COLLECTION, DIRECT AMPLIFICATION, AND ALTERNATE GENOTYPING METHODS FOR DNA RECOVERED FROM PIPE BOMBS AND FIREARMS

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DEDICATION

"*You are on the eve of complete victory. You can't go wrong. The world is behind you."*

– Josephine Baker

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ABSTRACT

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Improvised explosive devices (IEDs) and firearms have often been used to cause mass destruction and harm within communities. When a crime has been committed with these weapons, it is critical that a suspect or suspects be identified quickly. There are several approaches that can be used to identify a potential suspect, but few are as discriminatory as DNA analysis. Short tandem repeat (STR) analysis via capillary electrophoresis (CE) has proven to be robust method of human identification (HID); however, DNA remaining on touched or handled items is likely to be poor in quality and quantity, which can make STR profile interpretation extremely difficult or hinder it completely. Therefore, this study explores various collection strategies, novel genetic markers, and alternate analysis methods to increase the likelihood of retrieving usable genetic information from these challenging items. This was accomplished is four separate phases.

The first study compared the informativeness of alternate genetic markers and analysis methods to standard STR analysis. PVC pipe bombs and copper wires were constructed and spiked with known quantities of biological material to recover mock low-template DNA from the surface PVC pipes and traces of blood from the ends of copper wires. DNA collected from PVC pipe fragments underwent traditional STR analysis as well as insertion/null (INNULs) analysis via CE and single nucleotide polymorphism (SNP) analysis via massively parallel sequencing (MPS) for HID. Blood from copper wires were analyzed using ancestry informative SNPs (AISNPs) via MPS. The results of this

study showed that a complete INNUL profiles had a higher power of discrimination when less than 14 STR alleles were called. SNP analysis via MPS were the most discriminatory of all the methods tested when the same DNA concentration input was used. In addition, AISNPs correctly predicted the ancestry for five of the six blood samples tested.

In the second study, three swab types and a swab storage device called the SwabSaver® were evaluated. Traditional cotton swabs, nylon-flocked swabs, and layered cotton paper swabs were used to collect DNA from handled assault rifle magazines and were then extracted or stored at room-temperature in a centrifuge tube or SwabSaver® device for one or two months. While nylon swabs resulted in higher DNA yields overall, swab storage had a greater impact on STR profile completeness, and swabs stored in the SwabSaver® device resulted in profiles comparable to swabs that were not stored.

The third study examined two swab substrates and two direct amplification strategies for DNA collected from common pipe bomb substrates. PVC and steel pipes, electrical tape, and copper wires were spiked with known quantities of epithelial cells and swabbed with a conventional cotton swab or a microFLOQ® Direct swab (nylon-flocked swab). Swabs either underwent traditional DNA processing, direct amplification, or a pre-treatment strategy prior to STR amplification. The results of this proof-of-concept study showed that direct amplification using microFLOQ® Direct swabs was the most successful as it had the shortest processing time and resulted in the most complete STR profiles.

Lastly, the fourth and final study examined two sub-sampling strategies for direct amplification using microFLOQ® Direct swabs. The first strategy involved DNA collection from firearms using a regular-tipped nylon swab, which was then sub-sampled using a microFLOQ® Direct swab. Rather than collecting from the larger swab head, the

second strategy involved sub-sampling directly from post-blast pipe bomb substrates. A microFLOQ® Direct swab was used to collect from the fragments first and was followed with a more thorough collection with a regular-tipped nylon swab. All microFLOQ® Direct swabs underwent direct amplification while regular nylon swabs underwent traditional processing. Traditional DNA processing resulted in higher yields overall; however, sub-sampling from the larger swab head did result in partial and complete profiles. Sub-sampling from the pipe bomb substrates was mostly unsuccessful, which was likely due poor DNA deposition by the handler and high heat and pressure from the explosion.

KEYWORDS: Forensic DNA; Short tandem repeats; “Touch” DNA; Direct amplification improvised explosive devices; Firearms

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ABBREVIATIONS

AIM	Ancestry informative marker
AISNP	Ancestry informative SNP
AN	Ammonium nitrate
CE	Capillary electrophoresis
CODIS	Combined DNA Index System
DNA	Deoxyribonucleic acid
ESS	European Standard Set
FBI	Federal Bureau of Investigation
HID	Human identification
IED	Improvised explosive device
INDEL	Insertion/deletion polymorphism
INNUL	Insertion/null polymorphism
LINE	Long interspersed nuclear element
LT-DNA	Low-template DNA
MPS	Massively parallel sequencing
qPCR	Quantitative polymerase chain reaction
PCR	Polymerase chain reaction
RE	Retrotransposable element
RMP	Random match probability
RNA	Ribonucleic acid
SBS	Sequence-by-synthesis
SDS	Sodium dodecyl sulfate
SINE	Short interspersed nuclear element
SNP	Single nucleotide polymorphism
STR	Short tandem repeat
SVA	SINE-VNTR-*Alu* element

GLOSSARY

Allele	Alternative form of a gene or locus
Allelic drop-in	Contamination from an unknown source
Allelic dropout	Failure to detect an allele within a sample or failure to amplify during PCR
Biallelic	Only two alleles are observed at a genetic locus
Capillary electrophoresis	An electrophoretic technique for size-based separation of DNA molecules through a glass capillary
Deoxyribonucleic acid	Double stranded genetic material of many organisms
Polymerase chain reaction	A process that yields millions of copies of a DNA target region
Power of discrimination	The potential power of a marker to differentiate between any two people at random
Random match probability	The chance of a specific profile occurring in a specific population based on observed allele frequencies for that population
Short tandem repeats	Repeat sequence units 2 to 6 base pairs in length
Single nucleotide polymorphism	A single base pair variation

CHAPTER I

Introduction

The field of forensic biology has made many advancements in DNA evidence processing and profiling since its beginnings. Advancements in DNA chemistries have been introduced over the last 30 years and have increased the likelihood of producing DNA profiles from challenging pieces of evidence. However, evidence recovered from pipe bomb and firearm components can cause additional complications that may prevent a DNA profile from being produced. While it has been documented that biological material can be recovered from touched and handled items, “touch” DNA quantities may not be sufficient enough to produce a reliable DNA profile or generate genetic information for investigative leads. Furthermore, DNA can degrade from high heat and pressure, or inhibitory substances may be present during processing that hinder or prevent DNA profile quality and interpretation. Alternative collection strategies and amplification methods could aid the processing these difficult samples and increase the likelihood of retrieving sufficient amounts of usable DNA. Alternate DNA markers such as SNPs, INNULs, and mitochondrial DNA may also be used to complement or in lieu of STR analysis for human identification with such difficult samples. In addition, other genotyping methods have the potential to provide information beyond human identification, such as biographical ancestry and external physical traits that can narrow down a suspect pool when traditional routes fail to produce informative leads.

Firearms and Ammunition

Firearms are weapons designed to discharge a projectile by the action of an explosive (18 U.S.C. § 921(a)(3)), and are commonly used in the proceedings of crimes such as robbery, assault, and homicide. As a result, these weapons are routinely submitted to laboratories for forensic analysis. There are three general categories of firearms: handguns/pistols, rifles, and shotguns [1]. Of the three types, handguns are the most commonly owned by civilians, as approximately 42% of families in the United States have at least one gun in their households [2]. While the types of guns differ (i.e. guns with a single-shot action can only be loaded with and fire one shot at a time), there are three general components that each share. Firstly, the action is a combination of parts responsible for loading, firing, and unloading a firearm. The stock is the handle of a gun made of wood or synthetic material. Traditional handgun stocks are short and intended to be fired with one hand whereas rifles and shotguns may allow for shoulder support during operation [3]. Lastly, the barrel is the metal tube from which the projectile is ejected. Barrels can be smooth or rifled. Firearm barrels with rifling, such as rifles or handguns, have spiraled lands and grooves engraved [4]. The rifling causes the projectile to spin as it is ejected, and it keeps the projectile on a straight and stable trajectory [1, 5, 6].

Cartridges are ammunition rounds comprised of a case, primer, propellant (i.e. gun powder), and a projectile(s). These are commonly referred to as “bullets”; however, the bullet is only one component of a cartridge, specifically the projectile. Repeating firearms also contain a magazine where additional cartridges can be stored for subsequent firing operation cycles. Once a cartridge is chambered, an external force is required to start the chemical reaction that propels the bullet forward through the barrel. This external force is

typically provided by a firing pin that strikes a percussion primer charge that initiates the main propellant charge of the cartridge. Pulling and releasing the trigger of a gun releases the spring loaded firing mechanism (striker, hammer or other mechanism) which causes this primer strike by the firing pin [3].

Improvised Explosive Devices

Improvised explosive devices (IEDs) are weapons commonly used in terrorist crimes to cause bodily harm or death, property damage, or instill fear in communities [7-12]. In 2017, approximately one-third of all bombs in the United States could be attributed to IEDs [13]. There are several forms of IEDs, including but not limited to vehicle-borne IEDs (VBIEDs), postal bombs, and pipe bombs [7, 8, 10]. The type of IED chosen for a particular attack depends on a variety of factors, such as the intended target, the amount of damage desired, and ease of access to the necessary supplies. Pipe bombs are widely used because the materials are readily available, assembly is simple, and they can cause a considerable amount of harm and damage [9, 14]. Common pipe bomb materials include a metal or plastic casing filled with explosive powder, end caps, a fuse to initiate the chemical reaction, and adhesive tape or glue to attach the components. Additional components such as cell phones, or batteries can be used as switches to ignite a fuse [10, 15].

Explosive Powders

Explosive materials are categorized by the speed at which the chemical reaction burns to cause an explosion. Low explosives require an ignition stimulus, such as a spark or flame, to initiate the reaction. Once initiated, the powders burn and decompose, which releases gas, vapors and heat, causing an increase in temperature. This reaction is able to self-propagate and eventually leads to an explosion [16]. This chemical reaction for high

explosive powders occurs faster than low explosive powders, which affects the power of the explosion [10, 11, 17]. Low explosives burn at a rate of approximately 1500 m/s or less, whereas high explosives burn at a higher rate (> 1500 m/s), resulting in a more powerful explosion [18, 19].

Because the reaction is much slower, low explosives do not generate the same amount of heat and damage as high explosives [10, 11]. Therefore, low explosives tend to burn, or deflagrate, instead of detonate [6]. Low explosive powders must also be contained in order to generate enough pressure to explode [10]. Smokeless powder generates temperature between 2500 ˚C and 3000 ˚C, and is the most commonly used low explosive in IEDs [11] and as propellants in gun ammunition [4]. Two common types of smokeless powder are single-base or double-base powder [10]. Single-base smokeless powder is solely comprised of nitrocellulose, whereas double-base smokeless powder is made up of nitrocellulose and nitroglycerine [6].

High explosives are not required to be contained in order to detonate and will explode when subjected to shock and/or pressure. They are categorized into primary and secondary explosives. Primary high explosives (generally used as detonators) can be triggered by a flame, shock, or friction whereas secondary high explosives require a detonator in order to explode [6]. High explosive powders are used in primers to initiate firing bullets. Pulling the trigger of a gun causes the firing pin to release and strike the primer compound located in the rim or a primer cap. The primer compounds ignite, releasing hot gases and high pressure, and causes the propellant (low explosive powder) to burn. The increasing heat and pressure from both powders then forces the projectile through the barrel [3, 6].

Ammonium nitrate fuel oil (ANFO) and ammonium nitrate aluminum powder (ammonal) are examples of a third category of high explosives called blasting agents [20]. Blasting agents require a detonator and a secondary explosive booster in order to initiate an explosion [6, 10]. Ammonium nitrate (AN) is found in many garden fertilizers. Due to its wide availability, it is frequently used as an oxidizer when manufacturing homemade explosives [10, 17, 21]. Fertilizer-grade AN can be found in the form of small white beads called prills and are intended to release nitrogen to fertilize the soil. Prills found in fertilizer-grade AN are smoother than explosive-grade prills, and are not optimized to be used in explosives [17]. Regardless, it is still effective enough to detonate an IED when mixed with a fuel source, such as aluminum powder, forming a binary explosive.

Binary explosives are a type of high explosives that are comprised of two inert chemicals, an oxidizer and a catalyst, which form an explosive only when combined [20, 22]. Tannerite® (Tannerite® Sports LLC), is a brand of binary explosive marketed as exploding targets for licensed gun enthusiasts. Due to its wide accessibility, there has been some concern that binary explosives, such as Tannerite® may provide unrestricted access to a source of AN for homemade IEDs [23]. Tannerite® is a proprietary mix of AN (oxidizer), aluminum powder (catalyst), and other chemicals [24, 25]. Once the oxidizer and catalyst are mixed, the chemicals are no longer stable and will explode if initiated by a high energy impulse, or mixed with another explosive such as smokeless gunpowder to initiate the reaction [24].

The Federal Bureau of Investigation (FBI) has also documented cases of misuse, including the arrest of an individual from Minnesota who detonated 45 kg of Tannerite® with a 50-caliber rifle. The shock produced from the explosion was felt at a nuclear plant

almost 2 km away [23]. There is some concern within various branches of law enforcement that Tannerite® may be used to manufacture IEDs because of loose purchasing restrictions, and the FBI issued an Intelligence Bulletin stating these concerns in 2013 [23]. Since the publication of this Intelligence Bulletin, there have been a handful of instances involving Tannerite®, including a bombing in 2016 [26] and a mass shooting in 2017 [27]. However, in both cases it could neither be confirmed or denied that the Tannerite® found was used or intended to be used as an explosive. Currently, there are no published empirical studies concerning Tannerite® as an explosive agent within pipe bombs. However, one case reported post-blast injuries caused by the explosion in a case where Tannerite® was misused [25]. The victim in this study placed the target in a lit charcoal grill and initiated the explosion with a firework while in close proximity to the device when the target detonated. He suffered a variety of injuries including burns, abrasions, and ruptured tympanic membranes. In another case report, shrapnel from an exploding target made with Tannerite® killed a bystander [28]. Both reports demonstrate the potential danger of Tannerite® to cause extreme bodily harm and death.

Suspect Identification

After an explosive event, it is important for crime scene personnel to approach the scene with caution as several issues may arise. The IED may remain intact and crime scene personnel must take extra precautions to safely deactivate the IED while preserving the physical evidence [10, 17]. If an explosion occurs, evidence collection becomes much more complex. In addition to post-blast fragments from the bomb casing, other components such as batteries, fuses, tape, and electrical wires may be collected as evidence [9, 10, 17]. IED containers, such as vehicles, envelopes, and backpacks can also complicate collection as

the containers themselves are of evidentiary value [7, 8, 12]. Furthermore, the radius of the dispersion of these fragments depends on the magnitude of the blast and can affect post-blast collection methods.

Similar caution should be considered when firearms are involved. While firearms have several uses, including hunting and self-defense, they can also be used to harm and terrorize communities. Unfortunately, firearms are commonly used to commit violent and non-violent crimes, such robbery, assault, and homicide. According to data from the Bureau of Justice Statistics, 66% of homicides involving a single victim and 79% involving multiple victims were attributed to firearms from 2002-2011 [29]. Gun-related evidence, including cartridge or shell casings, bullets, gunshot residue (unburned powder), or the weapon itself may remain at the crime scene [1, 4, 5]. It is crucial that the scene is secured quickly as firearm and ammunition components may be widely dispersed and evidence can easily be contaminated or lost if not documented quickly [4, 5].

Fingerprint, toolmark, and residue analyses are commonly performed in cases involving IEDs and firearms [7, 14, 17, 30]. Toolmark evidence from ammunition, such as an indent from the firing pin and striations from rifling can be used to determine if the projectile was fired from the same gun and what type of weapon was used. Gunshot residue (GSR) from a suspect's hands or clothing can undergo chemical analysis to determine if a suspect was in close proximity to a fired weapon [4, 6]. Similarly, residues from IEDs can be used to indicate what type of explosive was used [31]. Toolmark and chemical analyses can determine which materials were used to manufacture the device and generate investigative leads. However, they are not as discriminatory as fingerprints and DNA evidence [31]. Historically, fingerprint analyses tend to be more probative than DNA

testing in these cases because the success of recovering enough good quality DNA for genotyping is severely affected by the nature of touched items, heat from firing or an explosion causing DNA degradation, and co-extracted inhibitors such as soot and humic acid found in soil [11, 12, 14].

"Touch" DNA

In 1997 van Oorschot et al. demonstrated that DNA profiles could be generated from fingerprints [32]. Since then, many studies have also shown that "touch" DNA can provide genetic profiles from a multitude of commonly handled items such a drinking glasses [33] and doorknobs [34, 35], to more specific items of evidence such as mobile phones [36, 37], cables [38, 39], knives [40-42] firearms and bullet cartridges [37, 39, 41, 43-48], and bomb substrates [9, 49-53].

When an item such as a pipe bomb or firearm is handled, the amount of DNA transferred varies and can greatly affect the quality of a genetic profile [54]. Typically, only a few cells are transferred when trace quantities (below 250 pg) of DNA are encountered on touched items [54, 55]. It was initially believed DNA collected from touched items originated solely from nucleated epithelial cells [56]. It was later proposed that extracellular, cell-free DNA may be a key source of collected genetic material [57]. The outer most layer of skin, the *stratum corneum*, consists primarily of flat, anucleate corneocytes [58]. These cells flake off of the body through a process called desquamation [59], and can be transferred after handling an item. Because these cells are anucleate, it has been suggested that the source of transfer DNA is extracellular [60]. This has been supported in a study by Kanokwongnuwut et al. using microscopic analysis of fingerprints and fluorescent dyes [37]. In this study, volunteers handled a variety of items of different

substrates. These items were then stained with a human-specific nucleic acid dye and DNA was visualized using a fluorescent microscope. Anucleate cellular material were observed through fluorescent detection, and DNA profiles were produced. This further indicates that extracellular DNA is likely bound to shed cells. The origin of this cell-free DNA is thought to be derived from sweat [61] or other biological fluids. It has also been suggested that the extracellular DNA becomes bound to the surface of anucleate corneocytes during cell differentiation [60]. However, it is unlikely that there is a single source of "touch" DNA, but rather a combination of nucleated epithelial cells, anucleate corneocytes, and cell-free DNA [62].

Some studies have also suggested that individuals may constitute different "shedder" types [63-67]. One notable study from Lowe et al. assessed differences in DNA deposition between individuals. Each subject washed their hands and gripped a sterile plastic tube for 10 seconds. The tubes and the hands of the participates were swabbed after deposition, and DNA profiles were generated once the DNA was extracted. Noticeable differences were observed in profile completeness when there was a 15-minute delay between handwashing and handling the tube. The authors then used this time point to classify shedder type; those who produced full profiles 15 minutes after handwashing were "good" shedders while those who only produced incomplete profiles were considered "poor" shedders [63]. In contrast, a study conducted by Phipps and Petricevic applied similar methods but did not have the same success classifying "good" or "poor" shedders, as the profiles produced from the subjects were not consistent over a five day period [64]. Other factors that have been cited as contributing to one's shedder status include the type of substrate [68-72], age [73-75], and gender [74, 76, 77]. Although the amount of DNA

transferred to an object when touched is highly variable, it is unlikely that high quantities of DNA (> 1 ng) will be recovered for identification purposes [54, 78, 79].

DNA Collection Methods

The ability to adequately recover DNA from challenging samples is imperative in order to provide reliable identifications and/or investigative leads. There have been a number of studies exploring various DNA collection methods to optimize recovery from "touch" and low-template samples, exploring a wide array of collection devices, collection techniques, and substrates [41, 49, 80-84].

The most common collection devices are swabs [84, 85] and adhesive tapes [86, 87]. Swabbing is frequently employed at crime scenes where trace quantities of DNA remain [41, 80, 88]. Traditionally, cotton swabs have been most commonly used by law enforcement to collect biological material from crime scenes or buccal swabs for databasing purposes. However, in an effort to improve DNA yields, swabs have also been made of alternative materials for forensic collection.

Foam swabs, such as such as those from Puritan®, and nylon swabs such as the 4N6FLOQSwabs™ and microFLOQ® Direct swabs (Copan Italia) have been proposed as a better alternatives to traditional cotton swabs due to concerns of DNA becoming trapped in the tightly wound cotton fibers (Fig. 1.1) [89]. Foam swabs consist of a porous, sponge-like head and nylon swabs are comprised of tiny fibers that are flocked around the applicator. Nylon fibers absorb fluid through capillary action, but the fluid remains near the surface as opposed to becoming trapped inside a tightly woven core [85, 89]. Several studies have examined the efficiency of both swab types with forensically relevant evidence such as trace DNA [9, 82, 85, 90-93]. One such study compared the recovery and

extraction efficiency of five different swab matrices and found that nylon 4N6FLOQSwabs® were the most effective for both. However, none of the swabs tested had an extraction efficiency over 50%, indicating that much of the DNA was still retained in the swabs [92].

Likewise, the CEP® swab (now called SimpleSwab™ — Gentueri, Inc.) is another alternative to traditional swabs (Fig. 1.1). Though they are made of cotton, they are not tightly wound around an applicator. Instead, the swab head brush is comprised of layers of untreated (no lysing agent) absorbent paper [85]. The brush also ejects from the applicator, reducing the risk of contamination by cutting off the swab head. Studies conducted using this swab are limited [85, 94, 95]. One study by Verdon et al. compared the collection efficiency of several collection devices including CEP® swabs, but found that it performed the worst and ultimately dropped it from the study [85]. While other studies demonstrated some success using these swabs [94, 95], further research is needed to truly assess its effectiveness as a DNA collector.

Fig. 1.1 From left to right: traditional cotton swab, regular-tipped 4N6FLOQSwab™ (left) microFLOQ® Direct swab, SimpleSwab™ (CEP®) consisting of layered cotton paper with split swab head.

Adhesive tapes have been predominately used to collect trace amounts of DNA on fabric and other porous substrates [80, 96]. Studies have examined a wide variety of tapes and different methods of recovering DNA from the adhesive [80, 82, 86, 88, 97, 98]. A small cutting of tape can be placed directly into extraction buffer [88, 99, 100] or swabbing the adhesive may be necessary as the adhesive may interfere with the DNA extraction process [98, 101]. However, this additional swabbing step increases the risk of losing DNA and contamination. Tapes such as SceneSafe FAST™ mini-tapes (SceneSafe™) are specifically designed to recover trace quantities of DNA [80, 82]. One study found that these mini-tapes collected higher quantities of DNA from unwashed shirts compared to collection with cotton, foam, and nylon swabs [82]. Adhesive tapes that dissolve in water have also been considered. A limited number of studies have examined the use of

dissolvable tapes to increase DNA recovery, but the viscosity of the lysate in some studies hindered downstream analysis [41, 82].

DNA Processing

Short Tandem Repeats

Short tandem repeats (STRs) are repeating units within the human genome that are 2 to 6 base pairs in length, and are currently the standard genetic marker used in forensic crime laboratories for DNA-based human identification (HID) [44, 102-104]. STRs have proven to be a robust method for HID due to their high discrimination power (as they multi-allelic) [102, 103] and relatively small size (< 450 bp) [105]. In addition, current STR kits are highly sensitive and can amplify relatively low amounts of DNA (> 100 pg) [68, 106]. Indeed, most commercial STR kits are optimized to produce high quality STR profiles using 0.5 ng to 1 ng of DNA (or approximately 200 cells) [107].

In 1997, the FBI introduced a set of 13 “core” STR loci that could be used for comparisons using the Combined DNA Index System (CODIS), national DNA databank, to aid with identifying suspects across the United States [108]. These loci continued to be the standard until 2017 when an additional seven loci were implemented, which not only increased the power of discrimination, but also allowed for better compatibility with international databases including the European Standard Set (ESS) and reduced the likelihood of adventitious database matches [109, 110]. Currently, there are three manufacturers that produce STR kits that contain all 20 core loci: Thermo Fisher Scientific (GlobalFiler® and GlobalFiler® Express), Promega, (PowerPlex® Fusion and Fusion 6C), and QIAGEN (Investigator® 24plex QS and 24plex GO!). In addition to the expanded core loci and the sex determination marker amelogenin, both Thermo Fisher Scientific STR kits

contain three autosomal loci and one Y-INDEL [111, 112], Promega's PowerPlex® Fusion includes four autosomal markers [113], while PowerPlex® Fusion 6C, encompasses the same markers as PowerPlex® Fusion plus an additional autosomal locus and two Y-STRs [114], and both QIAGEN kits have one autosomal STR and a Y-STR [115]. The QIAGEN kits also contain two quality sensors (QS) that serve as internal PCR controls. The QS markers assist with determining if amplification was successful and can also infer DNA quality (inhibited or degraded) [115].

When analyzing low-quality and low quantities of DNA, a variety of stochastic effects can occur including heterozygote peak height imbalance, allele and/or locus drop-out, allele drop-in, and exaggerated stutter [54, 116, 117]. These stochastic effects can negatively impact DNA profile quality and interpretation. While other approaches, such as increased number of PCR cycles, post-PCR clean up, and/or longer injection times during capillary electrophoresis (CE) have been used to help overcome some issues with genotyping minute DNA quantities [118], these practices can also exaggerate stochastic effects [119, 120].

Stochastic effects may also occur when amplifying degraded and inhibited DNA, as these types of samples mimic low-template samples and produce similar artifacts. STR markers in commercial kits can be up to 450 base pairs, and therefore when DNA degrades the smaller markers (< 250 bp) tend to be less susceptible to PCR failure than the large amplicons [121, 122]. This scenario can result in preferential amplification of shorter amplicons during PCR and/or drop-out of longer amplicons [44, 123]. Any trace amounts of DNA on post-blast IED fragments or fired bullets are also likely to be degraded from heat produced by the explosion. [7, 9]. Other environmental insults such as humidity [124],

UV radiation [125], and microbial activity [126] can also cause DNA to degrade and prevent the generation of high quality DNA profiles.

Similarly, inhibitors can also cause amplification issues and affect downstream data analysis. Common inhibitory compounds encountered in forensic casework are often biological (collagen, hematin, melanin, and calcium) or environmental (humic acid and metal ions) [127]. These inhibitors are commonly observed in samples from mass disaster events and human remains, but can be encountered in any evidence sample, including those involving IEDs and firearms (i.e. residual explosive powder).

One mechanism of inhibition is interfering with cell lysis during extraction [127]; however, inhibition during the PCR is more common [128]. For example, humic acid is found in soil and is often found with buried skeletal remains but may also be encountered if an IED is buried. When humic acid is co-extracted with DNA, it binds to specific sequences in DNA and prevents the polymerase from binding to the DNA template during amplification [129]. Metal ions like copper (Cu^{2+}) can also prevent proper DNA amplification. Copper is often found in electrical wires used during the manufacturing of IEDs [9, 130] and are a component in brass firearm cartridges [44, 131, 132]. Unlike humic acid, copper can interact directly with DNA and cause conformational changes and alter the charge [130]. Copper can also cause oxidative damage and lead to DNA fragmentation [133]. These changes not only hinder amplification but also CE [130], all of which produce similar stochastic effects seen with degraded DNA [123].

Alternate Molecular Markers

Another approach to retrieving more information from challenging DNA samples is to employ alternative molecular markers. Alternate DNA markers such as mitochondrial DNA, mini-STRs, and bi-allelic markers may aid investigations, as their effectiveness with genotyping low-template and highly degraded DNA samples has already been demonstrated, including identifying victims of the September 11, 2001 World Trade Center attacks [134]. Continual development in DNA technology could improve results from challenging DNA evidence and may provide investigative leads when other forms of evidence fail or are not available.

