THE RECOVERY AND ANALYSIS OF DNA FROM FIRED CARTRIDGE CASINGS

By

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ABSTRACT

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Firearms, particularly handguns, are used in the commission of various crimes in the United States, though frequently the only evidence that a gun was used is cartridge casings. Thus, deeper investigation of the utility of DNA analysis for fired cartridge casings has been attempted. Previous studies have explored the probability of developing latent fingerprints and generating STR profiles from unfired and fired cartridge casings in attempt to identify the individual who loaded the gun. In each of these instances casings were washed to remove DNA from prior handlers. In reality, however, criminals will not wash the cartridges before firing them, thus the primary focus of this study was utilizing un-cleaned casings. A cumulative swabbing method was compared to a single swabbing method to determine which generated higher DNA yields and more complete STR profiles consistent with the loader. A consensus method for determining STR profiles was also compared to the two swabbing methods. Thirty volunteers handled 10 cartridges each and loaded them into the magazine of a gun; cartridges were fired and casings were collected and swabbed. DNAs were extracted, quantified, and amplified for STR typing. There was no difference in DNA yield between the swabbing methods, though cumulatively swabbing resulted in the greatest percentages of consistent alleles and profiles. The consensus profiling method had a higher rate of consistency than the single swabbing method but did not outperform the cumulative swabbing method.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

History of Firearms

Carman (1955) defined a firearm as any device that expels a projectile through the use of sudden explosion. The projectile and explosive are contained within the ammunition, which in turn is contained in the firearm. The primary explosive for years has been a combination of chemicals, which collectively generate high heat and pressure in a very short amount of time. Gunpowder as first developed was a mixture of sulphur, charcoal, and saltpeter (potassium nitrate), intended for use in firecrackers but soon proved to be an extremely powerful tool in combination with projectiles (Carman, 1955; Peterson, 1961). Gunpowder generates up to six-times its mass in gaseous pressure. This expansion of gases inside the chamber of the firearm is used to drive projectiles, such as cannonballs, lead pellets, and bullets, at a high rate of speed in a specific direction (Peterson, 1961). Figure 1 shows the anatomy of a modern cartridge. The bullet and propellant are contained within the cartridge case. The rim contains the primer which when struck by the firing pin of a gun generates a spark to ignite the propellant, which in turn displaces the bullet as gaseous pressure builds up behind it.

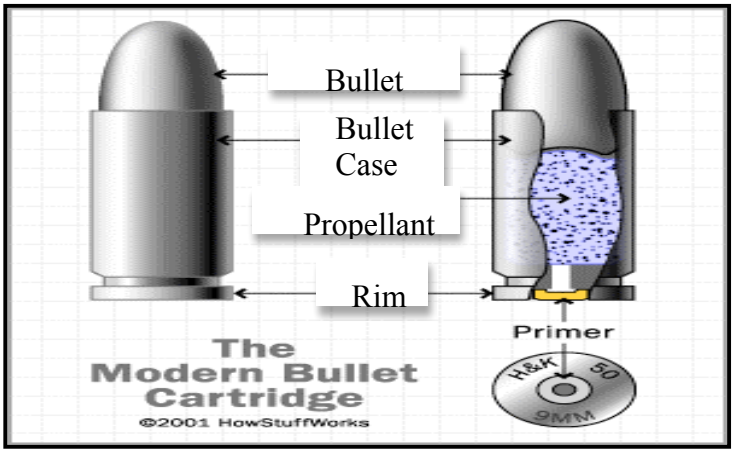

Figure 1. Anatomy of a modern bullet cartridge. Taken from HowStuffWorks 2001. http://static.howstuffworks.com/gif/revolver-bullet.gif. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Following the development of gunpowder, firearms have advanced immeasurably; gunsmiths developed mechanisms such as the matchlock, wheel lock, and flintlock, which worked in similar manners, all for the purpose of generating enough spark to ignite the gunpowder in the flash pan, ultimately resulting in an explosion and the firing of the projectile (Ricketts, 1962). Since 1918 firearms have been improved upon by simplifying them, making them more 'user friendly' (Roads and Hogg, 1983). After World War II large, 'heavy hitting' weaponry, such as the Boys Anti-Tank Rifle, ceased to exist as its civilian use could not be rationalized. Instead, lightweight arms for military parachutists and other infantry specialists became much more practical and were developed using a combination of already accepted devices (Roads and Hogg, 1983). It was the availability of semi-automatic weaponry to the public that seemed to change civilian dependency on firearms, especially handguns. Increased civilian use translated into increased numbers of firearm related deaths.

As gun technology improved so did the ease of use, and self-protection became the primary reason for owning a handgun in the US (OJJDP, 1996). In 2009, nearly 14,000 murders occurred in the United States. Just over 9,000 (67.1%) of those involved the use of firearms (FBI, 2010; Guardian News, 2011), 80% of which resulted from handguns, even though they account for only 34% of firearms in the U.S. (LCAV, 2011). Aggravated assaults and robberies, among other crimes, also involve firearms. In 2009, 42.6% of robberies and 20.9% of aggravated assaults were committed using a firearm (FBI, 2010). This is likely because the United States is one of the only modern industrial nations that is preserving a gun culture where it is legal for large portions of the population to own firearms (OJJDP, 1996). However, since peaking in the late 1980's gun-related crimes have decreased; from 2005 to 2009 there was a

10% drop in firearm use related to violent crime, yet guns still pose a major threat to the safety of all Americans (OJJDP, 1996; FBI, 2010).

Techniques Used for DNA Analysis

That guns are used to commit crimes is a given, however what is not so clear is how to successfully identify the people loading and/or firing weapons to commit those crimes. The ability of law enforcement to identify an assailant may lay in their capacity to utilize DNA. When an individual holds or loads a gun skin cells may be deposited on the grip, hammer, trigger, magazine, or cartridges; any surface that the 'shooter' comes in contact with has the potential to harbor touch DNA. Degraded and low copy number (LCN) DNA (or the presence of less than 100pg of DNA, Gill *et al*. 2000) are often encountered in forensic work. The most important tool used in these instances is the polymerase chain reaction (PCR), which amplifies a specific segment of DNA, resulting in over a billion copies and ensuring enough DNA for downstream procedures. Today, this entails amplification of short tandem repeats (STRs), which are the standard method used to produce highly discriminating DNA profiles. STRs are regions of DNA with repeating sequence units 2 – 6 base pairs long. The number of repeats in each STR marker is variable among individuals, and it is this variability that is used to differentiate and identify individuals. A clean and easily interpretable STR DNA profile is shown in Figure 2. If this profile were obtained from a piece of evidence it would be compared to a known or suspect sample to see if the evidence profile and the suspect profile are both 10, 11 at the D7 locus, 14, 15 at the D3 locus, etc. After a profile has been compared at each locus a random match probability is determined, calculating the probability that the evidence sample belongs to

someone other than the suspect. Often the random match probability from a full STR profile exceeds one in a hundred trillion.

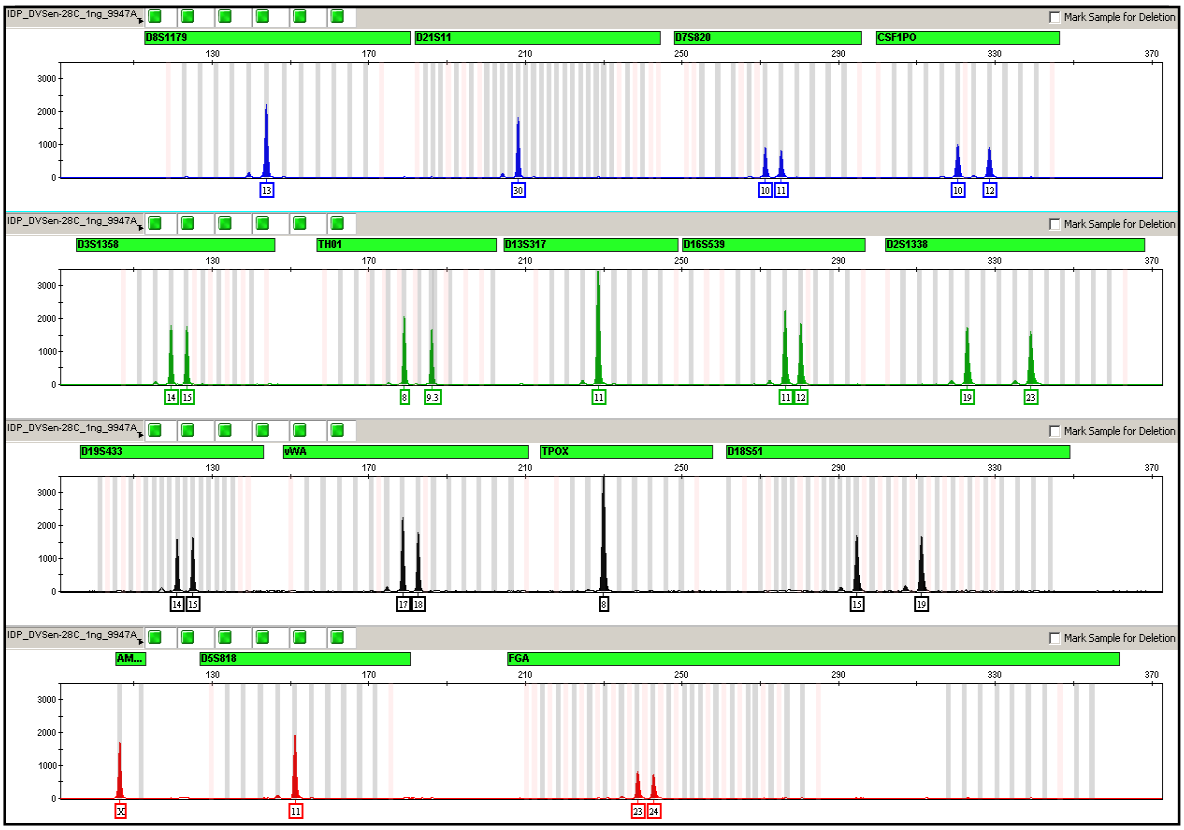

Figure 2. An example of an easily interpretable STR profile generated from control DNA, using an Identifiler™ Plus PCR Amplification Kit. The x-axis represents the size of the amplicon (bp) and the y-axis represents relative fluorescent units (signal intensity). Applied Biosystems Identifiler™ Plus Manual, 2009. The text is not meant to be readable, but is for visual reference only.

However, such a profile is often not obtained in forensic casework because the DNA is too degraded or is LCN. Degradation frequently results from exposure to the elements or other environmental conditions.

Though researchers have had great success performing STR analysis and generating DNA profiles from LCN DNA, there are several difficulties that may be encountered. In general, three artifacts often occur in STR profiles from LCN DNA. The first is 'drop-in', or the presence of alleles that are not native to the sample. Allelic drop-in can create the appearance of

a mixture, which may cause incorrect profile interpretation. The second artifact is 'drop-out' (Figure 3) or the absence of alleles that should be present but have failed to amplify as a result of unequal sampling of the two alleles from a heterozygous individual, which is often encountered when few DNA molecules are utilized during amplification (Walsh *et al*. 1992). Similar to drop-in, drop-out can be difficult to interpret. For instance, if an individual is heterozygous but one allele did not amplify then the analyst can easily interpret that individual as homozygous at that locus.

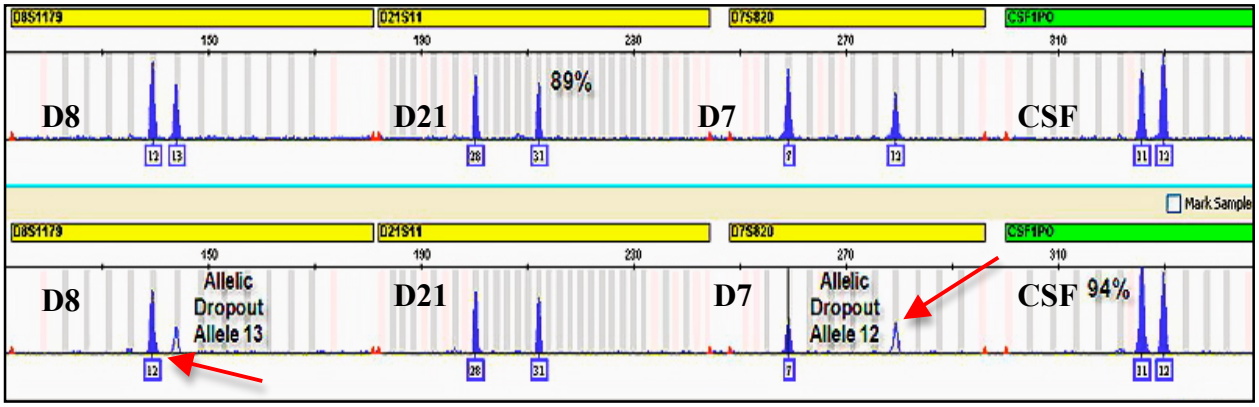

Figure 3. Example of four loci from a known sample (defendant) and the same four loci from an evidence sample. The D21 and CSF loci both show the same STR alleles, 28, 31 and 11, 12 respectively, between the defendant and evidence samples. The D8 and D7 loci, however, are exhibiting allelic dropout (red arrow), where the defendant is a 12, 13 at D8 but the evidence sample only shows a 12, similarly, the defendant is a 7, 12 at D7 but the evidence shows only a 7, indicating that the 13 and 12, respectively, were not amplified during PCR. The majority of text is not meant to be readable, for visual reference only. Taken from Applied Biosystems 2009. http://marketing.appliedbiosystems.com/images/all_newsletters/forensic_1109/articles.html

The last of the three common artifacts is 'stutter,' defined by the presence of peaks four bases (1 repeat unit) smaller (or sometimes larger) than an allele in the STR electropherogram. Stutter, exemplified in Figure 4, results from DNA slippage during DNA replication (Hauge and Litt, 1993) and can be much more extreme in LCN DNA, with stutter peaks equal to or exceeding the size of the alleles native to that individual. Similar to drop-in, stutter peaks can give the false appearance of mixtures.

