Evaluation of PowerPlex® Fusion for the Recovery of DNA from Cartridges and Shell Casings

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Abstract

Due to the limited amount of DNA deposited on a cartridge and the potential degradation effect of the firing process, it can be extremely difficult to obtain an interpretable DNA profile. With the introduction of STR megaplexes, such as PowerPlex® Fusion, that are more sensitive, contain smaller loci, and have optimized master mixes, less DNA is needed to generate an interpretable profile. PowerPlex® Fusion contains nine STR loci that are under 215 base pairs. In addition, the PowerPlex® Fusion optimized master mix is able to overcome higher concentrations of known inhibitors than other STR kits on the market [1]. The intention of this research was to evaluate PowerPlex® Fusion for the quantity and quality of DNA profiles produced from cartridges and casings at the Palm Beach County Sheriff's Office (PBSO).

In total, 36 cartridges and 36 shell casings were tested during the course of this study. Quantification values ranging from 0-0.00015 ng/µl were obtained and the total amount of DNA amplified was between 0 pg and 2.3 pg. The DNA results were as follows: 50% of the samples tested yielded no DNA profile, 44% yielded extremely partial profiles with only 1-5 called loci, and 6% yielded a partial DNA profile ranging between 6 and 11 called loci. Drop-in events were also regularly observed and only 18% of samples tested matched the expected volunteer profile. None of the partial profiles obtained met the laboratory's interpretation guidelines.

An additional timed experiment was conducted to try and determine the minimum amount of time a person would have to hold a cartridge to be able to obtain an interpretable profile. Two volunteers held three cartridges for each time interval of 30 seconds, 1 minute, 1.5 minutes, and 2 minutes. The volunteers repeated the two minute time point and those cartridges were fired. Quantification values ranged from 0 ng/µl to 0.0072 ng/µl, with amplification values

from 0-108.1 pg. Of the 28 profiles obtained, one sample from the 1.5 minute time point produced a full profile with 12 alleles above the stochastic threshold, however, there was not a strong correlation observed between the amount of time a cartridge was held and the amount of DNA recovered.

The results of the evaluation indicate that while it is possible to obtain genetic information from cartridges and casings, the use of PowerPlex® Fusion did not generate DNA profiles that met PBSO's current interpretation guidelines. If these samples were actual case evidence, there would not be any CODIS (Combined DNA Index System) qualifying profiles, which is an important element of forensic DNA testing. In addition, there was no difference observed between cartridges and casings with regard to the amount of DNA recovered or the quality of the profile obtained. Until a technology and methodology can be validated in the PBSO laboratory to provide quality, interpretable DNA data from cartridges and casings, PBSO will not accept cartridges and casings for STR testing.

Introduction

Fingerprints and firearms analyses are often used to link suspects to casings and cartridges recovered from a shooting scene. DNA typing has been an area that has been unable to routinely use casings/cartridges to help identify a suspect due to low levels of DNA deposited and possible degradation of DNA due to the firing cycle. The type of DNA deposited on these casings/cartridges is often referred to as touch DNA. Touch DNA is DNA deposited on an object by simply touching the object. The very nature of touch DNA results in low levels of DNA deposited on the object. In the past, STR or Short Tandem Repeat testing has not been able to produce reliable profiles from touch DNA due to the lack of sensitivity of the STR typing kit. In

recent years, STR typing kits have become available that can produce a profile with as little as 100 pg of DNA [1].

New STR megaplexes are designed to have an increased sensitivity in order to amplify touch DNA with greater success when compared to previous STR kits. These megaplexes can overcome high levels of inhibitors, and also operate in a similar manner as mini-STR kits with an increased capacity to amplify damaged or degraded DNA. The megaplex kits have more loci than previous kits, including loci with a shorter amplicon length. The shorter amplicon length may be amplified even though it is severely degraded [1].

A study performed by Karger et al reported a method to analyze the DNA on bullets that pierced a victim when multiple guns were used. By generating a DNA profile from the bullet that pierced a person, an identification of the weapon where the bullet originated could be made using traditional firearms identification techniques. An identification of the weapon where the bullet originated from and a generated DNA profile from the bullet could lead to the identity of the person who handled or fired that specific weapon. This research could be used to identify which participant in a gun fight delivered a fatal bullet. In this study, fourteen bullets were recovered from calves and the bullets were swabbed with moistened fiberglass applicators for DNA processing. A bovine specific DNA fragment was targeted during amplification. DNA was obtained from all the recovered bullets [2]. This study did not attempt to analyze the bullets, casings, or cartridges for the presence of the person handling the gun.

A study performed by Horsmann-Hall et al reported the generation of DNA profile using a double swabbing technique. The study examined at profiles obtained from fired casings and unfired cartridges. An individual handled cartridges for 30 seconds prior to loading and firing. A

total of five fired and five unfired samples were collected and swabbed using a double swab technique. The casings were swabbed with 40 µl of Type I (ultrapure) water on the first swab, followed by a second dry sterile swab. In addition, simulated casework samples were evaluated. A total of 30 samples were collected and swabbed with the same double swab technique described above. The samples were amplified with PowerPlex® 16 (Promega Corporation, Madison, WI) and MiniFiler™ (Life Technologies, Carlsbad, CA). No DNA profiles were obtained from either the fired or unfired samples for PowerPlex® 16, whereas MiniFiler™ produced DNA profiles with 13 called alleles for both the fired and unfired samples. For the simulated casework study, 3.3 alleles were observed using MiniFiler™ and 1.9 alleles were observed using PowerPlex® 16. The data suggests that the firing cycle degrades the DNA on the cartridge leading to preferential amplification. Allelic drop-out and degradation effects were also observed thus further supporting that the firing cycle degrades the DNA [3].

A 2009 study conducted by the Forensic Laboratory for DNA Research was able to generate profiles from cartridges, bullets, and casings (CBCs) when using the QIAamp® DNA Mini kit (Qiagen, Hilden, Germany) after a 30 minute incubation followed by swabbing the CBC. Visual data suggested that over time the CBCs would begin to oxidize in the ATL buffer, producing copper ions that would turn the ATL buffer blue. If the CBCs were in the lysis buffer for a long period of time, the CBC itself would begin to turn blue. These studies demonstrated that limiting the initial incubation step to 30 minutes produced optimum results. The preferred amplification kit was PowerPlex® 16 with the option of extending the profile using MiniFiler™ when a sample produced a full PowerPlex® 16 profile. Over the course of 6 years, 4,085 individual CBCs from 616 cases were analyzed. From these samples, a reproducible DNA profile was obtained in 26.5% of the cases and 6.9% of the individual CBCs. 84.1% of the

individual CBCs profiles obtained were single sourced. Fifty-one samples produced full PowerPlex® 16 profiles and 33 of those samples could be extended to 16 or 17 alleles using MiniFiler™. The data provided did not distinguish between casings and cartridges for the purpose of their study [4]. The data also indicated that the high temperatures reached during the firing cycle would severely damage or degrade any DNA present on the casing. STR typing of degraded DNA can lead to preferential amplification of amplicons resulting in allelic dropout of larger amplicons. Low-level contamination was observed throughout the study [4].