Mini-STRs

In 1995, it was demonstrated that smaller PCR amplicons resulted in higher profiling success after attempting to identify remains from a massive fire in Waco, Texas [121, 135]. Shortly afterwards, several studies explored reducing the size of existing STRs reducing [122, 136-140]. One such paper by Butler et al. specifically examined reducing the amplicon sizes of the 13-core CODIS loci using a novel set of primers for use with highly degraded DNA to create a set of "mini-STRs". Mini-STRs use primers that anneal as close to the repeat region as possible, thereby reducing the amplicon length [139]. Compared to traditional STRs, mini-STRs are < 250 bp, making them less susceptible to allele and locus drop-out as larger targets are less likely to remain intact in highly degraded samples [123]. Several studies have shown that mini-STRs have successfully generated profiles from challenging samples when traditional STRs failed [122, 138, 141-144]. However, due to the restriction in size (< 250 bp), fewer mini-STRs can be simultaneously separated and detected during CE. Reducing the number of loci for analysis also decreases

the power of discrimination for identification purposes [145]. Therefore, mini-STR systems like AmpFLSTR® MiniFiler (Thermo Fisher Scientific) can be used to supplement traditional STR analysis when genotyping difficult samples [142]. More recently, newer "megaplex" STR kits such as the Globalfiler® PCR Amplification Kit, PowerPlex® Fusion 6C, and the Investigator® 24plex QS Kit have incorporated the maximum number of mini-STR amplicons into their expanded panel designs.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) analysis has been traditionally relied upon when low level and/or extremely degraded samples require analysis due to the failure of other markers such as STRs [146]. Hundreds of mitochondria reside within each cell and can contain thousands of copies of mtDNA compared to nuclear DNA (nDNA) [146], and can therefore provide probative genetic information in trace or degraded samples when analysis of nDNA fails [147-149]. MtDNA is also circular in nature, where nDNA is linear, and therefore mtDNA is thought to be less susceptible to degradation relative to nDNA [150]. Unlike nDNA, mtDNA is inherited maternally and does not undergo recombination [148, 151]. Because mtDNA is maternally inherited and is identical among maternal relatives (barring mutation), the power of discrimination when conducting mtDNA testing for human identification purposes is greatly reduced compared to STR typing [148]. MtDNA has been used in several forensic applications such as identifying skeletal remains from mass disasters, missing persons, and historical cases [150, 152, 153]. One study conducted by Foran et al. investigated the use of mtDNA typing with IEDs. However, the appropriateness of the methods, specifically the use of nested PCR, used to obtain usable

data was questioned as nested PCR can cause false peaks that are indistinguishable from true alleles [154].

Forensic analysis typically focuses on the noncoding region of the mitogenome. Within this control region, lies hypervariable regions 1, 2, and 3 (HVI, HVII, and HVII, though forensics primarily analyzes the first two). The hypervariable regions mutate at a much higher can then be identified via Sanger sequencing and compared to the Revised Cambridge Reference Sequence (rCRS) for identification purposes [146, 155]. Furthermore, advancements in sequencing technology also allow a more in-depth analysis of the mitogenome [148, 156, 157]. Panels such as the Precision ID mtDNA Control Region Panel (Thermo Fisher Scientific) [158] and the PowerSeq™ CRM Nested System (Promega) [159] analyze the entire control region. These advancements also allow researchers and practitioners to look beyond the control region and interrogate the whole mitogenome (coding and noncoding regions) using panels such as the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific) and the soon-to-be released ForenSeq™ mtDNA panel (Verogen).

Bi-allelic Markers

Single Nucleotide Polymorphisms

SNPs are single base variations in the genome that also enable shorter amplicons (< 150 bp) [145], lower stutter, and lower mutation rates compared to STRs [104]. These characteristics make SNPs well suited for use with challenging samples, such as low-template and degraded DNA [103, 160]. Because SNPs are bi-allelic, more loci are required to have comparable discrimination power to STRs [104]. Approximately 50 SNP loci produce analogous likelihood ratios to 12 STR loci [123]. However, SNPs have also

been assessed to bolster the statistical strength of inconclusive or incomplete STR results [119, 145, 161]. Studies such as these have demonstrated the ability of SNPs to provide probative genetic evidence either alone or in tandem with conventional STR methods.

SNPs are used in several forensic applications including individualization [119, 162, 163], determination of biogeographical ancestry [164-167], and predicting physical traits through phenotyping [104, 166, 168-173]. Employing SNPs for DNA phenotyping can also suggest additional information about an individual's external characteristics, such as eye color or skin color, in cases where the physical appearance is unknown [168, 169, 173-175]. This information can be a valuable tool in forensics when applied to cases where eyewitness testimony is unreliable, or no database "matches" for identification are made. SNPs are therefore a valuable tool for HID and forensic intelligence purposes when analyzing highly degraded samples, including skeletal remains from mass disaster victims and missing persons cases [176], and possibly from evidence recovered from weapons, firearms, and explosives.

SNPs analysis methods include Sanger sequencing, minisequencing or, more recently, massively parallel sequencing (MPS). Although Sanger sequencing via CE has been the standard technology for over 20 years, it can be time consuming and expensive [177]. More commonly used in forensics are single base extension (SBE) reactions [162, 178]. While it is advantageous that this method is compatible with technology already available in forensic laboratories, the throughput is relatively low. In addition, there are several clean-up steps, which can make the process laborious [179]. Newer technologies such as MPS have emerged that are altering the future of SNP typing, and forensic DNA analysis in general. These advancements may be particularly beneficial with samples

recovery from touched items, firearms, and IEDs, particularly when traditional analyses fail.

Insertion/Deletion Polymorphisms

INDELs are alternative markers that may be beneficial to LT-DNA analysis and highly degraded samples. INDELs are length variations created by the insertion or deletion of one or more nucleotides in a DNA sequence [103, 180, 181]. INDELs are bi-allelic, do not create stutter peaks, have low mutation rates, and have the potential for small amplicon sizes less than 200 bp [103, 160, 182]. They also have the ability to provide biogeographical-ancestral information, which is potentially advantageous in crimes, including those involving firearms and IEDs [103, 183]. INDELs have predominately been explored in medical research to determine any contributions on human traits and diseases [180, 184]. However, there is potential for them to be implemented within the forensic community for HID and ancestry purposes [103, 181, 183]. Due to their abundance in the human genome and the ability to generate small amplicons [180, 184], they are potentially advantageous when amplifying low-template and other challenging DNA samples. This may be particularly useful when recovering DNA from post-blast pipe bomb fragments when high quality and quantity DNA is not expected.

Presently, the Investigator® DIPplex Kit (QIAGEN) is the only commercial INDEL kit available. However, previous studies have created robust in-house INDEL multiplexes to analyze high quality and degraded DNA samples [103, 160, 185]. Pereira et al. developed a multiplex comprised of 38 bi-allelic INDELs and demonstrated success with identifying degraded samples from skeletal remains and paraffin-embedded tissues. INDEL analysis produced more complete and higher quality profiles compared to a

commercial STR kit thus demonstrating their potential with challenging DNA evidence [160].

Retrotransposable Elements and Insertion/Null Polymorphisms

Retrotransposable elements (REs) are repetitive mobile genetic elements that are able to insert into other areas of the genome. REs are found in both plants and mammals, and make up roughly 40% of the human genome [186]. There are two classes of REs: long terminal repeat (LTR) retrotransposons, which are less abundant in humans, and non-LTR retrotransposons [187]. Non-LTR retrotransposons in the human genome include long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), and SINE-VNTR-*Alu* elements (SVAs) [188]. Unlike other transposable elements, REs transpose using RNA intermediates [186] and therefore must utilize reverse transcriptase which has a very high error rate. Because of this high error rate, REs are highly diverse throughout the genome [187-190]. LINEs are large elements that range from 4-6 kb in size and contains two open reading frames (ORFs), one of which encodes for the reverse transcriptase need for autonomous replication [186-188, 190]. Smaller than LINEs, SINEs are typically no more than 500 bp in size [186, 191]. While SINEs also require an RNA polymerase for amplification, they do not contain ORFs. Instead, SINEs are non-autonomous and must "borrow" ORFs from LINEs in order to replicate [189]. The most common SINE are *Alu* elements [102]. In fact, *Alu* repeats are the most abundant mobile element, making up approximately 10% of the human genome [186].

The repetitive nature of REs have made them an important in cancer and genetic disease research [189, 192], but have more recently been re-examined for forensic purposes. REs were initially problematic for forensic applications because of their size (>

400 bp) and the size difference between the insertion and null (INNUL) alleles would cause issues in a multiplex [102]. However, novel primers have been designed to overcome these issues and generate small amplicons, making them compatible with current forensic DNA technology [193]. These primers were used to create the InnoTyper® 21 kit (InnoGenomics, LLC), a novel multiplex system that containing twenty INNULs and one sex determination marker all under 125 bp [194], which is particularly advantageous when amplifying low-quantity and degraded DNA. Similar to SNPs and INDELs, these bi-allelic markers do not create stutter peaks and have low mutation rates [102, 193]. In addition, INNULS are also able to provide bio-ancestral information [102], which may be useful when recovering DNA from post-blast fragments when no identification is made, and further intelligence may be of assistance during an investigation.

Alternate Amplification and Genotyping Strategies

Direct Amplification

Standard DNA processing includes collection, extraction and purification, quantification, and amplification steps. Several studies have reported substantial DNA loss during the extraction process [78, 195-197]. It has also been suggested that cell-free DNA specifically is discarded and lost during the wash steps [57, 195, 198]. As a result, low level "touch" DNA samples would be the most vulnerable samples as any additional DNA loss could result in reduced or no DNA available for amplification. Furthermore, a small volume (2 μL) of DNA extract is required for DNA quantification, which also decreases the amount of template available for amplification [199]. Therefore direct amplification strategies have been designed to reduce the amount of time at the bench and DNA loss by

eliminating extraction and quantification steps, as well as reducing the risk of contamination events [200-202].

Direct amplification is widely used for processing reference samples (buccal swabs and FTA® cards), as the DNA is obtained from a known source, is relatively high quality DNA and the risk of having insufficient quantities of DNA and producing a poor-quality is minimal [112, 203]. There are many direct amplification kits on the market designed specifically for reference samples including GlobablFiler® Express (Thermo Fisher Scientific), Investigator® 24plex GO! (QIAGEN), and PowerPlex® 18D (Promega). A small cutting from the collector, such as a reference swab or a punch from an FTA® card (Whatman®) is placed into the amplification reaction for rapid processing. More recently, microFLOQ® Direct Swabs have been described as an optimal collection method for direct amplification of reference samples [35, 204]. Unlike other collectors, no cutting or punch is necessary as the entire swab head breaks off into PCR strip tubes.

When precious samples are involved (i.e. “touch” samples) it is crucial that measures are taken in order to prevent DNA loss, and it has been questioned if DNA extraction is necessary. As it is unlikely that large quantities of DNA will be obtained from touched items, and DNA may be lost during extraction and quantification, direct amplification has been suggested as a method to maximize the amount of DNA available for PCR [35, 200, 205, 206]. The first use of direct amplification for casework-type samples was described in 2010. In this initial study, a small cutting of fabric was placed directly into the amplification reaction, and full STR profiles were able to be produced without prior extraction or quantification [200]. There are now numerous studies that explore the utility of direct amplification with evidentiary samples [35, 94, 95, 202-204,

207-210], including IED substrates [51, 52] and brass cartridge casings [205]. This approach has the potential to increase DNA template in PCR without the stochastic effects associated with increasing the number PCR cycles or increasing the injection time during CE [201, 211]. However, direct amplification is not widely accepted for use with non-reference samples due primarily to the FBI Quality Assurance Standard 9.4 requiring DNA quantification [212].

Though studies have demonstrated success with directly amplifying DNA from challenging samples, there are concerns about using this approach for casework-type evidence. PCR Inhibitors such as copper and other contaminants can remain because purification steps have been bypassed. This can cause stochastic effects such as extreme peak height imbalance or complete amplification failure [204, 213].

Massively Parallel Sequencing

CE continues to be the method of choice for separating and detecting amplified DNA fragments for HID. However, there are limitations with this technology including the limited number of fluorescent dye channels and the required size separation between loci [214]; both of which restrict the number of loci that can be multiplexed. Over recent years, the forensic community has embraced massively parallel sequencing (MPS) technologies. MPS platforms of forensic interest include the MiSeq FGx™ Forensic Genomics System (Verogen®), the Ion Torrent Personal Genome Machine (PGM™) System, the Ion GeneStudio S5 System (Thermo Fisher Scientific), and the GeneReader® (QIAGEN).

All three platforms use a variation of sequencing-by-synthesis (SBS) technology [215, 216]. The MiSeq FGx™ (Fig. 1.2) and GeneReader® (Fig.1.3) systems employ fluorescent tags to detect when a nucleotide is incorporated. One key distinction between

the two platforms is that the Miseq ensures that all template strands incorporate a labeled nucleotide whereas the GeneReader® only provides enough to achieve detection [215]. Conversely, Ion Torrent sequencing uses a semiconductor based platform that detects a change in pH when a nucleotide is incorporated and the signal is translated to a base call (Fig. 1.4) [179].

Unlike CE, MPS does not utilize dye channels and requires no size separation between amplicons [214]. In addition, it is able to analyze large numbers of samples in parallel [217], allowing for a higher throughput of information compared to CE-based sequencing [218]. Several studies have explored the utility of MPS for the deeper interrogation of the mitogenome [148, 157, 219-223], STRs [224-230], greater resolution of DNA mixtures [222, 231-236], and the identification of previously unreported variations of alleles [237, 238]. In addition, MPS may also be used to probe biological samples using various SNP panels to indicate ancestral origin, kinship and lineage [239-244], hair, eye and skin color [239, 245], and individualization [219, 239, 246, 247].

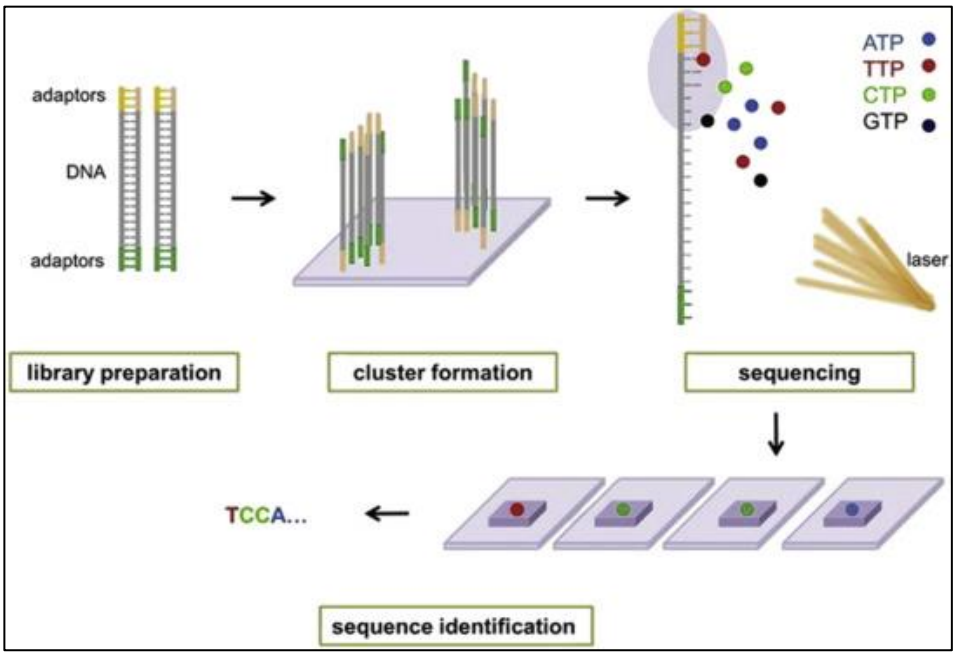

Fig. 1.2 Illumina® MiSeq FGx sequencing-by-synthesis (SBS) chemistry whereby bases are called when fluorescence is detected once a nucleotide is incorporated [248].

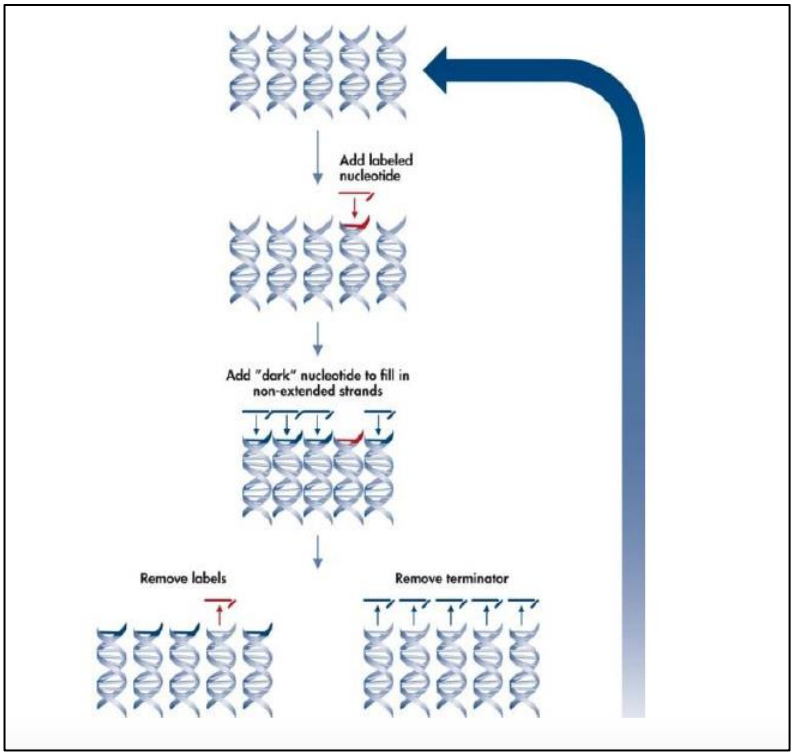

Fig.1.3 QIAGEN GeneReader® sequencing-by-synthesis chemistry whereby bases are called when fluorescence is detected once a nucleotide is incorporated [249].

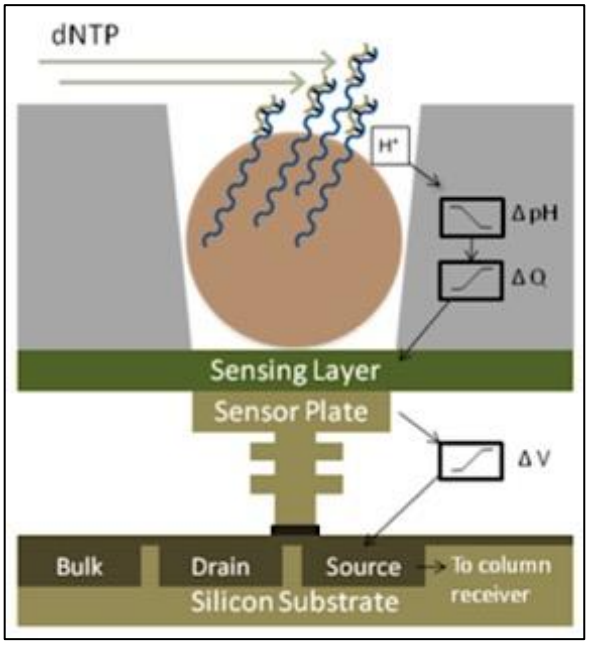

Fig. 1.4 Ion Torrent semiconductor sequencing whereby nucleotide incorporation causes a proton to be released and the change in pH is detected [250].

The MiSeq FGx™ and Ion Torrent systems have marker panels designed specifically for HID, including various STR and/or SNP HID markers. MPS has already shown potential with highly degraded DNA samples [214, 251, 252]. One such study reported the ability of the Ion PGM™ System to sequence damaged and degraded low-level DNA samples from ancient remains [253]. Though promising results for degraded samples have been reported, these panels may require relatively large amounts of DNA (1–10 ng) [179]. Studies have reported high partial SNP profiles (> 90% complete) with as little as 100 pg of DNA [163, 254, 255] including one study conducted by Thanakiakrai et al. [53]. In this study, buccal cells and control DNA were spiked in varying quantities (500 – 8 pg) onto several common IED substrates. The DNA was extracted and analyzed using the traditional STR-CE approach and MPS via the MiSeq FGx™. The authors reported full profiles with as little as 250 pg and stochastic variation appearing around 100 pg. However, other studies observed better reproducibility is achieved with at least 0.5 ng of DNA [256,

257]. In order for this technology to be successful analyzing touch DNA samples such as may be recovered from firearms and IEDs, greater sensitivity may be needed as it is likely that DNA yields will be much lower than 0.5 ng.

Using an MPS approach for mtDNA analysis for very challenging DNA samples is also beneficial. When analyzing mtDNA, MPS can be used to sequence the entire mtDNA genome relatively quickly, and better identify true heteroplasmies, or point mutations, within a sequence due to a much deeper coverage than traditional sequencing methods [152]. Success with mtDNA analysis via MPS has already been demonstrated with low-level DNA quantities from ammunition. Holland et al. reported degradation effects from the metal casings but were able to obtain 80-96% mtDNA profile completeness using MPS [131].

Another forensic advantage of MPS is the use of SNPs to predict ancestral information. When traditional analyses fail to yield probative results, ancestral information could be used to provide investigative leads [178, 258]. While the ability to predict ancestry already exists, MPS has the ability to examine higher numbers of ancestral informative markers (AIMs) from more samples. This information may also be useful when analyzing low amounts of DNA. However, more research analyzing low-template DNA samples with MPS technologies is still needed to determine the viability of these methods for low-level and/or degraded samples [252].

Statement of the Problem

Due to the accessibility of materials and ease of assembly, pipe bombs will likely continue to be the most commonly used IED by U.S. domestic terrorists. In addition, the United States also experiences high levels of gun violence and gun-related violence,

especially relative to other countries [259]. While other forensic analysis methods can be employed after a gun-related crime or an explosive event, DNA has the ability to provide information for identification and investigative purposes. Finding new, improved, and more informative techniques for DNA collection, extraction, and genotyping can provide more probative investigative leads as to the identity of the manufacturer or user of such devices. When an item such as a weapon is touched, the amount of DNA transferred varies and can greatly affect the quality of a genetic profile [54]. Typically, only a few cells are transferred and trace quantities of DNA are recovered from touched items, such as mobile phones and firearms [54, 55]. As it is unlikely to recover high quality/quantity DNA from these, it is necessary to optimize DNA recovery and genotyping methods from these challenging samples.

Recovery and isolation of DNA from touched items and compromised evidence frequently results in loss of DNA and poor downstream analysis. These unreportable or failed results may prevent investigators from being able to identify a vital person of interest, suspect, or possible terrorist. Various methods currently used to collect and process DNA from such challenging samples are often inadequate, or existing protocols are not optimized for maximal recovery of trace amounts of DNA from various substrates commonly encountered with explosive devices and firearms. While cotton swabs and tape-lifts are routinely used to collect DNA from handled items [41, 80, 87], it has been posited that DNA may get trapped in the tightly woven fibers or the sticky adhesive and is not being released during the extraction process [89]. In addition, common purification and automated silica-based extraction methods routinely employed in crime labs have been shown to result in significant DNA loss, and therefore development of new protocols to

improve the efficiency of DNA collection, isolation, and amplification for “touch samples” that improve downstream genotyping is needed [260]. To this end, research that explores more direct sampling and amplification strategies for “touch” evidence is required for the transition of technology and implementation of these improved methods into crime laboratories to achieve higher success rates from such challenging evidence.

Although short tandem repeats (STRs) have been the primary genetic marker used for human identification (HID) for over 15 years in the field of forensic genetics, commercial STR kits continue to evolve to include more (and smaller size amplicon) loci to improve sensitivity of detection, tolerance to PCR inhibitors, reduce the chances of adventitious matches, and increase the power of discrimination (PD) [106, 113, 261]. Sensitivity of a STR system is critical for success when genotyping questioned samples in crime laboratories such as “touch” evidence when DNA is frequently in very low amounts [262]. However, despite this increased sensitivity, DNA amplification can still result in a variety of stochastic effects that complicate the interpretation of electropherograms from “touch” DNA samples [54, 116, 117]. Therefore, it is vital to optimize protocols and explore newer devices, technologies, and methods that will maximize the amount of DNA collected from touch evidence (and retained during the analytical processes) in order to provide the most probative information for criminal investigative leads. Such advancements include alternate collection devices, faster and more efficient elution processes, direct amplification, and alternate (and more sensitive) marker systems.

In addition, newer technologies such as next-generation or massively parallel sequencing (MPS) also allow for higher throughput of samples and can mine deeper information from each sample. MPS panels can be highly multiplexed (~200 loci) and

contain small amplicon loci (< 200 bp) proving advantageous when genotyping degraded DNA. Additionally, alternative markers such as insertions/nulls (INNULS) have also demonstrated potential for interrogating low quantity/quality DNA because of the small amplicon size (< 125bp) and sensitive assay design. However, little work has been done to investigate how well these alternate genotyping systems may work when coupled with more effective DNA collection methods, more direct sampling, and alternate genotyping strategies from “touch” evidence.

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CHAPTER II

Analysis of DNA from post-blast pipe bomb fragments for identification and determination of ancestry[1]

This dissertation follows the style and format of *Legal Medicine*.

[1]E. Tasker, B. LaRue, C. Beherec, D. Gangitano, S. Hughes-Stamm, Analysis of DNA from post-blast pipe bomb fragments for identification and determination of ancestry, Forensic Sci. Int. Genet. 28 (2017) 195-202.

Abstract

Improvised explosive devices (IEDs) such as pipe bombs are weapons used to detrimentally affect people and communities. A readily accessible brand of exploding targets called Tannerite® has been identified as a potential material for abuse as an explosive in pipe bombs. The ability to recover and genotype DNA from such weapons may be vital in the effort to identify suspects associated with these devices. While it is possible to recover DNA from post-blast fragments using short tandem repeat markers (STRs), genotyping success can be negatively affected by low quantities of DNA, degradation, and/or PCR inhibitors. Alternative markers such as insertion/null (INNULs) and single nucleotide polymorphisms (SNPs) are bi-allelic genetic markers that are shorter genomic targets than STRs for amplification, which are more likely to resist degradation. In this study, we constructed pipe bombs that were spiked with known amounts of biological material to: 1) recover "touch" DNA from the surface of the device, and 2) recover traces of blood from the ends of wires (simulated finger prick). The bombs were detonated with the binary explosive Tannerite® using double-base smokeless powder to initiate the reaction. DNA extracted from the post-blast fragments was quantified with the Quantifiler® Trio DNA Quantification Kit. STR analysis was conducted using the GlobalFiler® Amplification Kit, INNULs were amplified using an early-access version of the InnoTyper™ 21 Kit, and SNP analysis via massively parallel sequencing (MPS) was performed using the HID-Ion Ampliseq™ Identity and Ancestry panels using the Ion Chef and Ion PGM sequencing system. The results of this study showed that INNUL markers resulted in the most complete genetic profiles when compared to STR and SNP profiles. The random match probabilities calculated for samples using INNULs were lower than

with STRs when less than 14 STR alleles were reported. These results suggest that INNUL analysis may be well suited for low-template and/or degraded DNA samples and may be used to supplement incomplete or failed STR analysis. Human identification using SNP analysis via MPS showed variable success with low-level post-blast samples in this study (<150 pg). While neat DNA samples (6 µL input as recommended) resulted in < 50% of SNP calls, samples that were concentrated from 15 µL to 6 µL (15 µL was added for STR and INNUL typing) resulted in more complete SNP profiles. Five out of six blood samples recovered from the wires attached to the pipe-bombs resulted in the correct ancestry predictions.