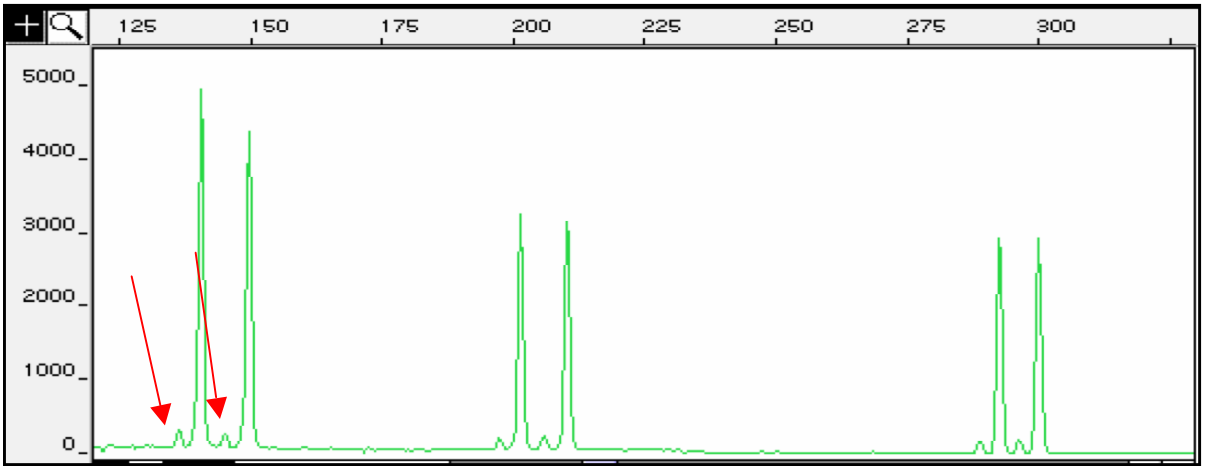

Figure 4. An example of stutter in an STR profile. The tall green peaks are alleles that belong to the handler. The red arrow points to a stutter peak for the first set of alleles. Taken from Butler 2005. http://www.cstl.nist.gov/biotech/strbase/training.htm. Text is not meant to be readable, but for visual reference only.

Another challenge encountered when working with forensic samples is various substances that inhibit PCR, resulting in little or no DNA amplification. Inhibitors can obstruct cell lysis in preparation for DNA extraction, can degrade the DNA, or may compete or interfere with elements of the PCR reaction, such as the polymerase, thereby preventing enzymatic activity and amplification (Wilson, 1997). Overall, there is a loss of alleles and thus a loss of genetic information.

Processing of Cartridge Cases by the Michigan State Police

Cartridge casings are often collected from crime scenes where a firearm has been used, and are placed in evidence bags for transport to the crime laboratory (Sgt. Ryan Larrison, personal communication). Once received into evidence by a Michigan State Police (MSP) forensic laboratory, casings are generally submitted to the DNA unit for analysis (Sgt. Ryan Larrison, personal communication). There, DNA analysts practice a cumulative swabbing technique, where presumably related casings are swabbed successively using a single pair of

swabs (David Bicigo, personal communication). DNA is purified from the swabs via organic extraction, quantified with Plexor® HY, and commercial kits Profiler™ Plus and Cofiler™ are used for STR amplification. Casings are returned to the evidence bag and no further processing is pursued if amplification does not occur (Heather Clark, personal communication).

There are potential advantages and disadvantages with the cumulative swabbing method utilized by the MSP. It is logical that the DNA yield from swabbing several casings at once would be greater than swabbing casings individually, thereby resulting in more informative STR profiles. Swabbing multiple casings at one time has the added benefit of being more cost and time effective than processing casings individually. Alternatively, this swabbing technique may lead to increased PCR inhibition due to an accumulation of inhibitors on the swabs. Further, if casings collected from a scene were not all handled by a single DNA contributor, there is potential for cross-contamination leading to an increased chance of mixtures. Lastly, DNA may be left behind on casings that are swabbed consecutively, illustrated in Figure 5.

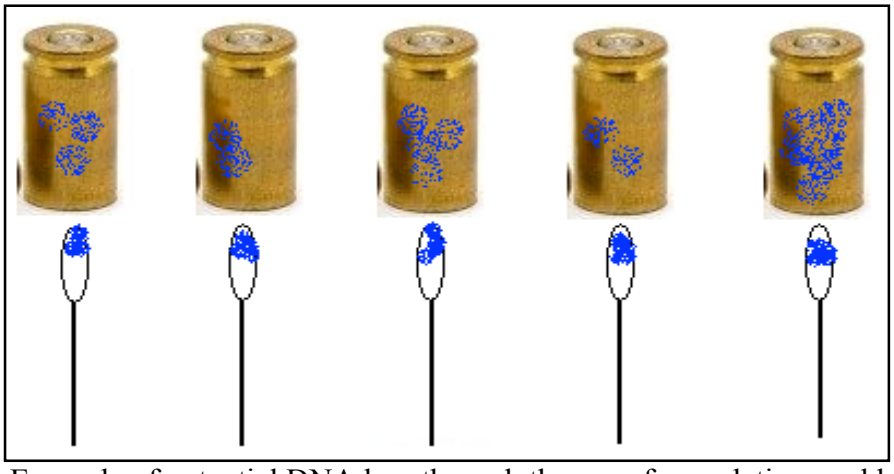

Figure 5. Example of potential DNA loss through the use of cumulative swabbing. The blue represents skin cells on both casings and swabs. By the time the last casings is reached there is an accumulation of DNA on that casing from previously swabbed casings, and less on the swab that will be subjected to extraction.

DNA Recovery from Fired Cartridge Casings

Researchers have started to examine the utility of DNA recovery and analysis from spent cartridge casings more rigorously because current DNA analysis and fingerprinting methods are generally unsuccessful. Spear *et al.* (2011) tested how often they could obtain fingerprints from unfired and fired cartridge cases, as well as the likelihood of obtaining a DNA profile from the same casings. Forty-eight casings made of brass, nickel-plated brass, and aluminum were examined. Bloody, eccrine, and oily prints were placed on the casings prior to firing or processing. Bloody fingerprints were treated with amido black, and the eccrine and oily prints were subjected to cyanoacrylate fuming. Casings were swabbed, and DNA was extracted and amplified using Profiler™ Plus. Overall, there were no useable prints on any of the smaller, fired casings regardless of the type of print or processing method. In contrast, 6 of 36 bloody prints were developed from the larger 9mm and 45 caliber casings, three oily prints were deemed useable from unfired casings, while no useable eccrine prints were recovered. Only three DNA profiles were obtained from the casings tested, all of which were developed from bloody prints, two unfired and one fired.

Horsman-Hall *et al.* (2009) investigated whether amplifiable DNA could be obtained from unfired and fired cartridge casings. The firearms and cartridges were first cleaned and then handled for 30 seconds. A gloved firearms examiner loaded the cartridges into the chamber of a gun, not using a magazine. As cartridges were fired the casings landed on a paper covered floor and were collected using a broom and the wooden end of a sterile swab. Buccal swabs were collected from all handlers for reference samples, and each casing was swabbed using a double swab technique. DNA was extracted using a DNA IQ™ Isolation System for Large Volumes with 0.4% Sarkosyl, and quantified using commercial kits from Promega and Stratagene. STR amplification was attempted using kits from Applied Biosystems (MiniFiler™ and Identifiler™)

and Promega (PowerPlex 16® BIO). The MiniFiler™ amplification kit resulted in a significantly greater number of alleles, overall, than either Powerplex 16® BIO or Identifiler™, the latter of which resulted in no alleles in any of the casing samples. The majority (54%) of fired cartridge casings generated no profile, 26% produced less than half of the possible alleles, 19% resulted in more than half the alleles, and 1% resulted in a full profile.

Goals of this Study

Cartridges are often the only evidence that a firearm was used at a crime scene, and they are handled for only a brief period of time during loading. Since casings are objects from which touch DNA may potentially be collected, the goals of this study were to utilize un-cleaned cartridges and different methodologies for recovering and analyzing DNA from fired cartridge casings, in order to maximize the chances of obtaining useful genetic information from a perpetrator. The effectiveness of two swabbing methods and the utility of DNA analysis for identifying an individual who loaded or shot a firearm were also assessed. DNAs were extracted, quantified, amplified using Identifiler™ Plus, and STR profiles developed. Results generated using the cumulative swabbing technique were compared to those developed from the single swabbing method, to determine which maximized DNA yield and allelic consistency, while limiting cross contamination (mixtures) and PCR inhibition. A consensus profiling method, combining STR profiles from five single swabs, was also used to evaluate the two swabbing methods based on the number of alleles consistent with the handlers' profiles.

MATERIALS AND METHODS

Cartridge Casing Collection

Remington®, 40-caliber, brass cartridges were purchased from a local sporting goods store. Prior to cartridge distribution, ten cartridges were selected at random, swabbed, and DNA was extracted to determine whether background DNA was present. Cartridges were divided into sets of ten, each of which were placed in paper bags and labeled 1 – 33. Volunteers from the Michigan State Police Bridgeport and Northville crime laboratories selected a number at random and were provided the bag of cartridges corresponding to that number. The volunteers loaded all ten cartridges into the magazine of a gun without excessive handling. The cartridges were fired until each magazine was empty and casings were caught either in heat-seal grade plastic bags contained in a wide rimmed container as they were ejected, or in the netting around the shooting hole of a firing tank. Casings were then placed back into their respective paper bags. A new heat-seal bag was used for each set of casings to avoid cross contamination. Volunteers selected a letter and submitted a buccal swab that was labeled with the letter and placed in a culture test tube; in total 33 sets of casings and corresponding buccal swabs were collected. The MSU Institutional Review Board cleared all experiments for human participation (IRB#10-766).

Cumulative and Single Swabbing of Collected Cartridge Casings

Casings were swabbed using a double swab technique (Sweet *et al*. 1997) wetting the first swab with 100µL of 5% SDS. Each set of casings was randomly divided in half. As illustrated in Figure 6, five of the casings were cumulatively swabbed using one pair of swabs consecutively, while the remaining five casings were swabbed individually, each with its own pair of swabs.

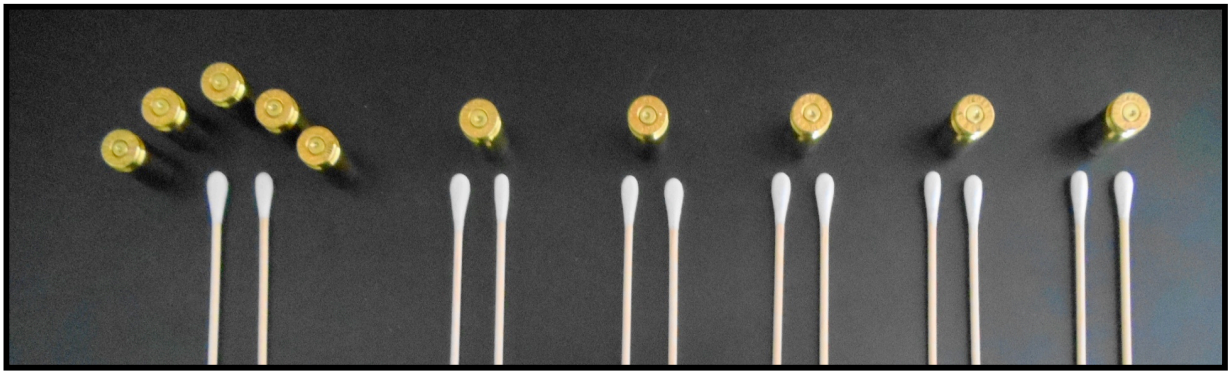

Figure 6. Cumulative swabbing is shown on the left, where 5 casings were swabbed with a single pair of swabs. The five un-grouped casings on the right were swabbed singly, each with its own pair of swabs.

DNA Extraction and Isolation

Swab heads were clipped off with wire cutters and placed in a 1.5mL microcentrifuge tube with 500µL of digestion buffer (20mM Tris—pH 7.5; 50mM EDTA, 0.1% SDS) and 6µL of proteinase K (20mg/mL). The tubes were vortexed and incubated overnight at 56°C. Swab heads were removed from the digestion solution and placed in spin baskets and centrifuged for 2 minutes at 1,000 relative centrifugal force (rcf). The liquid collected was added to the digestion solution and swab heads were discarded.