Data from the San Diego Police Department (SDPD) demonstrated the ability to obtain DNA profiles from cartridges using a specialized lysis buffer with Proteinase K and submerging the cartridges in the lysis buffer. The SDPD lysis buffer contained 10mM Tris-HCl, 10mM EDTA, 50mM NaCl, and 2% SDS. The initial lysis step was limited to 30 minutes due to prevent oxidation effects of the buffer eroding microscopic striations on casings or cartridges. The SDPD used 800 rounds for the study, with half being loaded directly from the box and the other half being carried for two days prior to loading. Two hundred cartridges from each group, handled and unhandled, were loaded into the weapon and fired, the other 200 cartridges were loaded into the weapon and then unloaded. From the fired casings and unfired cartridges, 100 casings and 100 cartridges were swabbed with a single nanopure water moistened cotton-tipped swab and half were subject to submersion of the sample in lysis buffer, this experiment design can be seen in Figure 1.

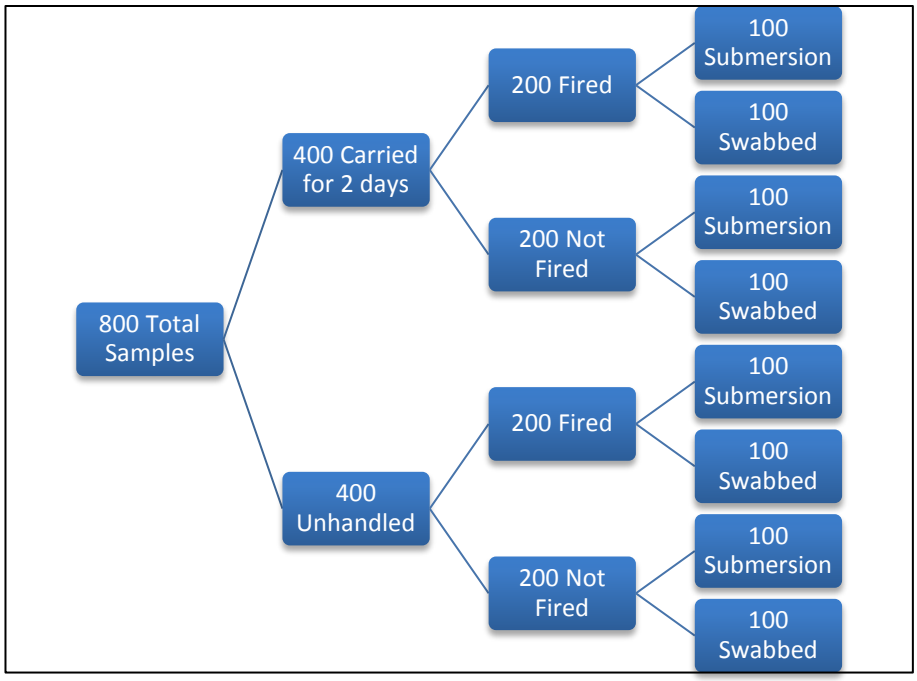

Figure 1 *Experimental design of SDPD*

Interpretable DNA profiles were obtained from 23.6% of the swabbed samples and 34.7% of the submerged samples. In addition, only 21.8% of casings produced interpretable DNA profiles compared to 36.25% of the cartridges. Of the profiles obtained 39.9% were mixtures, including 31% of cartridges loaded directly from the box. From the profiles obtained, 97.4% matched the volunteer who handled and loaded the cartridges. The study also examined 91 casings and one cartridge from casework requests. The results showed that 26.3% of the casings requested for analysis produced an interpretable profile [5]. QIAGEN® developed an extraction protocol based on the results from SDPD. The QIAGEN® protocol follows the same procedure as the SDPD protocol, but QIAGEN® recommended using diluted ATL buffer instead of SDPD's specialized lysis buffer [6].

Wan et al examined the collection of DNA using a dry-lifting or tape-lifting method. Individuals handled cartridges for one minute; the cartridges were then fired and collected. For half of the cartridges and casings in the study, two pieces of tape were placed on the cartridge or

casing in order to cover the entire surface of the item. The pieces of tape were submerged in a 2 ml tube containing a master mix of ATL buffer and Proteinase K. As a baseline, the other half the cartridges were swabbed using a water moistened swab. The amplification kit used during the study was Identifiler® Plus (Life Technologies, Carlsbad, CA), which targets 1.0 ng for amplification [7]. Twelve samples that yielded the highest amount of DNA were amplified. From the profiles that were produced, an average of 6 alleles was called from the swabbed samples with a range of profiles with 0-15 alleles. From the profiles produced by the tape lifted samples, an average of 10 alleles were obtained with profiles ranging from 0-16 alleles [8].

The Palm Beach County Sheriff's Office (PBSO) reported 882 shootings in 2015.There were 828 shootings reported in which an unknown assailant was involved. PBSO has attempted STR testing of cartridges and casings in previous years with kits such as PowerPlex® 16, but failed to obtain interpretable DNA profiles. The preferred extraction method for these types of samples was double swabbing with a water moistened swab. The introduction of new STR megaplexes, such as PowerPlex® Fusion (Promega Corporation, Madison, WI), that have increased sensitivity and increased ability to overcome inhibitors paired with the development of an effective extraction method to use with unfired cartridges and fired casings could result in new probative evidence for PBSO shootings.

Two different extraction methods used in the studies reported herein included tape-lifting and submersion were tested following QIAGEN's® recommended submersion protocol and Wan's recommended tape-lift protocol. These methods were used in order to assess the viability and practicality of their use in a casework setting, as well as the ability of the new PowerPlex® Fusion megaplex to recover DNA from these types of samples. Each method was measured by their respective quantitation results and the quality of the STR profile produced, in reference to

the number of alleles called and if the Relative Fluorescent Unit (RFU) height was above PBSO's validated stochastic threshold. In addition, a timed experiment was conducted to attempt to determine the minimum amount of time a person would have to hold a cartridge to be able to obtain an interpretable DNA profile.