Keywords: forensic science; DNA typing; improvised explosive devices; short tandem repeat; insertion/null polymorphism; single nucleotide polymorphism; massively parallel sequencing

Introduction

Improvised explosive devices (IEDs) are weapons commonly used in crimes to cause bodily harm or death, property damage, or instill fear in communities [1-6]. There are several forms of IEDs, but pipe bombs are the most commonly used devices due to the free availability of materials, such as explosive powder, and ease of assembly [1, 2].

Ammonium nitrate and aluminum powder are commonly used to manufacture homemade explosives [3, 4]. While materials such as smokeless gunpowder are more commonly used in pipe bombs [5], newer commercial products such as Tannerite® (Tannerite® Sports LLC, Pleasant Hill, OR) may also warrant concern. Tannerite® is a patented brand of binary reactive targets comprised of ammonium nitrate and aluminum powder, and it is marketed as shot indicators for licensed gun enthusiasts. Due to the wide accessibility and no purchasing restrictions for Tannerite® within the United States, these exploding targets have recently been identified as a potential explosive powder for use as IEDs [6].

When an item such as a pipe bomb is touched by a suspect (during assembly or placement), the amount of DNA transferred from the individual to the device varies and can greatly affect the quality of a resulting genetic profile [7-11]. Typically, only a few cells are transferred and trace quantities of DNA or low-template DNA (LT-DNA) are recovered from touched items, such as mobile phones and firearms [2, 7, 12-15]. Low-template and/or degraded DNA samples often produce incomplete and/or poor quality genetic profiles due to stochastic effects such as allele and/or locus drop-out, allele drop-in, and peak height imbalance, or no amplification [7, 16-20].

While several studies have examined various ways to improve the recovery and genotyping of low-template and degraded DNA samples [12, 16, 17, 19, 21-24], few have focused specifically on recovering DNA from IED post-blast debris [1, 25, 26]. Furthermore, the studies that have assessed DNA recovery from post-blast fragments only focused on using standard DNA collection and genotyping methods [1, 25-27].

Several methods have been suggested to improve LT-DNA analysis including increasing the number of PCR cycles, increasing the injection time during capillary electrophoresis, and reducing the PCR reaction volume [20]. However, these methods may (in some cases) exaggerate stochastic effects and result in increased stutter peaks, peak height imbalance, and drop-in alleles [19]. Another approach to retrieving more genetic information from challenging DNA samples such as those recovered from IEDs is to employ alternative molecular markers. These alternative genetic markers are insertion/null (INNUL) markers or single nucleotide polymorphisms (SNPs). INNULs are retrotransposable elements consisting of long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) [28, 29]. INNULs are bi-allelic markers that do not possess stutter peaks and have low mutation rates. Due to their abundance in the human genome and the ability to generate small amplicons, they are potentially advantageous when amplifying LT-DNA and degraded samples. The InnoTyper® 21 kit (InnoGenomics Technologies) used in this study is a commercially available small amplicon (60 – 125 bp) DNA typing system that has demonstrated some success with forensic samples [30-34]. SNPs are single base variations in the genome that also enable shorter amplicons (< 150 bp) [35], no stutter, and lower mutation rates compared to STRs [36]. As with INNULs, these characteristics make SNPs well suited for use with

challenging samples, such as low-template and degraded DNA [37, 38]. Furthermore, SNPs analyzed via massively parallel sequencing (MPS) has also shown some success with retrieving information from challenging DNA samples [39-41].

The aim of this study was to compare the success of genotyping DNA recovered from post-blast pipe bomb fragments using standard short tandem repeats (STR), and alternate markers such as INNULs, and SNPs via MPS analyses for human identification (HID) and ancestry.

Materials and Methods

Biological Samples

Buccal swabs collected from a single male Caucasian donor were collected. Blood added to the wires was collected from three ethnically different individuals (Asian, Caucasian, and African-American).

Epithelial Cell Suspension

A method for creating a homogeneous cell suspension from buccal swabs was adapted from a previous study examining the success of DNA recovery from post-blast bomb fragments [1]. This method was chosen to ensure a controlled number of epithelial cells was added to each device. Briefly, buccal cells were collected from one donor using three cotton swabs, with each swab being placed in a 1.5 mL tube with 1 mL of 1 X phosphate buffered saline (PBS) (pH 7.4) and briefly vortexed. The swabs were removed and centrifuged (12,400 × g) for 3 min to pellet the cells. The supernatant was removed, 1 mL of nuclease-free water was added, and vortex and centrifugation steps were repeated to wash the cells. The cells were finally re-suspended in 1 mL 1 X PBS solution. This process was followed in parallel for all three buccal swabs. Finally, the cell suspensions

were then combined into one tube. Cells were stained by adding 15 µL of 1 % Methylene Blue (Kordon®, LLC, Hayward, CA) to 15 µL of cell suspension and counted with a hemocytometer (Gizmo Supply Co., Fountain Valley, CA) under a Leica DM 750P compound light microscope (Leica Microsystems, Wetzlar, Germany) at 100 X magnification using a standard cell counting method [42]. Three cell counts were performed and averaged to estimate the total concentration of cells in suspension. The same cell suspension was used to spike all pipe bombs used in this study.

Pipe Bomb Preparation

Polyvinyl chloride (PVC) pipes (60 mm diameter) were cut to 20 cm lengths and a hole (1 cm diameter) was drilled in the center of one end cap (N = 13). The pipes and end caps were washed with Alconox® detergent (Alconox, Inc. White Plains, NY), soaked in a 15 % bleach solution for 30 min, wiped with 70 % ethanol, and UV-treated in a cross-linker (UVP, LLC., Upland, CA) for 10 min, rotating each pipe after 5 min. The pipe bomb casings were partially assembled by applying several coats of Oatey® Purple Primer (Oatey®, Cleveland, Ohio) and Fast Set Heavy Duty Gray PVC Cement (Oatey®) to one end of the pipe and on the inside of the intact end cap. The end cap was twisted onto the pipe end and held in place for 30 s. Four circles were engraved on each end cap and three on the pipe shaft to denote where the epithelial cells would be spiked (Fig. 2.1). These engraved circles assist with post-blast identification of cells' original location. A 20 µL aliquot of cell suspension (approx. 42 cells/µL) was added to each circle and left to dry in a sterile hood overnight.

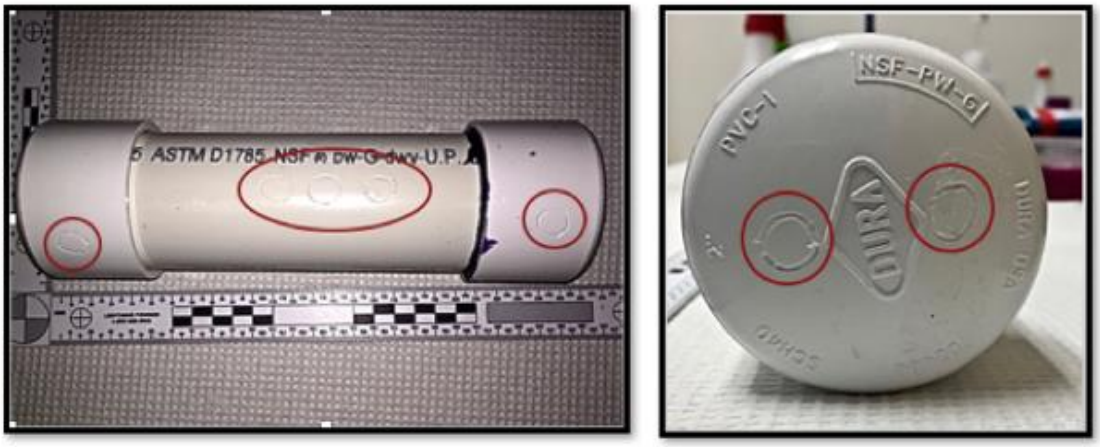

Fig. 2.1 Location of engraved circles on pipe bombs where biological material was deposited. Four circles were engraved on each end cap (two on the end and two on the join), and three were engraved on the shaft.

Wire Preparation

Insulated copper wire was cut into 8 cm segments and cleaned with 15 % bleach, 70 % ethanol, and then treated in the UV cross-linker for 10 min. The ends of the wires (approx. 5 mm) were stripped with sterile pliers and spiked with 10 µL of neat blood from one of three sources of blood and left in a sterile hood to dry overnight.

Controls

In addition to the 10 test bombs, one positive control and two different negative controls (no biological material added) pipe bombs were used in this study. The positive control was spiked with epithelial cells, but not detonated. Only one of the negative controls was detonated. The detonated negative control monitored any DNA contamination occurring during the bomb preparation, detonation and evidence collection. The other negative control was included to ensure that the decontamination process was successful.

Bomb Assembly, Detonation, and Collection

Montgomery County Fire Marshalls filled each pipe bomb with 113.4 g Tannerite® binary powder and 28.5 g of wrapped double-base smokeless powder. One wire was attached to the pipe shaft of each bomb with electrical tape (Fig. 2.2 A). Detonation was conducted in a secure location in Montgomery County, Texas. Each bomb was placed in one corner of a cement reinforced stall (approx. 1.5 m x 2 m). A sand bag was placed over each pipe bomb to minimize the dispersion of fragments (Fig. 2.3). After detonation, the pipe bomb fragments were collected and placed in sterile evidence bags at the scene using sterile forceps and sieves that were cleaned with 15 % bleach and 70 % ethanol. The wires were placed in separate evidence bags to avoid cross-contamination with bomb fragments. All fragments were transported to a dedicated low-template DNA pre-PCR laboratory for sample collection and processing. Reference buccal swabs were collected from all personnel involved in the experiment for elimination purposes.

Fig. 2.2 (left) Location on bomb inside stall (right) sandbag on top of bomb before detonation.

Fig. 2.3 Construction of pipe bomb with wire attached (left), and (right) post blast fragments from one bomb (right).

DNA Extraction

Cotton swabs treated with 30 µL of 2% sodium dodecyl sulfate (Sigma-Aldrich, St. Louis, MO) were used to swab pipe bomb fragments with evident intact or partial circles (engraved to indicate areas where epithelial cells were placed), and both ends of the copper wires. One swab was used per circle on the plastic casing or wire. DNA from the swabs was extracted using the QIAamp® DNA Mini Kit (Qiagen Inc., Hilden, Germany) following the buccal swab protocol with one modification [43]. An additional centrifugation step with a spin basket was added before the washing steps (6000 × g for 1 min) to maximize the recovery of lysate from the head of each swab. The swab was discarded after this step and the remaining steps were performed according to manufacturer instructions with a final elution of 70 µL.

DNA from pipe bomb fragments with no evidence of engraving, and too small for swabbing were extracted using a soaking approach with the Qiagen DNA Blood Maxi Kit (Qiagen Inc., Hilden, Germany) using a modified version of the whole blood protocol [44]. This soaking protocol was adapted from Montpetit and O'Donnell [45]. For each bomb, small fragments were collected and placed into two to eight 50 mL conical tubes

(depending on the number of small fragments). A cocktail of 10 mL 1 X PBS, 500 µL of Qiagen Protease, and 12 mL of Buffer AL were added to one of the tubes. Each tube was inverted 15 times, shaken vigorously for 1 min, and incubated for 10 min at 70 ˚C. Then, the liquid was poured into the next tube containing more small fragments from the same bomb, and the inversion, shaking, and incubation steps were repeated until all small fragments from the same bomb were extracted. The digestion buffer was then decanted from the plastic fragments into a clean 50 mL tube and 10 mL ethanol was added, inverted 10 times and shaken for 10 s. Approximately half of the solution (16 mL) was transferred into the QIAamp® DNA Blood Maxi spin column and centrifuged for 3 min (1,850 x g). The filtrate was discarded and the remaining solution from the tube (digestion buffer and ethanol) was transferred onto the same column and centrifuged. The final elution volume was 1 mL. These samples were transferred to 1.5 mL tubes and concentrated to 70 µL using the CentriVap® Centrifugal Concentrator (Labconco Corporation, Kansas City, MO) at 50 ˚C for 2.5 hr.

DNA Quantification

DNA extracts (2 µL) were quantified via real-time PCR using the Quantifiler® Trio DNA Quantification Kit (ThermoFisher Scientific, Waltham, MA) on a 7500 Real-Time PCR System (ThermoFisher Scientific) using cycling conditions recommended by the manufacturer [46]. Degradation was assessed using the Degradation Index (DI) and inhibition was evaluated using the Internal PCR Control (IPC) [46]. Samples with an IPC target < 2 ΔC_T (compared to the average of the 5 ng, 0.5 ng and 0.05 ng standards) were considered uninhibited [46].

STR Analysis

Neat DNA (15 µL) was amplified in a 25 µL reaction volume using the GlobalFiler® PCR Amplification Kit (ThermoFisher Scientific) on a GeneAmp® PCR System 9700 (ThermoFisher Scientific) according to manufacturer's recommendations [47]. Amplified DNA products were separated on the 3500 Genetic Analyzer (ThermoFisher Scientific) using a 36 cm capillary array (ThermoFisher Scientific) with POP-4 polymer (ThermoFisher Scientific), a 5 s injection time at 15 kv. Data analysis was performed using GeneMapper® ID-X v 1.4 software (ThermoFisher Scientific) with an analytical threshold of 150 RFUs and a stochastic threshold of 600 RFUs. STR success was determined by the number of reportable alleles detected, the heterozygote peak height ratio (PHR) and random match probabilities (RMPs). RMPs were calculated using allele frequencies from the GlobalFiler® PCR Amplification Kit User Guide [47]. Tests for statistical significance was determined using *Student's t-test*. A p-value of 0.05 was set for statistically significant differences.

INNUL Analysis

Neat DNA (16 µL) was amplified in a 25 µL reaction volume using an early access version of the InnoTyper™ 21 Kit (InnoGenomics Technologies LLC, New Orleans, LA) using manufacturer's recommendations [48]. Amplified DNA products were separated on the 3500 XL Genetic Analyzer (ThermoFisher Scientific) using a 36 cm capillary array with POP-4 polymer. Data was analyzed with GeneMarker® HID software (SoftGenetics, State College, PA, USA) with an analytical threshold of 50 RFUs and stochastic threshold of 600 RFUs. RMPs for the epithelial "touch" samples were calculated using allele

frequencies from the Caucasian population [49]. INNUL analysis was not performed on the blood samples.

SNP Analysis via MPS

For the pipe bomb fragments, four DNA extracts were used for HID analysis via MPS and compared to STRs and INNULs data. For the copper wires, the two samples from each blood source with the highest DNA concentrations and highest STR success were chosen for ancestry determination.

A master mix for amplification was prepared by adding 4 µL of 5X Ion AmpliSeq™ HiFi Mix (ThermoFisher Scientific) and 10 µL of HID-Ion AmpliSeq™ Identity Panel or HID-Ion AmpliSeq™ Ancestry Panel (ThermoFisher Scientific). The library preparation was performed according to manufacturer's protocol (35). For the DNA samples amplified with the AmpliSeq™ Identity panel, two different volumes of DNA extract were used: 1) 6 µL of neat DNA extract, and 2) 15 µL (the same volume used for STR and INNUL typing) was first to 6 µL using the CentriVap® Centrifugal Concentrator prior to amplification for library preparation.

The libraries were then diluted to 20 pM and pooled together in a single tube (25 µL) and added to the Ion Chef™ System (ThermoFisher Scientific). Two barcoded 316 semiconductor chips and reagents of the Ion PGM™ Hi-Q™ Chef Kit were placed on the Ion Chef™ to perform the template preparation. The chips were then placed on the Ion PGM™ for sequencing. The Torrent Suite™ Software v4.6 (ThermoFisher Scientific) and HID SNP Genotyper plugin v4.3.1 (ThermoFisher Scientific) was used for analysis for both panels using a default threshold of 10 reads.

Results and Discussion

The ability of traditional STR analysis to genotype degraded and LT-DNA from post-blast pipe bomb fragments was assessed via the completeness and quality of STR profiles. The comparative success rates of INNUL markers and SNPs (via MPS) for HID were also examined. Furthermore, the effectiveness of an MPS SNP-based ancestry panel to predict ancestry of blood recovered from post-blast wires originating from three ethnically distinct individuals was also investigated.

Recovery from Post-blast Fragments

The degree and pattern of damage and fragmentation varied greatly among the pipe bombs, with some devices experiencing extensive damage while others remained relatively intact (Fig. 2.2B). This variation may be due to differences in the effectiveness (or the intensity) of the explosion. One common observation was that relatively large end cap fragments were recovered, particularly from the sides of the end caps. This pattern suggests that the shaft of the bombs is less protected from the blast damage, and therefore fragmented into smaller pieces when compared to the end caps.

DNA Quantification

Pipe Bombs

From the ten test bombs detonated, a total of 83 fragments were recovered with evidence of either a full (59) or partial engraved circle (24). The samples were grouped for analysis by location (end cap or shaft), and by circle type (full or partial circle). If the location of the engraving could not be identified it was labeled “Undetermined”. Detectable amounts of DNA were recovered from 44 out of 83 fragments (53 %) ranging from 0.0002

– 0.023 ng/µL. Forty-four percent of the full circle fragments resulted in no DNA being detected while 54 % of samples with partial circles yielded no DNA for analysis.

As expected, significantly more DNA on average was recovered from the control bomb that was not detonated than from post-blast fragments originating from the end caps or shaft of the pipe bombs (0.015 ng/µL compared to 0.004 ng/µL and 0.003 ng/µL respectively) ($p < 0.01$). However, no notable difference in the average DNA concentration extracted from full circles recovered from end caps or pipe shaft fragments was observed. Bille et al. [1] recovered greater quantities of DNA from the pipe shaft rather than the end caps. This difference may be due to the different materials used to build the pipe bombs (galvanized steel pipes versus PVC pipes).

In addition to low DNA concentrations only three of the 43 samples were assessed as being severely degraded (DI > 10). The remaining samples were considered to be mildly or moderately degraded (DI range from 1 to 10). However, on average the post blast samples were assessed as being more degraded than the control bomb (average DI value of 6.09 compared to 1.94). Although more severe DNA degradation was expected, the DI assessment may have been underestimated due to the low DNA concentrations. Moreover, none of the samples were inhibited according to the real-time PCR data. Due to the low DNA concentrations, the maximum input volume of neat DNA extract (15 µL) was added to the PCR reactions for all samples for STR and INNUL analysis. No amplifiable DNA was recovered from the post-blast fragments without evidence of circle engravings.

Copper Wires

A wire spiked with blood from a donor of either Asian, Caucasian, or African-American ancestry was attached to each bomb. Quantifiable amounts of DNA were

detected (0.001 – 0.081 ng/µL) for all but one sample. In addition, no presence of human DNA was detected in either negative control, indicating that the decontamination process was sufficient, and no contamination occurred during the collection and extraction processes. Furthermore, none of the samples appeared to be significantly degraded and, PCR inhibition was not detected (based on the DI and IPC data).

STR Analysis

The success of STR typing for the blood on post-blast fragments and copper wires was evaluated using the number of alleles reported that were concordant with reference samples and heterozygote PHRs.

Pipe Bombs

Thirty-five out of 44 pipe bomb fragments that had quantifiable amounts of DNA (> 0.0002 ng/µL), resulted in STR allele calls ranging from 2 – 100 % of alleles being reported. Eight of the 44 samples failed to amplify and two samples (4 %) generated full STR profiles (Suppl. Fig. 3). The average STR success for DNA recovered from intact circles from the end caps was similar to those recovered from pipe shafts (28 % and 23 %, respectively).

Allele drop-out rates for each STR locus were also calculated to determine which STR loci were most susceptible to failure. Consistent with mild to moderate levels of DNA degradation, a slight increase in the rate of allelic drop-out was observed as the average size of the markers increased (Fig. 2.4). Considering the low quantities of DNA amplified in these samples, high rates of allele drop-out were expected. Although the slight increase in drop-out as the size of the markers increased may be indicative of some mild degradation, the allele drop-out rates observed in this study is thought to be primarily due

to low quantities of amplifiable DNA. As expected, the number of alleles detected increased as the amount of DNA increased (Fig. 2.5).

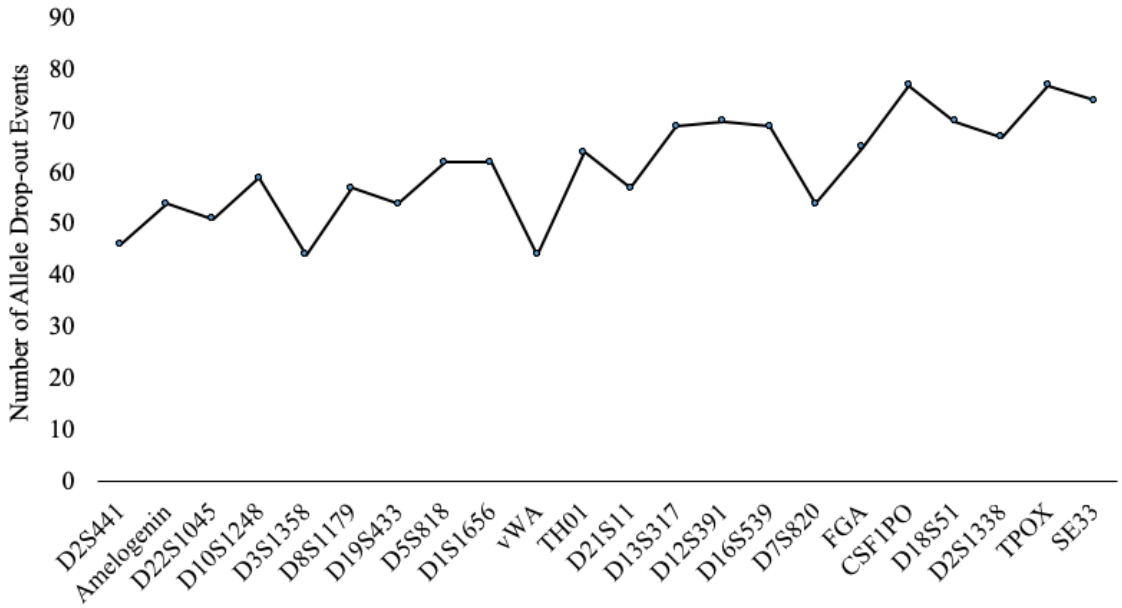

Fig. 2.4 Allelic drop-out rates at each locus in the GlobalFiler® kit from 43 post-blast fragment. The two Y-markers (DYS391 and Y-INDEL) were excluded from this analysis these markers are not autosomal.

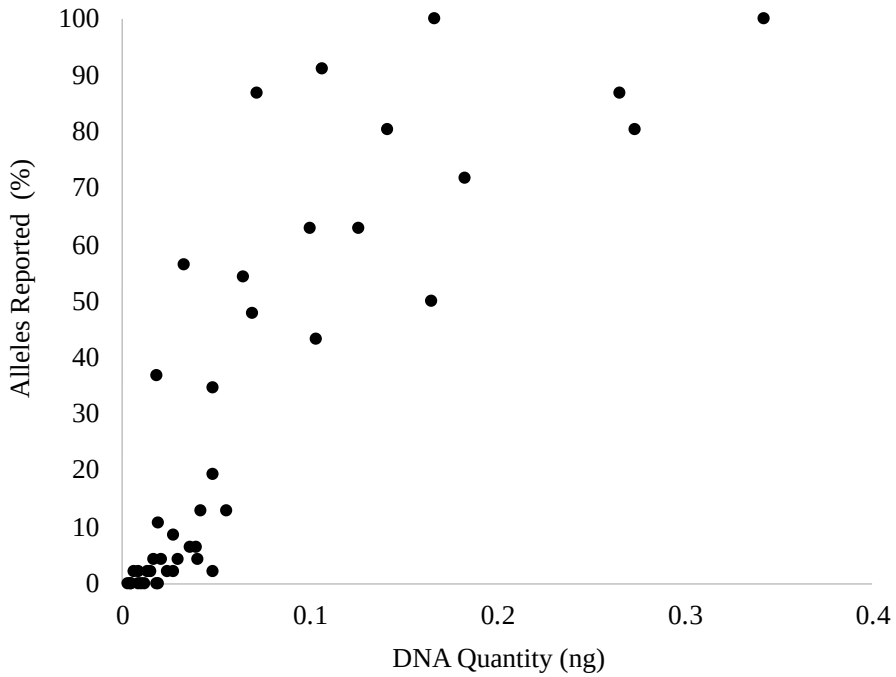

Fig. 2.5 Relationship between the number of alleles detected (as a percentage) and the amount of DNA amplified using the GlobalFiler™ kit. The two Y-markers (DYS391 and Y-INDEL) were excluded from this analysis these markers are not autosomal (N = 43).

Heterozygote peak height imbalance was observed across the majority of the STR profiles, with an average PHR of 0.19 for all 35 samples. Low peak height ratios were expected as these were low-template samples, experiencing stochastic effects such as amplification bias and allele drop-out. Many studies have reported high number of STR artifacts such as allele drop out, increased stutter peaks, and peak height imbalance when LT-DNA samples are used for STR typing [12, 19, 20]. Only one event of increased stutter was observed. This stutter peak was in the -4 bp position of the D1S1656 locus at a peak height of 12.5 % of the true peak, just exceeding the stutter filter at this locus (12.2 %).

Copper Wires

Seven out of nine wire samples produced full STR profiles, one sample yielded 82 % correct calls, and one failed STR analysis. No evidence of DNA contamination was observed with any of the samples.

INNUL Analysis

The InnoTyper™ 21 Kit contains 20 autosomal INNUL markers and amelogenin for sex determination. INNULs have been proposed as an alternate human identification system. INNULs have the potential to recover additional genetic information from challenging samples such as highly degraded skeletal and casework samples as amplicons are smaller than STRs (up to 450 bp compared to 125 bp). Twenty-five DNA extracts which resulted in varying STR success (ranging from 2 % to 87 % reported alleles) were chosen for INNUL analysis.

Genotyping using INNUL markers resulted in a high degree of success with nine of the 25 samples resulting in complete genetic profiles, and eight samples with between 80 % and 99 % of alleles being reported. Only six samples resulted in < 70 % of alleles being reported, and one sample completely failed INNUL analysis (Fig. 2.6).

