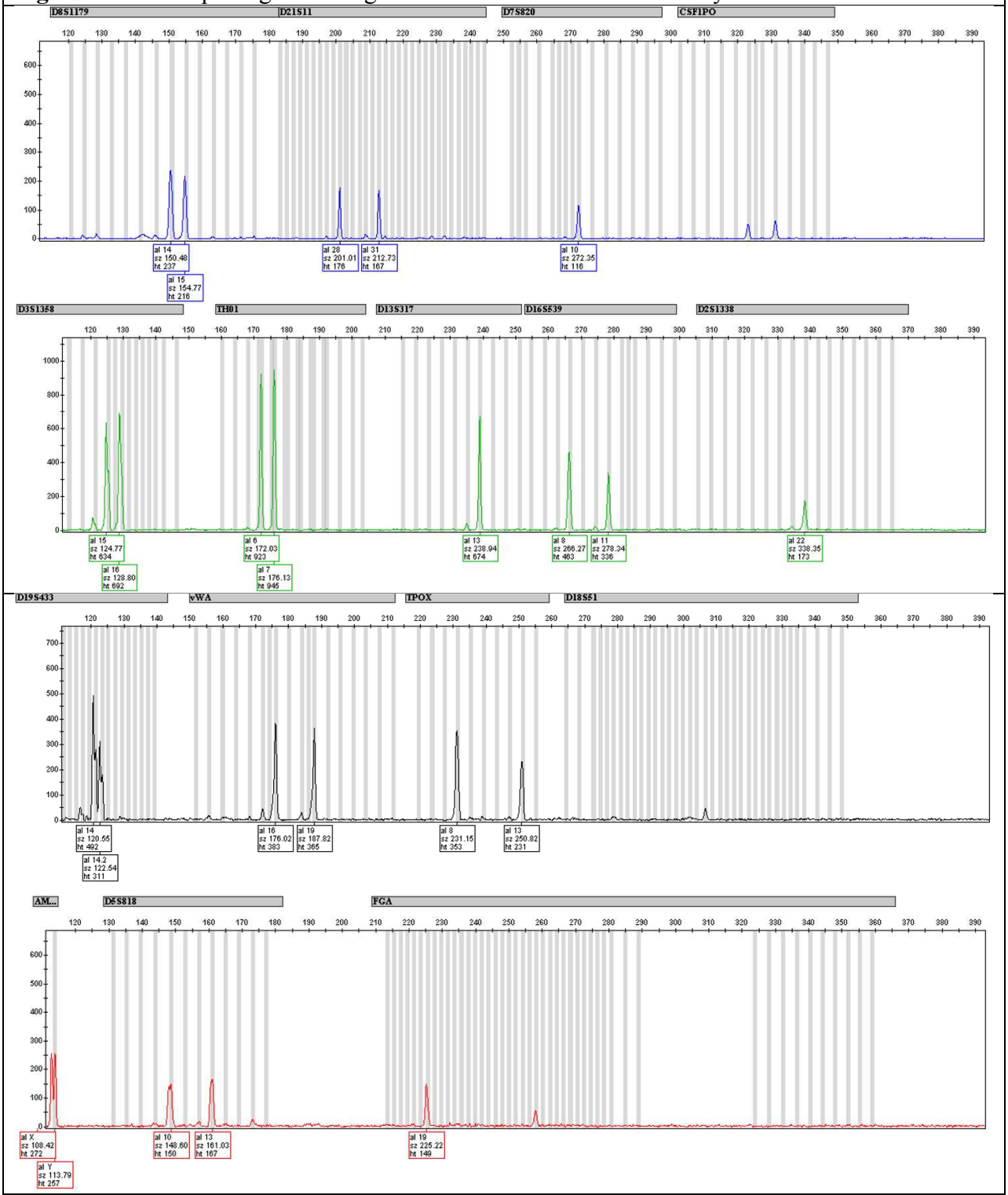
All 16 loci represented.