DNA extractions were performed by adding 600µL of phenol to the digestion tube, vortexing, and centrifuging at top speed (21,000 rcf) for 5 minutes. The aqueous layer was removed and added to 600µL of chloroform, vortexed, and centrifuged at 21,000 rcf for 5 minutes. The aqueous layer was removed and placed in a 30Kd Millipore Amicon® column. Columns were centrifuged at 14,000 rcf for 10 minutes and filtrate was discarded. DNAs were washed using two cycles of 300µL of TE (10mM Tris; 1mM EDTA, pH 7.5) and centrifuged at 14,000 rcf for 10 minutes. Columns were inverted over a clean 1.5mL Amicon tube and centrifuged at 1,000 rcf for 2 minutes.

Zymo-Spin™ IV-HRC columns were prepared per manufacturer's protocol. DNAs were added to the column in a 1.5mL microcentrifuge tube. Columns were centrifuged at 8,000 rcf for 1 minute and filtered DNAs were stored at 4°C. Buccal swabs were extracted and cleaned in the same manner, including the Zymo-Spin® column to keep processing consistent, and were diluted 1:100 for downstream analysis. During the extraction process two casing samples, #4 and 14, were compromised, as they were cross-contaminated.

DNA Quantitation

A Quantifiler™ Human DNA Quantification kit was used to determine DNA concentration. DNA (1.2µL) was mixed with 6.3µL of primer mix and 7.5µL of reaction mix. Tubes were vortexed and placed in the Bio-Rad iQ5 Thermal Cycler using cycling conditions: 95°C for 10 minutes, followed by 95°C for 15 seconds, and 60°C for 1 minute for 40 cycles. The real-time PCR software was used to compare cycle threshold (C_T) values for each reaction to those of two sets of 8 standards, containing known DNA concentrations as per the manufacturer's instructions. A standard curve was produced and used to determine DNA quantities for the casing reactions.

STR Typing of Cartridge Casing and Buccal Swab DNAs

Five microliters of each DNA extract, combined with 4µL of mastermix and 1µL of primer mix, were amplified using an AmpFℓSTR® Identifiler™ Plus amplification kit in an AB 2720 Thermal Cycler with cycling parameters: 95°C for 11 minutes, cycled 28 times at 94°C for 20 seconds, 59°C for 3 minutes, and was held at 60°C for 10 minutes. One microliter of

amplified product was mixed with 9.85µL of HI-DI formamide and 0.15µL of AB GeneScan™ – 500 LIZ™ Size Standard. Amplified products were electrophoresed on an AB 3500 Genetic Analyzer with the following instrument protocol:

Oven Temperature: 60°C

Run Time: 1330 seconds

Run Voltage: 19.5 kVolts

Injection Time: 8 or 12 seconds

Injection Voltage: 1.6 kVolts

Capillary Length: 50 centimeters

The 8 second injections did not generate as many informative genetic profiles as the 12 second injection, so the 8 second data are not represented in this study other then when the two injection times were directly compared. Data were analyzed with GeneMapper® Software Version 4.1 at a threshold height of 50 RFUs for all dyes.

Determination of Profile Categories

Electropherograms were evaluated for completeness relative to the STR profile of the reference buccal swab. The same categories used by Horsman-Hall *et al.* (2009) were applied: no profile was the complete absence of alleles, weak partial profiles contained seven or fewer loci with alleles, strong partial profiles contained eight or more loci with alleles, and a full profile had alleles at all 16 loci. Note that profile designation was not based on whether the alleles were consistent with the handler, just if alleles were present.

Construction of Consensus STR Profiles using Single Swab Allele Calls

Consensus profiles were generated using the five STR profiles from the individual casing swabs based on consensus profile guidelines generated in the Forensic Biology Laboratory at Michigan State University. Each locus in the single swab profiles was examined and 'true' peaks were separated from artifacts. The base (largest) peak at each locus was determined and peak height was recorded; an allele was considered 'Major' if it was less than or equal to 75% of the base peak height; 'Minor 1' alleles were between 33 – 74% of the base peak; and 'Minor 2' alleles were less than or equal to 32% of the base peak. 'Major' alleles were given 1 point; 'Minor 1' alleles were given 0.5 point, and 'Minor 2' alleles were given no points. For an allele to be included in the consensus profile its point value from all five swabs had to be 2 or greater, which was lowered from 3 in the original guidelines for this study.

Statistical Analyses of DNA Yields, Profile Types, and Allelic Consistency

DNA quantities were tested for normal distribution using R software (www.r-project.org) and the Shapiro-Wilk test. A Mann-Whitney U test was performed using R at a significance level of α=0.05 to determine whether the quantity of DNA obtained differed between the cumulative or single swabbing method. Prior to statistical analysis DNA quantities that were three or more standard deviations from the mean were removed; 11 values were withheld. Also removed from swabbing comparisons were DNA quantities of 0.0pg/µL, in attempt to decrease the variation. A Kolmogorov-Smirnov test using R was used to determine if there was a difference between the two swabbing methods in terms of profile type: (full profile, strong partial profile, weak partial profile, no profile).

STR profiles generated from the cartridge casings were compared to reference profiles to assess if they were consistent with the volunteer who loaded the magazine. An inconsistent

allele call was noted in two situations: when none of the handler's alleles were in the locus or when a locus had extraneous alleles (e.g., if the handler's profile was a 6, 9.3 but the casing produced a 6, 8, 9.3). An inconsistent profile on the other hand, was when at least one locus was composed only of erroneous alleles.

A Mann-Whitney U test was performed in R to determine whether there was a significant difference (α=0.05) between the number of consistent and inconsistent allele calls among swabbing methods or the consensus profiling method. The consensus profiling method and the cumulative swabbing method were also compared to determine which generated a greater number of alleles consistent with the handler.

Finally, STR profiles from the 8 and 12 second injection times were examined. The number of alleles that differed between the two, as well as the number of alleles consistent with the reference profiles, were compared. As these data were normally distributed, a t-test was performed (α=0.05) using R, to evaluate whether the consistency of allele calls with the handler was dependent on injection time.

RESULTS

Cartridge Casing Collection

Heat-sealed plastic bags held inside a large diameter circular container were better suited for casing collection than allowing casings to fall in the net of a shooting tank. When using the circular container new plastic bags for each individual reduced the chance of cross-contamination and casings were always caught as they were ejected from the weapon. The net attached to the firing tank could not be changed so the risk of contamination from outside sources was higher and occasionally casings fell out of the netting onto the floor during firing or while transferring casings back into the paper bags.

DNA Yields: Cumulative Versus Single Swabbing

Initial PCR experiments on DNA extracted from cumulative and single casing swabs showed inhibition (no amplification of the Quantifiler™ internal positive control), which was overcome by cleaning DNAs with the Zymo-Spin™ column. The 10 randomly selected casings that were tested for background DNA yielded 0.0pg/μL. Raw DNA yields for cumulative and single swabbings are shown in appendix A. The cumulative swabbing method did not produce significantly higher DNA yields than those generated using the single swabbing method ($p = 0.88$) when all 0.0pg/μL values and outliers were removed from the data set[1]. The mean DNA yields for the cumulative swabs were 38.23 ± 141.52pg/μL as compared to the mean yield of 7.67 ± 9.55pg/μL for the single swabs (Figure 7).

[1] There was no significant difference in DNA yields between cumulative and single swabbing methods prior to values of 0.0pg/μL and outliers being removed.

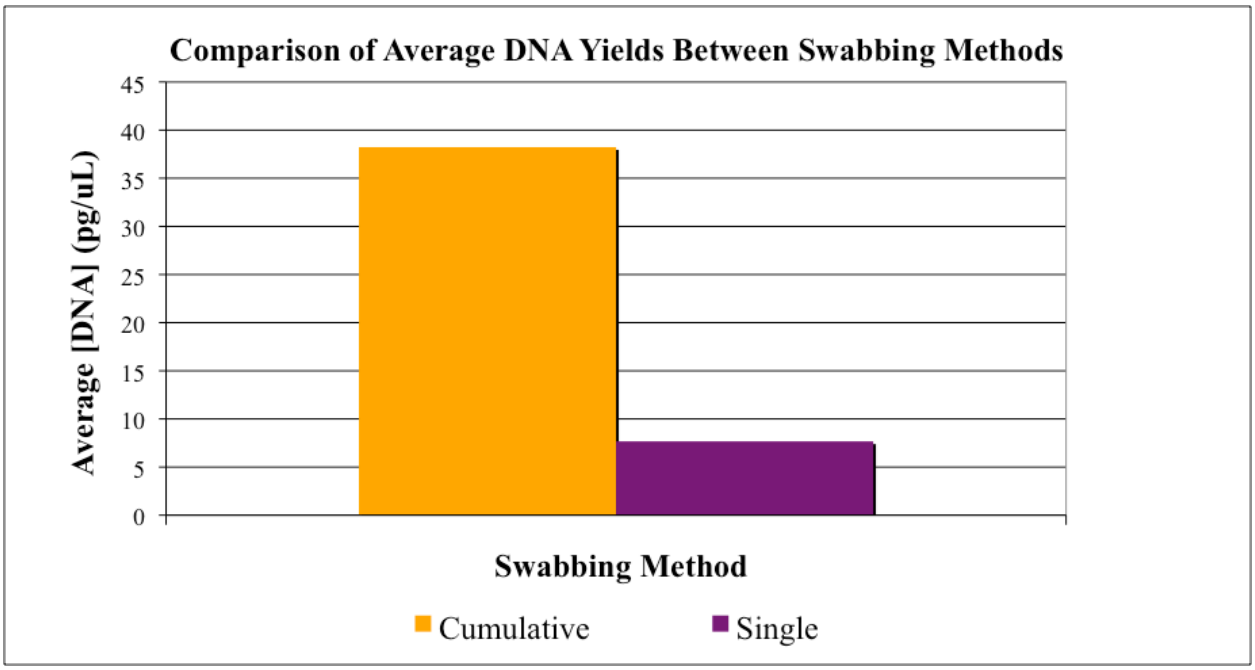

Figure 7. Histogram depicting the mean DNA yields between cumulative (n=20) and single (n=51) swabbing methods. The quantities represented are greater than 0.0pg/µL and less than 1000pg/µL as any quantity outside this range was at least three standard deviations from the mean.

STR Profile Designation

Most of the STR results developed using the cumulative swabbing method were weak partial profiles (67.7%), followed by strong partial profiles (25.8%), and no profiles (6.5%), while no full profiles were generated (Figure 8). Similarly, the majority of results developed using the single swabbing method were weak partial profiles (74.2%), followed by strong partial profiles (15.5%), no profiles (7.1%), and full profiles (3.2%). Results from the consensus profiling method showed a similar pattern, where 64.5% were weak partial profiles, 16.1% were strong partial or no profiles, and 3.2% were full profiles. Three of the full profiles from single swabs were from the same individual, and the lone full consensus profile was produced from that individual as well. The frequency of each type of profile did not depend on swabbing method, based on the test statistic of 0.06 being less than the critical value of 0.24. The same test

demonstrated no significant difference between profile type generated using the consensus profiling method and the single swabbing or cumulative swabbing methods.

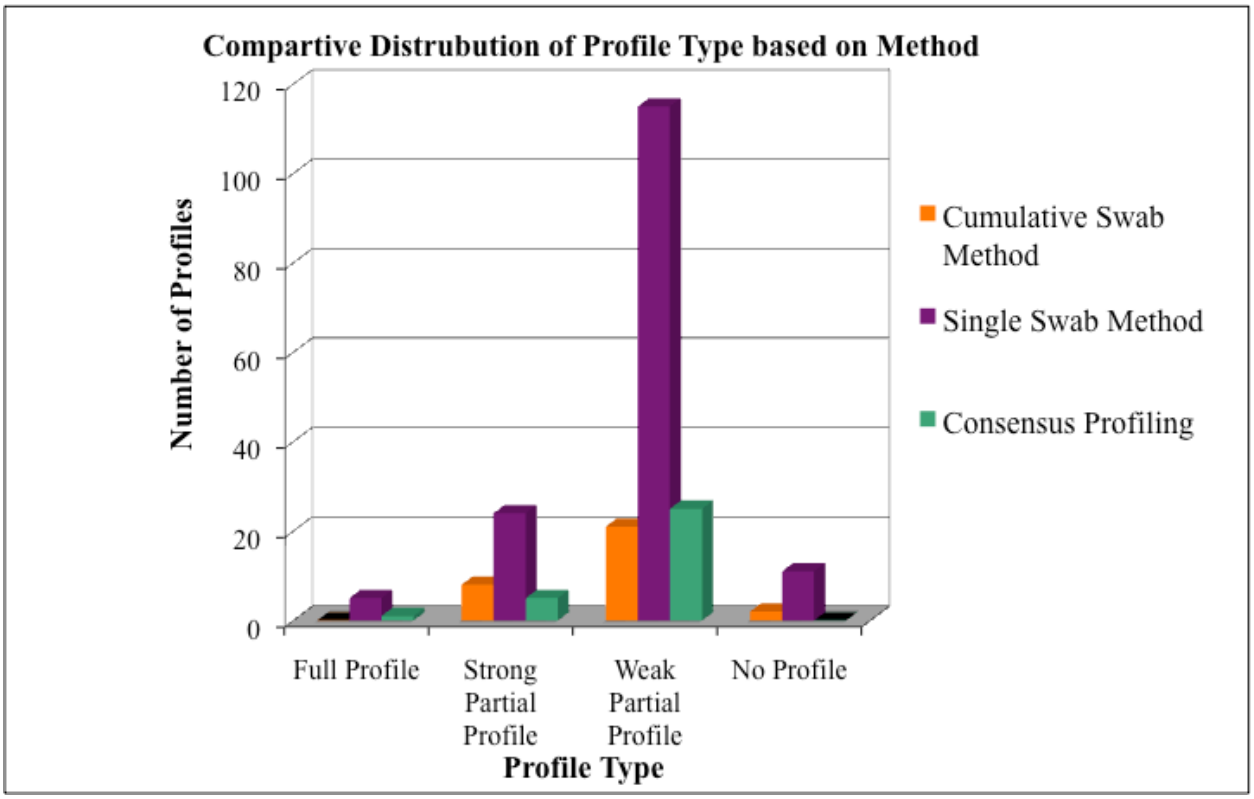

Figure 8. Histogram showing the total number of each type of profile obtained.