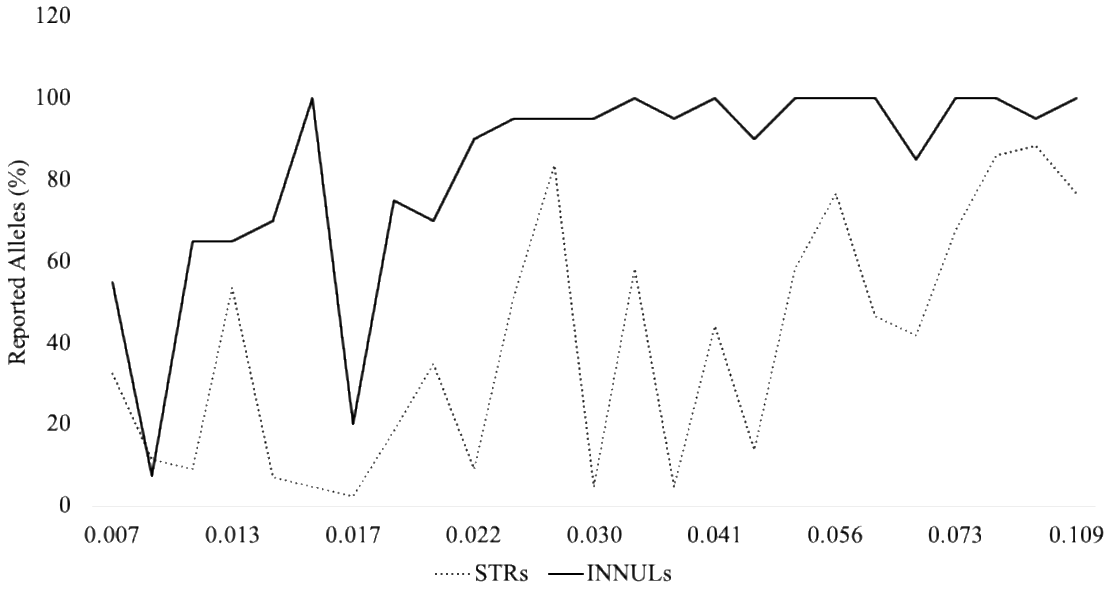

Fig. 2.6 Number of alleles reported (as a percentage) from 25 samples genotyped using STRs and INNULs ranked by increasing DNA quantity in PCR (ng). 15 µL and 16 µL of neat DNA was amplified in for STR and INNUL analysis, respectively.

DNA samples recovered from post-blast pipe bomb fragments showed significantly more complete genetic profiles when amplified with the INNULs than with the STR markers ($p < 0.001$) (Fig. 2.6). Six samples that had previously performed poorly or failed STR analysis (< 70 % of STR alleles reported) resulted in complete INNUL profiles. Compared to STRs, INNULs resulted in more complete profiles for all but one sample. These data suggest that like mini-STRs, INNULs can also amplify shorter DNA targets and may be more sensitive than STR panels to achieve greater genotyping success with lower quantities of DNA [24, 50-53]. In this study, a complete profile was produced from as little as 39 pg when STR typing of the same sample produced 7 % alleles. The results from this study support previous works [54, 55] demonstrating that INNULs show greater sensitivity to lower quantities of DNA and are more successful with degraded samples compared to STRs.

Despite more complete profiles being generated using INNULs compared to STRs, the RMPs for samples using STRs were lower than those generated by INNULs (Fig. 2.7). This is to be expected because INNULs are bi-allelic and are therefore less variable (lower power of discrimination) compared to STRs. In this study INNULs only produced lower RMPs when less than fourteen STR alleles were detected.

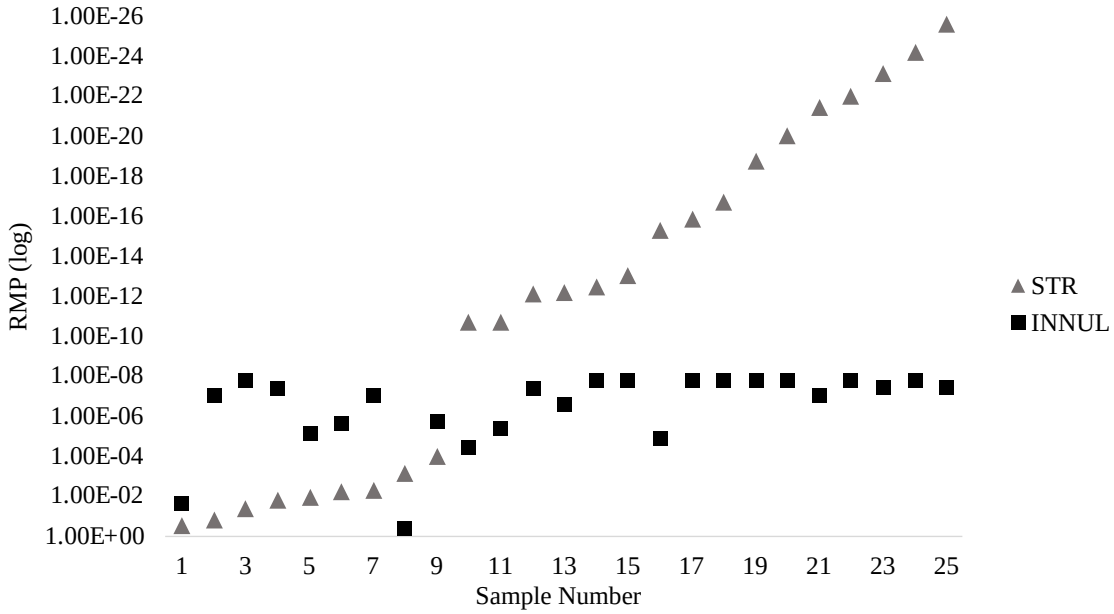

Fig. 2.7 Comparative RMPs of STRs and INNULs for each sample tested ranked in order of increasing STR alleles detected (N = 25). Horizontal dashed line represents the RMP for a complete INNUL profile (1×10^{-8}).

SNP Analysis via MPS

Human Identification

The HID-Ion AmpliSeq™ Identity Panel includes 124 SNPs encompassing 90 autosomal SNPS and 34 Y-SNPs. This panel has been proposed as a possible solution for genotyping highly degraded samples such as those that may be encountered with post-blast bomb fragments [39, 41].

MPS showed varying success in recovering DNA profiles from post-blast pipe bomb fragments. Two comparisons were performed based on the fact that STR and INNUL analyses can be performed with 15 µL sample input volume, but only 6 µL can be used for the initial PCR during library preparation for the HID-Ion AmpliSeq™ Identity Panel. First, 6 µL of neat DNA extract (0.03 – 0.12 ng) was analyzed, and second 15 µL of extract was concentrated to 6 µL in order have the same DNA input as was used for STR typing

and INNUL analysis (0.04 – 0.27 ng). None of the four neat DNA samples generated SNP profiles with more than 50 % correct SNP calls and one completely failed analysis (Fig. 2.8A). However, three of the concentrated samples resulted in > 70 % of SNPs called, and one resulted in a full SNP panel (Fig. 2.8B).

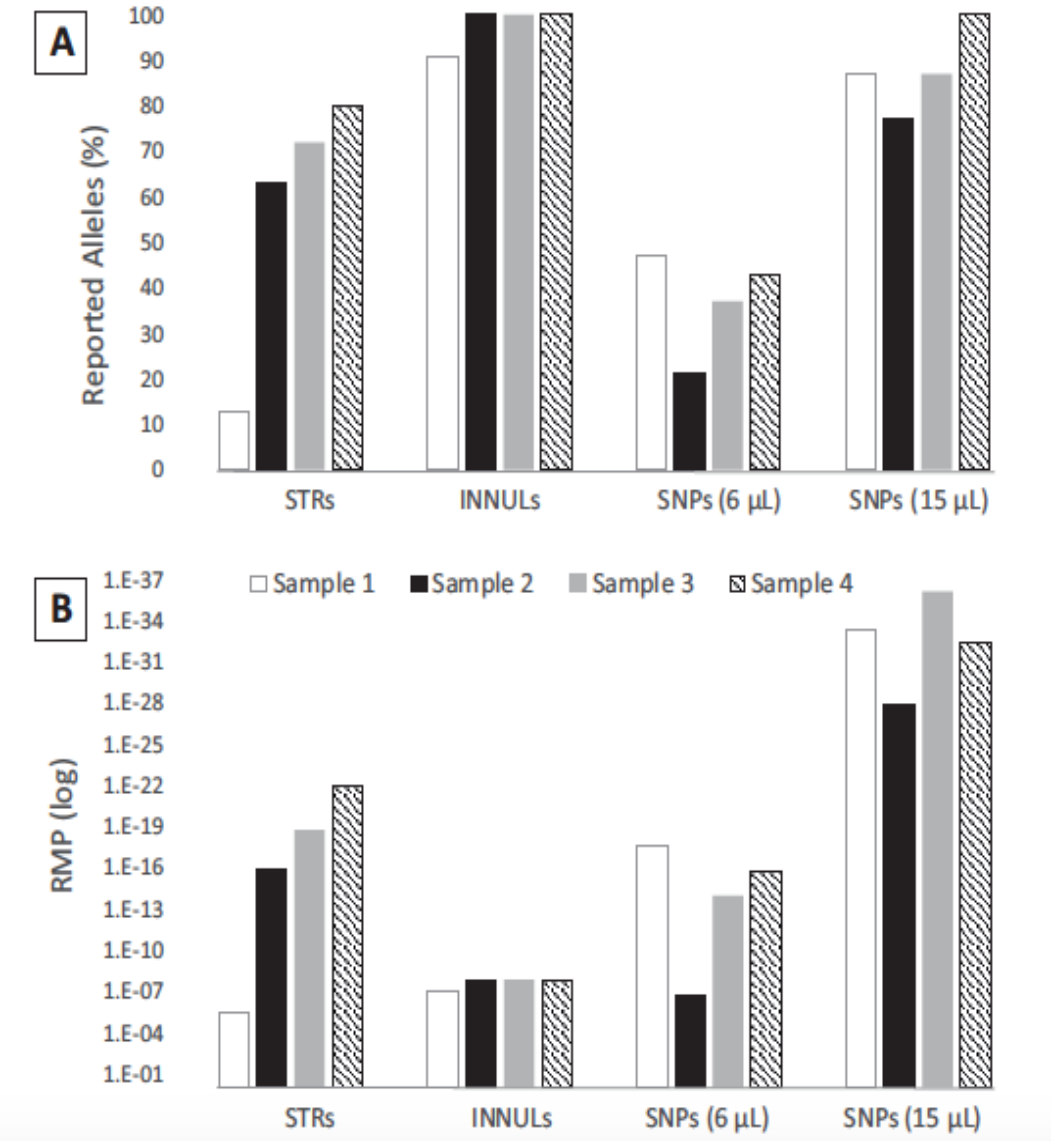

Fig. 2.8 (A) Comparative success and (B) RMPs of STRs, INNULs, and SNPs via MPS with the four samples tested. Sample 1: 0.003 ng/µL, Sample 2: 0.01 ng/µL, Sample 3: 0.012 ng/µL, Sample 4: 0.018 ng/µL. STR and INNUL analysis was performed with 15 µL of neat DNA extract. SNP typing was performed using 6 µL of neat DNA extract, and with 15 µL concentrated to 6 µL for amplification.

The relative success of the three HID methods (STRs, INNUL, and SNP via MPS) were compared (Fig. 2.8A). INNULs and concentrated samples and SNPs outperformed STRs in terms of the percentage of alleles reported in each system, indicating that these methods may be well suited for recovering DNA from post-blast pipe bomb fragments for HID purposes. The concentrated samples and SNPs resulted in the lowest RMPs compared to the other analysis methods tested in this study (Fig. 2.8B) demonstrating that SNP analysis via MPS offers great potential in identifying samples with low levels of DNA and moderate degree of degradation.

Ancestry Prediction

The ability to predict the ancestry (of the donor) from DNA recovered from the copper wires attached to each pipe bomb was assessed using the HID-Ion AmpliSeq™ Ancestry Panel which incorporates 165 autosomal ancestry informative SNPs (AISNPs). Wires were spiked with blood originating from Asian, Caucasian, or African-American donors.

Five out of six samples returned the correct ancestry predictions, with one sample (African-American) failing analysis. However, the confidence of all predictions was considered low. The two DNA samples for the Asian population were the most successful with 82% and 53% correct SNP calls (Table 2.1). All of the samples were below the target DNA input (1 ng) with input ranging from 52–485 pg, and therefore may explain the low confidence in the ancestry prediction.

Table 2.1 Percentage of correct AISNP markers called and ancestry prediction for each sample

Ancestry	Sample Number	DNA Input (ng)	Reported SNP Calls (%)	Ancestry Prediction
Asian	1	0.104	83	East Asian
	2	0.052	53	East Asian
Caucasian	3	0.485	15	European
	4	0.276	35	European
African - American	5	0.11	1	Inconclusive
	6	0.353	8	African

Conclusions

In this study we examined the comparative success of STR and alternative markers (INNULs and SNPs via MPS) to genotype DNA recovered from post-blast pipe bomb fragments from explosives detonated with Tannerite® for identification and ancestry purposes. Based on the DNA quantitation and STR results, DNA identification was equally successful when DNA was recovered from the end caps or from the pipe shaft of PVC pipe bombs. However, the majority of STR profiles were of poor quality and produced less than 30 % allele calls regardless of the location. In addition, stochastic effects such as heterozygote peak height imbalance and allelic drop-out were frequently observed, highlighting the difficulties of recovering DNA and generating reliable STR profiles from low-template and moderately degraded samples.

While the InnoTyper™ 21 Kit was more sensitive than STR analysis, being able to generate more complete genetic profiles, and resulted in a higher power of discrimination for some LT-DNA samples, STRs became more discriminatory when more than fourteen STR alleles were reported. Therefore, INNUL analysis could be used to supplement poor quality STR profiles or when STR typing fails. In addition to post-blast evidence, INNULs could be of equal benefit to other more typical challenging forensic casework samples such as skeletal, low-template, and highly degraded DNA samples. Because the InnoTyper™ 21 workflow is akin to STR analysis, INNUL analysis could be implemented in crime laboratories without requiring new equipment or software. InnoTyper® 21 has no peer-reviewed developmental validation study published to date. Best practices would dictate that prior to implementation of the assay for genotyping samples, labs should ensure that a developmental validation has been published in a peer-reviewed journal, and laboratories should perform internal validations as would be the case for any other genotyping method [56].

MPS technologies need additional optimization for analyzing extremely challenging samples, particularly with LT-DNA samples. The samples from post-blast fragments had variable success when analyzed via MPS for HID and ancestry prediction purposes. However, concentrating LT-DNA extracts was shown to be a viable method to enhance the quality of SNP profiles.

Although some of these alternate technologies are still in their infancy compared to STR typing methods for forensic purposes, the application of alternative markers for analyzing DNA recovered from post-blast pipe bomb fragments has potential to provide additional information about a suspect, particularly when traditional analyses fail.

Acknowledgements

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CHAPTER III

Efficacy of "Touch" DNA Recovery and Room-Temperature Storage from Assault Rifle Magazines

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This dissertation follows the style and format of *Legal Medicine*.

E. Tasker, C. Mayes, S. Hughes-Stamm, B. LaRue (2019).

Submitted to *Legal Medicine*.

Abstract

Crimes committed with assault rifles are becoming increasingly prevalent in the United States. In the absence of other evidence, DNA analysis can often provide informative leads. Unfortunately, any DNA transferred to rifle components left behind at a crime scene is likely to be low in quantity and/or quality. Furthermore, collected evidence is unlikely to be processed immediately and may require storage. Long-term storage can subject DNA to damage and degradation, which ultimately affects DNA profile interpretation and may prevent the identification of potential suspects.

This study assessed the ability of a new swab storage device, the SwabSaver®, to preserve "touch" DNA from AR-15 magazine rifles using three different collection devices. Three volunteers loaded bullet cartridges into plastic polymer and aluminum AR-15 magazines. DNA was collected with traditional cotton swabs, layered cotton paper swabs, or nylon-flocked swabs. Collection devices were then stored at room-temperature for up to two months in either the SwabSaver® device or an empty centrifuge tube. The results suggest that substrate and swab type had less of an effect on profile completeness than storage type. Furthermore, SwabSaver® storage yielded DNA quantities comparable to "touch" DNA extracted after 24 hours.

Keywords: "touch" DNA; assault weapons; DNA collection; DNA storage

Introduction

According to the Federal Bureau of Investigation's Uniform Crime Reporting program, firearms were responsible for approximately 73% of known homicides in the United States in 2017 [1]. Violent crimes committed with firearms have a number of residual items that are likely to be discarded at crime scenes (i.e. bullet cartridge casings, magazines) [2]. During preparation, the perpetrators of these crimes have had direct contact with these components during magazine loading. This physical contact can result in DNA being transferred to these discarded items, and therefore that DNA can generate investigative leads [3].

While it is possible to recover DNA from touched items [3], genotyping success using short tandem repeats (STRs) can be negatively affected by low quantities of DNA [4, 5]. In addition, DNA evidence may not be collected or processed immediately, which can cause larger DNA fragments to degrade and result in partial DNA profiles [6].

Biological materials collected on swabs are typically air-dried or placed in porous material, such as cardboard or paper, to prevent bacteria and mold growth. Removing oxygen and moisture from the environment prevents breaks in the DNA molecule caused via oxidation and hydrolysis, which could render those swabs unusable [7]. Gentueri, Inc. (Madison, WI; formerly Fast Forward Forensics, LLC) has recently been developed a novel device called the SwabSaver® for room-temperature storage of DNA collected on swabs (Fig. 3.1). The device consists of a polypropylene plastic tube with a color-indicating desiccant in the cap. Once swabbing is complete, the swab head is broken off into the tube. Traditionally, swabs wet from a biological fluid or pre-moistened prior to DNA collection need to be air-dried prior to storage. However, the SwabSaver® eliminates this step and

removes moisture using a desiccant and reduces the likelihood of DNA degradation by-hydrolysis. Drying biological material is a well-established method for preserving DNA [8], and there are collection devices that employ similar methods to drying swabs, such as the Bode SecurSwab line of swabs (Bode Cellmark Forensics, Lorton, VA). However, unlike the SecurSwabs, the SwabSaver® is universal, and can be used with virtually any collection device.

Fig. 3.1 SwabSaver® storage device before (left) and after (right) moisture is absorbed.

This study examined the efficacy of a prototype version of the SwabSaver® to preserve "touch" DNA recovered from aluminum and plastic polymer AR-15 magazines when three different swabs were used to collect the DNA (traditional cotton swabs, layered cotton paper swabs, and nylon flocked swabs). DNA was extracted from the swabs after three different time intervals: no storage (time zero), one month, and two months.

Materials and Methods

Substrate Preparation and DNA Collection

Initial storage studies were performed with the SwabSaver® storage devices by a third-party company using high quantity samples (buccal swabs) prior to this study to ensure DNA was indeed preserved (data not shown).

Three plastic polymer and three aluminum AR-15 magazines (N = 6 total) and rifle ammunition cartridges were cleaned with 20% bleach, rinsed with dH_2O followed by 70% reagent ethanol, and UV cross-linked for 20 min (Fig. 3.2). Biological samples were collected in accordance with the Sam Houston State University International Review Board Guidelines (#2016-09-31948). Volunteers (N = 3) rubbed their hands (unwashed) together for 10 s to help evenly distribute epithelial cells across both palms prior to handling [9, 10]. Each volunteer was timed while loading 20 cartridges into each rifle magazine. Hand rubbing was repeated prior to loading the cartridges into each magazine for a total of six magazines (three plastic polymer and three aluminum magazines) per day (Appendix A). In order to avoid the uneven deposition of cells on one magazine substrate over the other, participants loaded magazines in alternating substrate order. All cartridges and magazines were cleaned as previously described after DNA collection, and the handling process was repeated on another day.

Once the six magazines were loaded with cartridges, “touch” DNA was collected with one of the three swab types: a traditional cotton swab (Puritan®, Guilford, ME), the SimpleSwab2™ swab (Gentueri, Inc.), or the 4N6FLOQSwabs™: Genetics (Copan Italia, Bresica, Italy). A traditional double-swab technique was used for collection with cotton and nylon-flocked swabs [11]. During preliminary studies, it was determined that only

SimpleSwab2™ could be used because cotton paper layers absorb too much liquid during extraction; therefore, a modified double swab technique was used. Because the SimpleSwab2™ head is divided into two halves (Fig. 3.3), one half was used for wet swabbing and the remaining half was used dry. Cotton swabs and the SimpleSwab2™ were moistened with 30 µL of 2% SDS and 60 µL was used nylon-flocked swabs. Wet swabbing was followed by dry swabbing, and then both wet and dry swabs were stored in the SwabSaver® device (not air-dried) or microcentrifuge tubes (air-dried for 24hr prior to storage) at room-temperature for one or two months. Swabs that were not stored (time zero) were air-dried for 24 hrs and extracted the following day.

Fig. 3.2 Plastic polymer (left) and aluminum (right) AR-15 rifle magazine.

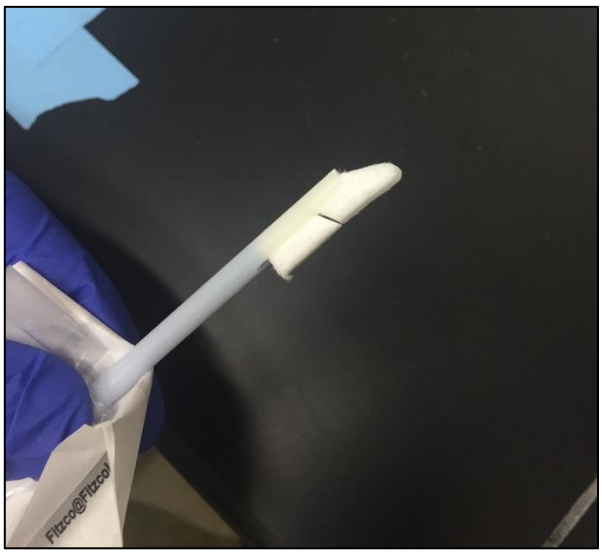

Fig. 3.3 SimpleSwab2™ with divided swab head.

DNA Extraction and Purification

DNA from swabs were lysed using the QIAamp® DNA Investigator kit (QIAGEN, Valencia, CA) following the "Isolation of Total DNA from Surface and Buccal Swabs" protocol according to manufacturer's instructions [12]. Prior to purification, swabs were placed in a spin basket and centrifuged at maximum speed (13,300 x g) for 1 min. The spin basket and swab heads were discarded, and the lysates were purified on the QIAcube® using the "Surface and Buccal Swab" protocol with a 60 µL elution volume (QIAGEN).

DNA Quantitation

DNA extracts were quantified using the PowerQuant® System (Promega, Madison, WI) and analyzed with PowerQuant® Analysis Tool v. 1.0.0.0 using the default thresholds for internal PCR control (IPC) shift (> 0.3) and degradation (> 2) [13].

PCR Amplification and STR Analysis

DNA extracts (15 µL) were amplified in a 25 µL reaction volume using the PowerPlex® Fusion 6C System (Promega) on an Applied Biosystems™ ProFlex™ PCR

System (Thermo Fisher Scientific, Waltham, MA) [14]. Amplified products were separated via capillary electrophoresis on an Applied Biosystems 3500 Genetic Analyzer using a 36 cm capillary array with POP-4 polymer and a 24 s injection time at 13 kV. Data analysis was conducted using GeneMapper® ID-X v 1.4 software (Thermo Fisher Scientific) with an analytical threshold of 175 RFU and a stochastic threshold of 400 RFU.

Data Analysis

Overall STR success was determined by the percentage of reportable alleles by comparing the experimental profiles to the volunteer's reference profiles (pulled from in-house database). Mixed effects models were used to evaluate the effects of the variables (substrate type, storage type, storage time, and swab type) on DNA quantities and STR success. Volunteers were used as the random effect in all models, as the quantities of DNA deposited were highly variable. Statistical models and subsequent analyses were performed using software packages in R [15] and RStudio [16]: "lme4" and "lmerTest" were used to generated mixed effects models [17, 18] and "userfriendlyscience" was used for all post hoc analyses [19]. Graphs were created in Microsoft Excel or in RStudio using "ggplot2" and "ggpubr" [20, 21]. Results were considered statistically significant for p-values < 0.05.

Results and Discussion

Time Handled

Each volunteer (labeled A, B, and C) was timed while loading the cartridges into the magazines to assess if handling time affected the quantity of DNA collected. While Volunteer A took the most time to load the rifle magazines, the overall DNA quantity was no greater than volunteer's B or C (Fig. 3.4). It was therefore determined that the amount of time handling the rifle magazines had no apparent effect on the amount of DNA

deposited regardless of the swab type used ($p = 0.1429$). This result supports other studies that have also observed individual variability to deposit cells, regardless of the length of time an item was contacted/handled [9, 22, 23].

While no internal studies have been performed, it is possible that Volunteer B is a "high shedder" relative to the other two participants. Notably, this was the only male participant. In a study conducted by Linacre et al. (2018), researchers attempted to quantify cellular deposits from participants and classified them as "heavy", "intermediate", or "light" shedders by visualizing shed cells from thumbprints [24]. Interestingly, female participants classified as "intermediate" produced partial profiles, while male "intermediate" shedders produced complete STR profiles. Similarly, a study conducted by Goray et al. (2016) found the propensity to shed cells differed between males and females [25]. It is possible the increased deposition of cells by males may explain the higher DNA quantities obtained from Volunteer B in this study, but this cannot be stated definitively.

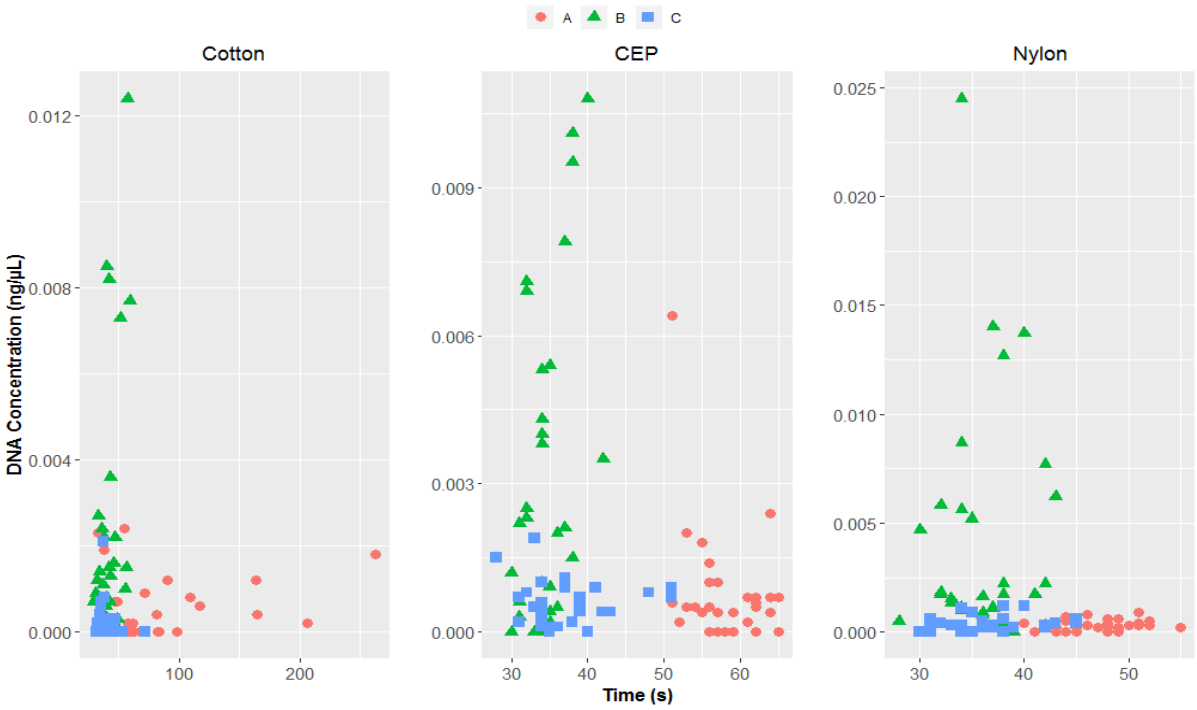

Fig. 3.4 Amount of time handled relative to DNA concentration (ng/µL) for all time points (graphed in Rstudio with "ggplot2" package).

