Research paper

Direct PCR amplification of DNA from human bloodstains, saliva, and touch samples collected with microFLOQ® swabs

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ABSTRACT

Previous studies have shown that nylon flocked swabs outperform traditional fiber swabs in DNA recovery due to their innovative design and lack of internal absorbent core to entrap cellular materials. The microFLOQ® Direct swab, a miniaturized version of the 4N6 FLOQSwab®, has a small swab head that is treated with a lysing agent which allows for direct amplification and DNA profiling from sample collection to final result in less than two hours. Additionally, the microFLOQ® system subsamples only a minute portion of a stain and preserves the vast majority of the sample for subsequent testing or re-analysis, if desired. The efficacy of direct amplification of DNA from dilute bloodstains, saliva stains, and touch samples was evaluated using microFLOQ® Direct swabs and the GlobalFiler™ Express system. Comparisons were made to traditional methods to assess the robustness of this alternate workflow. Controlled studies with 1:19 and 1:99 dilutions of bloodstains and saliva stains consistently yielded higher STR peak heights than standard methods with 1 ng input DNA from the same samples. Touch samples from common items yielded single source and mixed profiles that were consistent with primary users of the objects. With this novel methodology/workflow, no sample loss occurs and therefore more template DNA is available during amplification. This approach may have important implications for analysis of low quantity and/ or degraded samples that plague forensic casework.

1. Introduction

Nylon flocked swabs, such as 4N6 FLOQSwabs® (Copan, Brescia, Italy), were designed to maximize DNA collection and elution efficiency. In contrast to traditional fiber swabs, 4N6 FLOQSwabs® consist of short nylon fibers arranged in perpendicular fashion at the tip of an applicator shaft. Because these flocked swabs have no internal absorbent core to disperse and entrap the specimen, the sample remains close to the swab head surface, which facilitates analyte release and elution [1,2]. Studies have shown that 4N6 FLOQSwabs® outperform traditional fiber swabs in terms of DNA recovery [3–5].

One of the newest anatomic and ergonomic designs of 4N6 FLOQSwabs® is the microFLOQ® Direct swab (co-developed by the French Gendarmerie Forensic Research Institute, IRCGN™ and Copan). The fibers of microFLOQ® Direct swabs are arranged in the same manner as 4N6 FLOQSwabs® but are treated with a lysing agent for direct amplification, eliminating the need for DNA extraction and quantification [6]. Use of these swabs can enable DNA profiling from sample collection to final result in less than two hours. Additionally, due to the small dimension of the microFLOQ® swab head, only a minimal portion of a stain is collected, and thus there is far less sample consumption than traditional swabbing methods. Efforts that reduce sample consumption allow for more evidence to be retained for re-testing or post-conviction testing, if desired.

The purification step currently used in forensic DNA casework is time-consuming and labor intensive. Additionally, column-based purification methods result in loss of DNA, which could affect successful typing of degraded or low copy number samples [7–14]. Direct amplification with the miniaturized microFLOQ® offers the combined benefits of eliminating the purification step from sample processing, increasing laboratory throughput capacity, and consuming less sample to obtain a result. This study investigated the efficacy of direct amplification of DNA from bloodstains, saliva stains, and touch samples using microFLOQ® Direct swabs and the GlobalFiler™ Express system (Thermo Fisher Scientific, Waltham, MA). Comparisons in performance were made to traditional (manual extraction) methods to assess if improvements or changes could be made to the standard workflow in forensic casework by using microFLOQ® Direct swabs.

2. Materials and methods

Blood, saliva, and touch samples from four different individuals were collected and anonymized in accordance with methods approved by the Institutional Review Board of the University of North Texas Health Science Center in Fort Worth, Texas USA.

2.1. Human blood and saliva (buccal cell) samples

Whole human blood samples were collected in EDTA-treated tubes and stored at 4°C until use. Blood dilutions (10%, 5%, 1%) were prepared with physiological saline. Human saliva was collected in sterile conical tubes and diluted to 10%, 5%, and 1% with molecular grade water. Ten microliters of each blood dilution, neat saliva, and each saliva dilution were pipetted onto sterile glass microscope slides and allowed to dry overnight.