When examining both swabbing and the consensus profiling methods, the majority of full, strong partial, and weak partial profiles were inconsistent with the handler (cumulative swabbing 26.7% of the time, consensus profiling 33.3% of the time, and single swabbing 40% of the time). On the other hand, several of the weak partial profiles consistent with the handler contained minimal information, having single or few alleles. The majority of these occurred at amelogenin, where an X call was always consistent with the handler, even if a Y should also have been present (Appendix B). In contrast, only two of the five full profiles were consistent with the handler, two remaining full profiles contained alleles consistent with the handler at 75% of loci, and one had consistent alleles at fewer than 50% of loci. Twenty-eight percent of strong

partial profiles were consistent with the handler, all of which were developed using the cumulative swabbing method. The greatest percentage (68.7%) of consistent profiles were in the weak partial category; 23.8% of which were generated using cumulative swabbing, 15.7% were generated using the single swabbing method, and 29.2% were developed using consensus profiling. There was no significant difference in the frequency of consistent profiles between full and strong partial profiles ($p = 0.35$), nor between full and weak partial profiles ($p = 0.85$). There was a significant difference between strong partial and weak partial profiles, ($p = 0.047$). Two of the high DNA quantities removed as outliers resulted in no STR profiles, the largest outlier generated a strong partial profile, although with a majority of inconsistent alleles, and the remaining eight produced weak partial profiles, one of which was consistent with the handler (Appendix A).

Consistent and Inconsistent Allele Calls Based on Swabbing Method

The majority (56 – 61%) of profiles across all three swabbing/profiling methods had no allele calls (Figure 9). Those consistent with the handler ranged from 22 – 31%, while inconsistent calls ranged from 13 – 17%. The cumulative swabbing method produced significantly more consistent alleles ($p = 0.02$), and significantly fewer inconsistent calls ($p = 0.05$) than single swabs. In contrast, there were no significant differences in consistent or inconsistent calls between cumulative swabbing and consensus profiles ($p = 0.257$ and 0.48 respectively), or between single swabs and consensus profiles ($p = 0.269$ and 0.34 respectively).

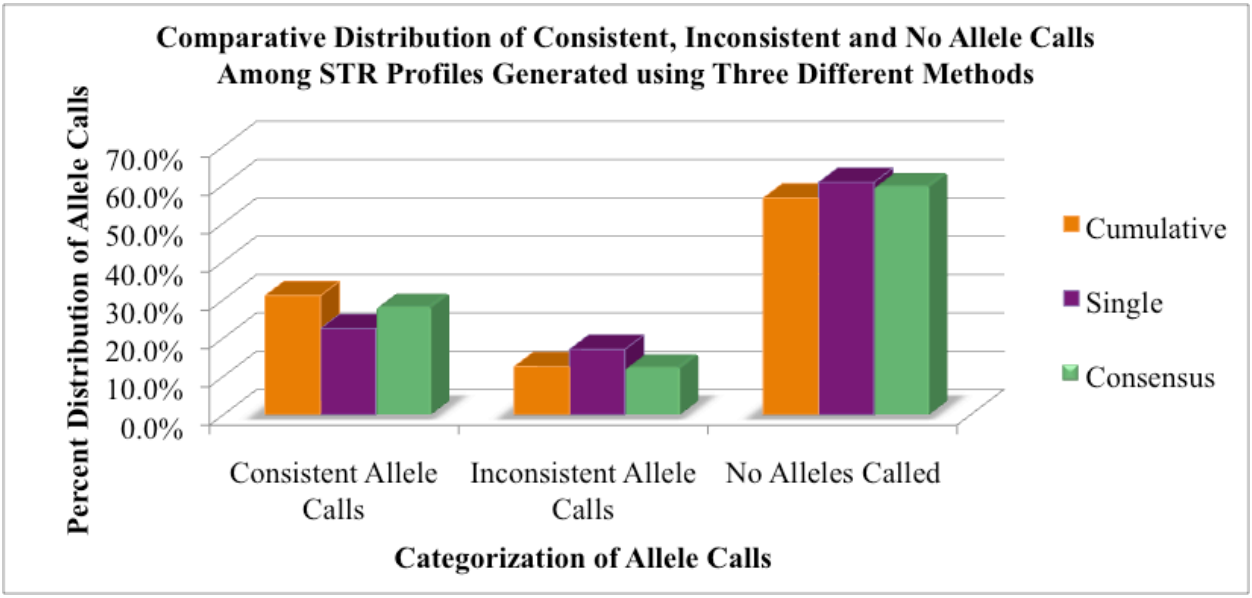

Figure 9. Histogram portraying the percentage of consistent (22 – 31%), inconsistent (13 – 17%), and no allele (56 – 61%) calls for the cumulative swabbing method (n=31), the single swabbing method (n=155), and the consensus profiling method (n=31).

Of all the profiles inconsistent with the handler 46 (21.2%) were different by only one allele 32 (14.7%) were different by two alleles, and 23 (10.6%) were different by three alleles and 165 (78.2%) were different by four or more alleles. Among the single allele inconsistencies, 7 (15.2%) were generated using the cumulative swabbing method, 27 (58.7%) were produced using the single swabbing method, and 12 (26.1%) from the consensus profiling method. Cumulative swabbing produced 7 (21.9%) profiles inconsistent by two alleles, single swabbing generated 24 (75%) and consensus profiling produced 1 (3.1%). One (4.3%), 16 (69.6%), and 6 (26.1%) profiles with three allelic inconsistencies were developed using cumulative, single and consensus profiling, respectively.

Differences in Allele Calls Generated from 8 and 12 Second Injections

The 12 second injections resulted in more allele calls than 8 second injections, wherein 41 of 180 single/cumulative swabs had no results for 8 second injections, yet had allele calls after the longer injection. Allele calls changed between the two injection times in 39 of the 180 samples (Table 1), 10 of which were derived from the cumulative swabbing method, and 29 from the single swabbing method. The 12 second injection aided in 'picking-up' a handler's allele in 19 instances, however in 13 instances a consistent allele was lost. Likewise, one or more new inconsistent alleles appeared after a 12 second injection in 24 loci, most of which were in FGA. Overall there was no significant difference in consistent allele calls between the two injection times ($p = 0.117$).

Table 1. Allelic differences observed between 8 and 12 second injection times. Shown are the samples and loci where allelic differences existed between the two injection times and what alleles were called after each injection time. The numbers highlighted in yellow indicate alleles that were consistent with the handlers' profile.

Sample	Locus	8 second inj.	12 second inj.
2C	FGA	29, 46.2	23
5F	FGA	25.2, 22	17
9A	FGA	23, 25.2	22
9C	FGA	19, 32	17, 22
11F	FGA	20, 47.2	20, 22
8C	FGA	17, 18	21, 25
17A	FGA	22, 28.2	22, 23
17E	FGA	46.2	23
17F	FGA	46.2	18, 28.2
12A	FGA	43.2	17, 18
20D	FGA	16	23, 23.2
16D	FGA	32	22, 28.2
22A	FGA	24	17, 44.2
18E	FGA	48.2	16, 16.2
23A	FGA	25, 45.2	17, 17.2
21B	FGA	27, 49.2	23.2
29D	FGA	30.2	24, 25
29E	FGA	24.2, 30	25

Table 1 (cont'd)

26F	FGA	18, 34.2	24
11A	D5	11, 13	11, 15
8D	D5	13	11, 12
8E	D5	7	11, 12
3D	D5	12	11, 13
6B	D5	12, 13	9, 12
11C	D21	31	28, 32
9C	D21	29, 30	27
8C	D21	27, 30	29
17A	D3	14, 16	18
19F	D3	16, 17	15
26A	D3	14, 16	17
7C	D19	13, 14.2	14.2, 15
8D	D19	10.2, 11	12, 13
17A	D8	14, 15	10
1D	D8	14	13, 17
8F	D7	10	8, 9, 12
7C	D7	8, 10	8, 11
8C	D16	9, 12	11
8F	TPOX	8, 11	8, 12
12A	vWA	14, 17	16
None	D18	0	0
	THO1	0	0
	D13	0	0
	D2	0	0
	CSF	0	0

DISCUSSION

Cartridge casings are a common form of evidence found at crime scenes in the United States, but standard methods for identifying the loader or shooter of the firearm are rarely successful. Historically, crime laboratory attempts to enhance latent prints from cartridge casings have met with little or no success, creating a need for different methods of analysis. Researchers have found that, under very controlled conditions, obtaining DNA profiles from fired cartridge casings is possible (Horsman-Hall *et al*. 2009; Spear *et al*. 2011). The present study aimed to utilize and load cartridges directly from the store shelf, as a shooter would do, and analyze the DNA remaining on the casing after it was fired. The recovery method currently used by the DNA unit of the MSP, cumulatively swabbing casings (Heather Clark, personal communication), was compared to an alternative recovery method, single swabbing and development of a consensus profile, in an attempt to determine which technique was more efficient for retrieving DNA from fired cartridge casings as well as which maximizes the genetic information obtained.

When cartridge casings are collected at a crime scene and subjected to DNA analysis, the goal is to gain as much genotypic information as possible. The fact that the type of profile (full, partial, etc.) obtained in this study did not correlate with the swabbing method used indicates that the recovery method is not as important as other factors in gathering a substantial amount of genetic information. In this study, the mean DNA yield from cumulative swabbing was much greater than single swabbing (Figure 7); however the standard deviations for the two methods were so large that the mean yield based on swabbing method was not statistically different. Alternative variables in obtaining genetic data range from the propensity of a person to leave cells on a cartridge to PCR inhibitors, all of which would affect the quantity of DNA recovered

and/or the quality of resultant STR profiles. Given that greater DNA yields would be expected to result in a stronger, more complete STR profile, it was surprising that there was no association between DNA yield and profile quality. In fact, the few swabs that produced improbably high DNA quantities generated only weak partial or no profiles, with the exception of a single strong partial profile (which was not consistent with the handler) thus those quantitative values may have been unreliable. In contrast, both strong and weak partial profiles were generated from some DNAs that quantified at zero, and it was not uncommon to have no STR profile generated from DNAs that quantified at greater than zero. Full profiles, however, were only derived from DNAs that quantified above zero, yet all were well below the threshold of what is considered LCN DNA. This indicates that the amount of DNA from casings estimated using Quantifiler™ did not translate well to the number or quality of alleles generated. The amplicon size of the Quantifiler™ target locus is smaller (62 bases) than those amplified using Identifiler™ Plus (100+ bp); therefore, Quantifiler™ should overestimate DNA quantity due to shorter DNA templates being more abundant in degraded DNA. This was apparently not the case in this study. A possible explanation is that DNA does not degrade uniformly and that the Quantifiler™ locus is more susceptible to degradation than the STR loci; a factor that has been noted previously for certain loci in degraded DNA (Foran, 2006). In this regard, it may be worthwhile quantifying DNAs using larger sized amplicons that better mimic STR loci, and in particular, target one or more loci that actually exist in Identifiler™, as these could better estimate the concentration of the loci to be assayed.

The number of casing profiles consistent with a handler correlated with the profile type. In this study, weak partial profiles, which were obtained most frequently, resulted in the largest number and percentage of handler profiles. In general this was because a profile that contained a

small number or even a single handler allele was categorized as completely consistent, even though the genetic information was minimal. Likewise, a profile that had a large number of handler alleles was classified as inconsistent owing to a few or even a single allele that did not come from the handler.

An important finding from this research was that the majority of profiles inconsistent with the handlers, no matter the recovery method, were only dissimilar by one, two, or three alleles. This is noteworthy because these profiles could still be probative given that the majority of information within them was consistent with the handler, and thus could be used for comparison to known profiles. Further, all results completely inconsistent with the handler were from weak partial profiles, which often resulted from as little as a single allele. This indicates that a greater variety of profile categories need to be developed, as the four used here are too vague and encompass too wide a variety of results, making them rather uninformative. Attempting to fit profile classification into only two categories (consistent or inconsistent with a handler) likely created a misleading dichotomy, wherein relatively good profiles were categorized as poor, while those with virtually no information were classified as good. However, since continuums of alleles were consistent/inconsistent with the handlers' profiles, attempting to place each result into a precise category would be unwieldy (or perhaps impossible), thus was not attempted in this research. An alternative approach to analyzing this type of data may be developing a random match probability (RMP) for each profile. If every profile were evaluated in this manner then a greater number of handler alleles would result in a stronger profile. On the other hand, erroneous alleles and mixtures would make the RMP weaker; overall, the RMP is more informative in terms of each profile than lumping profiles into different 'blanketing' categories.