Figure 13. Electropherogram Using Dacron Swabs Combined With Lysis on Steel.



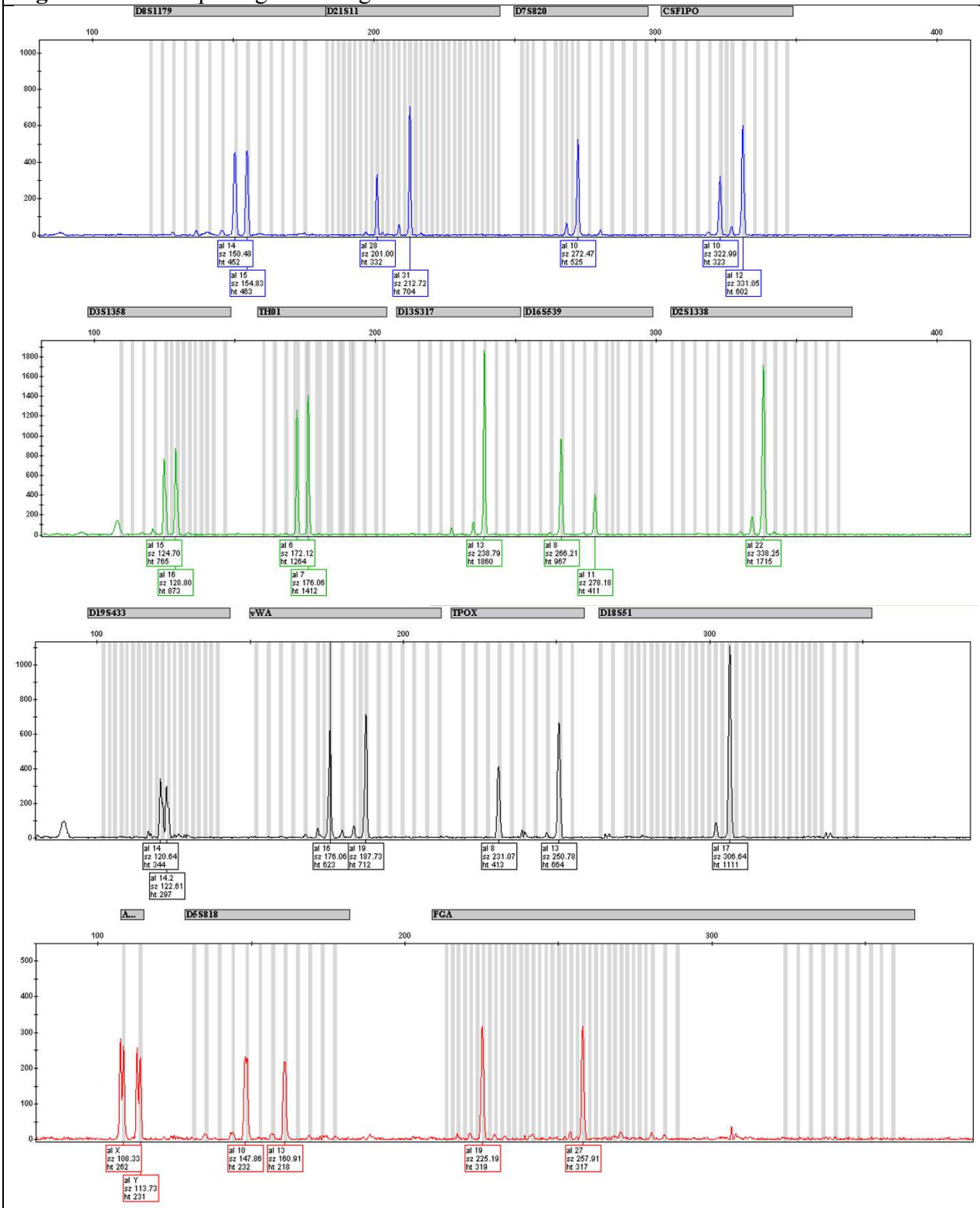
14 out of 16 loci represented. CsF1PO and FGA not reported.