2.2. Touch samples

Casual contact (touch) samples were obtained from a variety of surfaces, including four computer keyboards, three door handles, two computer mouses, two cell phones, and a necklace.

2.3. Swabbing of bloodstains, saliva stains, and touch samples

Bloodstains, saliva stains, and touch samples were collected with microFLOQ® Direct swabs (Copan, French Gendarmerie Forensic Research Institute) according to the "microFLOQ® Wet or Dry Traces Collection Procedure" (unpublished, copy provided by the manufacturer). In this procedure, each microFLOQ® Direct swab was either used dry or moistened with 1 μl of molecular grade water. The swab head was rubbed across the stain or surface in a subsampling manner to collect a very limited portion of the sample. For comparison studies after swabbing with a microFLOQ® Direct swab, the remainder of each stain was collected with a 4N6 FLOQSwab® (Copan) according to the "DNA Wet or Dry Sample Collection with 4N6 FLOQSwabs®" protocol (unpublished, copy provided by the manufacturer). One side of the 4N6 FLOQSwab® was moistened with 30 μl of molecular grade water and rolled over the surface to collect the majority of the stain. The dry side of the swab then was rolled over the same surface until the sample was completely collected. 4N6 FLOQSwabs® were allowed to dry overnight prior to DNA extraction.

2.4. DNA extraction

Bloodstains and saliva stains collected with 4N6 FLOQSwabs® were extracted using Nucleic Acid Optimizer (NAO®) Baskets (Copan) [15] and the QIAamp® DNA Investigator Kit (Qiagen, Valencia, CA) [16]. Incubation of swab heads was performed in NAO® Baskets and modifications were made to the QIAamp® extraction protocol according to Copan's recommendations. Modifications included performing sample lysis in NAO® Baskets under static conditions, with no vortexing or shaking during the first two incubation steps.

2.5. DNA quantification

The quantity of DNA recovered from 4N6 FLOQSwabs® was determined using the Quantifiler® Human DNA Quantification Kit and an ABI 7500 Real-Time PCR System (Thermo Fisher Scientific), according to manufacturers' recommendations [17].

2.6. PCR amplification of microFLOQ® Direct swabs

After stain collection, the tips of the microFLOQ® Direct swabs were snapped off into PCR strip tubes and amplified using the GlobalFiler™ Express PCR Amplification Kit (Thermo Fisher Scientific) [18]

according to Copan's "Direct DNA Analysis with the microFLOQ® Collection Device" protocol (unpublished, copy supplied by the manufacturer). In this protocol, the volume of sample solution required by the kit manufacturer was replaced with molecular grade water, PCR master mix was added directly to the tubes, and immediate amplification was performed on an ABI GeneAmp® 9700 PCR System (Life Technologies, Foster City, CA) for 28 cycles, following manufacturer's recommended conditions. For each batch of reactions, positive control DNA (007) was amplified in the presence of a microFLOQ® Direct swab to assess potential inhibition from the lysing agent incorporated into the swab head fibers. Negative controls containing only the microFLOQ® swab head also were included.

2.7. PCR amplification of manually extracted 4N6 FLOQSwabs®

Amplification of autosomal STRs, Y-STRs, and a Y-indel was performed using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) and 1 ng input DNA. For samples with less than 1 ng of DNA available for testing, the maximum recommended volume (15 μl) was added to the amplification reaction. Thermal cycling was performed on an ABI GeneAmp® 9700 PCR System (Life Technologies) for 29 cycles, according to the manufacturer's recommendations [19].