PowerPlex® Fusion is one of the current megaplex kits on the market. It is a 5 dye, 24 loci STR multiplex system for human identification. The system includes all 13 of the current CODIS core loci and 12 core European Standard Set loci. The system also includes a male-specific locus to identify any null Y allele results for Amelogenin. Penta D, Penta E, D2S1338, and D19S433 loci are included in the multiplex to help increase the power of discrimination. The PowerPlex® Fusion System had been validated at PBSO to amplify between 0.25 -0.50 ng of DNA in 10 µl of reaction mix. The reaction mix can accommodate up to 15 µl of sample or water resulting in a total reaction volume of 25 µl. The system is sensitive enough to produce full STR profiles from as little as 100 pg of DNA [1]. This kit, not only has a higher sensitivity for DNA, but it also contains 9 loci that are under 215 base pairs aiding in the recovery of degraded DNA. The kit contains a specialized master mix that is able to overcome higher levels of known inhibitors, such as hematin, humic acid, and tannic acid. The PowerPlex® Fusion System was optimized for amplification on the Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler. The PowerPlex® Fusion System was run on the Applied Biosystems® 3500xL Genetic Analyzer.

Methods

For the purpose of this research, a cartridge is referred to as the unfired portion of a single unit of ammunition consisting of the case, primer, and propellant with a projectile. A casing is

referred to as the portion of the cartridge ejected from the firearm after firing or the base of the cartridge [9]. The term handled, in this research, simply means that the cartridge or casing was held in a volunteer's hand.

Prior to extracting any of the samples used in the research, an initial test of the methods was conducted using extra cartridges and casings. The cartridge or casing was handled for 2 minutes, to ensure enough DNA was deposited on the sample to produce a profile. The sample then underwent the submersion, tape-lift, or traditional swabbing method to compare the recovery from each extraction method. This test ensured that the extraction methods, to be used in the study, were able to recover DNA and were compatible with PBSO's current instrumentation and protocols. The preliminary tests also served to compare the newer extraction methods, submersion, and tape-lift, to the traditional double swabbing method in the amount of DNA recovered.

A total of 72 cartridges were tested throughout the experiments. From this total, 36 of the cartridges were from a .38 Smith and Wesson revolver and 36 were from a 9 mm semiautomatic Glock. The ammunition used in the experiment was American Eagle .38 special centerfire for the revolver and Winchester 9 mm Luger for the semiautomatic.

Three volunteers loaded 6 cartridges into the revolver and fired. The other 18 cartridges were loaded 6 at a time by the volunteers, but were not fired. The same three volunteers repeated the process with the semiautomatic cartridges. The cartridges and the casings were collected and placed into labeled evidence bags. The shedder/non-shedder status of the volunteers was unknown.

For each extraction method, a blank cartridge was added to the sample list for each run. The blank cartridge was processed directly from the box of ammunition without any handling. Blank cartridges were added to each extraction to determine if there was DNA present on the cartridge from the manufacturer. The blank cartridge for each run was alternated between a 9mm cartridge and a 38 special cartridge.

For the submersion method, diluted ATL buffer was prepared by taking one part Buffer ATL and adding two parts TE^{-4}. From the total number of samples, 18 of the casings and 18 of the cartridges were subjected to the submersion method. The casing or cartridge was placed standing up in a flat bottom 5 ml screw cap tube. A master mix of 850 µl diluted ATL buffer, 34 µl Proteinase K, and 1 µl of carrier RNA (cRNA) per sample was prepared and 885 µl of the master mix was distributed to each sample tube. Care was taken when pipetting the master mix into the tubes with casings, to ensure no master mix ended up in the inside of the casing. The tubes were placed in a 56˚C incubator for 30 minutes. Following incubation, each cartridge or casing was removed from its respective sample tube and swabbed with a Buffer ATL moistened cotton-tipped swab. The entire head of the swab was removed and placed in a Nucleic Acid Optimizer™ (NAO) spin basket placed in a click fit tube. The buffer from the 5 ml flat bottom tube was also placed in same NAO™ spin basket as the swab.

The casing or cartridge was rinsed with deionized water and dried with a Kimwipe and set aside in the advent that future analysis or samples were conducted. The literature reported the formation of crystals on the surface of the casings and cartridges when the buffer was left on the surface [5]. The formation of these crystals could impede the identification of microscopic marks left by the firearm during the firing process, making it more difficult for firearms examiners to analyze the casings or cartridges.

The sample cutting and the lysate were placed on an Eppendorf Thermomixer® at 56˚C for 90 minutes. Following the second incubation, the samples were centrifuged for 2.5 minutes at maximum speed. The NAO™ spin baskets were removed from the original click fit tube and placed into another click fit tube. The samples were then centrifuged for 2.5 minutes at maximum speed. The large volume of the buffer necessitated 2 separate centrifugations in order to completely remove the liquid from the spin basket and the swab.

The sample cuttings were retained for any further testing. The lysates from each centrifugation were combined into a single click fit tube. The lysate was placed on the EZ1® XL Advanced and run using the Large Volume Protocol. Each sample was eluted in 40 µl of TE^{-4}.

The remaining 36 samples were collected using the tape-lift method. Prior to DNA extraction, a double sided piece of tape (Scotch® Re-stickable Dots) was placed on a glass slide and subjected to ultraviolet radiation for 30 minutes to remove any extraneous DNA. For this extraction method, the casings and cartridges were handled with rubber tipped tweezers to prevent leaving any microscopic tool marks. Each casing or cartridge was rolled across the tape dot multiple times to ensure all DNA present on the sample was removed, as seen in Figure 2.

Figure 2 *Processing a casing using the tape lift method. Each casing was rolled across a double-sided tape dot with rubber tipped forceps.*

The tape dot was swabbed with a Buffer ATL moistened cotton-tipped swab. The head of the swab was cut and placed in an NAO™ spin basket in a click fit tube. A master mix was made consisting of 190 µl Buffer ATL, 10 µl of Proteinase K, and 1 µl of cRNA per sample and 200 µl of master mix was placed into each NAO™ spin basket. The samples were placed on an Eppendorf Thermomixer® at 56˚C for 15 minutes. Following incubation, the samples were centrifuged at maximum speed for 5 minutes. The swabs were retained for further testing and the lysate was placed on the EZ1® XL Advanced and run using the Trace Protocol [10]. Each sample was eluted in 40 µl of TE^{-4}.

Timed Study

For the additional timed study, two volunteers held three 9mm cartridges, one at a time, for a predetermined amount of time. The time points used during the experiment were 30 seconds, 1 minute, 1.5 minutes, and 2 minutes. For the 2 minutes time point, the volunteers held

six instead of three cartridges. Three of the cartridges from each volunteer from the 2 minute time point were fired through the original 9mm Glock used in the main study. One of the volunteers used in this study was a known shedder, determined by past empirical data.

The casings and cartridges from the timed experiment were extracted using the tape lift method described above, with the exception of using two swabs instead of just one swab. The tape lift extraction method was chosen for this additional experiment due to the laborious and time-consuming nature of the submersion extraction method. Since both of these methods yielded approximately the same amount of DNA, the less labor-intensive method that reduced the chance of contamination was chosen.