Figure 14. Electropherogram Using Cotton Swabs Combined With Lysis on Aluminum



14 out of 16 loci represented. CsF1PO and D18S51 not reported. FGA is partial, one allele reported.

Figure 15. Electropherogram Using Cotton Swabs Combined With TE^{-4} on Brass Plated Metal.



All 16 loci represented.

CHAPTER V

DISCUSSION

Low copy number DNA, or touch DNA, is becoming more prevalent in crime scenes. Due to the phosphate backbone of DNA, the structure and function of DNA is dependent upon metal cations. However, this dependency creates a problem when attempting to recover low amounts of touch DNA from metals, due to the ionic bond that is created between metal cations and the phosphate group. Previous studies have researched various methods to recover touch DNA from metal bullets and casings, generally revolving around the type of buffer solution. However, a study reporting on the various buffer solutions combined with collection method has yet to have been published. The goal of this study was to apply various buffer solutions to multiple collection tools to determine which combination yielded the best recovery rate from copper, brass, steel, tin, aluminum and brass plated metals.

5.1 DNA Yield

A high throughput method is required for low copy number DNA. An amount of DNA as low as 100 pg/μL may be used for STR analysis and genetic profiling. If a sample does not contain sufficient DNA or if there are inhibitors present in the sample, a genetic profile will not

be generated. Both the 7500 Quantifiler and the 3130xl genetic analyzer (Applied Biosystems, Foster City, CA) have systems in place for high throughput analysis. The 7500 Quantifiler kit used in conjunction with the ABI7500 real time thermal cycler uses SDS software to obtain fluorescence emission data from a camera and then uses algorithms for data analysis.[53] The 3130xl genetic analyzer collects emitted fluorescence, separates it by wavelength and then focuses it onto a charged coupled device to use algorithms for analyzing the data and generating electropherograms.[54] The electropherogram is used to visualize the loci present in the DNA sample.

Published studies have shown that approximately 0.5 ng of DNA, or 500 pg/µL DNA is ideal to generate a full genetic profile.[55] During this study very few samples achieved that recovery amount. Because it is difficult to recover the ideal amount of touch DNA from metals, it is imperative that a valid and thorough purification process is utilized to recover DNA that can be amplified. This study also used samples with a recovery amount of touch DNA lower than the ideal amount of 500 pg/µL to determine if full profiles could be generated from the lower amounts.

The Promega DNA IQ bead extraction kit contains all of the necessary products required for purification of the DNA, including a lysis buffer. Using this kit for silica bead extraction maintained the same methodology throughout the study. Two variables were applied to each set of experiments on each metal. The lysis buffer contained in the kit was used with DTT added as a reducing agent to break disulfide links within and between cellular structures facilitating DNA release and also to prevent oxidation. The SDS/Pro-K buffer and the Sarkosyl/Pro-K also contained DTT as the reducing agent. These three buffers are able to lyse open the cells for the release of DNA in a soluble form. The buffer TE^{-4} is able to solubilize the DNA while still protecting it from degradation, which is why DNA is stored in TE^{-4} indefinitely.

5.1.1 Statistical Comparison of DNA Yield

Determining which combination of collection method and buffer solution yielded the best recovery was very dependent upon the metal the DNA was deposited on. Although statistically the brass plated metal using Sarkosyl and tape lift showed the best recovery of DNA among all metals, some of the other metals did not work well with Sarkosyl and tape lift, but rather had more consistent DNA recovery using another collection method. Each of these metals were also analyzed individually to determine if any significant difference existed. One-way ANOVAs were used to compare the buffers and collection tool per metal to see if these statistics lined up with the overall multiple comparison.

For Aluminum, the cotton swab combined with DTT/Lysis was the most consistent with recovery from every sample. The average yield was 117.37 pg/µL with 71.84 pg/µL being the lowest amount recovered. Considering the recovery yield was higher than other samples that generated a full STR profile, it is not surprising that a nearly full STR profile was generated. Since cotton swabs yielded the most consistent and highest recovery regardless of buffer, a one-way ANOVA was performed specifically to compare the Cotton across the buffers on Aluminum. Not surprisingly a p-value = 0.0391 was produced from the DTT/Lysis buffer when cotton was used on Aluminum.