2.8. DNA detection, separation, and analysis

Amplified products were size-separated and detected on an ABI 3500xl Genetic Analyzer (Life Technologies) using 1 μl PCR product, 9.5 μl Hi-Di™ formamide, and 0.5 μl GeneScan™ 600 LIZ® Size Standard v2.0 (Thermo Fisher Scientific). One microliter of allelic ladder was included at least once per injection on the 96-well plate. Samples were denatured at 95 °C for 5 min and then immediately cooled on ice for 5 min. Electrophoresis was performed on a 36-cm capillary array with POP-4™ polymer (Life Technologies) using standard injection parameters (1.2 kV, 24 s). STR data were sized and typed with GeneMapper® ID-X Software Version 1.4 (Life Technologies) using manufacturer validated analytical thresholds. For analysis, total RFUs observed were directly reported for homozygous loci and by adding the peak heights of each allele for heterozygotes.

3. Results and discussion

3.1. Dry swabbing versus wet swabbing with the microFLOQ® system

The "microFLOQ® Wet or Dry Traces Collection Procedure" offers instructions for collecting evidentiary stains both with and without moistening the swab head. Direct amplification studies with bloodstains and saliva stains were carried out using both methods. Minimal results were obtained with dry swabbing (data not shown), presumably due to the inefficiency of dry fibers to retrieve and retain the cellular components of the stains for subsequent analysis. Markedly improved signal was observed when microFLOQ® Direct swab heads were moistened prior to stain collection, and therefore all subsequent studies were performed using the wet collection procedure.

3.2. Modifications to manual DNA extraction (static lysis)

NAO® Baskets were used instead of traditional spin baskets and incubation steps were performed under static conditions. The NAOb® Basket is an alternative to the traditional spin basket and reduces sample manipulation during DNA extraction. Incubation and lysis of the sample occurs entirely within the chamber of the NAO® Basket. After incubation, the NAO® Basket (designed with a collapsible grid bottom) can be used for a one-step collection of sample eluate from the swab head. With traditional spin baskets, the swab head is transferred manually to the basket and then subjected to centrifugation to recover remaining liquid (and DNA) trapped within the fibers of the swab. This
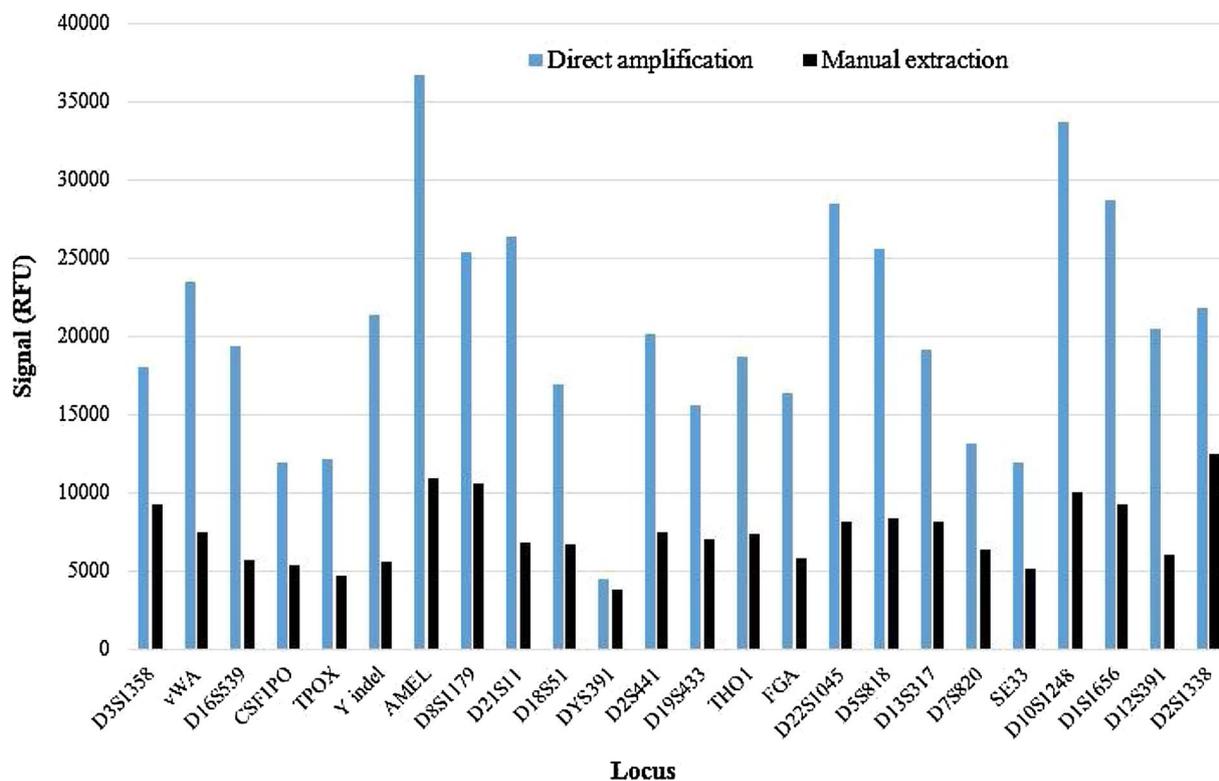
Fig. 1. Comparison of average signal (RFU) per locus from DNA in 5% bloodstains after direct amplification using microFLOQ® swabs (n = 30) and GlobalFiler™ Express versus manual extraction of 4N6 FLOQSwabs® (n = 30) using the QIAamp® DNA Investigator Kit and amplification of 1 ng DNA with the GlobalFiler™ kit. Standard deviation (SD) values are provided in Supplementary Table 3.