No Storage

Regardless of swab or substrate type, DNA quantities for samples extracted after 24 hrs were low for all volunteers (0 – 0.016 ng/µL). Several of these extracts were flagged for degradation; however, it is possible that the low quantities of DNA caused this to inaccurately flag as the PowerQuant® Analysis Tool flags for degradation when the DI is "Undetermined". Possible inhibition was flagged in only one of the swab samples, but this was not reflected in the STR profile.

Not only were DNA concentrations highly variable between volunteers, but some intra-variation was also observed (Fig. 3.5). As expected, STR success was consistent with the amount of DNA recovered and available for amplification; volunteer B had the highest overall average of alleles reported (48%) and Volunteer C had the least (8%). No drop-in alleles were observed with these replicates.

A linear mixed effects model was calculated to determine which of the two factors (swab type or substrate type) had the greatest effect on STR success for swabs undergoing immediate extraction. ANOVA results from this model revealed that swab type had a significant impact on STR success ($p < 0.01$), whereas substrate type was not a significant factor (Fig. 3.6). STR profiles generated from DNA collected with traditional cotton swabs were the least informative compared to SimpleSwab2 ($p < 0.05$) and nylon-flocked swabs ($p < 0.01$); there was no statistical significance between SimpleSwab2™ and nylon swabs. While it has been suggested that nylon-flocked swabs readily release DNA into solution more efficiently than traditional cotton swabs [26, 27], few studies have explored the benefits of SimpleSwab2™ swabs [28, 29] with touched items. Considering the relative success compared to traditional cotton swabs in this study, SimpleSwab2™ swabs warrant further exploration to assess their utility with a wider range of challenging DNA evidence.

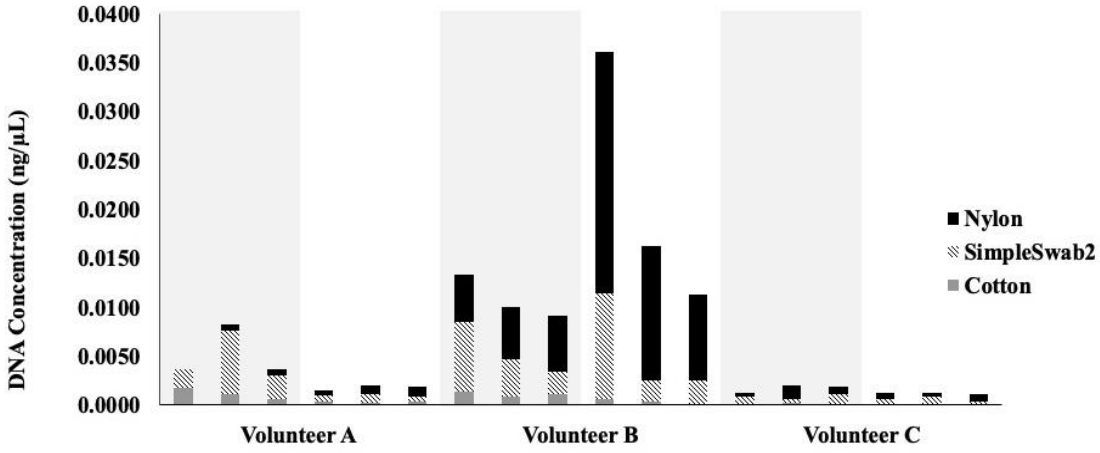

Fig. 3.5 DNA concentrations for replicates (n = 3) from all volunteers for DNA collected from plastic polymer (gray shading) and aluminum (white shading) AR-15 magazines and extracted after 24 hrs.

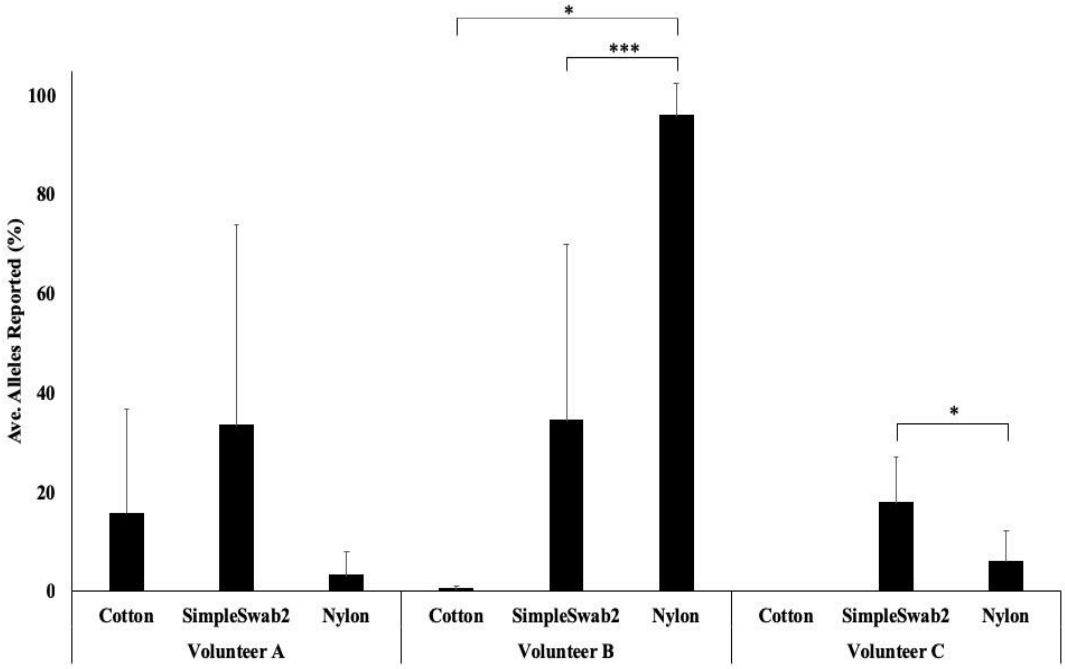

Fig. 3.6 Average percent of alleles reported for swabs extracted after air-drying for 24 hrs (n = 3 per swab type). Statistical significance determined with Welch ANOVA and Games-Howell post hoc test (Volunteer A & B) or Welch t-test (Volunteer C). Error bars represent standard deviation. *$p < 0.05$, ***$p < 0.001$

Swab Storage v. No Storage

Swabs that did not undergo immediate extraction were stored at room-temperature in the SwabSaver® device or air-dried for 24 hrs and placed into microcentrifuge tubes for one or two months. While some stored samples were flagged for inhibition, there no evidence inhibition in the STR profiles. However, the majority of stored samples were flagged for possible degradation by the PowerQuant® Analysis Tool, which was reflected in the STR profiles (Fig. 3.7).

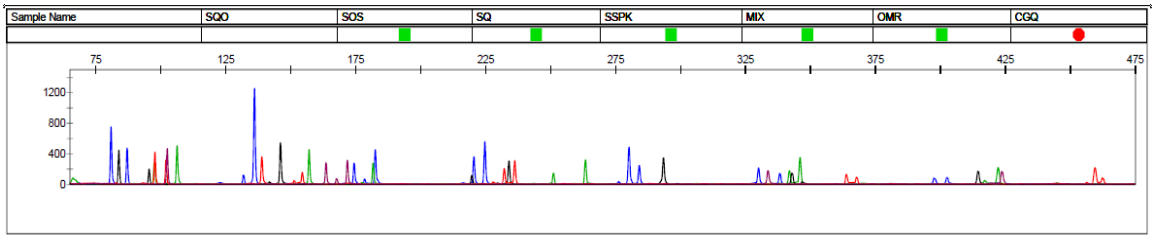

Fig. 3.7 Example of profile produced from swabs stored for 2 months in a SwabSaver® (0.0053 ng/µL) showing evidence of degradation.

Substrate and extraction negatives were processed to ensure that substrates were adequately cleaned between handling and that contamination did not occur during the extraction process. These negatives resulted in 0 ng/µL DNA concentrations. Two instances of contamination were observed, with both cases attributed to pre-existing contamination on the SwabSaver® tube (positively identified as the same non-donor source) and these data were removed from statistical analysis. However, this occurred in less than 2% of SwabSavers® used in this study. No drop-in alleles were observed for swabs stored in centrifuge tubes.

A linear mixed effects model was used to determine the effects of the type of storage, the length of time in storage, collection method, and substrate type had on STR success. A one-way ANOVA revealed that substrate type ($p < 0.0001$) and the storage method used ($p < 0.0001$) significantly affected profile completeness. No significant difference was observed whether stored for one or two months; therefore, these data were combined.

When focusing on substrate type, a Games-Howell post hoc test determined that centrifuge tube storage produced significantly fewer alleles compared to immediate extraction ($p < 0.05$) or storage in the SwabSaver® ($p < 0.01$) for plastic polymer magazines (Fig. 3.8); there was no significant difference in STR results whether swabs were processed

immediately or stored for DNA collected from the aluminum substrate. Several studies have examined the effect substrate has on collection and downstream STR analysis [30-32]. The increased STR success with plastic compared to the aluminum magazines is likely due to the rougher texture and porous nature of the plastic magazine. These data confirm that DNA was preserved in the SwabSaver® device and provided comparable results to air-dried swabs. It also suggests that they could provide a viable room-temperature storage option. This is particularly important as DNA collected from swabs may not be processed immediately, which can negatively impact the quality of DNA extracts and downstream analysis.

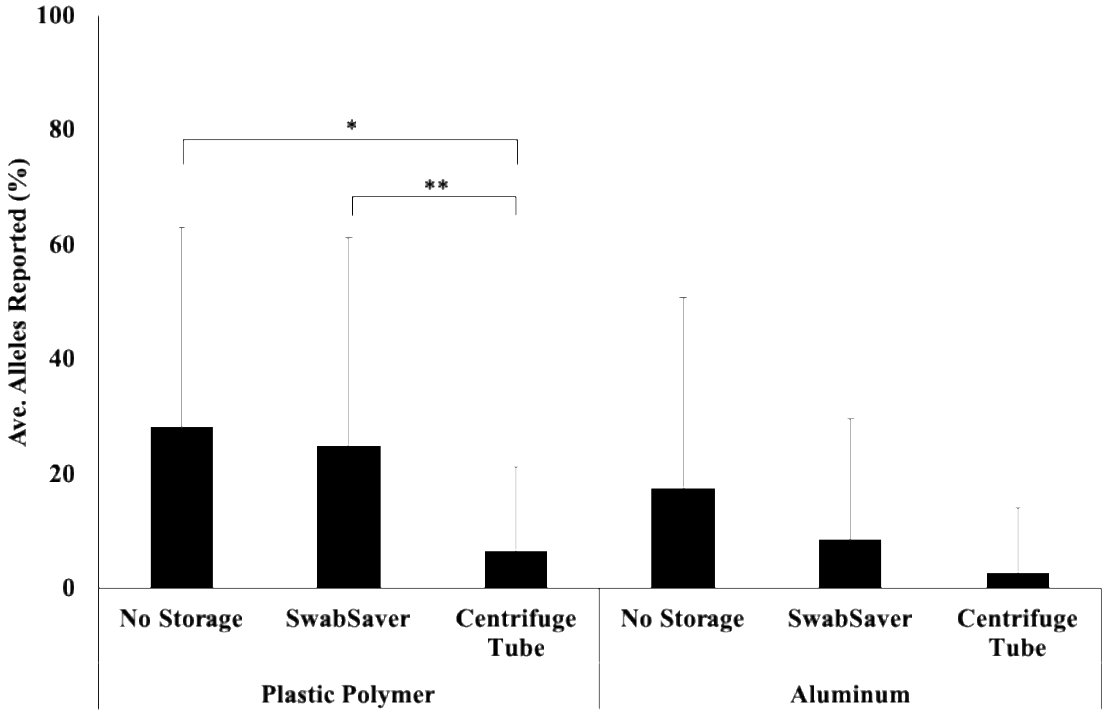

Fig. 3.8 Average percentage of alleles reported for each storage method (1 and 2-month data combined) for both substrates. Statistical significance determined by Welch ANOVA and Games-Howell post hoc analysis. Error bars represent standard deviation. *$p < 0.05$, **$p < 0.01$

Conclusions

This study assessed the ability of the SwabSaver®, a novel room-temperature swab storage device, to preserve "touch" DNA collected from two common AR-15 rifle magazine substrates. The SwabSaver® successfully preserved "touch" DNA for up to two months at room-temperature and produced statistically similar results to swabs that were immediately extracted (no storage). Furthermore, the type of swab used did not significantly impact DNA quantities after storage. Although this is a preliminary study, these data suggest that the SwabSaver® can be a practical, room-temperature alternative for preserving and storing swabs used to collect "touch" evidence for DNA analysis.

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CHAPTER IV

Collection and direct amplification methods using the GlobalFiler™ kit for DNA recovered from common pipe bomb substrates[1]

This dissertation follows the style and format of *Legal Medicine*.

E. Tasker, C. Mayes, B. LaRue, S. Hughes-Stamm. (2019)

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Abstract

When analyzing DNA from exploded pipe bombs, quantities are often in trace amounts, making DNA typing extremely difficult. Amplifying minute amounts of DNA can cause stochastic effects resulting in partial or uninterpretable profiles. Therefore, the initial DNA collection from "touch" evidence must be optimized to maximize the amount of DNA available for analysis.

This proof-of-concept study evaluated two different swab types with two direct amplification strategies to identify the most effective method for recovering DNA from common pipe bomb substrates. PVC and steel pipes, electrical tape, and copper wire spiked with epithelial cells were swabbed with cotton or microFLOQ® Direct Swabs and amplified directly or via a pre-treatment prior to STR amplification.

Not only was the microFLOQ® Direct Swab protocol the quickest method with the least risk of contamination, but in combination with direct amplification, the microFLOQ® Direct Swabs also generated the most complete STR profiles.

Keywords: forensic science; forensic biology; "touch" DNA; polymerase chain reaction; direct amplification; short tandem repeats

Introduction

Pipe bombs are explosive devices that are relatively easy to construct. As a result, they are often the chosen method by domestic and international terrorists to cause harm and destruction in communities [1]. After an explosive event, identifying a suspect in a timely manner is critical. While there are several approaches to identify a potential suspect [1, 2], few methods are as individualizing as DNA identification. Unfortunately, any trace DNA left on post-blast fragments is unlikely to be high in quantity or quality, which can impede downstream DNA analysis. In addition, DNA is often lost during DNA processing (collection, extraction, and quantification), further decreasing the likelihood of producing a quality DNA profile for generating an identification or investigative lead [3].

Newer and alternate sample collection and amplification methods may be more effective than traditional casework protocols when processing "touch" and other challenging DNA samples, and therefore warrant investigation. Direct amplification of DNA in a forensic context was first demonstrated in 2010 [4]. Since then, several studies have explored various direct amplification methods; however, few studies have applied this approach to substrates commonly used to manufacture improvised explosives [5-7]. Direct amplification bypasses the DNA extraction and quantification steps by placing the collected sample directly into the PCR [4, 8, 9]. This reduces DNA loss, increases the amount of starting template available for amplification, and increases the likelihood of generating more complete profiles [9]. This approach holds great potential for crimes involving weapons and improvised explosives, as high quantities of DNA are unlikely to be recovered. However, direct amplification from the swab does not allow for DNA quantitation and may also consume the entire sample depending on the type of swab used.

STR kits specifically designed for direct amplification include the GlobalFiler® Express (Thermo Fisher Scientific), PowerPlex® 18D (Promega, Madison, WI), and the Investigator® 24plex GO! (Qiagen®, Valencia, CA). However, these kits are intended for processing blood or buccal reference samples with ample amounts of high quality DNA [10]. Some traditional casework STR kits also provide alternate protocols for direct PCR [11, 12]. However, many laboratories may prefer to use the same STR kit for all samples (casework and reference). Therefore, using a standard case-working STR kit for all sample types (including a direct amplification approach) may be more practical and cost effective for forensic laboratories by eliminating the need to purchase and validate additional specialty STR kits (i.e. direct amplification kits).

While cotton swabs are commonly used as collection devices and undergo a full DNA extraction prior to amplification, microFLOQ® Direct Swabs (Copan Italia, Bresica, Italy) were developed to be placed directly into a PCR to decrease the likelihood of DNA loss and contamination [13]. Unlike the tightly woven fibers of cotton swabs, the small swab head consists of nylon-flocked fibers treated with a proprietary lysing agent which helps release DNA into solution [14]. While this potentially increases the risk of amplifying impurities and inhibitors (by eliminating purification steps), sample processing time is shortened as fewer steps are employed. In addition, these swabs can be used in a subsampling manner to triage evidence and reduce sample consumption [13]. There are few studies that have examined the utility of microFLOQ® Direct swabs [13, 15-17]. However, none to date have compared the performance of the swabs to what is commonly used in forensic laboratories.

This study investigates two different collection devices and two direct PCR processing methods which aim to decrease DNA loss and capture more genetic information from handled items. Common pipe bomb substrates were spiked with controlled amounts buccal cells and collected with cotton or microFLOQ® Direct Swabs. Traditional DNA workflows and two direct amplification methods were tested, and completeness of profiles was used to evaluate overall success.

Materials and Methods

Epithelial Cell Suspension Preparation

To control the number of cells being deposited experimentally, an epithelial cell suspension was prepared from buccal swabs obtained from a single male donor in accordance with Sam Houston State University International Review Board Guidelines (#2016-09-31948). Using a modified method from University of Illinois, Chicago (*personal communication*), a single buccal swab was placed into a 2 mL microcentrifuge tube with 1 mL of 1X PBS (VWR, Radnor, PA). The tube was incubated at room temperature (24 ˚C) with shaking at 700 rpm for 30 min. After incubation, the tube was inverted several times and centrifuged in a spin basket at maximum speed (approx. 13 000 × g) for 1 min to pellet the cells. The swab and spin basket were discarded, and the liquid was decanted. Then, 1 mL of fresh 1X PBS was added and the tube inverted to resuspend the cells.

Pipe Bomb Substrate Preparation

Four common pipe bomb substrates were selected: PVC pipe, galvanized steel pipe, electrical tape, and copper wire. PVC and galvanized steel pipes were washed with Alconox® detergent (Alconox, Inc., White Plains, NY) and sterilized using 20% bleach

and 70% reagent alcohol. The ends of insulated copper wire were stripped (approx. 1 cm) and cleaned with 70% reagent alcohol. All four substrates were then UV-treated in a cross-linker (UVP, LLC., Upland, CA) for 20 min.

Ten aliquots (10 µL each) of diluted cell suspension (approx. 200 pg or 30 cells) were placed onto the surface of each substrate (adhesive side for electrical tape) and dried overnight. Dried cell spots were swabbed for 30 s with either a cotton swab (Puritan, Guilford, ME) treated with 30 µL of 2% SDS or a microFLOQ® Direct Swab moistened with 1 µL of dH_20. Swab blanks were also taken from each substrate and processed through each method to ensure that cleaning was sufficient. All swabs were dried for at least 24 hrs after swabbing before proceeding with extraction or amplification. Cotton swabs were air dried and microFLOQ® Direct swabs were placed back into the transport tube.

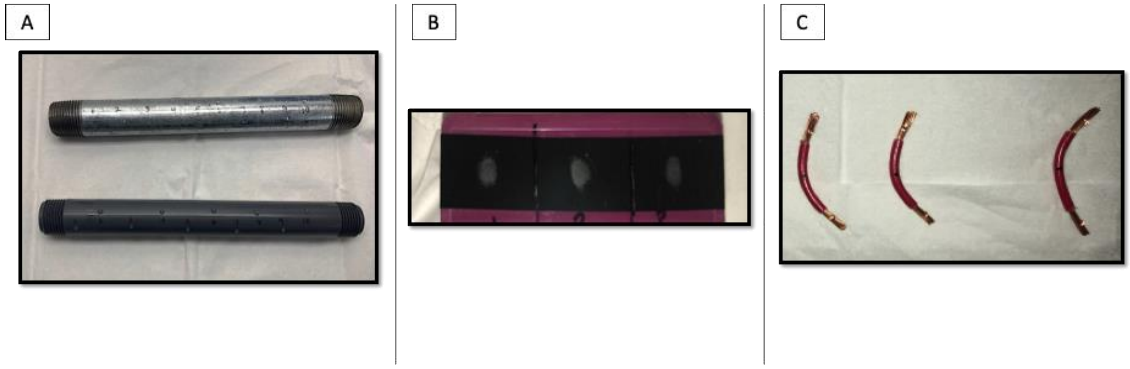

Fig. 4.1 Images of substrates spiked with epithelial cell suspension: (A) PVC and steel pipes**;** (B) electrical tape; (C) copper wires.

Sample Processing

Traditional Sample Processing (Controls) and Direct Amplification

For traditional DNA processing, cotton swabs (N = 10 per substrate) were extracted using the PrepFiler *Express*™ Forensic DNA Extraction Kit (Thermo Fisher Scientific) on the AutoMate *Express*™ Forensic DNA Extraction Instrument (Thermo Fisher Scientific)

using the “body fluids on swabs (buccal and other body fluids)” protocol [18]. In addition, a neat epithelial cell suspension (30 µL) was extracted in triplicate to determine the degree of DNA loss.

DNA Quantification

To estimate cell concentration of the suspension, a hemocytometer was used to visualize the cells using a 1:1 ratio of suspension and methylene blue to ensure intact cells are present, and cell counting was performed using standard counting methods [19]. The DNA concentration was also determined using the QuantiFiler® Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA). Triplicate aliquots of neat suspension (2 µL) was added directly to the qPCR mix and rigorously vortexed to shear the cells and release the DNA. The cell suspension was then diluted based on the qPCR quantitation values to approximately 0.04 ng/µL (6 cells/µL) and quantified using the previously described method to verify the DNA concentration.

The QuantiFiler® Trio DNA Quantification Kit was also used to assess DNA concentration, DNA degradation, and inhibition for the extracted swabs [20]. Replicates were averaged, and recovery was calculated by dividing the average DNA concentration of each substrate by the concentration of the epithelial cell suspension (0.04 ng/µL). The maximum volume of extract (15 µL) was then added to the GlobalFiler® reaction mix for amplification.

Direct Amplification

For direct amplification, a small portion of each cotton swab (approx. 1.2 mm) was sampled using a Whatman™ Uni-Core™ Punch (Fisher Scientific, Pittsburg, PA) or one

microFLOQ® Direct Swab head was added directly to a 0.2 L PCR tube containing 25 µL of GlobalFiler® reaction mix.

A pretreatment method for direct PCR was also explored to prevent consuming the entire sample and allow for DNA quantification and resampling if necessary. This is particularly important for the microFLOQ® Direct Swabs as the entire swab is used for amplification via the recommended protocol. Swab pretreatment was adapted from the GlobalFiler® Express direct amplification kit (Thermo Fisher Scientific) [21]. Swabs were incubated in either 400 µL of TE at 90 ˚C (cotton swabs) or in 40 µL of TE at room temperature (microFLOQ® Direct Swabs) for 20 min. The maximum volume of lysate (15 µL) was then directly added to 10 µL of GlobalFiler® master mix (25 µL total reaction volume).

DNA Amplification and Analysis

All samples were amplified using the GlobalFiler® PCR Amplification Kit (Thermo Fisher Scientific) using the standard 29 cycles on a ProFlex™ PCR System [22]. Amplified products were separated and detected on a 3500 Genetic Analyzer (Thermo Fisher Scientific) using a 36 cm capillary array with POP-4™ polymer (Thermo Fisher Scientific), and a 5 s injection time at 1.2 kV. STR analysis was conducted using GeneMapper® ID-X v 1.4 (Thermo Fisher Scientific) and in-house Excel workbooks with validated analytical and stochastic thresholds of 150 and 600 RFUs, respectively. Non-parametric univariate ANOVA with a Games-Howell post hoc and Welch t-tests were performed in RStudio [23] and graphed in Microsoft Excel. A confidence level of 0.05 ($p < 0.05$ = "*") was used for all statistical analyses.

Results

Traditional Processing – Cotton Controls

Neat cell suspension extracts (controls) and cotton swab extracts (N = 10 per substrate) were quantified to determine DNA concentration and DNA quality. The Quantifiler® Trio kit did not indicate the presence of PCR inhibitors, and no alleles were observed in STR profiles generated from all copper wire samples. Therefore, the copper substrate was removed from the study.

The percent DNA recovery was low for the remaining three substrate types (Fig. 4.2). More than half (52%) of the DNA from the neat epithelial cell suspension (control) was lost during the full extraction process. As may be expected, DNA loss was even more severe when collected from the PVC pipe, steel pipe, and electrical tape using cotton swabs, which resulted in 3%, 6%, and 14% DNA recovery, respectively. A one-way ANOVA and a Games-Howell post hoc test revealed that electrical tapes resulted in significantly higher DNA quantitation values compared to PVC ($p < 0.0001$) and steel pipes ($p < 0.0001$). No significant difference in DNA concentration between PVC and steel pipes was observed.

The STR profiles produced from all substrates resulted in less than half of the expected number of alleles being called, with electrical tape having the most alleles reported (Fig. 4.3). Not only did electrical tape samples yield the highest DNA concentrations, but also the most complete STR profiles and highest peak heights compared to PVC ($p = 0.0001$) and steel pipes ($p < 0.01$). No alleles were observed in any of the substrate negative controls, and no spurious drop-in alleles were observed in the experimental samples or any no template controls.