When DNA was spotted onto the brass plated metal, tape lift and Sarkosyl/Pro-K generated the most consistent recovery levels ideal for profiling. 100% of the replicate samples yielded a recovery rate of 135.34 pg/µL for the mean recovery. Dacron combined with DTT/lysis as well as cotton swabs combined with TE^{-4} recovered DNA from brass plated metals, however

the amounts recovered had a highly variable range. The statistics support tape lift with Sarkosyl being highly favored to recover DNA from brass plated metals. Sarkosyl used with tape lift on brass plated metal was maintained as the most significantly different among all of the metals and treatments.

When comparing the statistics for DNA recovery from tin, glass fiber swabs combined with Sarkosyl/Pro-K showed a significant difference in means, however this is attributed to the low recovery amounts when glass fiber swabs were used with the other buffers during recovery of DNA from tin. The raw data shows that the cotton swabs were very consistent among all of the buffers when recovering DNA left behind on tin. Dacron swabs also had a good recovery rate with DTT/Lysis with a mean yield of 93.31 pg/μL recovered. However, this may not have reported as statistically different because the statistics may have been skewed when using Dacron with other buffers due to some of the other buffers recovering an abnormally high amount (369.93 pg/μL) for a single sample, skewing the overall mean yield for that particular buffer, which is what was compared during the one-way ANOVA.

Comparing the statistics for the recovery of low copy DNA from steel, it is evident PCR squares with Sarkosyl/Pro-K buffer performed the best, with recovery from 100% of the samples and 80% of those having a recovery amount of 103.80 pg/μL or greater. The one-way ANOVA statistics were in line with the overall statistics. Although the recovery amount was enough for profiling, a genetic profile was not obtained from one of these samples. The reason for this may be there was not enough of the sample injected during the sample preparation for the genetic analyzer. The DNA may also have become degraded while it was suspended in TE^{-4} after the purification step while it was waiting to be genetically analyzed. Although a genetic profile was not generated from the DNA yielded from the PCR square and Sarkosyl combination, a nearly full profile was generated from the Dacron swab when used with Lysis. This was surprising considering this collection method had a mean recovery amount of 36.60 pg/μL with a 90%

recovery rate from the samples, an amount much lower than the ideal amount of 500 pg/µL for profiling.

Copper and brass are the two most difficult metals to recover low copy number DNA from due to what we believe are binding tendencies of DNA to the metals. Using the one-way ANOVA, there was a statistically significant difference in the mean yield of DNA from copper using PCR squares and Sarkosyl, however the amount of recovery is too low for genetic profiling. Glass fiber swabs did have a single sample with an abnormal recovery amount of 730.60 pg/µL when using the Sarkosyl/Pro-K buffer, however this was in only one sample. The remainder of the samples ranged from 1 pg/µL to 5.75 pg/µL for the glass fiber swab with any of the buffers on the steel. This may have skewed the statistics since the mean of all five samples is what was compared during the multiple comparison analysis. Cotton swabs with TE^{-4} did appear to be the most consistent when recovering trace amounts of touch DNA from copper, however the amounts were still below the 20 pg/µL level; too low for genetic analysis. Brass yielded extremely low recovery amounts, recovering DNA in only 25% of the samples. DNA recoveries were all below 40 pg/µL except for one sample that recovered 133.28 pg/µL when using a cotton swab with Sarkosyl/Pro-K. This may possibly be due to contamination. This sample was not STR profiled.