manual transfer step increases the risk of cross-contamination and results in potential loss of DNA. Aside from reducing sample manipulation, it has been reported that a 60% increase in DNA recovery can be achieved by processing samples with the NAO® Basket in comparison with standard procedures [15].

During DNA extraction with the QIAamp® DNA Investigator Kit, the protocol recommends vortexing the samples prior to incubation on a heat block and then subsequent shaking at 900 rpm for at least 1 h. Throughout the extraction protocol, the user is reminded that "to ensure efficient lysis, it is essential that the sample and buffer be thoroughly mixed to yield a homogenous solution" [16]. The NAO® Basket design is such that the tube lid does not close tightly. If the seal was tight, the collapsible grid would not open during the high speed centrifugation step. The NAO® Basket protocol employs a static lysis method without vortexing and without shaking during incubation. There does not appear to be any impact on recovery of DNA with the static lysis method (data not shown).

3.3. Assessment of inhibition from chemical treatment of microFLOQ® swab heads

Positive control DNA (007) was amplified in the presence of a microFLOQ® swab head for each batch of samples tested. All positive controls yielded full STR profiles and performed as expected, suggesting that the manufacturer's chemical treatment of microFLOQ® swab heads with a lysing agent does not interfere with or inhibit successful amplification of DNA.

3.4. Direct amplification of DNA from human bloodstains

Dilute (10%) bloodstains (n = 30) were collected with moistened microFLOQ® swabs and immediately amplified with GlobalFiler™ Express. Only seven samples yielded full STR profiles (Supplementary Table 1). Given that the blood samples were recently collected and

stored under controlled conditions in a refrigerator (and therefore the DNA contained in the samples should be nondegraded and of high quality), a higher success rate was expected. Two explanations for the lower success rate are that insufficient sample was collected with the microFLOQ® swabs and/or substantial inhibition was impacting amplification. While the most probable explanation is inhibition due to the presence of heme from the bloodstains, other factors could have contributed to the variation in observed results. The flocked design and open matrix of the microFLOQ® swab head results in a non-absorbent core. With this design excessive rolling, manipulation, or rubbing during swabbing can result in sample loss during collection and/or fiber loss from the swab head. Some of the partial profiles obtained could be the result of user variation. Non-optimal collection and/or sample loss could have resulted due to the small diameter of the microFLOQ® swab head (approximately 1 mm), minor differences in swabbing technique, differences in the amount of pressure applied, and/or due to variation in the area or quadrant of the stain that was subsampled for testing.