It is interesting to examine profile consistency at the allelic level, as the number of alleles from a handler is critical in real world applications. In this study allele consistency was significantly greater when casings were swabbed cumulatively than when swabbed singly, but this method only improved allele consistency slightly over consensus profiling. Since cumulative swabbing generated a greater percentage of handler alleles it is logical that it also resulted in the smallest percentage of inconsistent alleles. This indicates that swabbing five casings consecutively results in a higher percentage of the handlers' alleles being recovered. It is also possible that the increased recovery of handlers' alleles through cumulative swabbing swamps out the small amount of background DNA during amplification, which may not be the case with single swabbing since drop-in occurred with greater prevalence in the profiles developed using that method.

The purpose of consensus profiling was to filter out inconsistent alleles and the results illustrate that this technique generated a higher percentage of handler alleles, and a lower percentage of inconsistent alleles than the single swabbing method. Therefore, consensus profiling was effective at removing some, but not all, of the inconsistent alleles recovered through single swabbing. This method, however, was outperformed by cumulative swabbing for two possible reasons. First, because consensus profiling scored allele peaks based on intensity, 'Major' peaks occurring two or more times were automatically placed into the profile. This means that drop-in occurring twice as a 'Major' peak, was interpreted as part of the handlers' profiles resulting in a greater number of inconsistent profiles. Second, the score required for alleles to be included in the consensus may have been too low. The score was dropped to 2 for this study because alleles belonging to the handler often showed up only twice among the five single swab profiles. The consensus rules did lessen the subjectivity inherent in STR analysis,

though empirical rules developed to increase profile consistency would ideally be applicable to any type of STR profile, while still being modifiable depending on the amount and type of evidence obtained. The consensus profiling methodology applied in this study was developed using an entirely different sample type: post-deflagration IED components. The consensus criteria were, therefore, modified for these data based on the amount of genetic information obtained from the cartridge casings. Since profiles generated in this study often had few alleles, the number of alleles required for placement into the consensus profile was based on the most common number of alleles, two, present at each locus across five single swabs. Given that the consensus profile rules are influenced by sample type and size it would be worthwhile reinvestigating the consensus methodology using fired cartridge casings, to see whether the consensus method can be improved upon.

STR profiles containing fewer non-handler alleles would be forensically advantageous since drop-in or mixtures decrease a RMP. This could potentially be accomplished by re-amplifying extracts and performing multiple injections of the same sample, which should result in an increase in handler alleles and a decrease of inconsistent alleles (Taberlet *et al.* 1996; Whitaker *et al*. 2001). The more times an extract is injected onto the capillary and the results of those individual injections are combined, the better the chance of obtaining true alleles from the handler.

Regardless of the method used to generate STR profiles, it is important to understand the origin of extraneous alleles. Contamination is always a concern when working with LCN DNA because it has the potential to be amplified when LCN DNA is not (Gill and Kirkham, 2004). DNA other than the handler's may have been introduced post-firing, while they were being collected and put back into paper bags, but could also have been introduced during DNA

processing, though alleles were not consistent with the researchers involved. Since cartridges were not washed prior to firing the potential for contamination from pre-firing sources was higher than in previous studies that pre-cleaned cartridges before loading (Horsman-Hall *et al.* 2009), however if this were the case, contaminating DNA would likely have been detected on the cartridges that were swabbed for background DNA. Given that no alleles were generated from such swabs, contamination from outside sources was not likely the cause of extra (inconsistent) alleles. Although contamination may have occurred when casings fell on the floor, inconsistent alleles could not be traced back to any specific individual. This raises questions about real-world crime scenes where casings may be collected from a variety of locations, which could easily increase contamination, given the environmental conditions in which casings existed, and result in mixture profiles and erroneous allele calls during downstream analysis. One question that remains upon completion of this research is how various environmental conditions, such as precipitation, temperature, ground cover, etc., affect the quantity and quality of DNA recovered from fired cartridge casings. A study exploring the effects of environmental factors and collection methods on cartridge casing evidence would be useful for the advancement of forensic DNA analysis.

The fact that the same extraneous alleles were not present among profiles and that variable loci were affected indicates that they resulted from random drop-in rather than contamination. The effects of drop-in are often quite dramatic in extracts containing very small amounts of DNA, wherein sporadic peaks can be present at the same intensity as alleles belonging to the handler, giving the impression of a mixture (Taberlet *et al*. 1996; Gill *et al*. 2000). In this study, sporadic peaks frequently occurred at the FGA and D5 loci, which combined resulted in over 60% of inconsistent alleles. These loci are in the red channel in

Identifiler™, which also regularly shows the most noise among dyes, thus noise may be called as allele peaks when the RFU threshold is set very low, as was the case here (50 RFU). A possible remedy would be to increase the threshold in the red channel to greater than 50 RFU, which would decrease the amount of noise being called, but would also result in the loss of handler alleles. If the RFUs were increased to 100, more than half of the allelic data from the red channel in this study would have been lost.

Allelic drop-out was also common in this study, and undoubtedly caused interpretation problems. Using amelogenin as an example, there were several instances in weak partial profiles where only one allele existed, which was frequently an uninformative X, even though the individual was male. The reverse situation also occurred, where the Y locus amplified and X dropped out. Another common occurrence was X and Y peaks existing in a profile indicating that the handler was male, when in actuality the handler was female, thus the Y peak dropped in. For those reasons, peaks at amelogenin were not particularly informative, and of course, the same was often true of other loci.

Drop-in and drop-out were best demonstrated during the 8 and 12 second injection comparisons. In numerous instances alleles, both consistent and inconsistent with the handler, were called after each of the injections, though in most cases, alleles present from 8 second injections were not the same as those from 12 second injections. Three different scenarios existed; the 8 second injection had a handler allele that dropped out with the 12 second injection and an inconsistent allele dropped in, the 8 second injection had inconsistent alleles that dropped out and a handler allele was picked-up with the 12 second injection, or inconsistent alleles present after the 8 second injection dropped out after the 12 second injection and different inconsistent alleles dropped in. These circumstances are cause for concern considering different

results were obtained when analyzing the same sample but modifying only capillary injection time. If handler's alleles had been called consistently after going from the 8 second to the 12 second injection then it would seem that an increased injection time is a solution to obtaining handler allele's more often. This was not the case however, which indicates a need for optimization and a means of separating handler alleles from erroneous drop-in.

Perhaps the best approach for recognizing and eliminating drop-in in LCN samples was suggested by Taberlet *et al.* (1996), who recommended multiple amplifications of the same DNA extract. This was based on the probability of a given allele dropping in being approximately 5%, which meant that the probability that the same erroneous allele occurring in two separate amplifications was less than 1%. They and others have advised that replicates be evaluated to develop a "composite profile" where alleles are considered 'true' if they are present in each of the replicates (Taberlet *et al*. 1996; Whitaker *et al.* 2001). Development of a composite profile from several amplifications of a single extract should result in a decreased number of erroneous alleles, similar to the consensus profiling method used in this research. However, the consensus method was somewhat different because it looked at repeatability among DNA extracts rather than within a single extract. Using the consensus method, drop-in from cartridge casings handled by a single individual were often effectively filtered out. Drop-out, on the other hand, could potentially be addressed by strengthening the signal for that allele, including increasing DNA injection time or voltage during electrophoresis (Budowle *et al*. 2001; Horsman-Hall *et al*. 2009). The increased injection times tested in this study generally resulted in more handler alleles, but as noted, some drop-out and new inconsistent alleles also occurred.

Overall the methods tested in this study did slightly improve on results from earlier research of forensic DNA analysis from cartridge casings. Given that the majority of handler

profiles were partial, the forensic utility of swabbing cartridge casings becomes a concern, but in fact partial profiles can be very informative. As an example, they can be used for calculating RMPs as discussed previously, making each profile informative to a certain extent. Even if the probability of the evidence coming from the suspect or someone other than the suspect is 1 in 100 or 1 in 10,000 (as opposed to 1 in 100s of trillions found in a full profile), any such number can have value in court (Budowle *et al*. 1998). The more the guilt of the suspect can be supported by STR data, the more worth it has in the minds of the jurors. If quantitative information can be presented even without a full profile then it makes even limited STR data useful, as compared to fingerprinting or firearms examination where analysis cannot be upheld with numbers or statistics.

Partial profiles can also be used to search databases. The Combined DNA Index System (CODIS) is a US DNA database available to crime laboratories. It has two primary indices: convicted offenders and forensic casework. For profiles to be uploaded into the forensic casework index they must have at least 10 CODIS loci with genetic information, while the full 13 are required for convicted offenders (Budowle *et al*. 1998; FBI, 2011). Profiles then, remain in the database acting as a foundation to which others are compared.

Searching CODIS requires far fewer alleles than uploading a profile; in fact data from a single locus can theoretically be interrogated. This means that any of the results obtained in this study could have some utility. When a search is performed in CODIS using partial profiles, a partial match between two single source profiles may result, where the known profile contains all of the alleles of the questioned one, while the questioned profile contains a subset of the known alleles (FBI, 2011). Although partial profiles are not ideal, they may provide investigative leads or be useful for exclusions. In this regard, the single swabbing method could prove preferable to

the cumulative swabbing method in that the latter provides only one 'chance' for detecting alleles belonging to the handler of cartridges, whereas the single swabbing method provides several opportunities for handler alleles to show up multiple times among the swabs. This could be particularly important because excluding an individual requires only a single inconsistent allele between evidence and suspect profiles. If, for example a set of cartridge casings were submitted as evidence and an analyst was using the cumulative swabbing method, and the result was a profile containing alleles inconsistent with the suspect, there is no 'second chance' to obtain alleles consistent with the handler. It is possible; however, that a single swab of a casing would have picked up the true alleles and the suspect and known profiles would match at all loci. Thus, with regard to databases such as CODIS, partial STR profiles, though not ideal, can be helpful in generating leads in criminal cases and in exclusion of suspects.

In their study of DNA analysis from fired cartridge casings, Horsman-Hall *et al.* (2009) compared profile types between fired and unfired casings handled by five individuals. One of the unfired cartridges produced a full profile and the remaining 4 produced strong partial profiles. The fired casings, on the other hand, resulted in no full profiles (the authors did not address partial profiles in this section of the study, strong or otherwise). The authors also assessed DNA recovery from 5 types of fired ammunition using Minifiler™ and demonstrated a distribution of profile types, similar to the profile distribution of this research: after injecting DNA from 75 samples for 12 seconds onto an AB 3130, greater than 50% of casings resulted in no profiles, followed by weak partial profiles (26%), strong partial profiles (19%) and full profiles (1%). Identifiler™ produced no alleles for any of the 5 ammunition types. It is not surprising that Minifiler™ produced better results because it has an enhanced buffer to help overcome PCR inhibition, which also exists in Identifiler™ Plus that was used in the current

study. It is surprising, however, that in the majority of cases no profiles were obtained. In this study, volunteers handled cartridges for a much shorter time, yet weak partial profiles were often obtained. Unfortunately, Horsman-Hall *et al.* (2009) did not state whether their results were consistent or inconsistent with the handlers, so profile type results are the only possible comparison to the research presented here.

Horsman-Hall *et al.* (2009) also examined the recovery of DNA from firearm surfaces. This portion of their study does not relate directly to the experiments presented here, but it does raise an interesting point. They tested three surfaces, the ejection port, breechface, and barrel, which were swabbed in between sets of 3 shooters, for a total of nine swabs. When the shooter profiles were compared to the profiles obtained from the firearm surfaces, allelic drop-in consistent with a previous shooter was observed. This indicates that cartridges may pick up contaminating DNA from the gun surface that was left by a previous shooter upon loading or casing ejection. This could affect the current study because shooters shared guns and magazines without cleaning in between. Transfer of shooter DNA to other casings would not be surprising, especially when using the firing tank, where every cartridge casing was caught in the same net.

The results of this study raise several new questions that could be investigated. Optimizing protocols and instrumentation would be highly beneficial in increasing the numbers of handlers' alleles recovered and obtaining profiles with less noise and drop-in. To achieve such goals, cycle number during STR amplification should be adjusted to yield the greatest amount of DNA product possible; although this is difficult since stochastic effects are inherent with low copy number samples. Similarly, due to stochastic sampling and drop-in, multiple injections of the same DNA extract should be explored and applied in a manner similar to consensus profile analysis in an effort to eliminate noise and drop-in. With the results of this

study being relatively variable with regard to the 8 and 12 second injection times, capillary injection times for DNA extracts should be optimized as well. The longer injection time produced a greater number of handler alleles, but it is unclear if a longer injection is always better, or only if a sample is LCN. If capillary injection time affects overall allele recovery then it should be investigated in more detail.

Further research could also be performed on the DNA quantitation and identification methodologies themselves. One important undertaking, especially for LCN DNA work, is the development of a quantitative assay that uses STR loci rather than the current Quantifiler™ locus, as DNA quantitation would be more representative of the loci being assayed. Alternatively, testing the effectiveness of alternative identification methods, such as mitochondrial DNA analysis, could prove valuable in collecting data from fired cartridge casings.