Considering only the raw data, each metal had a different combination of collection tool and buffer solution that worked better than how that same combination worked on a different metal. For copper, cotton swabs with TE^{-4} were the most consistent recovery although the mean recovery was very low among the samples. For brass, the most consistent recovery was cotton combined with DTT/Lysis, having a 100% recovery among samples, although a very small mean recovery as well. When considering steel, using PCR squares and Sarkosyl performed the best, which is in line with the statistics on both the multiple comparison analysis and the one- way ANOVA. Cotton swabs used with DTT/Lysis on aluminum proved to have the best recovery, also in line with the one-way ANOVA. There was a consistent recovery above 100 pg/µL, as well

as a nearly full profile generated from one of these samples. For tin, Dacron and DTT/Lysis performed the most consistently recovering nearly 100 pg/µL from each sample. On brass-plated metals, using Sarkosyl and tape lift proved to have the best recovery with 135.34 pg/µL mean recovery, which is in agreement with both the multiple comparison analysis and the one-way ANOVA.

5.1.2 Complications

DNA is known to degrade over time. Although DNA has shown to remain stable in a soluble state suspended in TE^{-4} at a temperature of 4° C, it is possible the DNA continues to degrade while in the PCR tube. Results might have been less variable if the recovered DNA was quantified immediately after purification. During this study, some of the samples were amplified the following day after purification while others were amplified as long as two weeks after purification. This may have affected the outcome.

When a DTT/Lysis solution and a Sarkosyl/Pro-K solution was created fresh, the purification process appeared to produce better results than when a solution was used that was several days old (up to two weeks). For future experiments, the ideal step would be to make the buffer solution fresh daily or to only make precisely enough solution for the needed experiment.

Due to the low amount of recovery of touch DNA from each of the metals, the DNA was maximized during the genetic analyses. Substituting the nuclease-free water with additional amplified DNA sample during the Identifiler-Plus set up maximized the DNA per sample for the genetic analyzer.

High variations of DNA recovery occurred among each metal. This seems logical since metals are comprised of different elements and may have different binding strengths. Brass

followed the same trend as copper for failing to produce any reliable results regardless of the collection method or buffer solution used, which is expected since brass is an alloy of copper. Statistics from this study show that the recovery rate for touch DNA is not only dependent upon the type of collection method and buffer solution being used but also dependent on the particular metal upon which the touch DNA was deposited. When considering which buffer solution to use, the substrate composite should be considered as well.

5.2 Future Research and Recommendations

Considering some touched objects may results in less than 300 pg touch DNA being transferred to the object, [56] it is imperative to maximize the amount of DNA recovered from these items. Recovering as much as 100 pg/µL may yield a profile, however this study has also shown that is highly variable since a full profile might be yielded from as low as 40 pg/µL. This study only performed a spot analysis on a few samples of the total being amplified. Future research may focus on recovering lower than 100 pg/µL and genetically analyzing the amounts to determine if there is a significant statistical difference and what exactly is the ideal amount for genetic analysis. During any analyses of low copy DNA, the DNA would need to be maximized during the profiler set up, as was done for the few samples analyzed in this study. It would also be beneficial to study environmental effects, such as weather, on the amount of DNA deposited.

This study used a stock purchased 'naked' DNA, so the DNA was not encapsulated in cells. Future research may involve DNA in cells. It is difficult to determine the exact amount of DNA spotted onto a sample using cells, however a standard calculation for cells per µL could be determined and used throughout the study. The methodology for this would need to be adjusted for digestion time of the DNA prior to using the magnetic beads.

The novel glass fiber swab, which was being researched concurrently with this study, relies upon chaotropic salt buffer such as lysis as well as DTT to remove the denaturants. The DNA is expected to bind to the silica fibers on the swab, and the swab itself being the filter. A different methodology may be followed rather than creating a double tube filter to utilize the glass fiber swab more efficiently. Some of the DNA may have remained bound to the swab considering the glass fiber attracts DNA like the silica-coated bead do in the extraction kit used for this research.

Because there was no one combination of buffer solution and collection tool that worked best across all metals, future research ideas may revolve around the affinity of individual metals to DNA. Due to the variation of results, the strength of bond formed between each metal and the DNA phosphate group may play an important role in recovery.