Larger loci for each dye channel dropped out for the majority of samples and the typical "ski slope" effect representative of degraded or inhibited samples was observed in electropherograms (Supplementary Fig. 1). Additionally, split peaks were present in many of the samples (especially for the smaller loci in each dye channel), an observation often attributed to excessive amounts of DNA in the PCR [20–23]. To assess whether inhibition from heme was occurring during direct amplification, additional diluted bloodstains (5% and 1%) were prepared for analysis. Signal decreases across each dye channel from smaller to larger loci were observed with 10% bloodstains. However, with 5% bloodstains, full STR profiles were obtained from 28 out of 30 samples. Profiles were markedly more balanced than those obtained from 10% bloodstains and average signal (RFU) across all loci was greater than for 10% bloodstains. For 1% bloodstains, full profiles were obtained for all but one sample, although peak heights were lower than for 5% bloodstains. Moreover, the split peaks (i.e., incomplete adenylation) present with direct amplification of 10% bloodstains were not observed
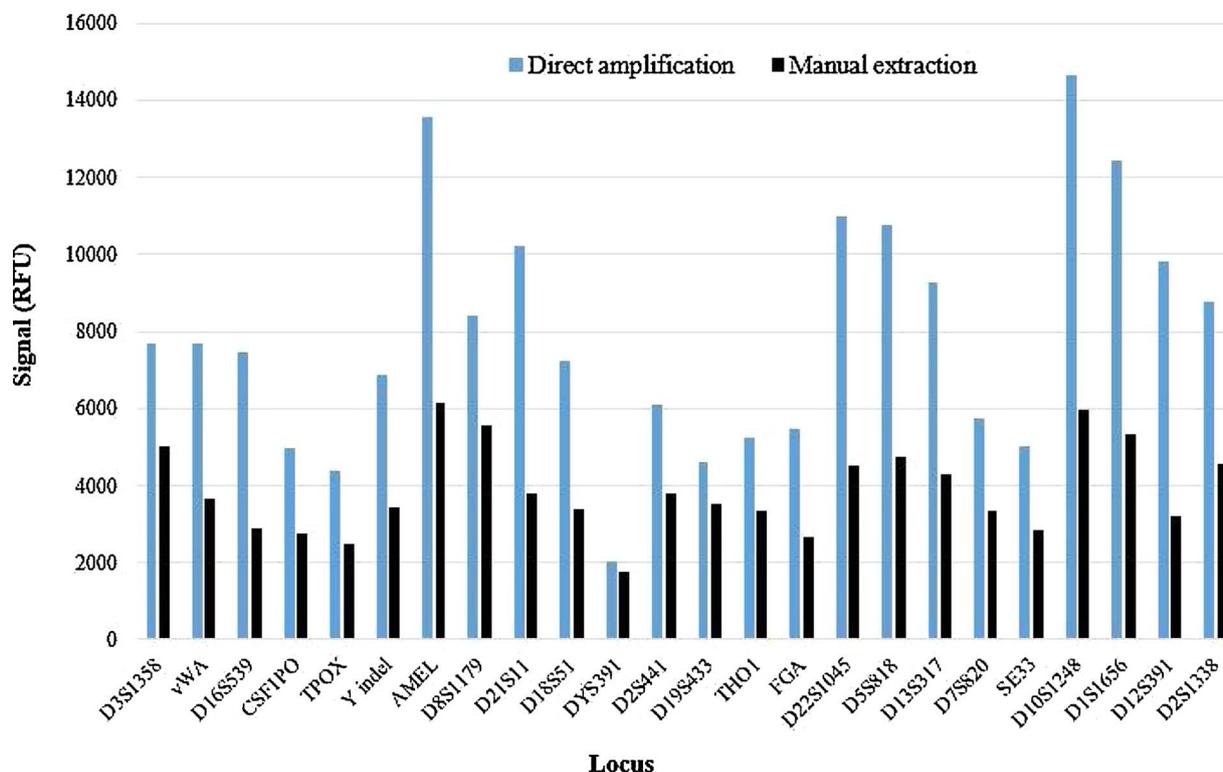
Fig. 2. Comparison of average signal (RFU) per locus from DNA in 1% bloodstains after direct amplification using microFLOQ® swabs (n = 30) and GlobalFiler™ Express versus manual extraction of 4N6 FLOQSwabs® (n = 30) using the QIAamp® DNA Investigator Kit and amplification of 1 ng DNA with the GlobalFiler™ kit. Standard deviation (SD) values are provided in Supplementary Table 4.