Following extraction, all samples were quantified on an Applied Biosystems® 7500 Real-Time PCR (Applied Biosystems, Foster City, CA) with HID Analysis Software using Promega® PowerQuant® (Promega Corporation, Madison, WI). The samples were amplified on the Applied Biosystems® GeneAmp® 9700 (Applied Biosystems, Foster City, CA) with PowerPlex® Fusion, following the DNA workflow and in accordance with PBSO's protocols. The samples underwent capillary electrophoresis on the Applied Biosystems® 3500 xl Genetic Analyzer (Applied Biosystems, Foster City, CA) with a 24 second injection time and a 1.2kV injection voltage and the data was analyzed with GeneMapper® ID-X v 1.3(Thermo Fisher Scientific, Waltham, MA).

The data was analyzed for the amount of DNA obtained from the extraction, the quality of the profile obtained, and each sample was assessed for contamination. The quality of the profile produced was dependent on the number of called alleles, the number of alleles that matched the volunteer who handled the samples, and the Relative Fluorescent Unit (RFU) height of the alleles. For the additional timed experiment, the data from each time point was also

compared to the other time points. Contamination was monitored throughout the experiment and at each step of the DNA workflow.

Results

Out of 89 total samples, including the blank cartridges, a profile was not generated in 50% of the samples tested. Figure 3A summarizes the results of these studies. DNA profiles were categorized regarding the number of loci obtained. None of the samples tested returned full profiles. Only 18% of the samples tested generated DNA profiles that matched the donor as illustrated in Figure 3B.

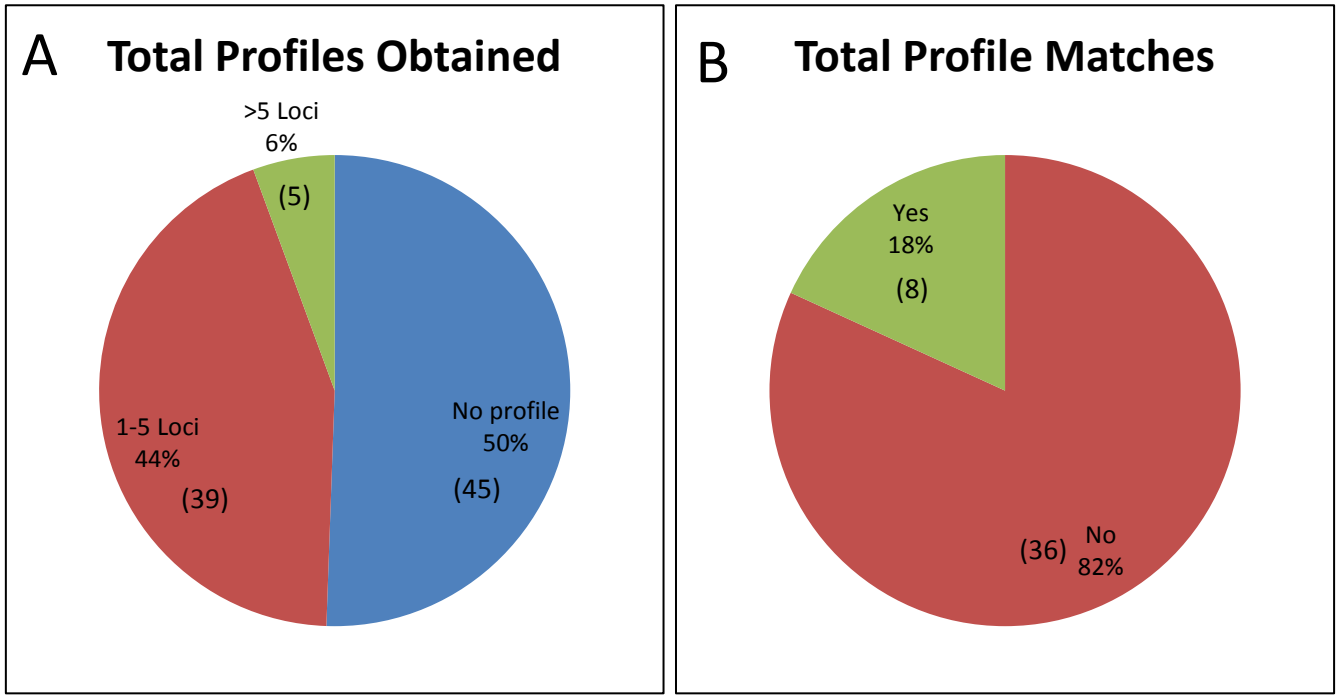

Figure 3A *Total percentage of profiles obtained from the 89 samples.* ***Figure 3B*** *Total percent of profile matches from the 89 samples used in the experiment.*

From the cartridges, in 47% of the samples tested a profile was not generated (Figure 4A) and only 32% generated a profile that matched the donor (Figure 4B).

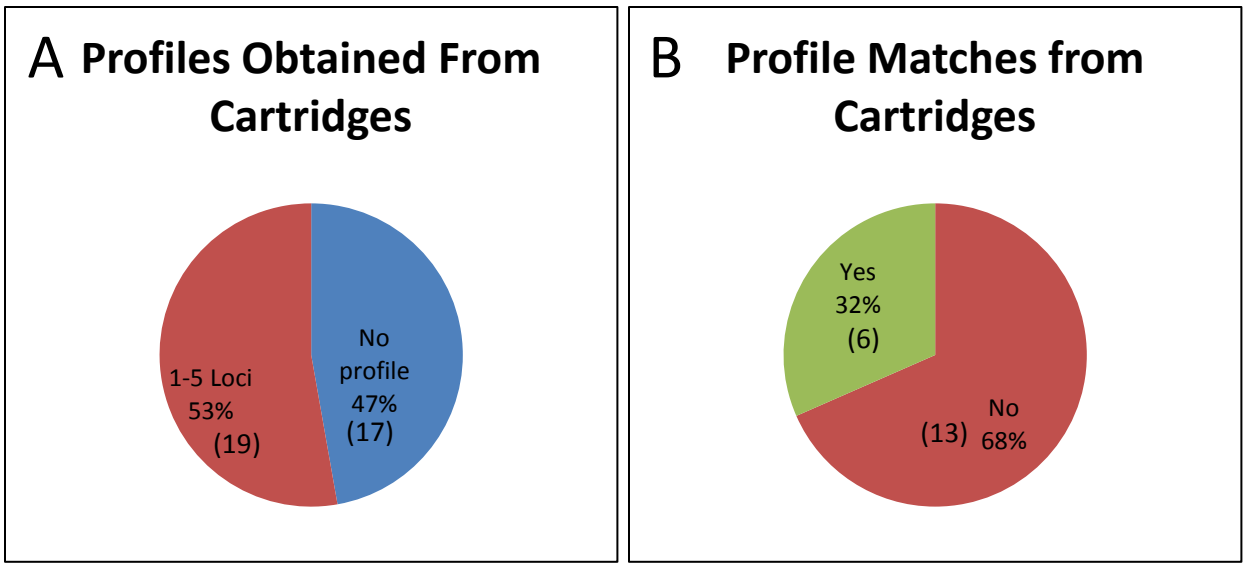

Figure 4A *Percentage of profiles obtained from the cartridges.* ***Figure 4B*** *Percentage of profile matches obtained from the cartridges*

Data from the casings showed that 56% of the samples produced no profiles (Figure 5A) and only 13% returned a profile that matched the donor (Figure 5B).