5.3 Conclusion

In conclusion, this study provided an understanding of what affects the recovery of touch DNA left behind on metals. This study clearly demonstrated that both swab collection type and buffer solution play a crucial role during the touch DNA collection process. Because no one combination performed the same across all six metals, there was no one statistically different combination that was better than the others overall. Statistically tape lift and Sarkosyl stood out as significantly different, and with a mean recovery of 135.34 pg/µL, this combination was the highest yielding. However, when comparing the raw data and one-way ANOVAs, it is evident that cotton consistently recovers an optimal amount near 100 pg/µL for touch DNA when compared to other recovery combinations across the various metals. Not only were the cotton swab samples the most consistent, these samples were also the most to generate a genetic profile. Although other buffer and collection tools yielded higher recovery of DNA, cotton generated a

profile, even if lower than 100 pg/μL was recovered. Choosing which method to use should be determined on the most reliable and consistent recovery results in order to optimize the probability of generating a profile.

Understanding how each solution and swab type performs on various metals will be beneficial to labs when they have limited samples to work with. They will be able to choose the most optimal method and solution in order to achieve the highest recovery of DNA, allowing them to have a better chance of obtaining a genetic profile.

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APPENDICES

Appendix A

Copper-Average yield per sample pg/µL

DNA IQ Kit with Lysis/DTT pg/µL	Cotton	Dacron	PCR square	tape lift	Glass fiber swab
Sample A	2.46	2.48	0.00	0.00	3.56
Sample B	6.97	0.00	0.75	0.00	1.28
Sample C	1.43	0.00	0.00	0.00	1.49
Sample D	3.69	1.35	0.00	1.41	1.28
Sample E	8.77	3.46	0.00	0.00	0.00
Average	4.66	1.46	0.15	0.28	1.52
DNA IQ Kit with TE-4 pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	5.03	0.00	2.72	0.00	0.00
Sample B	20.56	2.77	1.10	0.00	0.00
Sample C	7.68	0.00	0.00	86.17	0.00
Sample D	1.51	3.42	0.00	0.00	0.00
Sample E	38.87	0.00	0.87	0.00	0.00
Average	14.73	1.24	0.94	17.23	0.00
DNA IQ with SDS/ProK Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	1.01	0.00	0.00	0.00
Sample B	0.00	0.00	0.00	0.00	0.00
Sample C	8.06	0.00	0.00	0.00	0.00
Sample D	35.26	0.89	0.00	0.00	0.00
Sample E	12.34	0.00	0.00	0.00	0.00
Average	11.13	0.38	0.00	0.00	0.00
DNA IQ with Sarkosyl Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	0.00	2.14	0.00	632.56
Sample B	0.00	2.90	13.34	0.00	1.40
Sample C	0.00	3.16	0.58	1.27	0.00
Sample D	0.36	7.82	0.61	0.00	5.75
Sample E	2.89	14.14	8.16	0.00	0.00
Average	0.65	5.60	4.97	0.25	127.94

Appendix B

Brass-Average yield per sample pg/µL

DNA IQ Kit with Lysis/DTT pg/µL	Cotton	Dacron	PCR square	tape lift	Glass fiber swab
Sample A	5.94	1.09	0.00	0.00	1.36
Sample B	5.76	0.00	5.35	0.00	0.00
Sample C	1.11	0.00	0.00	1.47	0.00
Sample D	20.42	1.48	0.58	0.00	0.00
Sample E	5.37	0.00	0.00	3.39	0.00
Average	7.72	0.51	1.18	0.97	0.27
DNA IQ Kit with TE-4 pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	8.41	0.00	0.00	10.65	0.00
Sample B	2.01	0.00	0.00	0.00	0.00
Sample C	0.69	0.00	0.00	0.00	0.00
Sample D	0.00	0.00	1.27	1.35	0.00
Sample E	4.44	3.73	1.35	0.00	0.00
Average	3.11	0.75	0.53	2.40	0.00
DNA IQ with SDS/ProK Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	0.00	0.00	0.00	0.00
Sample B	0.00	0.00	0.00	0.00	0.00
Sample C	1.47	0.00	0.00	0.00	0.00
Sample D	0.34	0.00	0.00	0.00	0.00
Sample E	0.00	0.00	3.58	0.00	18.31
Average	0.36	0.00	0.72	0.00	3.66
DNA IQ with Sarkosyl Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	2.13	0.00	1.27	1.35
Sample B	0.00	0.00	7.64	0.00	2.48
Sample C	0.00	1.62	6.30	0.00	0.00
Sample D	0.00	0.00	0.00	1.39	0.00
Sample E	109.97	0.00	9.89	3.36	0.00
Average	21.99	0.75	4.77	1.21	0.77