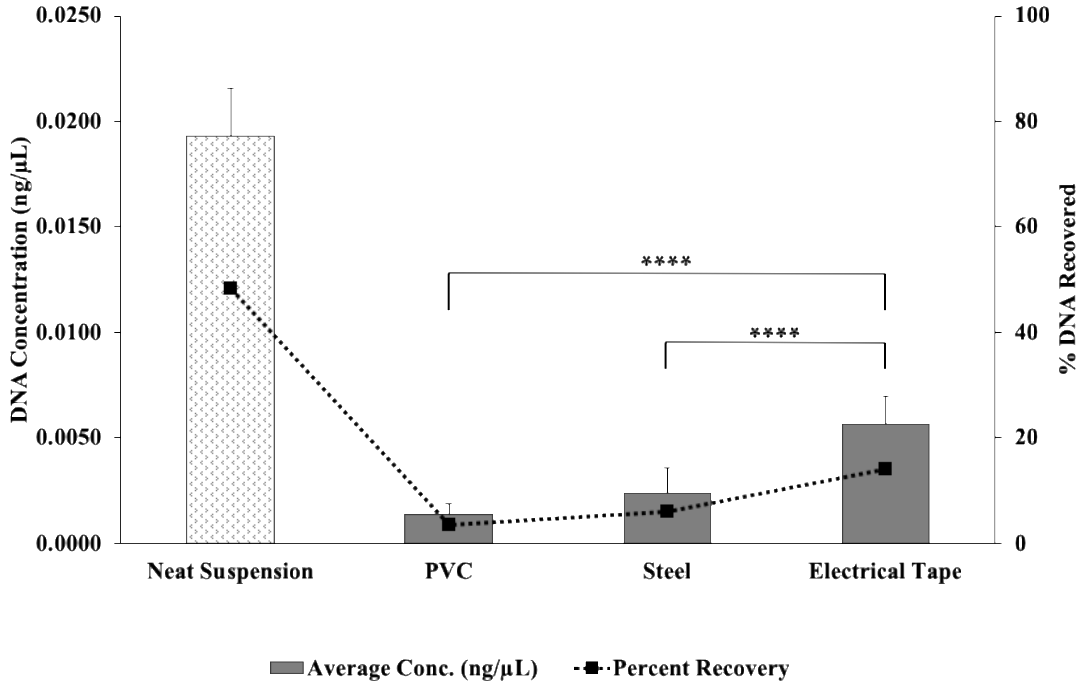

Fig. 4.2 Average DNA concentration for extracted cotton controls (N = 10 per substrate) and neat cell suspension (N = 3) extracted using the PrepFiler *Express*™ DNA Extraction Kit on the AutoMate *Express*™ extraction instrument. Statistical significance determined by ANOVA and Games-Howell post hoc test. Error bars represent standard deviation. ****$p < 0.0001$

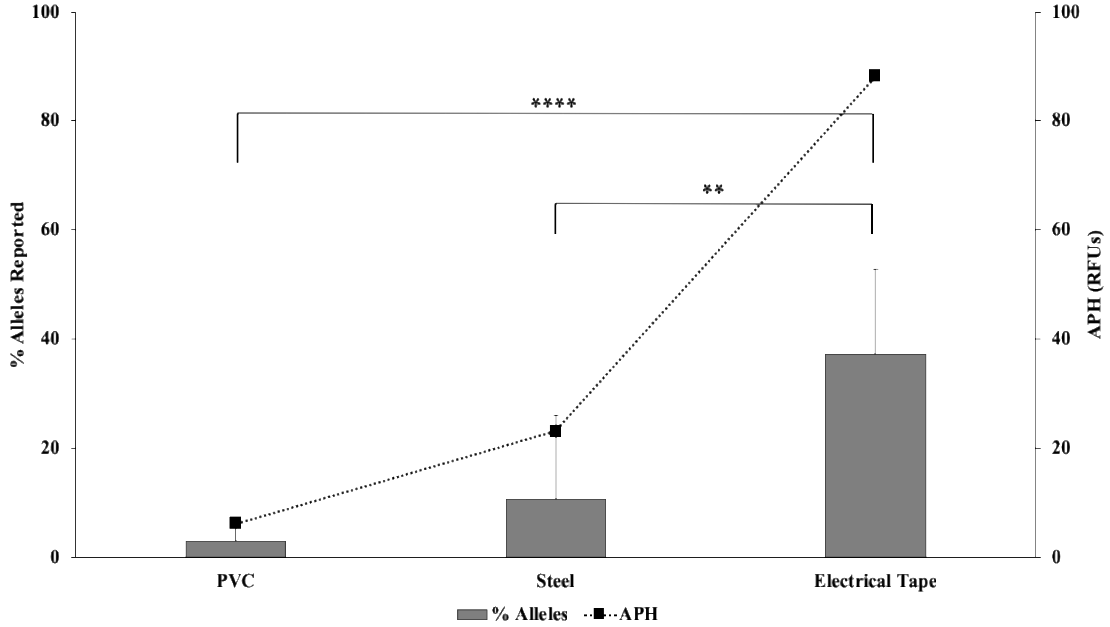

Fig. 4.3 Average percent of alleles reported and average peak height for control extracts (N = 10) per substrate. Statistical significances determined by ANOVA and Games-Howell post hoc test. Error bars represent standard deviation. **$p < 0.01$, ****$p < 0.0001$

Comparison of Sample Processing Strategies with Both Swab Types

Direct PCR and swab pretreatment strategies were tested with both cotton and microFLOQ® Direct Swabs to determine if sample processing time could be reduced while preserving (or increasing) the number of reportable alleles. Each swab type was processed with both methods and STR results were compared. As with the cotton control samples, all copper wire samples failed to amplify with both the direct and pretreatment methods and therefore were also removed from the study (Appendix B).

When DNA was collected using cotton swabs from all substrates, more alleles were recovered when swabs underwent a full extraction compared to the direct PCR and pretreatment methods (Fig. 4.4). Direct amplification of cotton swabs collected from PVC pipes failed to produce any STR results; therefore, only full automated extraction and swab pretreatment methods were compared. When compared to the extracted cotton swabs, a Welch t-test revealed significant differences for direct PCR and swab pretreatment ($p < 0.05$) for PVC pipes. A Games-Howell post hoc test also revealed significant differences for electrical tape ($p = 0.0001$), specifically between extraction and direct PCR, and extraction and swab pretreatment ($p < 0.001$ for both). Though the full DNA extraction provided more alleles for DNA from steel pipes than the other processing method, this increase was not statistically significant.

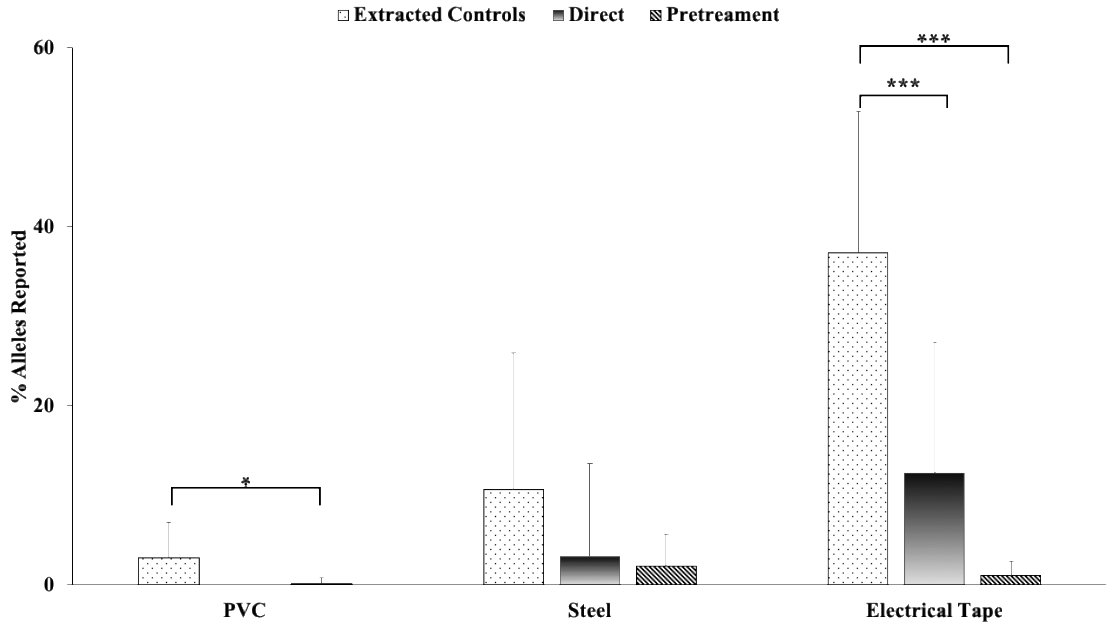

Fig. 4.4 Comparative STR results for cotton swabs grouped by substrate (N = 10 per substrate and treatment). Statistical significance determined by Welch t-test (PVC) and Games-Howell post hoc test (electrical tape). Error bars represent standard deviation. *p < 0.05, ***p < 0.001

Overall, samples collected using the microFLOQ® Direct Swabs showed the most complete STR profiles for the three substrate types regardless of the DNA processing method used (p < 0.0001) (Fig. 4.5), with direct amplification of the microFLOQ® Direct Swabs being the most successful method. When microFLOQ® Direct Swabs were placed directly into the PCR amplification more alleles were reported for the PVC (p < 0.001) and steel (p < 0.0001) pipes compared to pretreatment, with comparable results for the electrical tape. As with the cotton swabs, pretreating the microFLOQ® Direct Swabs diluted the DNA concentration and therefore reduced the amount of DNA for amplification.

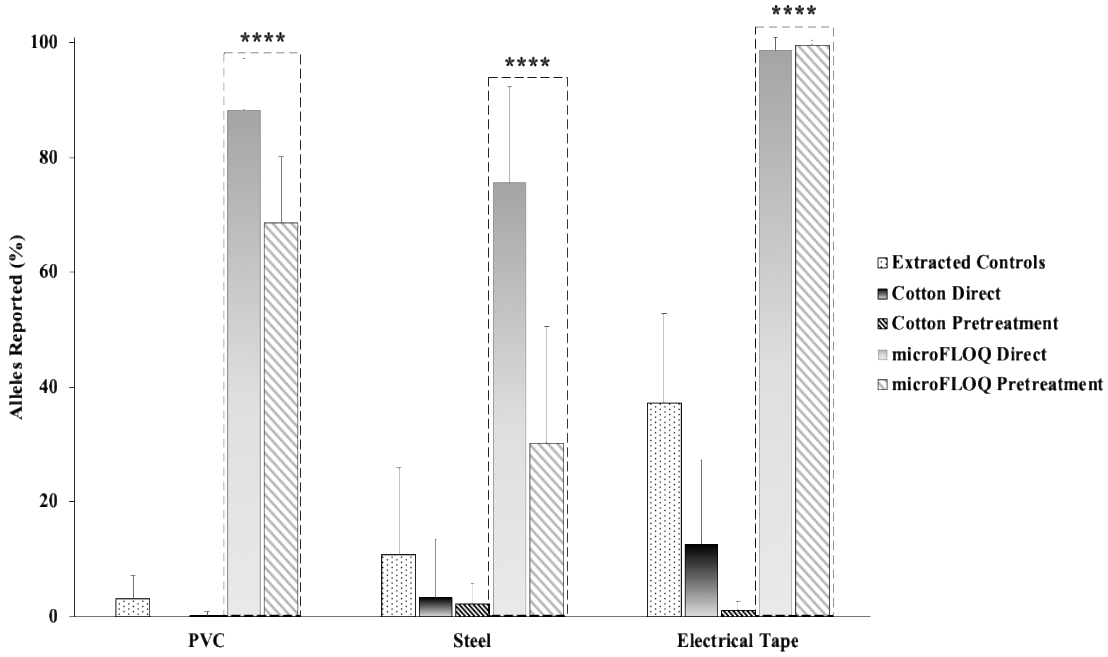

Fig. 4.5 Comparison of automated extraction, direct amplification, and pretreatment methods for both cotton and microFLOQ® Direct Swabs (N = 10 per method and substrate). Controls were cotton swabs extracted using the PrepFiler *Express*™ Forensic DNA Extraction Kit (N = 10 per substrate). Statistical significance determined by ANOVA and Games-Howell post hoc test. Error bars represent standard deviation. ****$p < 0.0001$

Directly amplifying microFLOQ® Direct Swabs from electrical tape did not result in a significant difference in reportable alleles compared to the pretreatment method. It should be noted that nylon fibers from the swab head adhered and remained attached to the adhesive surface of the tape after collection, but this did not seem to impact STR results. Average peak heights were the highest when microFLOQ® Direct Swabs were directly amplified ($p < 0.01$; Fig. 4.6). The success with electrical tape could be attributed to the adhesive surface with the epithelial cells likely clinging to the adhesive that was then collected by the swab head.

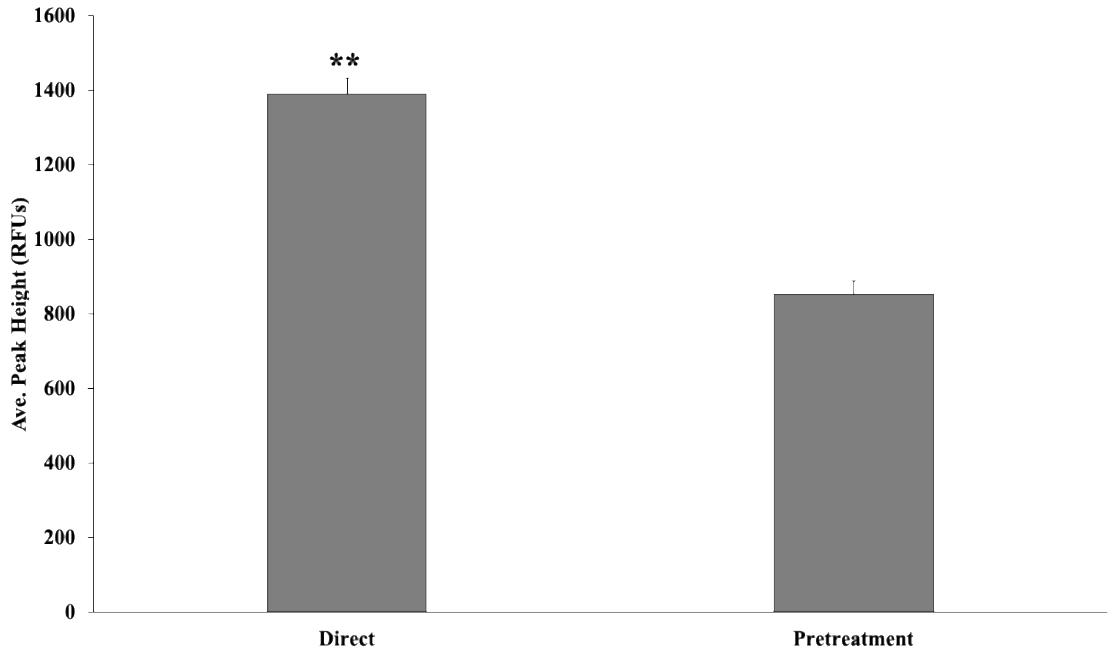

Fig. 4.6 Averaged peak height values for microFLOQ® Direct Swabs directly amplified and pretreated with TE for electrical tape samples. Statistical significance determined by Welch t-test. Error bars represent standard deviation. $^{**}p < 0.01$

Discussion

Large quantities of DNA were lost using traditional extraction methods. This loss was not unexpected, as other studies have documented significant DNA loss after DNA extraction [3, 24, 25]. Specifically, one study also observed sufficient DNA loss on the AutoMate *Express*™ for trace DNA quantities below 0.05 ng/μL [26]. Despite the expected DNA loss, in our study more DNA was recovered, and more alleles were reported from the electrical tape samples compared to the PVC and steel pipes. These data are consistent with a study by Mattayat et al. also demonstrating that electrical tape is a viable source of DNA from improvised explosives [27]. These results also suggest that electrical tape may be a rich source of DNA when touched, and therefore could be prioritized in relevant forensic casework. However, the relatively low DNA recovery and STR success rates overall

demonstrate that these traditional DNA extraction methods may not be the best approach for “touch” samples.

A similar study conducted by Phetpeng et al. explored the effectiveness of various DNA collection devices and moistening agents to recovery DNA from pipe bomb substrates, including electrical tape [28]. However, the overall DNA recovery was relatively low for electrical tape (0 – 58%) regardless of the collection device or moistening agent used, which the authors attributed to cells (from buffy coat) adhering to the substrate. While that study did investigate several cotton collectors and one nylon swab, all of the collection devices underwent traditional DNA processing. Therefore, it is possible that their low recovery was also attributed to substantial DNA lost during extraction in addition to cells adhering to the electrical tape. This may also explain the reduced recovery for DNA collected from electrical tape when extracted via traditional methods (37%).

Direct amplification with microFLOQ® Direct Swabs produced the most complete STR profiles of all the methods tested. While studies utilizing these swabs are limited, high STR success has been observed [13, 17]. However, both direct amplification and pretreatment methods with cotton swabs were less successful. While our swabbing technique ensured that only the tip of the swabs was used for collection, it is possible that the small portion (approx. 1.2 mm) sampled for direct amplification did not contain a sufficient amount of DNA. Although only a small portion was taken per swab in this study, the swabs could be re-sampled in an attempt to produce more complete profiles. For the pretreatment method, 400 µL of the incubation liquid was needed to adequately cover the swab head. This large volume also diluted the DNA recovered. Additional steps such as using a spin basket or concentrating the DNA, could be taken in order increase the amount

of DNA available for amplification. However, this added step will increase processing time, any PCR inhibitors, and the risk of contamination.

DNA collected from copper wires failed to produce results regardless of the method used. Although the QuantiFiler® Trio kit was used to access the quality of DNA extracts, it failed to indicate any inhibition for the copper wire extracts. Copper is a known PCR inhibitor [29] and was likely the cause of the failed profiles for all samples. However, considering 2 µL of extract is used for quantitation and 15 µL is amplified for STR typing, the level of inhibition was likely high enough in the STR reaction to prevent amplification despite not being detected during quantitation. Similar results were observed in other studies [28, 30] which further supports that PCR inhibition is a reasonable explanation for the results observed in this study.

Conclusions

The results of this study provide further support that traditional DNA processing may not be the most effective method for processing "touch" and low-template DNA samples for STR analysis. Collection of biological evidence from touched items using microFLOQ® Direct Swabs and processed via direct amplification resulted in the most complete STR profiles regardless of IED substrate type (copper wires excluded). In addition, direct amplification was successful using the routine casework GlobalFiler® kit, thereby eliminating the need to use a specific direct PCR kit for this type of evidence. This study also demonstrates that this faster and more streamlined method may be advantageous for processing challenging DNA samples, including those recovered from pipe bomb fragments and other weapons.

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CHAPTER V

Direct amplification and sub-sampling strategies using microFLOQ® Direct swabs for DNA recovered from handguns and exploded pipe bombs

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This dissertation follows the style and format of *Legal Medicine*.

Abstract

microFLOQ® Direct swabs are relatively new to the forensic market and have been introduced as a DNA collection device intended to accelerate DNA processing. The swab head is small relative to a traditional swab (approximately 1 mm in diameter), and ideally collection is performed in a sub-sampling manner. In cases where an item is regularly used or there is a visible biological fluid, direct amplification with microFLOQ® Direct swabs is a practical approach. However, biological material is not always evident or abundant, and sub-sampling in a very small surface area from the evidence may not yield results. Using a larger swab could increase the amount of DNA recovered by swabbing from more surface area, but DNA may be lost during traditional DNA processing methods.

In this study, two sub-sampling strategies for microFLOQ® Direct swabs were explored. The first strategy involved collecting DNA from firearms using a regular nylon-flocked swab, and then sub-sampling from the larger swab head using a microFLOQ® swab. Results were variable, but full STR profiles were produced using this approach. The second strategy examined sub-sampling directly from post-blast pipe bomb components (PVC pipe, electrical tape, and mobile phones) with microFLOQ® Direct swabs first and then using a regular nylon swab to collect DNA from a greater surface area. This sub-sampling approach was less informative compared to collection with regular nylon swabs, indicating that this approach is not ideal for substrates with presumed low quantities of DNA or the location of biological material on an exhibit is unknown.

Keywords: “touch” DNA; direct amplification; pipe bombs; handguns

Introduction

Firearms and improvised explosive devices (IEDs) have been used to cause harm, fear and destruction, with notable events including the Columbine High School shooting in 1999 and the Centennial Olympic Park bombing in 1996. DNA analysis using short tandem repeat (STR) markers is the current gold standard for human identification (HID); however, the amount of genetic material deposited onto a touched object is highly variable, and any DNA recovered is often low in quantity [1, 2]. There are also inter- and intrapersonal variability in a person's propensity to deposit cells [3-5]. DNA recovery from explosives is further complicated by high heat and pressure, causing damage and degradation [6].

Nylon-flocked swabs, such as the 4N6FLOQSwabs™ (Copan Italia, Bresica, Italy), have been explored as an alternative to traditional cotton swabs to increase the amount of DNA recovered from forensic evidence [7-9]. Regardless of the swab type used, a significant amount (> 50%) of DNA has reported being lost during the DNA extraction process [10-12]. One option is to skip the extraction and purification process and directly amplify the sample in order to maximize the amount of DNA available for amplification [13]. Recently, small nylon-flocked swabs called microFLOQ® Direct swabs (Copan Italia) have been developed specifically for direct amplification. A few of studies have demonstrated success with bodily fluids such as blood and saliva, as well as "touch DNA" [14-17]. While the microFLOQ® Direct swab are designed for direct amplification, the small swab head is not intended for collection from large surface areas. Instead, it is suggested these swabs are better suited for sub-sampling and possible triage of evidence [14].

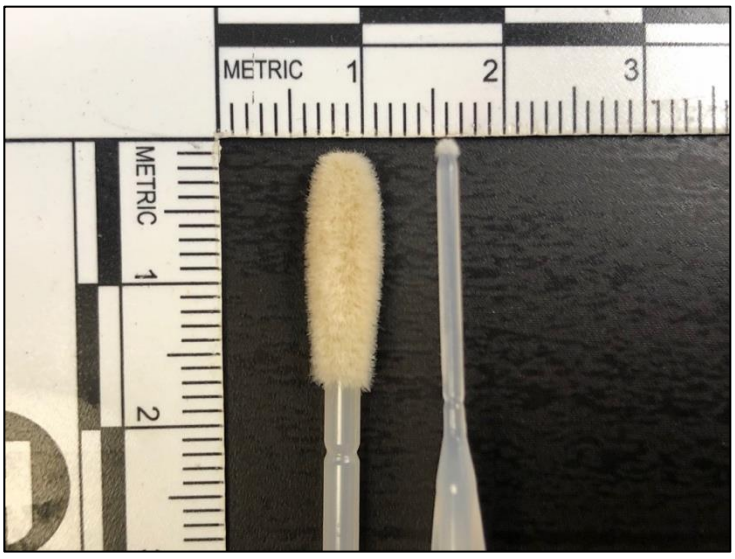

Fig. 5.1 Comparison of both nylon-flocked swabs: (left) regular-tipped 4N6FLOQSwab™; (right) microFLOQ® Direct swab.

This study explored two sub-sampling strategies using microFLOQ® Direct swabs with DNA collected from firearms and post-blast pipe bomb fragments. The first strategy investigated using the microFLOQ® Direct swab to sub-sample from the head of a regular 4N6FLOQSwabs™ that was first used to collect DNA from the evidence. The second strategy used microFLOQ® Direct swabs to sub-sample directly from the substrate.

Materials and Methods

Biological Material

All reference buccal swabs and "touch" DNA from donors were obtained in accordance with Sam Houston University International Review Board Guidelines (# 2016-09-31948).

Firearms

Shooting took place at a law enforcement firing range in Walker County, TX. Two officers from the Montgomery County Fire Marshal's Office (hereby referred to as Fire Marshal A and B) volunteered as shooters and provided their own handguns (N = 5 each;

Table 5.1). The handguns were not cleaned beforehand in order to avoid damaging the officers' personal weapons and to mimic a more realistic handling scenario. Officers stated that they were the only ones to handle their own guns during the previous few weeks. This arraignment also prevented us from collecting the firearms as evidence and, therefore DNA collection was performed on-site. Two tables were cleaned with 20% bleach, dH_2O, and 70% reagent ethanol, and an absorbent pad was placed over the table for DNA collection. Separate tables were used to collect from each officer's handguns to reduce the risk of cross-contamination.

Table 5.1 List of firearms used

Fire Marshal A	Fire Marshal B
SIG Sauer 1911 TACOPS Full-Size (45 mm)	CZ P-07 (9 mm)
SIG Sauer 1911 TALO (45 mm)	SIG Sauer 1911 (45 mm)
SIG Pro (40 caliber)	Smith & Wesson M&P9 Shield™ (9 mm)
SIG Sauer SP2022 (9 mm)	SIG Sauer P928 (9 mm)
SIG Sauer P320 (9 mm)	Smith & Wesson Model 60 (38 Special caliber)

Each officer fired 10 rounds per handgun. Once all 10 rounds were fired, the handgun was taken for DNA collection after the officer rendered it safe for handling. After collection was complete, the swab was placed back into the transport tube, the absorbent pad was replaced, and the process was repeated with all remaining firearms.

Pipe bomb preparation and evidence collection

Thirteen polyvinyl chloride (PVC) pipes (approximately 33 cm in length and 6 cm in diameter) were washed with a 1% detergent solution (Alconox Inc., White Plains, NY),

cleaned with 20% bleach, sterile water, 70% reagent ethanol, and UV-treated for an hour. Half of the endcaps (N = 13) were drilled with a 6 mm hole into the center, and then all endcaps (N = 26) were cleaned using the process described above. Mobile phones were also cleaned with bleach, water, and ethanol, and then UV-treated for 10 min in a crosslinker (UVP, LLC., Upland, CA). After cleaning, all substrates were placed into a clean container for transport.

Detonations occurred at a secure location near Montgomery, Texas. A metal shipping container was used to help contain the dispersion of fragments. The box was swept out and the metal base plate was cleaned with bleach, water, and ethanol. One fire marshal (Fire Marshal C) assembled ten pipe bombs without gloves and all additional volunteers wore the appropriate protective equipment to prevent contamination (i.e. gloves and faces masks). Pipes were filled with smokeless gun powder (Hodgdon Powder Company, Shawnee, KS) with an electric match attached. Two endcaps, one with and the other without a drilled hole, were secured onto the pipe using PVC primer and cement (Oatey®, Cleveland, OH). One mobile phone was then attached onto the body of the pipe using electrical tape. One bomb was placed onto a metal plate embedded the shipping container floor. The doors were then closed, and the bomb was detonated from a safe distance using an electric match. After detonation, volunteers wearing appropriate protective equipment collected the fragments into evidence bags using brooms covered in cloth and swept into a clean dustbin. Cell phones and pipe fragments were bagged separately when possible. After collection, any additional gunpowder and debris were swept out of the box and the metal plate was cleaned before placing the next pipe bomb. Once the remaining bombs were

detonated and fragments collected, the evidence bags were transported back to the laboratory for DNA processing.

Fig. 5.2 Interior of the shipping container. Arrow indicates location of the metal plate embedded in the container floor.

The remaining three pipes served as controls. One positive control pipe was used to confirm DNA was transferred to the substrate. This pipe was handled and assembled without gloves, but not filled with explosive powder nor detonated. Two negative controls, one of which was detonated, were assembled using gloves to ensure that the cleaning and collection processes were sufficient.

DNA Collection

Firearms Swabs

Regular-tipped 4N6FLOQSwabs™ with transport tube and Active Drying System (Copan Italia, Bresica, Italy) were moistened with one drop of sterile water (Teleflex, Morrisville, NC). DNA was then collected from the entire surface of the gun with special attention to the stock and trigger using a single wet swab. After collection, swabs were placed back into the transport tube and brought back to a dedicated low-template Pre-PCR laboratory for sample processing.

microFLOQ® Direct swabs moistened with 1 µL of autoclaved water were then used to sample from the heads of 4N6FLOQSwabs™ using circular motions. Because microFLOQ® Direct swabs do not undergo a purification process, efforts were made to avoid areas of the larger swab darkened with residual gunpowder to minimize possible PCR inhibition. Both swabs were placed back into their respective transport tubes after sub-sampling and dried overnight.

Pipe Bombs, Mobile Phones, and Electrical Tape

PVC fragments were sieved (approximately 1 mm x 1 mm mesh) to exclude minute fragments deemed too small to collect and process. A single microFLOQ® Direct swab was used to subsample from all remaining fragments and electrical tape (one swab per substrate). While each fragment was swabbed, collection using microFLOQ® Direct swabs was brief. A more thorough collection was performed with a moistened (50 µL of 2% SDS), regular-tipped 4N6FLOQSwabs™ over the same pieces of substrate. All swabs were then air-dried overnight.