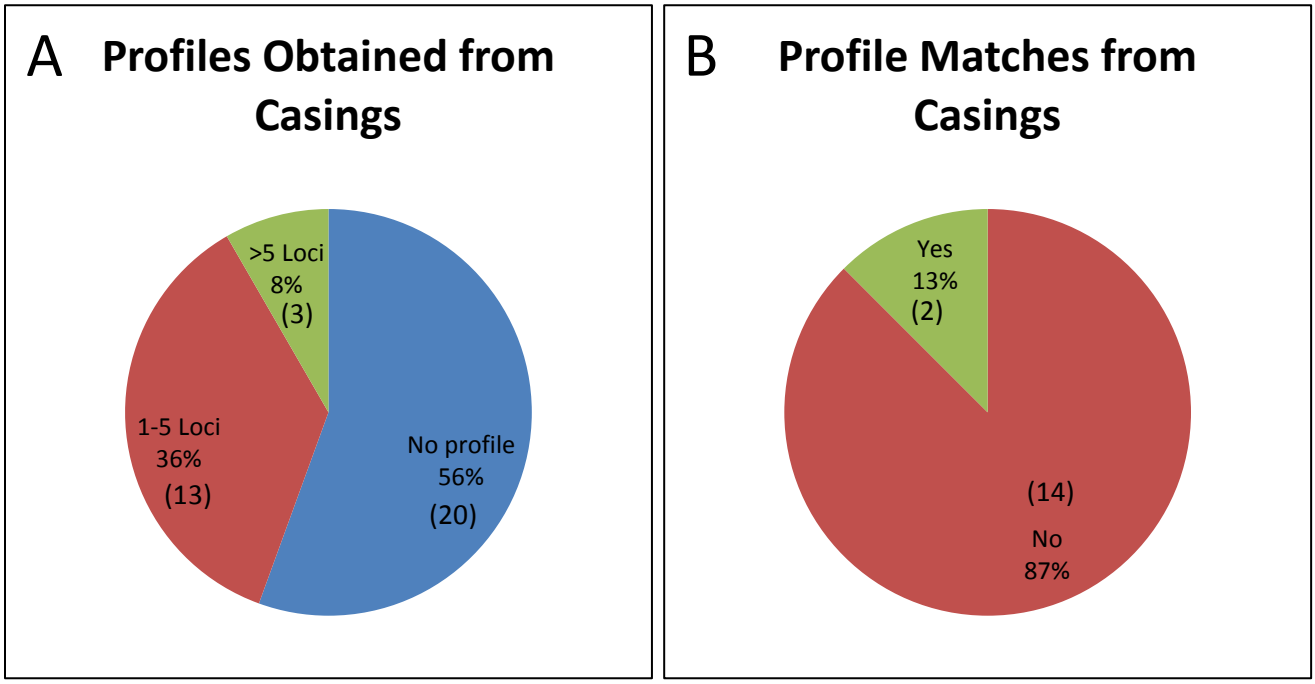

Figure 5A *Percentage of profiles obtained from the fired casings.* ***Figure 5B*** *Percentage of profile matches obtained from the fired casings.*

The blank cartridges for each extraction were alternated between the 9mm semiautomatic and the .38 special revolver, for a total of 19 samples. From these cartridges, 58% returned a profile (Figure 6).

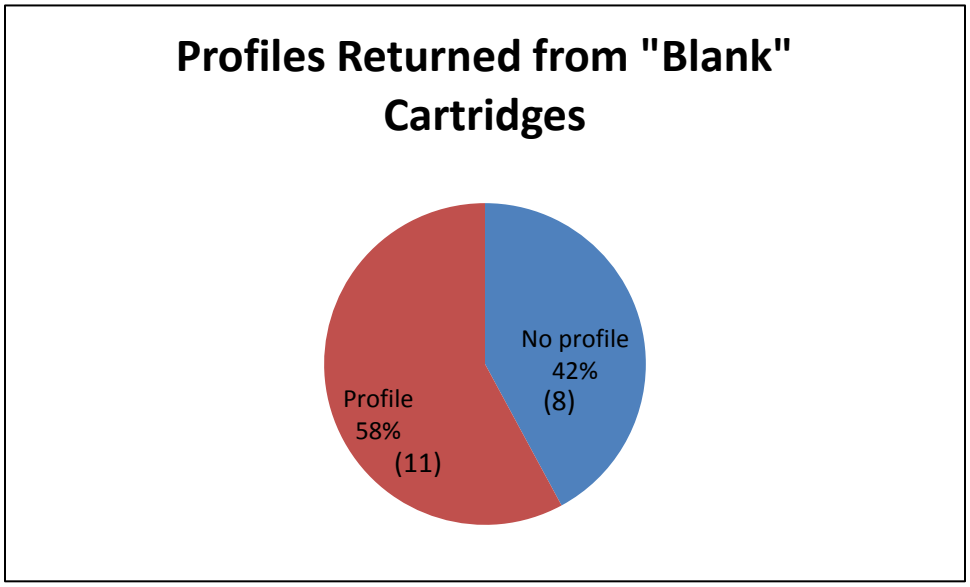

Figure 6 *Percentage of profiles obtained from "blank" cartridges.*

When comparing the different extraction methods and the quantification values obtained, both methods had 24 samples with 0 ng/µl of DNA. The submersion method had a slightly higher number of samples, four samples compared to two samples, which returned quantification values between 0.0001-0.0005 ng/µl as illustrated in Figure 7.