Maximizing cell collection is imperative in optimizing DNA yields and STR profiling, and various collection techniques should be investigated. For instance, a larger study comparing DNA recovery through cumulative or single swabbing would be helpful, particularly since the tendency of individuals to shed skin cells is highly variable (Lowe *et al*. 2002). Likewise, a soaking method, where fired cartridge casings are placed in a soaking solution to release all DNA from the surface, could increase DNA yields. However; PCR inhibition caused by gunshot residue from the inside of cartridge or the metal casing reacting with the reagents in the soaking solution might result. All of these could be explored.

As mentioned, the categorization system used to analyze the profiles in this study may have been too broad to be highly informative. Therefore, it would be helpful to explore a variety of ways to pull pertinent information out of the data obtained from the STR profiles. Using

random match probability calculations to determine the frequency of a DNA profile would be more informative than determining whether the profile fell into categories, such as weak or strong or full. Another means of indicating how useful a profile is would be to calculate the percent of the profile that was recovered, be it 100%, 95%, 50%, etc. There are likely several other ways to establish how genetically or forensically informative an STR profile is, but these two would work well when applied to the results obtained here.

Lastly, since this was a controlled experiment that occurred indoors away from the elements, the results may not be applicable to cartridge casings found at an outdoor crime scene. Therefore, the next step would be to investigate the effects of different environmental conditions, such as precipitation, ground condition (e.g., gravel, sand, asphalt), temperature, etc., on the quantity and quality of DNA recovered from cartridge casings under those circumstances.

Overall, profiles containing handler alleles, even if few in number, are useful and can aid in criminal investigations. Crime laboratories must recognize that successfully recovering and analyzing LCN DNA can be difficult but highly valuable. As criminals find ways to circumvent existing forensic technologies and analytical methods, it is important for researchers to continue pushing the limits. The development of new, more sensitive, and effective methods for analyzing LCN DNA are imperative in achieving greater success in producing genetic profiles from fired cartridge casings. Data from this study show that using Identifiler™ Plus, in combination with the swabbing and consensus profiling methods, generated handler alleles more often than extraneous alleles. The fact that cumulative, single swabbing, and consensus profiling all produced handler alleles demonstrates their utility and applicability to forensic casework.

CONCLUSION

DNA recovered from fired cartridge casings ranged from zero to LCN, which indicated that, although there is an ideal quantity of DNA for optimum STR results, lower quantities can still result in genetically informative profiles. Cumulatively swabbing cartridge casings resulted in greater DNA yields on average, although this did not correlate with the number of handler alleles recovered. No relationship existed between swabbing method and the resulting profile type: full, strong partial, weak partial, or none. However, cumulative swabbing generated a significantly higher percentage of handler's alleles than did individual swabbing.

Given that the recovered DNAs were LCN in nature and were subject to PCR inhibition, contamination, drop-in, and drop-out, steps should be taken to address sources of inconsistent alleles. Utilizing a DNA purification kit that removes PCR inhibitors and an STR amplification kit designed for LCN DNA would likely augment results. Multiple amplifications of extracts in combination with consensus profiling should be used to address the remaining sources of allelic inconsistency. Instituting these measures will assure that the greatest numbers of handler alleles are obtained. Though cross-contamination is a concern, based on the results of this study, the cumulative swabbing method appears to be best for recovering and analyzing DNA from fired cartridge casings.

APPENDICES

APPENDIX A. DNA QUANTITIES FOR CUMULATIVE AND SINGLE SWABS

Extracts identified with an "A" indicate quantities recovered using the cumulative swabbing method. Extracts identified with "B – F" indicate quantities recovered using the single swabbing method. All DNA yields are represented in pg/μL. Quantities highlighted in yellow indicate values that were removed as outliers, as they were three or more standard deviations from the mean DNA yield.

Table A.1. DNA quantities recovered from five fired cartridge casings using the cumulative swabbing method.

Sample ID	A
1	1.23
2	9.08
3	7.37
5	3.74
6	2.10
7	4.46
8	2.19
9	0.00
10	2.08
11	3.28
12	0.00
13	9.25
15	2.94
16	0.00
17	13.50
18	0.00
19	5.18
20	40.58
21	2.03
22	1.75
23	638.33
24	0.00
25	0.00
26	58916.67
27	2.99
28	2.00
29	0.00
30	0.00
31	32750.00
32	10.58
33	0.00

Table A.2. DNA quantities recovered from five fired cartridge casings, B – F, using the single swabbing method

Sample ID	B	C	D	E	F
1	0.00	0.00	0.00	0.00	0.00
2	0.00	2.95	1.17	0.00	586666667
3	2.88	0.00	12.08	0.00	37167
5	0.00	0.00	0.00	5.91	5.03
6	0.00	19.75	0.00	11.67	1.92
7	0.00	27.75	0.00	0.00	10.75
8	3.70	22.75	0.00	0.00	0.00
9	1.94	10.58	0.00	0.00	0.00
10	0.00	0.00	0.00	640833	3.91
11	7.66	0.00	3.20	0.00	1.46
12	0.00	0.00	0.00	90000000	0.00
13	3.20	0.00	0.00	0.00	0.00
15	0.00	0.00	5.91	0.00	0.00
16	2.28	4.56	0.00	1.40	1.13
17	0.00	32.42	0.00	0.00	0.00
18	0.00	0.00	0.00	12166667	0.00
19	2.68	2.36	3.46	1.76	0.00
20	7.67	50.75	27.92	15.25	3.79
21	0.00	0.00	0.00	0.00	0.00
22	5.61	1.53	0.00	0.00	6.82
23	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	5.91	0.00	0.00
25	0.00	0.00	10750000	4.07	3.64
26	0.00	0.00	0.00	0.00	0.00
27	0.00	2.43	6.08	0.00	0.00
28	0.00	5441667	0.00	0.00	0.00
29	0.00	0.00	1.32	0.00	662500000
30	0.00	5.35	3.90	0.00	0.00
31	10.00	0.00	0.00	0.00	182500000
32	0.00	2.69	5.07	1.48	0.00
33	8.03	0.00	0.00	0.00	0.00

APPENDIX B: ALLELE CALLS AT EACH LOCUS FOR ALL SETS OF FIRED CARTRIDGE CASINGS

Allele calls are shown at each locus for all sets of fired cartridge casings. The letter A indicates profiles developed using the cumulative swabbing method, while B – F denote those profiles generated using the single swabbing method. Profiles B – F were combined to develop the consensus profile for each set of 5 casings that were singly swabbed, these are shown in the last column of each table. The bolded numbers denote allele calls that were consistent with the handlers' profiles. (-) indicates that no alleles from the five single swabs were placed in the consensus profile. It should be noted that casing set #12 has allele calls but no numbers are bolded as there was no STR amplification from its corresponding buccal swab.

Table B.1. Alleles obtained at each locus for casing set #1.

Locus	1A Allele Call	1B Allele Call	1C Allele Call	1D Allele Call	1E Allele Call	1F Allele Call	Consensus
AMEL.	NONE	**X**	**Y**	**X,Y**	**X**	NONE	**X,Y**
D5	**12**	NONE	NONE	NONE	NONE	NONE	-
FGA	NONE	NONE	**19**	NONE	**23**	NONE	-
D19	**14**	NONE	NONE	16	**14**	NONE	-
vWA	NONE	NONE	NONE	17	NONE	NONE	-
TPOX	NONE	NONE	NONE	**8**	NONE	11	-
D18	NONE	**14**	**14**	NONE	NONE	NONE	**14**
D3	NONE	**17**	**17**	NONE	NONE	NONE	**17**
THO1	NONE	**6**	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	**12**	11	NONE	NONE	-
D16	NONE	NONE	NONE	13	**11**	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	13	**14**	NONE	13,17	NONE	NONE	-
D21	NONE	27	NONE	27	**29**	NONE	27
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	**11**	NONE	NONE	-

Table B.2. Alleles obtained at each locus for casing set #2.

Locus	2A Allele Call	2B Allele Call	2C Allele Call	2D Allele Call	2E Allele Call	2F Allele Call	Consensus
AMEL.	**X**	**X**	**X**	NONE	NONE	NONE	**X**
D5	**11**	NONE	NONE	**11**	**11**	NONE	**11**
FGA	NONE	NONE	**23**	NONE	NONE	45.2	-
D19	**15**	13, **14, 15**	**14**	NONE	NONE	NONE	**14**
vWA	**14**, 17, **18**	**14**	NONE	**14**	NONE	NONE	**14**
TPOX	**11**	**11**	NONE	NONE	NONE	NONE	-
D18	18	NONE	NONE	NONE	NONE	NONE	-
D3	17	**14, 15**	NONE	NONE	NONE	NONE	-
THO1	**9.3**	**6, 9.3**	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	**14**	**11**	NONE	NONE	-
D16	**12**	NONE	NONE	**11**	NONE	NONE	-
D2	NONE	NONE	**19**	NONE	NONE	NONE	-
D8	**15**	**15**	NONE	**15**	NONE	NONE	**15**
D21	NONE	NONE	NONE	**28**	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	**12**	NONE	NONE	NONE	**10**	NONE	-

Table B.3. Alleles obtained at each locus for casing set #3.

Locus	3A Allele Call	3B Allele Call	3C Allele Call	3D Allele Call	3E Allele Call	3F Allele Call	Consensus
AMEL.	**X,Y**	NONE	NONE	**X,Y**	NONE	**Y**	**X,Y**
D5	**11**	NONE	NONE	**11**,13	NONE	15	-
FGA	29.2	29,29.2	29.2	**21**,24,29.2	29.2	29	29,29.2
D19	NONE	12	NONE	13,**14**	**14**	**14**	**14**
vWA	**16**,17	18	NONE	18	NONE	NONE	18
TPOX	8	NONE	NONE	8	**9**	NONE	-
D18	14	NONE	NONE	12,13	NONE	NONE	-
D3	14,**16**	**16**	NONE	14,17	**16**	NONE	-
THO1	9	NONE	NONE	**6,8**	NONE	NONE	-
D13	NONE	NONE	NONE	9	NONE	9	9
D16	NONE	NONE	NONE	**12**,13	NONE	NONE	-
D2	25	NONE	NONE	19	NONE	19	19
D8	NONE	**13**	NONE	**13**	NONE	NONE	**13**
D21	NONE	NONE	NONE	30.2,32.2	NONE	NONE	-
D7	NONE	NONE	NONE	10	NONE	NONE	-
CSF	**12**	NONE	NONE	11	NONE	NONE	-

Table B.4. Alleles obtained at each locus for casing set #5.

Locus	5A Allele Call	5B Allele Call	5C Allele Call	5D Allele Call	5E Allele Call	5F Allele Call	Consensus
AMEL.	NONE	NONE	NONE	NONE	NONE	**X**	-
D5	**11**	NONE	NONE	10	15	15	15
FGA	**21**	NONE	NONE	NONE	NONE	17	-
D19	NONE	13	NONE	NONE	**14**	**14**	**14**
vWA	NONE	NONE	NONE	NONE	NONE	**17**	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	13, 17	-
D3	**17**	NONE	NONE	14	15, **16**	NONE	-
THO1	**8**	NONE	NONE	9	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	11	11	11
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	**13**	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	27	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	**12**	10	NONE	NONE	NONE	NONE	-

Table B.5. Alleles obtained at each locus for casing set #6.

Locus	6A Allele Call	6B Allele Call	6C Allele Call	6D Allele Call	6E Allele Call	6F Allele Call	Consensus
AMEL.	**X,Y**	**X**	**X,Y**	**Y**	**Y**	**X,Y**	**X,Y**
D5	NONE	**9,12**	NONE	NONE	**12**	**9**,11	**9,12**
FGA	NONE	NONE	30	NONE	NONE	18	-
D19	**14**	**16**	**14**,15.2	**14**	**14,16**	11,15,**16**	**14,16**
vWA	**17**	NONE	NONE	NONE	NONE	**17**	-
TPOX	NONE	NONE	NONE	NONE	**11**	NONE	-
D18	NONE	NONE	**15**	NONE	NONE	**21**	-
D3	**15**,16	**15,18**	14,**18**	16,**18**	**15**	NONE	**15,18**
THO1	**9.3**	**9.3**	NONE	NONE	6	7,**9.3**	**9.3**
D13	**11**	NONE	NONE	NONE	12	**11**,12	12
D16	NONE	NONE	NONE	NONE	NONE	12,**13**	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	**14**	NONE	NONE	NONE	**14**	**10,14**	**14**
D21	NONE	NONE	NONE	NONE	NONE	31.2	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.6. Alleles obtained at each locus for casing set #7.

Locus	7A Allele Call	7B Allele Call	7C Allele Call	7D Allele Call	7E Allele Call	7F Allele Call	Consensus
AMEL.	**X,Y**	**X**	**X,Y**	NONE	**X**	**X**	**X,Y**
D5	NONE	NONE	10,**11**	NONE	NONE	NONE	-
FGA	NONE	17	NONE	NONE	18	NONE	-
D19	**13**	14	14.2,15	NONE	**13**	**13**,15	**13**,15
vWA	NONE	17	14,**16**	NONE	NONE	NONE	-
TPOX	NONE	NONE	**8**,12	NONE	**8**	NONE	**8**
D18	NONE	NONE	13	NONE	17	NONE	-
D3	14,**18**	**18**	14,15,16	NONE	NONE	15	15
THO1	NONE	9	9	NONE	**6,9.3**	NONE	9
D13	NONE	NONE	9	NONE	12	NONE	-
D16	NONE	**11**	9	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	**13**	NONE	**13**	NONE	NONE	NONE	-
D21	NONE	NONE	29,**32.2**	NONE	NONE	NONE	-
D7	NONE	NONE	8,**11**	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.7. Alleles obtained at each locus for casing set #8.