Appendix C

Steel-Average yield per sample pg/µL

DNA IQ Kit with Lysis/DTT pg/µL	Cotton	Dacron	PCR square	tape lift	Glass fiber swab
Sample A	9.42	53.61	13.06	0.00	3.41
Sample B	17.85	36.77	6.58	10.37	0.00
Sample C	36.83	7.53	0.12	7.79	0.00
Sample D	45.02	34.21	14.58	18.51	0.00
Sample E	17.54	50.87	13.68	9.15	0.00
Average	25.33	36.60	9.61	9.16	0.68
DNA IQ Kit with TE-4 pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	16.95	8.49	0.00	0.00	0.00
Sample B	35.59	7.14	0.00	12.15	0.00
Sample C	27.95	16.27	2.50	0.00	0.00
Sample D	75.19	7.03	1.15	1.81	4.69
Sample E	21.53	6.98	9.81	2.41	0.00
Average	35.44	9.18	2.69	3.28	0.94
DNA IQ with SDS/ProK Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	11.19	0.00	0.00	0.00	0.00
Sample B	5.28	0.00	0.00	0.00	2.69
Sample C	2.53	0.00	0.00	0.00	3.16
Sample D	3.56	0.00	0.00	0.00	0.00
Sample E	3.66	0.00	0.00	0.12	4.03
Average	5.24	0.00	0.00	0.02	1.98
DNA IQ with Sarkosyl Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	118.62	106.39	0.00	23.95
Sample B	155.20	0.00	112.85	32.17	7.81
Sample C	0.00	0.00	161.94	18.93	8.59
Sample D	137.31	0.00	57.99	0.00	5.98
Sample E	0.00	91.58	192.48	41.59	2.23
Average	58.50	42.04	126.33	18.54	9.71

Appendix D

Aluminum-Average yield per sample pg/µL

DNA IQ Kit with Lysis/DTT pg/µL	Cotton	Dacron	PCR square	tape lift	Glass fiber swab
Sample A	90.62	45.01	0.00	12.14	7.77
Sample B	166.45	89.56	31.09	4.41	0.60
Sample C	116.85	59.37	4.35	26.26	2.91
Sample D	82.04	29.12	0.00	7.71	10.17
Sample E	130.88	0.23	0.00	20.20	0.53
Average	117.37	44.66	7.09	14.14	4.39
DNA IQ Kit with TE-4 pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	72.68	20.81	23.62	3.32	0.00
Sample B	95.97	320.03	5.08	0.00	0.00
Sample C	88.81	6.74	7.12	9.30	0.00
Sample D	71.05	9.19	7.51	15.59	0.00
Sample E	149.25	9.40	15.92	7.12	0.00
Average	95.55	73.23	11.85	7.07	0.00
DNA IQ with SDS/ProK Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	0.00	0.00	0.00	0.00
Sample B	0.00	133.05	0.00	0.00	0.36
Sample C	0.00	0.00	0.00	0.00	25.51
Sample D	106.35	0.00	0.00	0.00	7.02
Sample E	75.55	0.00	0.00	0.00	0.00
Average	36.38	26.61	0.00	0.00	6.58
DNA IQ with Sarkosyl Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	164.93	0.00	1.70	0.00	16.97
Sample B	0.00	0.00	0.00	16.13	31.90
Sample C	0.00	0.00	169.96	86.61	0.00
Sample D	0.00	0.00	0.62	0.00	9.51
Sample E	0.00	213.71	0.00	0.00	29.84
Average	32.99	42.74	34.46	20.55	17.64