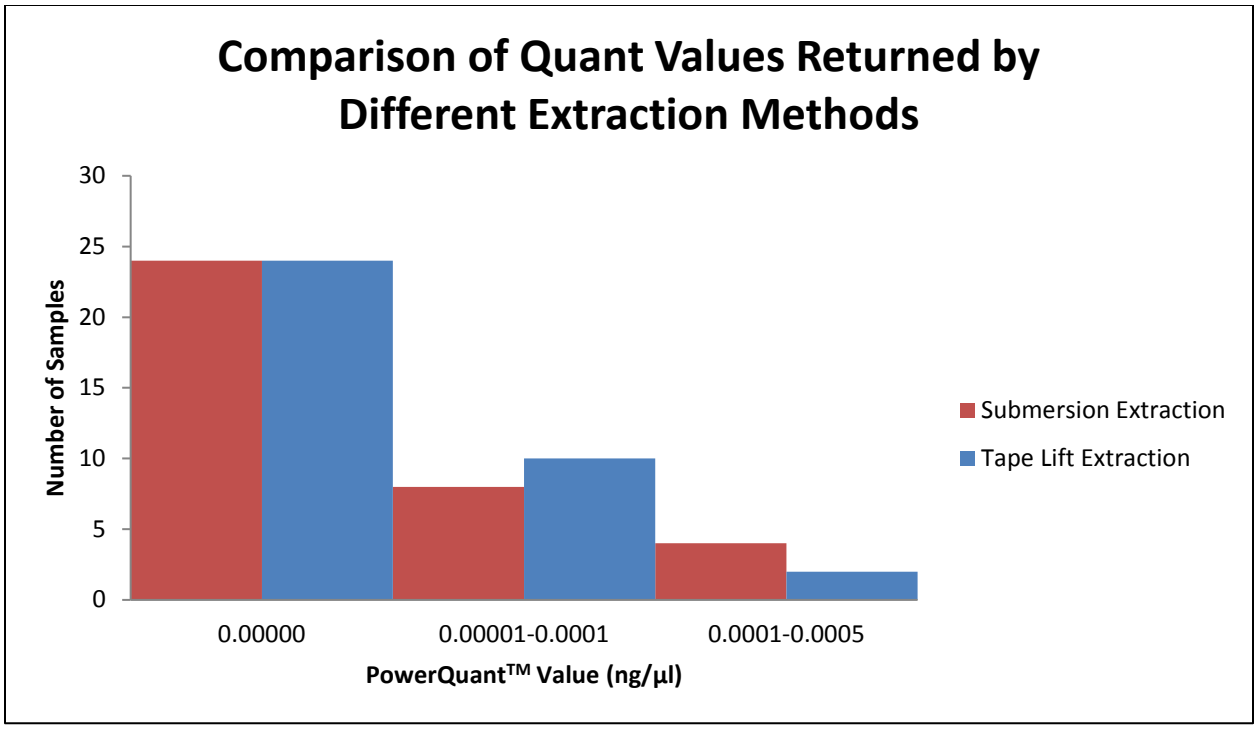

Figure 7 *Comparison of the quantification values returned from PowerQuant™ when using the different extraction methods.*

The data for the different extraction methods show that the submersion method was successful in generating profiles for 58% of the samples (Figure 8A), but only 29% of the samples tested generated profiles that matched the donor (Figure 8B).

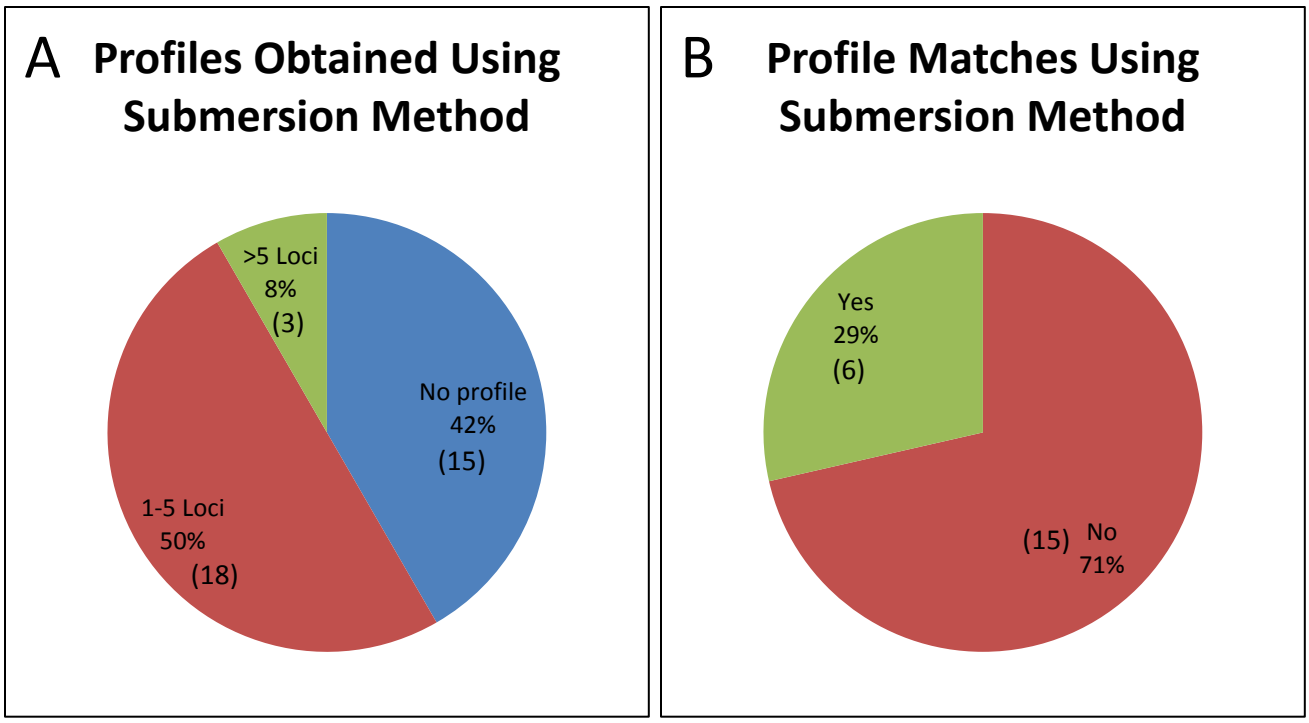

Figure 8A *Percentage of profiles obtained from the samples that underwent the submersion method.* ***Figure 8B*** *Percentage of profile matches obtained from the submersion method*

For the tape lift method, 61% of the samples did not generate a DNA profile (Figure 9A) and a profile was generated in only 14% of the samples that matched the donor (Figure 9B).