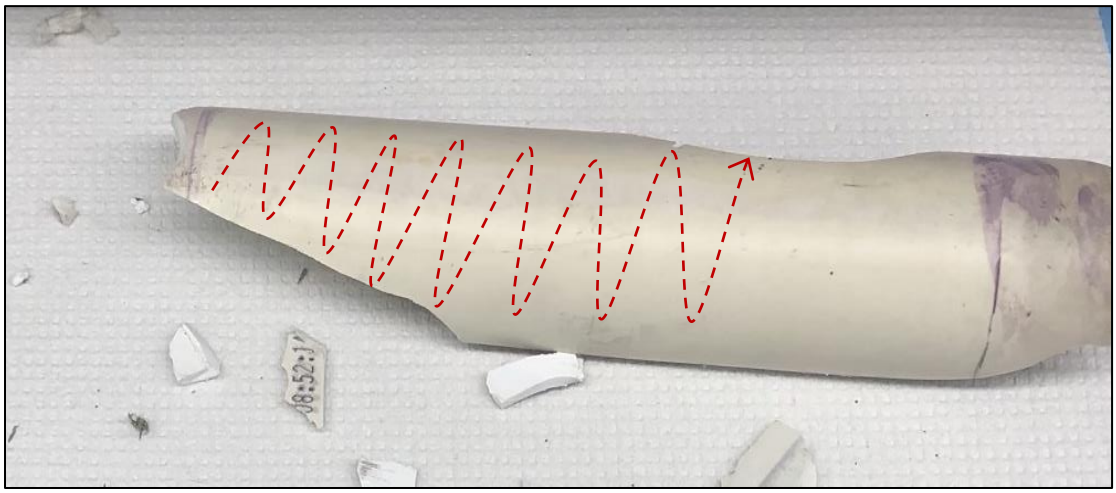

Fig. 5.3 Illustration of collection strategy using microFLOQ® swabs for sub-sampling directly from post-blast substrates.

DNA Extraction

All 4N6FLOQSwabs™ were extracted using Prepfiler® *Express* on the Automate *Express* (Thermo Fisher Scientific) according to manufacturer's instructions with an elution volume of 50 µL [18].

DNA Quantitation

DNA concentrations and overall DNA quality from swab extracts were determined using the Investigator® Quantiplex® Pro real-time quantification kit on the ABI 7500 real-time PCR system (Thermo Fisher Scientific) and analyzed using the Quantification Assay Analysis Tool v3.3. DNA quality was assessed using the default thresholds set for DNA degradation and inhibition (QIAGEN) [19].

DNA Amplification and STR Analysis

Autoclaved water (1 mL) was added to the transport tubes for microFLOQ™ Direct swabs, and vortexed for approximately 10 s to remove any soot and gunpowder and reduce the risk of PCR inhibition. The water was discarded after vortexing and the swab was placed back into the transport tube.

DNA from extracts were amplified and direct amplification of microFLOQ® Direct swabs was performed using the Investigator® 24plex QS kit (QIAGEN). Extracts were amplified according to manufacturer's protocol using a DNA input target of 0.8 ng. The maximum volume (15 µL) of amplification-grade water was used in place of extract volume for direct amplification. All samples were amplified using the recommended 30 cycles on the ProFlex™ PCR System (Thermo Fisher Scientific) [20].

Amplified products were separated and detected on a 3500 Genetic Analyzer using a 36 cm capillary array with POP-4™ polymer (Thermo Fisher Scientific) and the recommended injection time and voltage. STR analysis was conducted using GeneMapper® ID-X v 1.4 (Thermo Fisher Scientific) and in-house Excel workbooks with internally validated analytical and stochastic thresholds of 100 and 200 RFUs, respectively. The internal PCR control in the amplification kit (Quality Sensors QS1 and QS2) were used to assess amplification success and/or presence of PCR inhibition [20]. The number of reportable alleles and the average peak height ratios were used as metrics to compare the success of traditional sample processing and the sub-sampling methods.

Results and Discussion

DNA from Firearms

Quantification Results for 4N6FLOQSwabs™

DNA extracted from 4N6FLOQSwabs™ used to collect from the officers' handguns were quantified using the Quantiplex® Pro kit. Despite not cleaning the weapons prior to this study, the overall DNA concentrations were relatively low for both officers (< 200 pg/µL). DNA from Fire Marshal B resulted in higher concentrations on average (104 ± 45 pg/µL) compared to Fire Marshal A (45 ± 30 pg/µL). Furthermore, no evidence of

mixtures, degradation, or PCR inhibition were indicated by the Investogator® Quantiplex® Pro kit.

Traditional Processing vs. Sub-sampling with microFLOQ® Direct Swabs

Complete STR profiles were produced from all amplified extracts for Fire Marshal B (N = 5), and mostly complete profiles (93 – 98% reportable alleles) from Fire Marshal A for four of the five samples. Consistent with the quality assessment data during quantification, the QS markers did not indicate any inhibition but "ski-slope" peaks indicative of degradation were observed. However, one mixed DNA profile was observed for each officer. The contaminant alleles, which were above the analytical threshold (100 RFU), could not be attributed to any of the other volunteers. Contaminant alleles were observed at the majority of loci for Fire Marshal A, and a major contributor could not be determined due to the peaks having similar heights (Fig. 5.4). A clear major contributor could be attributed to the handler in the mixed profile from Fire Marshal B but not from the corresponding microFLOQ® sub-sample (Fig. 5.5). As the officers were not asked to clean their weapons, it is possible that someone other than the shooter handled the firearm previously and were the source of the extraneous alleles. The contaminant alleles could also be a result of secondary transfer, where DNA on a person or object is subsequently transferred to a secondary person or item [21].

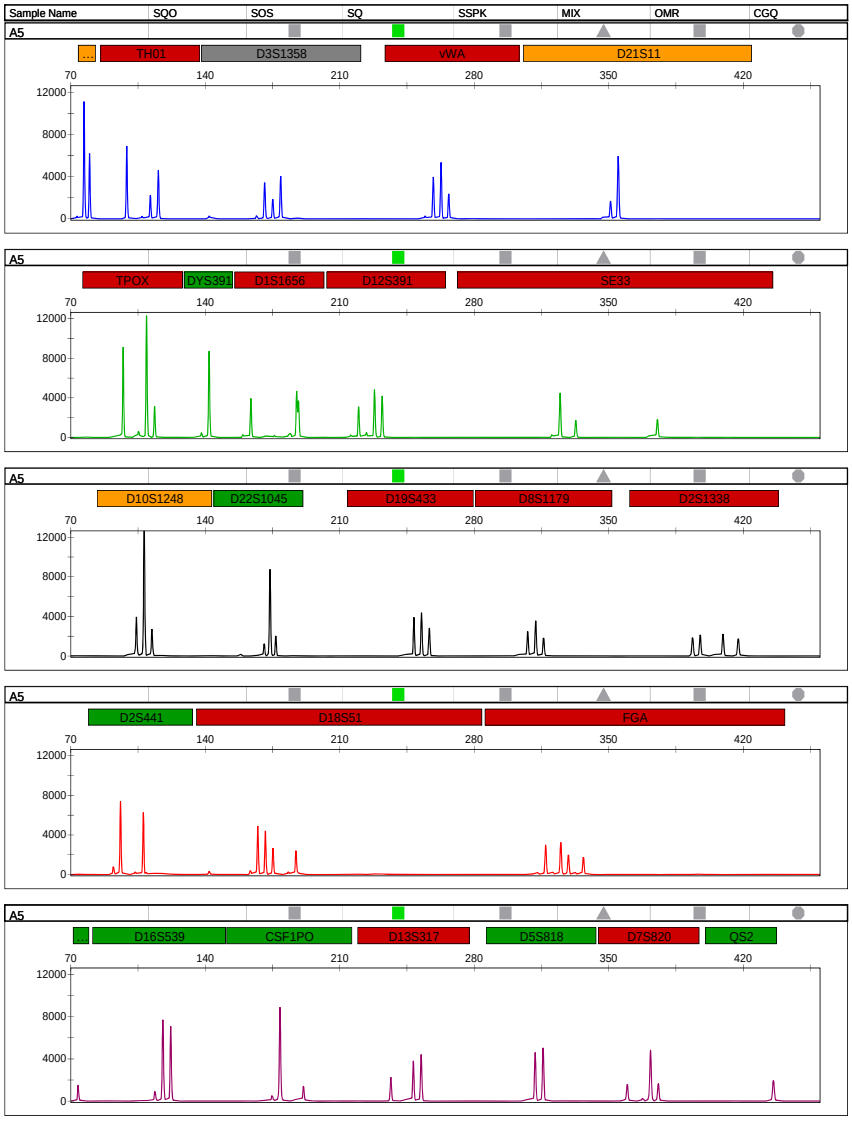

Fig. 5.4 STR profile from Fire Marshal A containing a DNA mixture.

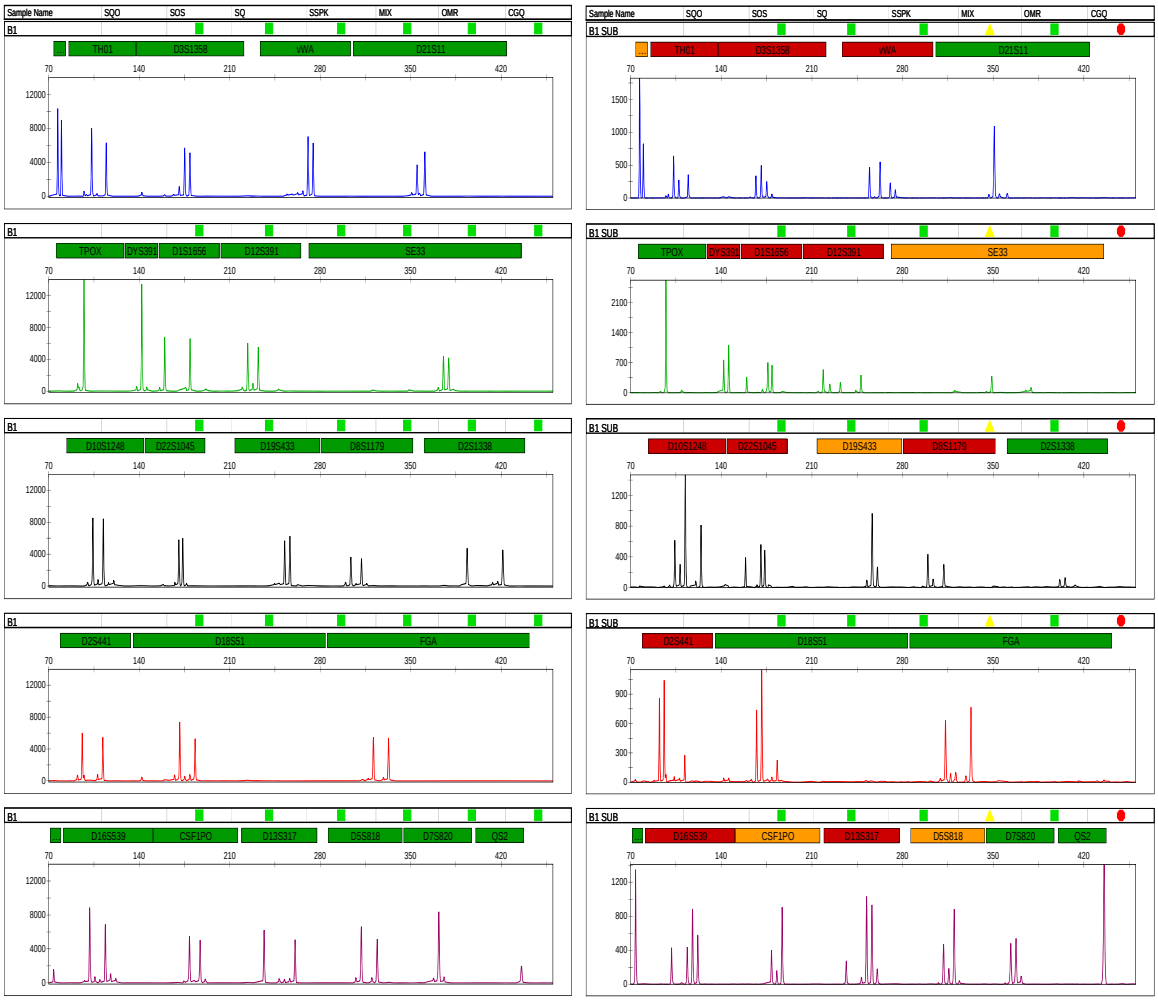

Fig. 5.5 Mixture STR profiles from Fire Marshal B: 4N6FLOQSwab™ (left) showing a clear major contributor and the microFLOQ® used to sub-sample (right) showing a complicated mixture.

Sub-sampling from the 4N6FLOQSwabs™ using the microFLOQ® Direct swabs had variable success (Fig. 5.6). This was especially noticeable for Fire Marshal A (N = 4), where the profile completeness ranged from 4 – 78%. In contrast, three of the four profiles produced from sub-sampling resulted in full STR profiles and the fourth had 95% of alleles reported for Fire Marshal B. These results may be expected due to the difference in DNA quantities recovered from weapons touched by the two donors, and therefore more DNA

was available for amplification from the items touched by Fire Marshal B. This result may also imply that Fire Marshal B had a higher propensity to shed epithelial cells relative to the other officer [4]. While sub-sampling produced mostly comparable STR profile completeness, there was a slight reduction in profile balance (average peak height ratios) compared to the 4N6FLOQSwabs™ (Fig. 5.6 C & D).

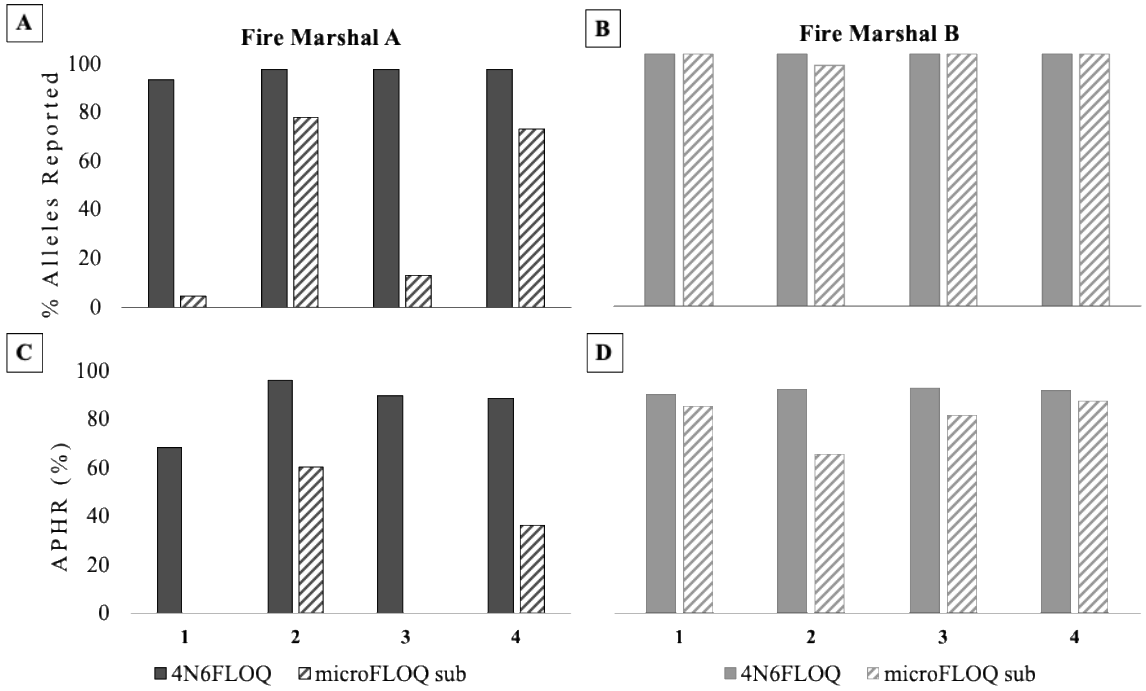

Fig. 5.6 Comparison of traditional collection and processing with 4N6FLOQSwab™ and sub-sampling with microFLOQ® Direct swabs (N = 4): Percentage of STR alleles reported for Fire Marshal A (A) and Fire Marshal B (B); average peak height ratios for Fire Marshal A (C) and Fire Marshal B (D).

DNA from Post-blast Pipe Bombs and Components

DNA deposited from Fire Marshal C was collected from post-blast PVC pipe fragments, electrical tape, and mobile phones. Sub-sampling occurred from the substrate using the mirocFLOQ® Direct swabs, and then a regular-tipped nylon swab was used for a more thorough collection. The collection differed for each bomb as the degree of

fragmentation varied greatly from extensive damage to minor damage (Fig. 5.7). Therefore, the swabbing regime was adapted to sample from as many fragments, and as many different locations as possible. The inconsistency observed in the degree of fragmentation across the 10 explosive devices in this study could also be expected in a real-world situation, and therefore was considered an acceptable reflection of case-work type evidence.

Fig. 5.7 Examples of the varying degrees of fragmentation observed.

Mobile Phones

Only nine of the ten phones were recovered post-blast, as one of the phones was lost during detonation. DNA concentrations from the extracted 4N6FLOQSwab™ ranged from 0.0001 – 0.0020 ng/µL for the remaining nine phones (Fig. 5.8). Partial STR profiles were produced for five of the nine 4N6FLOQSwabs™ with the percentage of alleles reported ranging from 8.9 – 97.8% (Fig. 5.8). Sub-sampling with microFLOQ® Direct swabs directly from the substrate was less successful. All but one microFLOQ® Direct swab failed to produce any reportable alleles. However, that one microFLOQ® swab resulted in more alleles and better peak height ratios than the extracted swab. That 4N6FLOQSwab™ had the second highest DNA concentration of 0.0018 ng/µL (Fig. 5.9),

and therefore more DNA available for sub-sampling using the microFLOQ® swab. However, the swab with the highest DNA concentration and most complete profile (0.0020 ng/µL; 97.8%) did not result in any reportable alleles using the sub-sampling approach with the microFLOQ® swab. Although it is unclear why this occurred, it could be presumed that when the small microFLOQ® Direct swab was used to sub-sample from the primary swab, it did not sufficiently collect enough amplifiable DNA due to the small surface area sampled, and simply by chance missed where the DNA was located on the larger swab head .

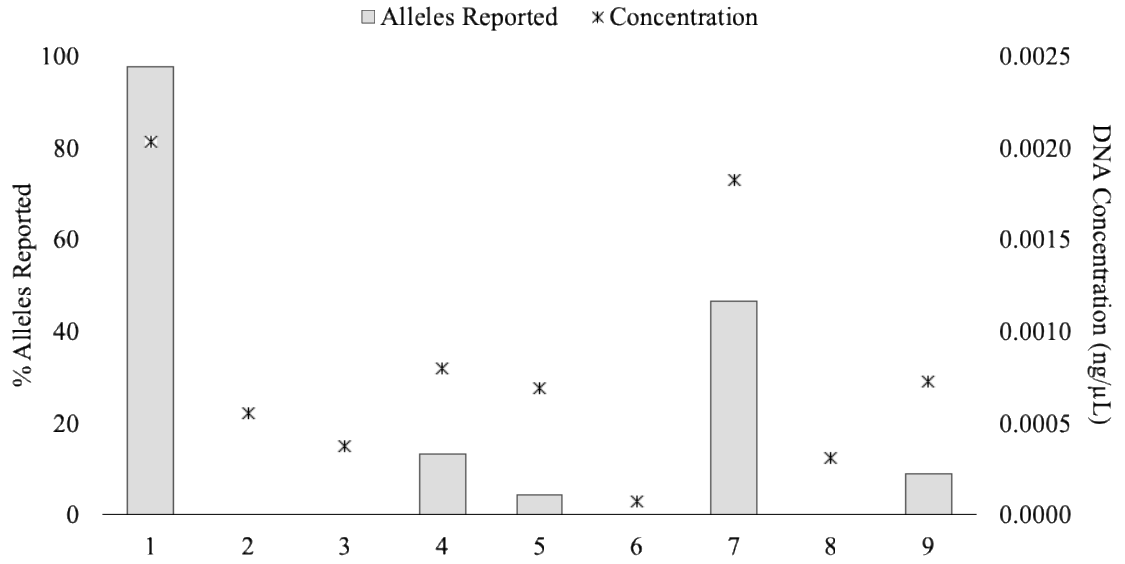

Fig. 5.8 Reported STR alleles (left y-axis) and DNA concentrations (right y-axis) for DNA collected for post-blast mobile phones using 4N6FLOQSwabs™.

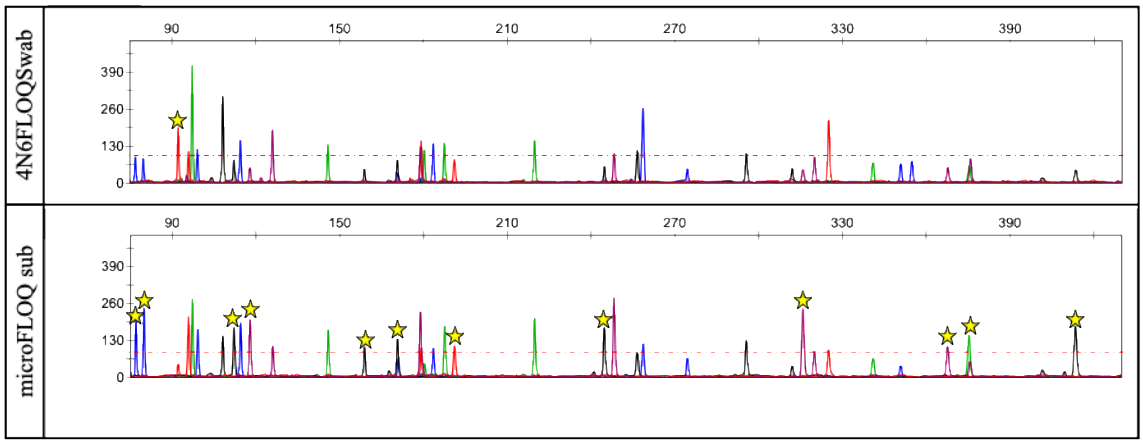

Fig. 5.9 Comparison of STR profiles from the same phone using both the 4N6FLOQSwab™ (top) and sub-sampling using the microFLOQ® Direct swab. Stars denote alleles not detected in the other profile.

The two negative control phones resulted in no reportable alleles. The positive control phone appeared to be single source profile from a male (no more than two alleles detected at a single loci); however, this profile did not correspond with any of the reference profiles of the fire marshal's or the volunteers. This may indicate that the pipe was not sufficiently cleaned and sterilized prior to the study, or contamination occurred at the scene. Although the detonations occurred in a secure location, there were other personnel who were not a part of this study but were in the vicinity and could be the source of the unknown profile.

PVC Fragments and Electrical Tape

In general, DNA recovery from post-blast pipe fragments and electrical tape was even less successful compared to DNA collection from mobile phones. Specifically, sub-sampling directly from the substrate using microFLOQ® swabs was unsuccessful, with only one allele detected from the PVC substrate and no alleles at all detected from electrical tape. One possibility for the lack of success with this sub-sampling strategy was the inclusion of a wash step. In a study conducted by Ambers et al., an agitation step was

evaluated in attempts to reduce possible PCR inhibition from bloodstains collected with microFLOQ® Direct swabs. It was determined that this step did not adversely affect STR profile completeness for 5% bloodstains, but a reduction in peak heights and complete amplification failure for 1% bloodstains. Therefore, it is plausible that the wash step in this current study may have also resulted in a reduction in STR profile quality and completeness.

Collection from electrical tape was difficult due to the adhesive, which may also account for the lack of results. The tape would often stick to itself or, occasionally, to the swab head. Many of the pieces also contained unburned gunpowder stuck to the adhesive, which also complicated collection using the microFLOQ® Direct swabs. Studies have explored ways to increase DNA recovery from electrical tape [22-25]. A preliminary study involving collection of cellular material using microFLOQ® Direct swabs from adhesive was also performed in-house (see CHAPTER IV). Direct amplification from the adhesive resulted in complete profiles for all replicates. However, this was a controlled study with known quantities of buccal cells (200 pg), which was greater than the DNA quantities obtained from the detonated pipe bombs, and the substrates were not subjected to the same extreme conditions in this current study. Therefore, the lack of results in this study were not unexpected.

Sub-sampling directly from an item may be more successful from items regularly and repeatedly used as demonstrated by Ambers et al., where complete profiles were produced from mobile phones and keyboards [14]. It is likely that similar results were not observed in this current study due to the high heat and pressure from the detonation, and the limited handling time with the pipe bomb substrates.

When traditional collection was performed with the 4N6FLOQSwabs™, some partial profiles were produced through traditional collection and amplification strategies (Fig. 5.10). DNA concentrations were low for all extracted swabs. Profiles from PVC fragments resulted in 3 – 9 reportable alleles whereas electrical tape only produced 1 – 3 alleles. The poor DNA recovery can be attributed to a variety of factors. Based on the minimal amount of DNA recovered from the positive control (not detonated), what little DNA that was deposited onto the test bombs was destroyed by the heat produced from the blast [6]. It could also suggest that the handler was not a "good shedder", which resulted in low DNA yields [4].

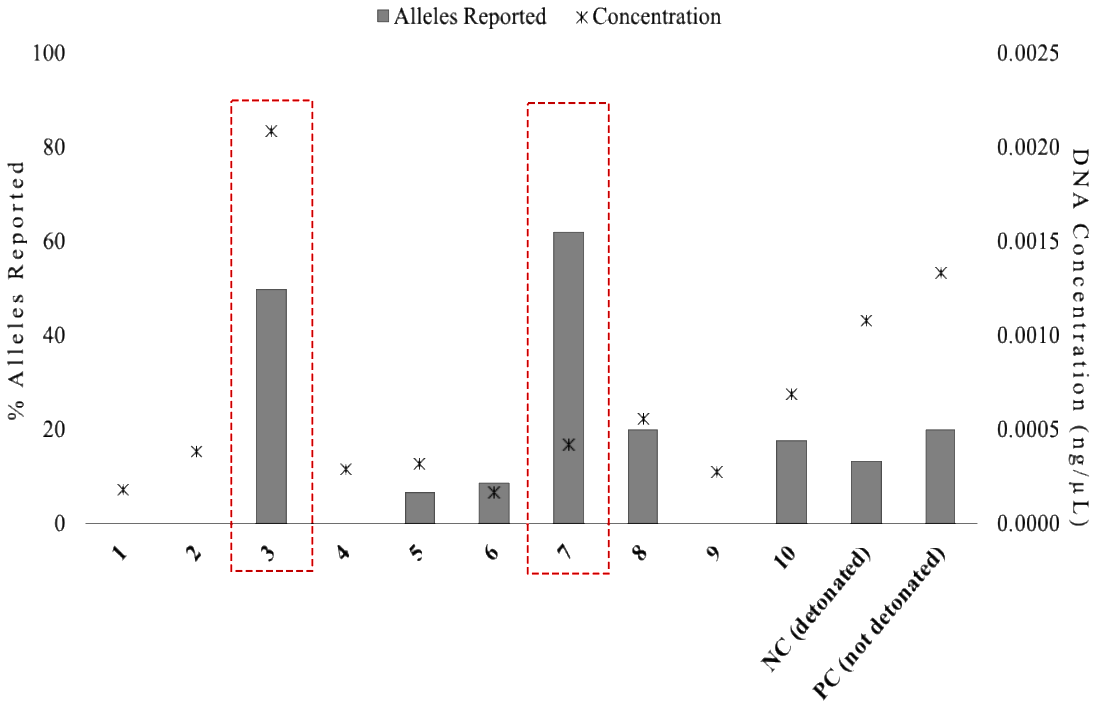

Fig. 5.10 Reported STR alleles (left y-axis) and DNA concentrations (right y-axis) for DNA collected for post-blast PVC fragments (N = 10), the detonated negative control (NC), and the positive control (PC) using 4N6FLOQSwabs™. Red boxes indicate contamination from someone other than the handler.