Appendix E

Tin-Average yield per sample pg/µL

DNA IQ Kit with Lysis/DTT pg/µL	Cotton	Dacron	PCR square	tape lift	Glass fiber swab
Sample A	73.88	76.21	5.65	21.24	2.72
Sample B	84.33	105.30	11.83	7.03	2.60
Sample C	92.86	89.47	16.71	20.57	7.21
Sample D	103.56	98.26	3.53	32.44	13.39
Sample E	80.52	97.31	1.40	55.65	0.00
Average	87.03	93.31	7.82	27.38	5.18
DNA IQ Kit with TE-4 pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	71.51	4.65	15.29	30.96	0.00
Sample B	32.63	11.65	6.11	2.20	0.00
Sample C	44.19	4.93	0.00	14.72	0.00
Sample D	39.60	3.87	12.07	105.44	0.00
Sample E	59.36	12.76	6.29	41.06	0.00
Average	49.46	7.57	7.95	38.88	0.00
DNA IQ with SDS/ProK Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	104.31	0.00	0.00	0.00	0.00
Sample B	128.44	0.00	0.00	0.00	7.95
Sample C	0.00	0.00	25.55	0.00	0.00
Sample D	0.00	0.00	10.04	0.00	2.62
Sample E	114.31	0.00	24.90	0.00	0.00
Average	69.41	0.00	12.10	0.00	2.11
DNA IQ with Sarkosyl Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	0.00	91.30	81.22	22.22
Sample B	0.00	329.01	0.00	0.00	46.18
Sample C	151.74	0.00	0.00	170.22	18.69
Sample D	55.56	0.00	3.19	26.60	26.35
Sample E	0.00	0.00	37.40	72.19	12.62
Average	41.46	65.80	26.38	70.04	25.21

Appendix F

Brass Plated – Average yield per sample pg/µL

DNA IQ Kit with Lysis/DTT pg/µL	Cotton	Dacron	PCR square	tape lift	Glass fiber swab
Sample A	78.56	142.24	6.27	18.52	0.00
Sample B	86.47	155.53	20.66	0.00	1.15
Sample C	93.14	103.69	2.71	5.95	5.14
Sample D	74.42	80.01	27.68	5.66	1.45
Sample E	0.00	93.99	14.41	22.74	5.78
Average	66.52	115.09	14.35	10.58	2.70
DNA IQ Kit with TE-4 pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	87.76	0.86	1.98	6.54	0.00
Sample B	105.50	2.50	2.01	2.73	0.00
Sample C	112.41	9.12	0.00	0.00	0.00
Sample D	61.96	17.44	0.00	0.00	0.00
Sample E	37.94	2.68	0.00	0.00	0.00
Average	81.11	6.52	0.80	1.85	0.00
DNA IQ with SDS/ProK Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	0.00	4.70	0.00	5.02
Sample B	7.12	0.00	0.00	0.00	5.92
Sample C	3.53	19.50	0.00	0.00	1.36
Sample D	3.05	0.00	0.00	43.15	0.00
Sample E	4.54	0.00	5.15	0.00	1.19
Average	3.65	3.90	1.97	8.63	2.70
DNA IQ with Sarkosyl Buffer pg/µL	**Cotton**	**Dacron**	**PCR square**	**tape lift**	**Glass fiber swab**
Sample A	0.00	0.00	148.61	146.51	22.11
Sample B	145.41	0.00	196.41	179.73	26.61
Sample C	131.00	0.00	0.17	135.16	17.25
Sample D	0.00	223.08	0.93	79.84	9.87
Sample E	42.45	0.00	0.00	135.46	22.90
Average	63.77	44.62	69.22	135.34	19.75

VITA

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