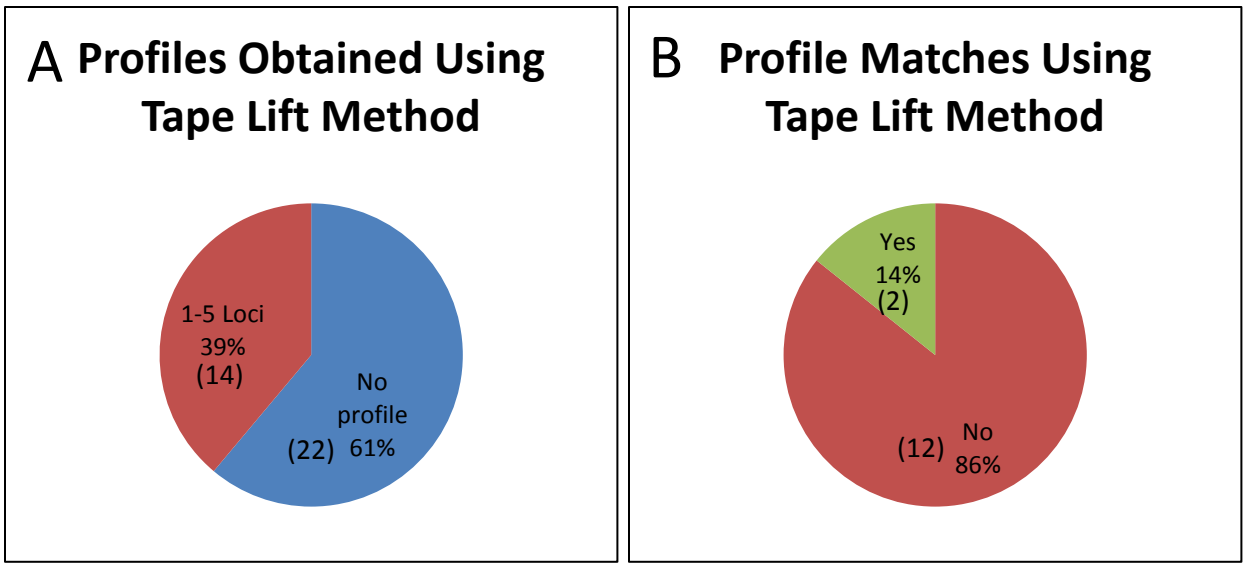

Figure 9A *Percentage of profiles obtained from samples that underwent the tape lift method.* ***Figure 9B*** *Percentage of profile matches that were obtained using the tape lift method.*

Timed Study

For the timed experiment, 68% of the samples tested provided profiles that matched the donor (Figure 10). Also, there did not appear to be a correlation between the amount of time a cartridge was held and the amount of DNA recovered from the cartridge (Figure 11).

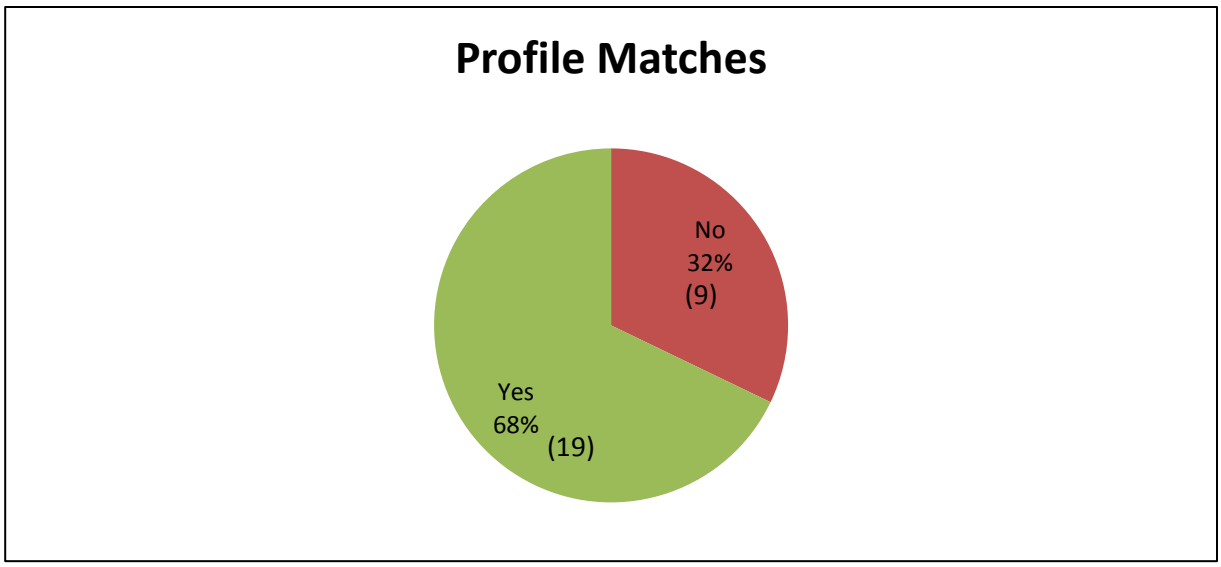

Figure 10 *Percentage of profile matches obtained from the timed study.*

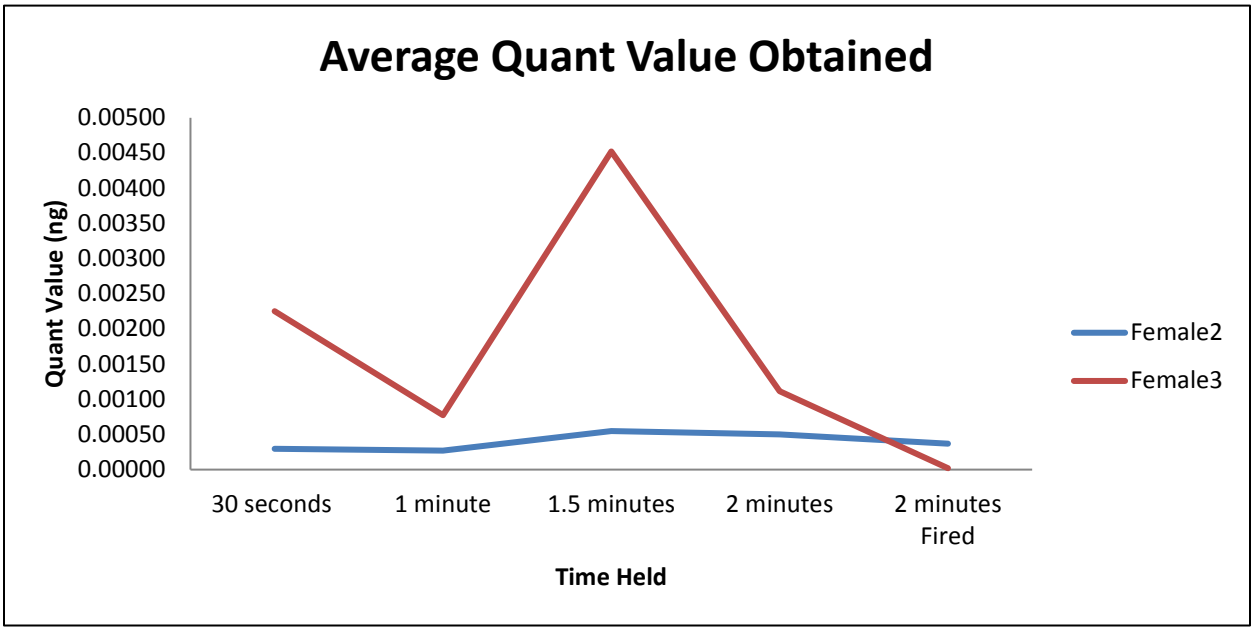

Figure 11 *Average Quant values obtained from the timed study at each interval. Three samples were analyzed at each time interval.*