Locus	8A Allele Call	8B Allele Call	8C Allele Call	8D Allele Call	8E Allele Call	8F Allele Call	Consensus
AMEL.	NONE	**X**	**X**	**X**,Y	**X**	**X**	**X**,Y
D5	NONE	11	**12**,13	11,**12**	11,**12**	10	11,**12**
FGA	NONE	23.2	21,25	NONE	17,**22**	21	21
D19	NONE	14.2,**15**	13	**12**,13	NONE	NONE	13
vWA	NONE	NONE	17	NONE	17	19	17
TPOX	**8**	NONE	NONE	**8**	11	**8**,12	**8**
D18	NONE	NONE	NONE	18	17	18	18
D3	**17**	**15**	**17**	NONE	16,**17**	**15**	**15,17**
THO1	NONE	NONE	NONE	9.3	**6**	NONE	-
D13	NONE	NONE	**12**	NONE	9,**11,12**	8,**12**	**12**
D16	**12**	**12**	11	NONE	9,11	11	11
D2	NONE	NONE	18	NONE	NONE	NONE	-
D8	**13**	**13**,14	**15**	NONE	NONE	**13**	**13**
D21	NONE	32.2	**29**	NONE	27,**29,30**	32	**29**
D7	NONE	9	9	NONE	**10**	8,9,12	9
CSF	NONE	**10**	**10**	NONE	**10,12**	NONE	**10**

Table B.8. Alleles obtained at each locus for casing set #9.

Locus	9A Allele Call	9B Allele Call	9C Allele Call	9D Allele Call	9E Allele Call	9F Allele Call	Consensus
AMEL.	NONE	NONE	**X**	**X**	**X**	**X**	**X**
D5	NONE	NONE	**11**	NONE	NONE	10	-
FGA	22	22, 22.2	17, 22	NONE	18, 21.2	NONE	22
D19	NONE	NONE	13	**12**	NONE	NONE	-
vWA	NONE	NONE	17, 18	NONE	NONE	NONE	-
TPOX	NONE	NONE	8, **11**	NONE	NONE	8	8
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	NONE	14	**17**	NONE	NONE	NONE	-
THO1	NONE	NONE	9.3	6	NONE	NONE	-
D13	NONE	NONE	11	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	13	13,15	NONE	NONE	NONE	13
D21	NONE	NONE	27	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.9. Alleles obtained at each locus for casing set #10.

Locus	10A Allele Call	10B Allele Call	10C Allele Call	10D Allele Call	10E Allele Call	10F Allele Call	Consensus
AMEL.	**X,Y**	NONE	**X**	NONE	**X**	**X**	**X**
D5	10,15	**11**,13	NONE	**11**	**11**	**11**	**11**
FGA	NONE	NONE	NONE	19,32	NONE	NONE	-
D19	**13**	**13**	NONE	NONE	14	**13**	**13**
vWA	17	NONE	NONE	NONE	NONE	NONE	-
TPOX	12	**11**	NONE	NONE	NONE	12	-
D18	17	18	NONE	NONE	NONE	13,16	-
D3	NONE	**15**	**15**	NONE	16	NONE	**15**
THO1	NONE	**9.3**	6	NONE	NONE	7	-
D13	NONE	NONE	NONE	13	NONE	NONE	-
D16	**11**	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	12,13	NONE	-
D21	29	NONE	NONE	29	NONE	27	-
D7	9	NONE	NONE	NONE	NONE	9	-
CSF	NONE	NONE	NONE	NONE	NONE	10	-

Table B.10. Alleles obtained at each locus for casing set #11.

Locus	11A Allele Call	11B Allele Call	11C Allele Call	11D Allele Call	11E Allele Call	11F Allele Call	Consensus
AMEL.	**X**	**X**	NONE	**X**,Y	**X**	**X**	**X**,Y
D5	**13**,15	15	**11**,15	**11**	NONE	**13**	**11**,15
FGA	NONE	25	NONE	**20**	**20**,21	**20,22**	**20**
D19	**13.2**	**13.2**	13	NONE	NONE	**13.2**	**13.2**
vWA	NONE	NONE	**18**	**15**	**15,18**	NONE	**15,18**
TPOX	11	NONE	**8**	**10**	**8**	**8**,11	**8**
D18	**19**	NONE	NONE	NONE	**15**	NONE	-
D3	**14**	**18**	**18**	**14**	**18**	16,17,**18**	**18**
THO1	NONE	**6**	**6**,9,9.3	NONE	NONE	**6**	**6**
D13	**11**,14	NONE	NONE	NONE	**8**	**11**	-
D16	NONE	**13**	NONE	NONE	**13**	NONE	**13**
D2	16	NONE	NONE	NONE	**18**	NONE	-
D8	**12**	NONE	10,11,**12**	NONE	**12,13**	**13**	**12,13**
D21	NONE	NONE	**28**,32	NONE	32.2	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	**11**	-

Table B.11. Alleles obtained at each locus for casing set #12. The known handler specimen for individual #12 did not generate any profile data therefore it cannot be known which of the allele calls from the swabbed casing were consistent with the handler.

Locus	12A Allele Call	12B Allele Call	12C Allele Call	12D Allele Call	12E Allele Call	12F Allele Call	Consensus
AMEL.	X	X	X	NONE	X,Y	NONE	X,Y
D5	10	NONE	NONE	NONE	NONE	NONE	-
FGA	17,18	NONE	NONE	NONE	NONE	NONE	-
D19	13,14,16	NONE	13	NONE	15	NONE	-
vWA	16	NONE	15	NONE	NONE	NONE	-
TPOX	NONE	8	NONE	NONE	NONE	NONE	-
D18	16,19	NONE	19	NONE	NONE	NONE	-
D3	NONE	NONE	NONE	17	NONE	NONE	-
THO1	9,9.3	NONE	NONE	5.3	NONE	NONE	-
D13	NONE	NONE	NONE	12	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	17	NONE	NONE	NONE	NONE	NONE	-
D8	13	NONE	15	NONE	NONE	NONE	-
D21	NONE	33.2	NONE	30	29	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.12. Alleles obtained at each locus for casing set #13.

Locus	13A Allele Call	13B Allele Call	13C Allele Call	13D Allele Call	13E Allele Call	13F Allele Call	Consensus
AMEL.	**X**	NONE	NONE	NONE	NONE	NONE	-
D5	**10**	NONE	NONE	NONE	NONE	**10**	-
FGA	32	33.2	33.2	33.2	33.2	33.2	33.2
D19	**13**,15	NONE	**14**	NONE	NONE	NONE	-
vWA	NONE	NONE	NONE	NONE	NONE	**17**	-
TPOX	NONE	NONE	**9**	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	**17**	12,13	**17**	NONE	**16**	**16**	**16**
THO1	NONE	NONE	NONE	**6**	NONE	NONE	-
D13	**12**	NONE	**11**	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	**24**	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.13. Alleles obtained at each locus for casing set #15.

Locus	15A Allele Call	15B Allele Call	15C Allele Call	15D Allele Call	15E Allele Call	15F Allele Call	Consensus
AMEL.	NONE	NONE	NONE	NONE	NONE	NONE	-
D5	NONE	NONE	15	NONE	**12**,16	**12**,16	**12**,16
FGA	NONE	NONE	NONE	NONE	NONE	NONE	-
D19	NONE	NONE	16	**14**	NONE	NONE	-
vWA	**18**	NONE	**19**	NONE	17	**18**	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	**16,17**	NONE	NONE	NONE	**16**	NONE	-
THO1	NONE	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	**10**	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.14. Alleles obtained at each locus for casing set #16.

Locus	16A Allele Call	16B Allele Call	16C Allele Call	16D Allele Call	16E Allele Call	16F Allele Call	Consensus
AMEL.	**X**	NONE	**X**	**X,Y**	NONE	**X**	**X,Y**
D5	**12**	NONE	NONE	**12**,13	NONE	13	13
FGA	NONE	27.2	21.2,28.2	**22**,28.2	NONE	28.2	28.2
D19	NONE	NONE	NONE	13,14	NONE	NONE	-
vWA	NONE	NONE	NONE	15,**16**	NONE	**17**	-
TPOX	11	NONE	NONE	11	**8**	**8**	**8**
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	NONE	NONE	NONE	**16**	NONE	**16**	**16**
THO1	NONE	NONE	NONE	9.3	NONE	**6**	-
D13	NONE	NONE	NONE	12	NONE	12	12
D16	NONE	13	NONE	9,**11**	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	10	**13**	**13,14**	**13**
D21	NONE	NONE	NONE	28	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	**10**	**10**	NONE	**10**	**10**

Table B.15. Alleles obtained at each locus for casing set #17.

Locus	17A Allele Call	17B Allele Call	17C Allele Call	17D Allele Call	17E Allele Call	17F Allele Call	Consensus
AMEL.	**X,Y**	NONE	**X,Y**	**X**	NONE	NONE	**X,Y**
D5	**11**	NONE	**11**,12	NONE	**11**	NONE	**11**
FGA	**22,23**	NONE	17,18,**23**	NONE	**23**	18,28.2	18
D19	**12**	NONE	13	**12**	**12**,15.2,18.2	14	**12**
vWA	**18,19**	NONE	17,**18**	**18**	**18**	NONE	**18**
TPOX	**8**	NONE	**8**,11	NONE	NONE	NONE	-
D18	NONE	NONE	**14**,17,18	NONE	NONE	18	18
D3	**18**	NONE	16,17	15,17,**18**	**18**	**18**	17,**18**
THO1	**7**	NONE	6,**7**,9.3	NONE	**7**	NONE	**7**
D13	NONE	NONE	8,**11,12**	NONE	NONE	NONE	-
D16	**9**	NONE	11	NONE	NONE	NONE	-
D2	NONE	NONE	**17**,25	NONE	NONE	NONE	-
D8	**10**	NONE	**10**,13,15	**10**	**10**	**10**,13	**10**,13
D21	**27**	NONE	**27,30**	NONE	NONE	NONE	-
D7	NONE	NONE	**9**,10	NONE	NONE	NONE	-
CSF	NONE	NONE	10	10,**12**	NONE	NONE	10

Table B.16. Alleles obtained at each locus for casing set #18.

Locus	18A Allele Call	18B Allele Call	18C Allele Call	18D Allele Call	18E Allele Call	18F Allele Call	Consensus
AMEL.	**X**	**X**,Y	**X**	NONE	NONE	Y	**X**,Y
D5	11,**12**	NONE	NONE	NONE	NONE	NONE	-
FGA	16.2,17	NONE	NONE	17	16,16.2	17.2	-
D19	NONE	13,**14**	NONE	10.2	NONE	NONE	-
vWA	NONE	**17**	NONE	NONE	NONE	19	-
TPOX	NONE	**9**	NONE	NONE	NONE	8	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	NONE	NONE	NONE	NONE	NONE	**17**	-
THO1	NONE	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	12	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	24	NONE	NONE	NONE	NONE	-
D8	10	13	NONE	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	8	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.17. Alleles obtained at each locus for casing set #19.

Locus	19A Allele Call	19B Allele Call	19C Allele Call	19D Allele Call	19E Allele Call	19F Allele Call	Consensus
AMEL.	**X,Y**	**Y**	**X,Y**	NONE	**X,Y**	**X**	**X,Y**
D5	**8,10**	NONE	11,12	**10**	**10**	NONE	**10**
FGA	**27**,28.2	**27**	18	43.2	**22,27**,44.2	44.2	**27**,44.2
D19	**16.2**	**16.2**	13	NONE	13,**16.2**	NONE	13,**16.2**
vWA	**16,20**	NONE	17	19	**16,20**	NONE	-
TPOX	**8**	NONE	**8**,11	NONE	**8**	NONE	**8**
D18	NONE	NONE	17	NONE	**19**	NONE	-
D3	**15,17**	14	16,**17**	**15**,16,**17**	**15,17**	**15**	**15,17**
THO1	**7,9.3**	NONE	6,**9.3**	**9.3**	6,7	6	6,**9.3**
D13	NONE	**8**	NONE	**8**	**8**,11	**8**	**8**
D16	NONE	NONE	NONE	NONE	**11**	NONE	-
D2	NONE	**17**	NONE	NONE	**17**	NONE	**17**
D8	**12,14**	**12**	13,15	NONE	**12,14**	NONE	**12**
D21	**29,31**	NONE	30	**29**	**31**	**29**	**29**
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	10,**12**	NONE	NONE	NONE	-

Table B.18. Alleles obtained at each locus for casing set #20.