Three contamination events were observed for the PVC fragments, all of which were matched to reference profiles in the in-house databases (red boxes in Fig. 5.10). Alleles belonging to the handler were also observed in the profile produced from PVC fragments from the detonated negative control. The purpose of this negative control was to assess contamination that occurred during post-blast evidence collection. As the handler was not permitted near the detonation area, it can be inferred that the contamination event took place during the assembly process despite wearing gloves and a face mask. This result highlights how easily DNA can be transferred to a touched item, or via secondary transfer by being in contact with other touched items. In addition, recovering a DNA profile from the handler in this control bomb confirms that small amounts of DNA were likely transferred to the other 10 devices, but then destroyed during the blast process, or not effectively collected during the swabbing process.

Conclusions

In this study, microFLOQ® Direct swabs were used to explore two sub-sampling strategies. Sub-sampling directly from post-blast pipe bomb fragments with microFLOQ® Direct swabs only yielded a few reportable STR alleles and therefore may not be the best approach for collecting "touch" DNA due to the extreme conditions and the trace amounts of DNA on the various substrates. However, sub-sampling from a regular-tipped 4N6FLOQSwabs™ showed more promise, with some complete STR profiles being obtained from firearms. Although more complete STR profiles were generated when touched items were sampled with the 4N6FLOQSwabs™, this study has shown some potential for a sub-sampling and direct amplification approach using microFLOQ® Direct swabs for faster processing and screening purposes, warranting further exploration. In

general, microFLOQ® Direct swabs may be best suited for sub-sampling directly from items that are handled frequently or when the location of biological material is known. Sub-sampling from a larger swab head may also be a viable approach for direct amplification, as the DNA collected from larger pieces of evidence will be concentrated onto a smaller surface, and therefore more likely to be sampled using the microFLOQ® Direct swabs.

Acknowledgements

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CHAPTER VI

CONCLUSIONS

The materials to manufacture pipe bombs are readily accessible at most hardware and sporting goods stores, and the construction process is relatively simple. For these reasons, pipe bombs are one of the most common improvised explosives used to cause terror and destruction. Similarly, firearms such as handguns are frequently used as weapons to commit a variety of crimes, including mass shootings, and often result in numerous injuries and/casualties. There are many forensic approaches that are used to identify person(s) of interest after an explosion or shooting, including chemical analysis, fingerprinting, and DNA analysis. DNA analysis using STR markers are currently the gold standard in forensics as they are robust and quite sensitive to low-quantities of DNA (< 100 pg). However, low-template, degraded, and inhibited DNA often result in stochastic effects that can hinder STR analysis. Other collection strategies may be better suited at maximizing the amount of DNA available for PCR amplification while some strategies involving more sensitive genetic markers may be more informative compared to STR analysis via CE. Therefore, the purpose of this study was to explore alternative collection, amplification, and analysis approaches to improve the genotyping success with low quality and quantity DNA retrieved from pipe bombs and firearms.

Two biallelic markers, INNULs and SNPs, were compared to STR markers and evaluated on their ability to detect minute quantities of DNA from post-blast pipe bomb fragments. While STR analysis did result in some partial profiles, both INNULs and SNPs were more informative to an extent. INNUL amplification using the InnoTyper21™ kit resulted in lower RMP values compared to STRs when fewer than 14 STR alleles were detected. However, the InnoTyper21™ consists of 20 autosomal markers that are less

polymorphic than STRs and may be better suited as a supplement to current STR analysis for challenging and compromised DNA samples. In addition, INNUL markers that are not genetically linked could be combined with STR markers in order to increase the power of discrimination.

SNP analysis via MPS using the what was initially less successful compared to STR and INNUL analysis because the MPS kit used requires a smaller input volume (6 µL) than the other marker systems (15 µL), and therefore less DNA was available. However, concentrating 15 µL of extract down to 6 µL allowed for the same quantities of DNA to be input for MPS and for a more accurate comparison of the three genetic markers. SNP analysis using concentrated DNA resulted in more complete SNP profiles and was more discriminatory compared to STRs and INNULs. Therefore, concentrating low-template DNA extracts may be a viable method to improve overall success with MPS analysis.

In addition to alternate DNA collectors, DNA storage conditions and different storage times were evaluated for "touch" DNA recovered from magazine assault rifles. The results from this study not only demonstrated that room-temperature storage of swabs using the SwabSaver® device produced similar numbers of STR alleles compared to swabs extracted after 24-hours, but that alternate collection devices may increase DNA recovery from touched items.

Collection with nylon swabs showed promise with "touch" DNA and warranted further exploration. A small nylon swab (microFLOQ® Direct) designed for direct amplification was explored as an alternative to conventional cotton collectors. In addition, two direct amplification strategies were examined to improve DNA recovery from common pipe bomb substrates and increase DNA processing times. Traditional DNA processing of

cotton swabs resulted in DNA loss during extraction and few STR alleles. Compared to cotton swabs, direct amplification of microFLOQ® Direct swabs produced more complete STR profiles, with the most complete profile being produced from electrical tape.

Based on this outcome, two sub-sampling strategies using microFLOQ® Direct swabs were applied to handguns and post-blast pipe bomb fragments. DNA was collected from handguns using a regular-tipped nylon swab, and then a microFLOQ® was used to sub-sample from the larger swab head. This sub-sampling strategy did provide some partial and complete STR profiles. The second approach sub-sampled directly from post-blast pipe bomb substrates but did not produce the same level of STR success observed with the other strategy. There are several factors that likely attributed to this difference, including the amount of time each substrate was handled, poor DNA deposition onto the substrate, and DNA degradation caused by extreme heat and pressure from the explosion. The level of STR profile completeness observed in this study was vastly reduced compared to the STR results from the first study (CHAPTER II); however, the location and quantities of DNA on the pipe bombs were known in the first study. This final study provided a more realistic scenario with true "touch" DNA and, therefore, the lack of STR results was not unexpected. In addition, concentrating trace quantities of DNA onto a larger swab head provided a smaller surface area for sub-sampling with the microFLOQ® Direct swabs and increased the amount of DNA available for amplification. Therefore, this could be a viable strategy to rapidly analyze trace DNA and other challenging DNA samples via direct amplification without risking DNA loss via conventional extraction.

In summary, the results of this study demonstrate that other approaches to traditional DNA processing can improve downstream genotyping success from weapons

such as firearms and explosive devices. STR markers have been and will continue to be the gold standard in forensic DNA typing; however, alternate genetic markers could supplement HID when STR analysis fails. In addition, novel collection devices such as microFLOQ® Direct swabs can increase the amount of amplifiable DNA and reduce the amount of time spent processing these samples. Although this study focused specifically on DNA from weapons, the methods could be applied to other challenging DNA samples that are often encountered in forensic casework.

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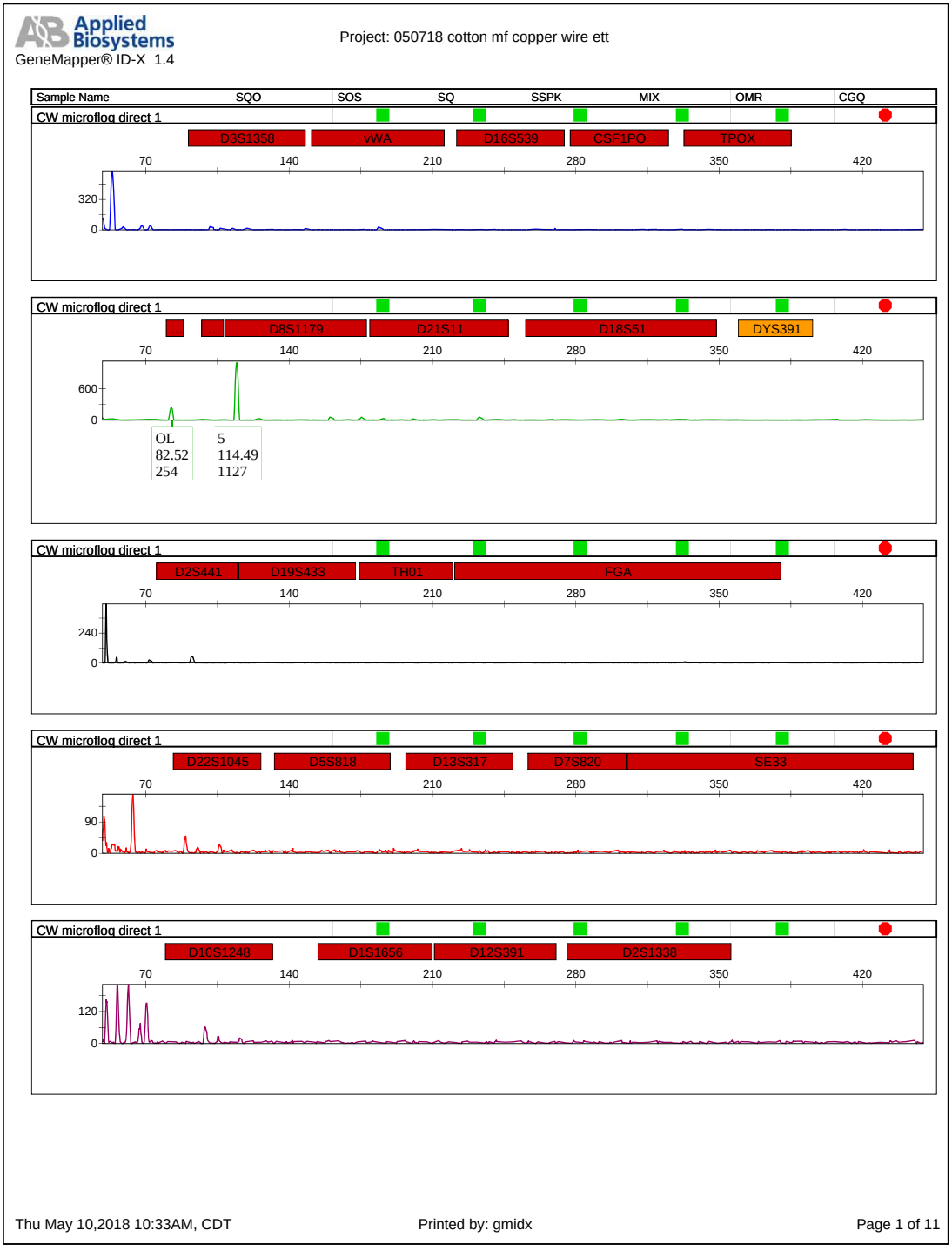
APPENDIX A

Appendix A

Volunteer	Substrate	Collection Date	Storage Time	Storage Type	Extraction Date	Time Handled (s)
A	Plastic	4/18/17	Time Zero	N/A	4/18/17	263
A	Plastic	4/18/17	Time Zero	N/A	4/18/17	164
A	Plastic	9/6/17	Time Zero	N/A	9/6/17	47
A	Plastic	4/25/17	1 Month	SwabSaver	5/25/17	54
A	Plastic	4/25/17	1 Month	SwabSaver	5/25/17	38
A	Plastic	4/25/17	1 Month	SwabSaver	5/25/17	33
A	Plastic	4/28/17	1 Month	Centrifuge Tube	5/29/17	70
A	Plastic	4/28/17	1 Month	Centrifuge Tube	5/29/17	57
A	Plastic	4/28/17	1 Month	Centrifuge Tube	5/29/17	61
A	Plastic	4/19/17	2 Months	SwabSaver	6/19/17	109
A	Plastic	4/19/17	2 Months	SwabSaver	6/19/17	71
A	Plastic	4/19/17	2 Months	SwabSaver	6/19/17	117
A	Plastic	4/21/17	2 Months	Centrifuge Tube	6/21/17	90
A	Plastic	4/21/17	2 Months	Centrifuge Tube	6/21/17	82
A	Plastic	4/21/17	2 Months	Centrifuge Tube	6/21/17	57
A	Aluminum	4/18/17	Time Zero	N/A	4/18/17	165
A	Aluminum	4/18/17	Time Zero	N/A	4/18/17	206
A	Aluminum	4/18/17	Time Zero	N/A	4/18/17	81
A	Aluminum	4/25/17	1 Month	SwabSaver	5/25/17	35
A	Aluminum	4/25/17	1 Month	SwabSaver	5/25/17	49
A	Aluminum	4/25/17	1 Month	SwabSaver	5/25/17	36
A	Aluminum	4/28/17	1 Month	Centrifuge Tube	5/29/17	61
A	Aluminum	4/28/17	1 Month	Centrifuge Tube	5/29/17	60
A	Aluminum	4/28/17	1 Month	Centrifuge Tube	5/29/17	57
A	Aluminum	4/19/17	2 Months	SwabSaver	6/19/17	98
A	Aluminum	4/19/17	2 Months	SwabSaver	6/19/17	83
A	Aluminum	4/19/17	2 Months	SwabSaver	6/19/17	82
A	Aluminum	4/21/17	2 Months	Centrifuge Tube	6/21/17	62
A	Aluminum	4/21/17	2 Months	Centrifuge Tube	6/21/17	67
A	Aluminum	4/21/17	2 Months	Centrifuge Tube	6/21/17	58

Example of collection table (Volunteer A).

APPENDIX B



Example of artifacts observed in electropherograms when directly amplifying DNA collected with microFLOQ® Direct swabs from copper wires.

APPENDIX C

Sam Houston State University
Consent for Participation in Research
Enhancing DNA Recovery from Low Template Samples and Improvised Explosive Devices.

Why am I being asked?

You are being asked to be a participant in a research study about the forensic identification of human DNA from post-blast bomb fragments conducted by Dr. Sheree Hughes-Stamm, the Department of Forensic Science at Sam Houston State University. You have been asked to participate in the research because you are eligible to participate. We ask that you read this form and ask any questions you may have before agreeing to be involved in the research. Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future relations with Sam Houston State University or the Forensic Science program. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

Why is this research being done?

The biological samples provided in this study will serve as mock crime scene evidence after an explosion. These materials will be used for research, and will provide researchers with the appropriate human samples for forensic analysis investigating the utility of various types of DNA markers for identification from “touch” samples.

Biological samples from living humans are required in order to simulate mock crime scene samples such as small amounts of blood, fingerprints and saliva on items of evidence. The ability to recover and retrieve genetic information from the minute amounts of human DNA from these types of samples is vital for forensic analysis.

What is the purpose of this research?

The purpose of this project is to evaluate the utility of alternate DNA markers compared to traditional DNA profiling methods for human identification and forensic intelligence purposes with “touch” and post-blast evidence. This project will also investigate various methods of enhancing low level DNA samples prior to genotyping.

What procedures are involved?

If you agree to be involved in this research, we may ask you to do one or more of the following things:

• Wipe the inside of your cheek with four swabs for approximately 30 seconds

• Collect saliva in a tube (approximately 1 mL).

• Have your venous blood collected by a qualified phlebotomist (approx. 10 mL).

• Place fingerprints on various items

Samples (and consent form with name) will be destroyed after a period of 4 years after the completion of the project. Samples will be disposed of via the standard pathological waste collection service.

Approximately *10-20 subjects* may be involved in this research at Sam Houston State University.

What are the potential risks and discomforts?

There is minimal risk for the participants. These may include:

• Mild irritation and bruising whilst getting blood drawn (arm).

There are no significant physical or psychological, legal or reputational risks to participation. If you feel uncomfortable at any time during the study, please notify Dr. Sheree Hughes-Stamm on 936 294 4359.

All collection procedures will be done at the same time and no future participation is required.

What other options are there?

There are no other options. Animal samples cannot be used for forensic human identification work.

What about privacy and confidentiality?

The only person who will know that you are a research participant is the principle investigator (PI) of this research project. No information about you, or provided by you during the research will be disclosed to others without your written permission, except:

• -if necessary to protect your rights or welfare (for example, if you are injured and need emergency care or when the SHSU Protection of Human Subjects monitors the research or consent process); or

• -if required by law.

When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity. If photographs, videos, or audiotape recordings of you will be used for educational purposes, your identity will be protected or disguised. Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law.

All samples are treated as anonymous, and are assigned a numbered code upon collection. No personal information is collected except the sex, ancestry, hair color, eye color and skin color of the participant. All personal information will only be accessed by the PI.

What if I am injured as a result of my participation?

In the event of injury related to this research study, you should contact your physician or the University Health Center. However, you or your third party payer, if any, will be responsible for payment of this treatment. There is no compensation and/or payment for medical treatment from Sam Houston State University for any injury you have from participating in this research, except as may by required of the University by law. If you feel you have been injured, you may contact the researcher, Dr. Sheree Hughes-Stamm at 936 294 4359.

What are the costs for participating in this research?

There are no research costs for which the subject is responsible.

Will I be reimbursed for any of my expenses or paid for my participation in this research?

The subject will not receive payment, remuneration or reimbursement for participation in this study.

Can I withdraw or be removed from the study?

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may also refuse to answer any questions you don't want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so.

The participant can withdraw from this study at any time for any reason.

Who should I contact if I have questions?

The researcher conducting this study is Dr. Sheree Hughes-Stamm. You may ask any questions you have now. If you have questions later, you may contact the researchers at: Phone: 936 294 4359.

What are my rights as a research subject?

If you feel you have not been treated according to the descriptions in this form, or you have any questions about your rights as a research participant, you may call the Office of Research and Sponsored Programs – Sharla Miles at 936-294-4875 or e-mail ORSP at sharla_miles@shsu.edu.

You may choose not to participate or to stop your participation in this research at any time. Your decision whether or not to participate will not affect your current or future relations with the University. Non-participation in this study will not result in any sanction. Participation is completely voluntary and you are under no obligation to participate. If you are a student, this will not affect your class standing or grades at SHSU. The investigator may also end your participation in the research. If this happens, your class standing or grades will not be affected.

If you are a staff person at SHSU, your participation in this research is in no way a part of your university duties, and your refusal to participate will not in any way affect your

employment with the university, or the benefits, privileges, or opportunities associated with your employment at SHSU.

You will not be offered or receive any special consideration if you participate in this research.

Agreement to Participate

I have read (*or someone has read to me)* the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research.

Consent: I have read and understand the above information, and I willingly consent to participate in this study. I understand that if I should have any questions about my rights as a research subject, I can contact Dr. Sheree Hughes-Stamm at 936 294 4359 or by email at shereehs@shsu.edu. I have received a copy of this consent form.

Your name (printed): _____ _____

Signature: _____

Date: _____

Assigned number:

VITA

Education

Sam Houston State University - Huntsville, TX *Aug. 2014 – Present*
Doctor of Philosophy in Forensic Science
Expected Graduation: Summer 2019

Wesleyan College - Macon, GA *Aug. 2010 – May 2014*
Bachelor of Arts in Biology

Work Experience

Sam Houston State University, Doctoral Teaching Fellowship *Fall 2017 – Present*
Facilitated online undergraduate courses "Introduction to Forensic Science" and "Fundamental of Forensic Biology"

Sam Houston State University, Graduate Assistant *Aug. 2015 – Present*
Assisted with the preparation of class and lab activities

Florida Department of Law Enforcement, Intern - Fort Myers, FL *June – Aug. 2015*

- Shadowed analysts during evidence examination and serology screening
- Trained on the Qiagen® EZ1 Advanced XL Robot
- Conducted two performance checks on the Qiagen® EZ1 Advanced XL Robot

Wesleyan College, Camp Counselor - Macon, GA *July 2013, 2014*
Math and science camp for rising 7th, 8th, and 9th grade girls at Wesleyan College. Duties included aiding professors with class assignments and experiments, and supervising students during free time and field trips.

Talbots, Sales Associate - Macon, GA *April 2012 – Dec. 2013*
Duties include greeting customers, aiding customers with finding items, cleaning, helping create displays/mannequins, ordering items for customers, and restocking merchandise.

Relevant Coursework

Graduate
Forensic Biology, Advanced Forensic DNA, Statistical Genetics, Quality Assurance, Forensic Statistics/Evidence Interpretation, Law and Forensic Sciences

Undergraduate
Molecular Biology, Genetics, Biochemistry, Statistical Methods, Forensic Biology

Relative Skills and Techniques

Extraction: organic extraction (PCIA), Chelex® DNA extraction, PrepFiler™ and PrepFiler™ *Express*, QIAamp DNA Investigator and DNA Mini, EZ1 DNA Investigator
Quantification: QuantiFiler® Duo and Trio, Investigator Quantiplex Pro, PowerQuant®
Amplification: IdentiFiler® Plus, GlobalFiler® and GlobalFiler® *Express*, NGM Detect™, Investigator 24plex QS, PowerPlex® Fusion 6C

Instrumentation

Qiagen® EZ1 Advanced XL and QIAcube® extraction robots, AutoMate Express™ Forensic DNA Extraction System; Applied Biosystems® 7500 and StepOne™ Real-time PCR systems, Applied Biosystems® GeneAmp® 9700, ProFlex™, and Veriti™ PCR systems; Applied Biosystems® 3500 Genetic Analyzer

Software

GeneMapper® ID-X Analysis Software, Microsoft Office, R Statistical Software

Publications in Peer-Reviewed Journals

Esiri Tasker, Carrie Mayes, Bobby LaRue, Sheree Hughes-Stamm. Collection and direct amplification methods using the GlobalFiler™ kit from common pipe bomb substrates. Sci. Justice (2019).

Esiri Tasker, Bobby LaRue, Charity Beherec, David Gangitano, Sheree Hughes-Stamm. Analysis of DNA from Post-blast Pipe Bomb Fragments for Identification and Determination of Ancestry. Forensic Sci Int: Genet. 2017;28:195-202.

Scientific Presentations

microFLOQ®: Collection and Direct Amplification Methods Using the GlobalFiler™ Kit from Common Pipe Bomb Substrates. September 2018. ***Esiri Tasker, BA****; Carrie Mayes, BS; Sheree Hughes-Stamm, PhD*. American Academy of Forensic Science. Baltimore, Maryland. (Oral Presentation)

microFLOQ®: Collection and Direct Amplification Methods Using the GlobalFiler™ Kit from Common Pipe Bomb Substrates. September 2018. ***Esiri Tasker, BA****; Carrie Mayes, BS; Bobby LaRue, PhD; Sheree Hughes-Stamm, PhD*. International Symposium on Human Identification. Phoenix, Arizona. (Poster Presentation)

Optimization of Direct Amplification Methods for DNA Samples from Common Pipe Bomb Substrates Using the GlobalFiler™ Kit. June 2018. ***Esiri Tasker, BA****; Carrie Mayes, BS; Sheree Hughes-Stamm, PhD*. Gordon Research Conference: Forensic Analysis of Human DNA. Newry, Maine. **(**Poster Presentation)

SwabSaver®: Room-Temperature Storage of "Touch" DNA Recovered from Assault Rifle Magazines. May 2018. ***Esiri Tasker, BA****; Sheree Hughes-Stamm, PhD; Bobby LaRue, PhD*. QIAGEN DNA Investigator Forum. San Antonio, Texas. (Oral Presentation)

Efficacy of DNA Recovery and Room-Temperature Storage from Assault Rifle Magazines. February 2018. ***Esiri Tasker, BA****; Sheree Hughes-Stamm, PhD; Bobby LaRue, PhD*. The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. Orlando, FL. (Poster Presentation)

Efficacy of DNA Recovery and Room-Temperature Storage from Assault Rifle Magazines. October 2017. ***Esiri Tasker, BA****; Sheree Hughes-Stamm, PhD; Bobby LaRue, PhD*. International Symposium on Human Identification. Seattle, WA. (Poster Presentation)

Analysis of DNA from Post-blast Pipe Bomb Fragments for Identification and Determination of Ancestry. July 2017. ***Esiri Tasker, BA****; Bobby LaRue, PhD; Charity Beherec, MS; David Gangitano, PhD; Sheree Hughes-Stamm PhD*. Association of Forensic DNA Analysts and Administrators. Austin, Texas. **(**Oral Presentation)

Analysis of DNA from Post-blast Pipe Bomb Fragments for Identification and Determination of Ancestry. September 2016. ***Esiri Tasker, BA****; Bobby LaRue, PhD; Charity Beherec, MS; David Gangitano, PhD; Sheree Hughes-Stamm PhD* International Symposium on Human Identification. Minneapolis, Minnesota. (Poster Presentation)

Analysis of DNA from Post-blast Pipe Bomb Fragments for Identification and Determination of Ancestry. June 2016. ***Esiri Tasker, BA****; Charity Beherec, MS; Bobby LaRue, PhD; David Gangitano, PhD; Sheree Hughes-Stamm PhD* Gordon Research Conference: Forensic Analysis of Human DNA. Waterville Valley, New Hampshire. (Poster Presentation)

Bodies, Bones and Bombs; Human Identification. 2016. *Esiri Tasker, BA; Charity Beherec, MS; Rachel Houston, MS;* ***Sheree Hughes-Stamm, PhD***. 2nd Human Identification Solutions (HIDS) Conference. Barcelona, Spain. (Oral Presentation)

Bodies, Bones and Bombs; Human Identification. 2016. *Esiri Tasker, BA; Charity Beherec, MS; Rachel Houston, MS;* ***Sheree Hughes-Stamm, PhD***. Human Identification University Series. Office of the Chief Medical Examiner, NYC, NY. (Oral Presentation)

HID & MPS for Post-blast bomb fragments and highly inhibited samples. November 2016. *Esiri Tasker, BA; Kyleen Elwick, BA; Bobby LaRue, PhD; Charity Beherec, MS; Rachel Houston, MS; David Gangitano, PhD;* ***Sheree Hughes-Stamm, PhD***. Summit Forum of Forensic Technology and Applications, China Association for Forensic Science and Technology. Foshan, Guangzhou, China. (Oral Presentation)

Memberships, Honors, and Activities

American Academy of Forensic Sciences, Student Affiliate

Saturdays at Sam

Volunteer to talk to students and parents about the different disciplines in Forensic Science.

WOW! A Day
One day a semester Wesleyan College students perform various volunteer services within the Macon, GA community.

Wesleyan Admissions Volunteer Experience (WAVE) Member
Duties included assisting with events/activities, calling, emailing, and touring prospective students.

Beta Beta Beta Biological Honor Society Treasurer, Sigma Lambda Chapter
Assisted in organizing events and fundraisers.

Continuing Education

- The Ethics of Stewardship and the Stewardship of Ethics (2015)
- Bloodborne Pathogens: Exposure in the Workplace (2014)
- Safety Training: OSHA Laboratory Standard (2014)