Discussion

A blue discoloration of the lysis buffer was observed with all of the samples that were subject to the submersion method, as was noted by the Forensic Laboratory for DNA Research in

the Netherlands. The intensity of the blue lysis buffer varied from sample to sample. The blue color change suggested the production of copper ions through the oxidation of the outside of the samples [4]. No correlation as to the effects of the blue lysis buffer on the DNA profile can be made from the data obtained. Evaluation of the quantification results shows that 33% of the samples extracted with the submersion method and 33% of the samples extracted with the tape lift method produced results that were above the laboratory's degradation threshold. The partial DNA profiles that were produced may have resulted from the low amount of DNA present on the cartridges and casings as opposed to adverse effects from the oxidation of the cartridges or casings.

The profiles that did not match the donor were possibly due to contamination by the processing analyst, drop in, an unidentified source, or any combination of the three. There was low-level contamination observed thought the experiment, but according to PBSO's current protocols, none of the samples would have met the laboratory's amplification threshold and continued through the DNA workflow after quantification. This low-level contamination was characterized by less than 5 alleles called, no alleles above the stochastic threshold of 1100 RFU, RFU heights barely above the analytical threshold of 130 RFU, and no contamination in the run negative controls. The laboratory's current protocol stops the DNA workflow of any sample that has a quantification value less than 0.001 ng/µl of DNA, a minimum of 15 pg of DNA is required for amplification. The highest quant value returned from any sample was 0.0004 ng/µl. In addition to the low-level contamination observed, two separate contamination events were observed. The classification of a contamination event occurred when there was contamination by the processing analyst in at least 75% of the samples for that run, slightly higher RFU heights than samples with low-level contamination, contamination of the run negative controls, and 5 or

more alleles called. Both of the contamination events were observed when extracting with the submersion method. These events could explain the high percentage of profiles returned and the higher percentage of profile returned without a match. Even with the contamination events, there was no sample tested that was above the laboratory's minimum amplification threshold of 15 pg.

It is also important to note that none of the profiles obtained produced alleles above the laboratory's stochastic threshold of 1100 RFU; therefore, all the profiles obtained would not be interpretable. All of the quantification values returned were less than the laboratory's amplification threshold, so none of these samples would have been amplified. The highest quantification value returned was 0.0004 ng/µl with the highest amplification value of 6 pg.

There were several profiles produced that did not appear to be the result of contamination or drop in. These profiles did not match any of the donors that participated in the study or the processing analyst. One theory is that the profiles are the result of DNA accumulated during the manufacturing process as human contact is typically involved in quality checking ammunition batches [9]. This conclusion seems unlikely given that all but one of the profiles obtain from the blank cartridges were due to low-level contamination. SDPD reported that during their investigation in possible mixture sources, less than 1% of manufactured ammunition would be subjected to human contact [5].

Timed Study

For the timed study, the correlation between the amount of time the donors held the cartridge and the amount of DNA recovered was not very strong. From this study, one full profile was obtained from a donor who was previously known to be a shedder. Two samples from the study did not return a profile, one sample from a cartridge at the two (2) minute time

point and one casing. The number of loci returned from the samples ranged from 0-23. In addition, the quantification values ranged from 0-0.0072 ng/µl with amplification values ranging from 0-108.1 pg.

From the timed study, one fired casing produced a possible three-person mixture with a male donor. This profile gave 22 out of the 24 loci and had a much higher quantification value than any other sample returning a value of 0.0011 ng/µl with an amplification value of 16.6 pg of DNA. For this reason, the quant values obtained from this time point by "Female2" are slightly elevated when compared with the average quant value from "Female3".The second swab from the fired casing that produced the mixture was extracted and analyzed. The profile produced from the second swab still indicated a mixture, but there were no male alleles called.

The partial male profile that was obtained from the first swab did not match any of the donors from the previous study. Also, there was no vendor contamination, as reaching out to QIAGEN® and searching their records did not produce a match. There was no match for the partial male profile from any of the staff at PBSO.

The results from the time study further support the data obtained from the Netherlands of the firing process damaging DNA [4]. The average amount of DNA recovered from the cartridges after being held for two minutes was 0.000805 ng/µl, while the average amount of DNA recovered from the casings was 0.000195 ng/µl. The decrease in the amount of DNA recovered could be attributed to the high heat achieved in the chamber of the gun during the firing process or the motion of the cartridge when firing.

Recommendations

It is recommended that two swabs be used when swabbing the tape dot or the sample. This allows one swab to be preserved for further testing. Also, future studies may be considered in which the efficacy of the SDPD in-house lysis buffer is compared with the QIAGEN® recommended lysis buffer when using the submersion method. Additional individuals should be added for future studies for a more robust sample size.

Conclusions

STR testing has become more sensitive from when the first STR kits were introduced for DNA testing. Recent advances in the STR kits commercially available to forensic DNA laboratories have increased the number of interpretable DNA profiles. In addition, the newer STR megaplexes are much more sensitive and robust in terms of overcoming degradation and inhibition than previous kits.

The different extraction methods examined posed their own challenges and difficulties with regard to processing the samples, but the submersion method was more laborious and time-consuming than the tape lift method. The submersion method required more awareness of the samples due to the multiple transfer steps involved, presenting an elevated risk of contamination. As a result of the multiple transfers, the instances of contamination observed in the study were much higher in samples that underwent the submersion method.

The combination of low quantification values, the increased risk of contamination, the observance of drop in, the non-concordant results with the expected profile, and lack of alleles above the stochastic threshold affected the ability to obtain reliable and interpretable profiles. Of

the samples processed in the study, 50% of the samples returned a profile, only 18% of the profiles returned matched the expected volunteer profile, and none of the samples tested met the laboratory's amplification threshold to continue in the DNA workflow past quantification. The results of the evaluation indicate that while it is possible to obtain genetic information for cartridges and casings, the use of PowerPlex® Fusion did not generate DNA profiles that met the laboratory's current interpretation guidelines. In addition, there was no difference observed between cartridges and casings with regard to the amount of DNA recovered or the quality of the profile obtained. Until a technology and methodology can be validated in the PBSO laboratory to provide quality, interpretable DNA data from cartridges and casings, PBSO will not accept cartridges and casings for STR testing.

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