Locus	20A Allele Call	20B Allele Call	20C Allele Call	20D Allele Call	20E Allele Call	20F Allele Call	Consensus
AMEL.	**X,Y**	**X,Y**	**X,Y**	**X,Y**	**X,Y**	**X,Y**	**X,Y**
D5	**12**	**12**	**12**	11,**12**	**12**	**12**	**12**
FGA	NONE	**23**	**26**	**23**,23.2	**23,26**	**23,26**	**23,26**
D19	**14**,15	**14**	**14**,15	13,14.2,15	**14**	**14**	**14**,15
vWA	**14,17**	**14,17**	**14**	**14,17**	**14**,16,**17**	**14,17**	**14,17**
TPOX	**8**	**8**	**8**	9,11	NONE	**8(NC)**	**8**
D18	NONE	**17**	NONE	14,16,**17**	**15,17**	**17**	**17**
D3	**14,17**	**14,17**	**14,17**	**14**,15,**17**	**14**,15,**17**	**14,17**	**14,17**
THO1	**6**	**6**	**6**	**6**,7,9	**6**,9.3	**6**,9.3	**6**,9.3
D13	**11,12**	**12**	**11**	10,**12**	**11**	**11,12**	**11,12**
D16	11,**12**	**12**	NONE	10,11	11	**12**	11,**12**
D2	**17**	**17**	**17**	**17**,18	NONE	25,**26**	**17**
D8	**12,13**	**12,13**	**12,13**	11,**12,13**,14	**12**	11,**12,13**	**12,13**
D21	**29**	**29**	**31**	**29**,33.2	NONE	**31**	**29,31**
D7	NONE	**12**	**12**	11	NONE	**12**	**12**
CSF	NONE	**11,12**	NONE	10,**12**	NONE	**11,12**	**11,12**

(NC) – indicates the allele was not called by the GeneMapper™ Software

Table B.19. Alleles obtained at each locus for casing set #21.

Locus	21A Allele Call	21B Allele Call	21C Allele Call	21D Allele Call	21E Allele Call	21F Allele Call	Consensus
AMEL.	NONE	**X**	NONE	**X**	NONE	**X**	**X**
D5	NONE	**13**	NONE	NONE	NONE	NONE	-
FGA	NONE	NONE	49.2	16.2	49.2	16.2	16.2,49.2
D19	NONE	14.2	NONE	**14**	NONE	NONE	-
vWA	NONE	NONE	NONE	NONE	NONE	NONE	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	18	-
D3	NONE	NONE	NONE	16	**19**	16	16
THO1	NONE	NONE	NONE	NONE	NONE	6	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	**9**	-
D2	21	**19**	NONE	NONE	NONE	NONE	-
D8	**11**	NONE	NONE	NONE	NONE	13,15	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	11	NONE	-
CSF	13	NONE	NONE	12	NONE	NONE	-

Table B.20. Alleles obtained at each locus for casing set #22.

Locus	22A Allele Call	22B Allele Call	22C Allele Call	22D Allele Call	22E Allele Call	22F Allele Call	Consensus
AMEL.	**Y**	NONE	**X**	**X,Y**	NONE	NONE	**X,Y**
D5	NONE	**12**	NONE	NONE	NONE	NONE	-
FGA	17,44.2	NONE	NONE	NONE	32.2	NONE	-
D19	**13**,16.2	NONE	NONE	NONE	NONE	NONE	-
vWA	NONE	NONE	NONE	**18**	NONE	NONE	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	16,17	**14,16**	NONE	**16**	NONE	NONE	**16**
THO1	**6**	7	NONE	**6**	NONE	**6**	**6**
D13	8	8	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	10	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.21. Alleles obtained at each locus for casing set #23.

Locus	23A Allele Call	23B Allele Call	23C Allele Call	23D Allele Call	23E Allele Call	23F Allele Call	Consensus
AMEL.	**X**	NONE	**X**	**X**	**X**	**X**	**X**
D5	**13**	**10**	NONE	NONE	NONE	**10**	**10**
FGA	17,17.2	17.2	17.2	17.2, 18	17, 17.2	19	17.2
D19	**13**	NONE	NONE	NONE	NONE	NONE	-
vWA	**16**	**16**	NONE	NONE	**16**	NONE	**16**
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	**17**	NONE	NONE	NONE	**17**	**17**
D3	**14**	**14,15**	NONE	NONE	NONE	**14**	**14**
THO1	**6,9**	**6**	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	**18**	NONE	NONE	-
D8	NONE	**13,14**	NONE	NONE	NONE	**13**	**13**
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	**11**	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.22. Alleles obtained at each locus for casing set #24.

Locus	24A Allele Call	24B Allele Call	24C Allele Call	24D Allele Call	24E Allele Call	24F Allele Call	Consensus
AMEL.	NONE	NONE	**Y**	NONE	NONE	**X,Y**	**X,Y**
D5	NONE	NONE	NONE	NONE	NONE	NONE	-
FGA	NONE	NONE	30,51.2	NONE	NONE	NONE	-
D19	NONE	NONE	NONE	NONE	NONE	NONE	-
vWA	NONE	NONE	**15**	NONE	NONE	**17**	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	18	NONE	NONE	-
D3	NONE	NONE	NONE	NONE	NONE	NONE	-
THO1	NONE	NONE	NONE	NONE	NONE	**9**	-
D13	NONE	NONE	9	NONE	NONE	**12**	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	NONE	14	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.23. Alleles obtained at each locus for casing set #25.

Locus	25A Allele Call	25B Allele Call	25C Allele Call	25D Allele Call	25E Allele Call	25F Allele Call	Consensus
AMEL.	NONE	NONE	NONE	NONE	**X**	**X**	**X**
D5	**11**	NONE	10,**11**	NONE	NONE	NONE	-
FGA	NONE	21.2,22	21.2	22	21.2	21.2	21.2, 22
D19	NONE	NONE	NONE	NONE	NONE	NONE	-
vWA	NONE	17	17	NONE	NONE	17,**18**	17
TPOX	**10**	NONE	**8**	NONE	NONE	NONE	-
D18	NONE	NONE	17	NONE	NONE	NONE	-
D3	NONE	NONE	NONE	NONE	NONE	16,**17**	-
THO1	NONE	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	**17,25**	-
D8	NONE	13	**14**	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.24. Alleles obtained at each locus for casing set #26.

Locus	26A Allele Call	26B Allele Call	26C Allele Call	26D Allele Call	26E Allele Call	26F Allele Call	Consensus
AMEL.	**X,Y**	**X**	**X**	NONE	NONE	**X**	**X**
D5	**12**	NONE	NONE	**11**,16	NONE	**12**	-
FGA	18	24.2	NONE	24.2	NONE	24	24.2
D19	NONE	NONE	NONE	**14**	NONE	NONE	-
vWA	NONE	NONE	NONE	NONE	**19**	NONE	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	**17**	NONE	NONE	NONE	NONE	15	-
THO1	NONE	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	NONE	13	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.25. Alleles obtained at each locus for casing set #27.

Locus	27A Allele Call	27B Allele Call	27C Allele Call	27D Allele Call	27E Allele Call	27F Allele Call	Consensus
AMEL.	**X**	NONE	**X**	NONE	NONE	**X**	**X**
D5	NONE	NONE	**12**	NONE	NONE	NONE	-
FGA	NONE	NONE	NONE	NONE	NONE	NONE	-
D19	NONE	NONE	13.2,**14**	**13**	**14**	**14**	**14**
vWA	15	17	**19**	13	NONE	NONE	-
TPOX	NONE	NONE	NONE	NONE	**8**	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	NONE	NONE	**16**	NONE	NONE	**15**	-
THO1	6,**9.3**	NONE	NONE	6	7	7	7
D13	NONE	13	NONE	NONE	NONE	11	-
D16	NONE	NONE	**11**	NONE	NONE	NONE	-
D2	NONE	**19**	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	NONE	NONE	-
D21	NONE	NONE	**32.2**	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	10	NONE	NONE	NONE	-

Table B.26. Alleles obtained at each locus for casing set #28.

Locus	28A Allele Call	28B Allele Call	28C Allele Call	28D Allele Call	28E Allele Call	28F Allele Call	Consensus
AMEL.	**X**	**X**	NONE	NONE	NONE	NONE	**X**
D5	**11**	**11**	NONE	NONE	NONE	**11**	**11**
FGA	NONE	NONE	NONE	18	NONE	NONE	-
D19	NONE	NONE	NONE	NONE	NONE	NONE	-
vWA	NONE	NONE	NONE	NONE	NONE	NONE	-
TPOX	NONE	NONE	**11**	NONE	NONE	NONE	-
D18	NONE	16	**17,18**	NONE	NONE	NONE	-
D3	**16,17**	NONE	NONE	NONE	NONE	**17**	-
THO1	NONE	9.3	NONE	NONE	9.3	NONE	9.3
D13	NONE	NONE	**11**	NONE	NONE	NONE	-
D16	NONE	9	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	**13**,15	NONE	NONE	15	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	9	NONE	NONE	NONE	NONE	-

Table B.27. Alleles obtained at each locus for casing set #29.

Locus	29A Allele Call	29B Allele Call	29C Allele Call	29D Allele Call	29E Allele Call	29F Allele Call	Consensus
AMEL.	NONE	NONE	NONE	**X**	NONE	**X**	**X**
D5	16	16	**11**,13,16	**11**,12	NONE	**11**,13	**11**,13,16
FGA	NONE	49.2	NONE	**24,25**	**25**	**25**	**25**
D19	NONE	NONE	NONE	NONE	**13**	12	-
vWA	NONE	17	NONE	NONE	NONE	**15**,17	17
TPOX	NONE	NONE	NONE	**8**,11	NONE	11	11
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	NONE	NONE	16	16	NONE	**15**	16
THO1	NONE	NONE	NONE	6	NONE	9	-
D13	NONE	**11**	NONE	NONE	NONE	12,14	-
D16	NONE	9	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	**13**	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	29	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.28. Alleles obtained at each locus for casing set #30.

Locus	30A Allele Call	30B Allele Call	30C Allele Call	30D Allele Call	30E Allele Call	30F Allele Call	Consensus
AMEL.	**Y**	NONE	NONE	**X**	NONE	NONE	**X**
D5	**12**	NONE	NONE	NONE	NONE	**10**	-
FGA	NONE	NONE	NONE	NONE	NONE	NONE	-
D19	NONE	NONE	NONE	NONE	13	13	13
vWA	NONE	NONE	NONE	NONE	**17**	NONE	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	18	NONE	-
D3	NONE	NONE	16	NONE	17	NONE	-
THO1	NONE	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	9	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	17	-
D8	**12**	NONE	NONE	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.29. Alleles obtained at each locus for casing set #31.

Locus	31A Allele Call	31B Allele Call	31C Allele Call	31D Allele Call	31E Allele Call	31F Allele Call	Consensus
AMEL.	NONE	NONE	**X**	NONE	**X**,Y	NONE	**X**,Y
D5	NONE	NONE	NONE	NONE	NONE	NONE	-
FGA	NONE	NONE	NONE	49.2	51.2	NONE	-
D19	NONE	NONE	NONE	NONE	NONE	NONE	-
vWA	NONE	NONE	NONE	NONE	NONE	NONE	-
TPOX	NONE	NONE	NONE	NONE	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	NONE	NONE	NONE	NONE	NONE	NONE	-
THO1	NONE	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	25	NONE	NONE	NONE	-
D8	NONE	NONE	NONE	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	NONE	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.30. Alleles obtained at each locus for casing set #32.

Locus	32A Allele Call	32B Allele Call	32C Allele Call	32D Allele Call	32E Allele Call	32F Allele Call	Consensus
AMEL.	**X**	NONE	**X**	NONE	NONE	**X**	**X**
D5	**10,12**	NONE	NONE	NONE	**10**	**12**,16	-
FGA	NONE	NONE	49.2	NONE	NONE	**22,24**	-
D19	**13,14**,15	NONE	**14**	NONE	14.2	NONE	-
vWA	NONE	NONE	NONE	NONE	NONE	**17,18**	-
TPOX	NONE	NONE	NONE	11	NONE	NONE	-
D18	NONE	NONE	NONE	NONE	NONE	NONE	-
D3	**14**,16	NONE	**17**	NONE	NONE	**14,17**	**17**
THO1	**8**	NONE	NONE	NONE	NONE	NONE	-
D13	NONE	NONE	NONE	NONE	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	**17**	-
D8	**13,14**	NONE	NONE	NONE	NONE	10,**13**	-
D21	NONE	NONE	NONE	NONE	NONE	**32.2**	-
D7	NONE	NONE	NONE	NONE	9	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

Table B.31. Alleles obtained at each locus for casing set #33.

Locus	33A Allele Call	33B Allele Call	33C Allele Call	33D Allele Call	33E Allele Call	33F Allele Call	Consensus
AMEL	Y	Y	**X**	**X**	**X**	**X**	**X,Y**
D5	12	9	NONE	NONE	NONE	NONE	-
FGA	NONE	21.2	34.2	50.2	25	26	-
D19	**13**	**13**	NONE	NONE	NONE	NONE	-
vWA	NONE	NONE	17	NONE	**16**	NONE	-
TPOX	NONE	NONE	NONE	8	NONE	NONE	-
D18	NONE	NONE	NONE	18	NONE	NONE	-
D3	NONE	**17**	NONE	NONE	**15**	NONE	-
THO1	**9.3**	**9.3**	NONE	9	NONE	NONE	-
D13	NONE	NONE	NONE	11	NONE	NONE	-
D16	NONE	NONE	NONE	NONE	NONE	NONE	-
D2	NONE	NONE	NONE	NONE	NONE	NONE	-
D8	NONE	**13**	NONE	NONE	NONE	NONE	-
D21	NONE	NONE	NONE	NONE	NONE	NONE	-
D7	NONE	NONE	NONE	**10**	NONE	NONE	-
CSF	NONE	NONE	NONE	NONE	NONE	NONE	-

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