with 5% bloodstains or 1% bloodstains. Average signal (RFU) per locus for each of the three blood dilution experiments are summarized in Supplementary Table 2. These results are indicative that inhibition occurred during direct amplification of 10% bloodstains and that the microFLOQ® swabs can collect sufficient sample to obtain high quality profiles from at least 1:99 diluted bloodstains.

3.4.1. Comparison of direct amplification of human bloodstains to the traditional (manual extraction) workflow

Since forensic casework samples often are low quantity and/or degraded, additional studies were performed to compare STR results from direct amplification to those obtained with traditional extraction and typing methods. For 5% and 1% bloodstains, higher signal (RFU) was observed for all loci after direct amplification compared with those that were collected, extracted, and amplified (with 1 ng input DNA) using the standard casework approach (Figs. 1 and 2). These results are promising, as the ability to obtain comparable (or higher) signal using direct amplification would save considerable time and resources, as well as potentially reduce the risk of cross-contamination due to less sample and tube manipulations compared with the manual extraction process. Additionally, the microFLOQ® swab subsamples a much smaller portion of a stain than a standard-sized swab head, preserving evidence for re-testing or future inquiries. Supplementary Tables 3 and 4 show the locus-by-locus signal and total average signal with standard deviation (SD) for each sample for both methods for 5% bloodstains and 1% bloodstains, respectively.

The higher standard deviation values observed with direct amplification may be attributed to several factors. Stochastic sampling likely is occurring due to the compact size of the microFLOQ® swab head and the small portion of the stain that is collected. Additionally, with direct amplification, samples are not quantified and normalized prior to STR genotyping. With the direct amplification approach it is unknown whether there is more than 1 ng of DNA present on the swab or if a concentration of sample on the small surface area of the swab

contributes to the increased signal compared with the standard manual method.

3.5. Direct amplification of DNA from human saliva

Neat saliva stains (n = 15) were collected and immediately amplified with GlobalFiler™ Express. Fourteen samples yielded full STR profiles. Although the profiles from neat saliva were noticeably more balanced than those from 10% bloodstains, a mild-to-moderate 'ski slope' effect still was present in many of the electropherograms. Also, similar to results obtained from bloodstains, evidence of split peaks (i.e., incomplete adenylation) was present for a large number of samples. Unlike blood samples which contain a known PCR inhibitor (heme), saliva has not been reported to intrinsically possess substances that would impact amplification. However, signal was lower than expected given the undiluted nature of the samples, indicating that perhaps some constituents in saliva may inhibit amplification to some degree. Additionally, the amount of input DNA could be excessive and may be overloading the GlobalFiler™ Express reaction.

To further determine if some level of inhibition was occurring, 10% saliva stains (n = 15) were prepared, collected, and analyzed. Complete STR profiles were obtained for all samples. More importantly, peak heights (RFU) for all loci increased compared with the results observed with neat saliva, which supports that some component(s) in neat saliva inhibits the GlobalFiler™ Express reaction or that the effective input DNA is excessive. Profiles from 10% saliva showed better interlocus balance and no evidence of the incomplete adenylation that was observed both with neat saliva stains and 10% bloodstains. Results and locus-by-locus signal (RFU) for each neat saliva and 10% saliva sample are summarized in Supplementary Table 5. Direct amplification of 5% saliva stains (n = 6) and 1% saliva stains (n = 6) showed no evidence of inhibition and resulted in complete, balanced profiles. Signal was higher for 5% saliva stains than for 1% saliva stains (Supplementary Table 6).
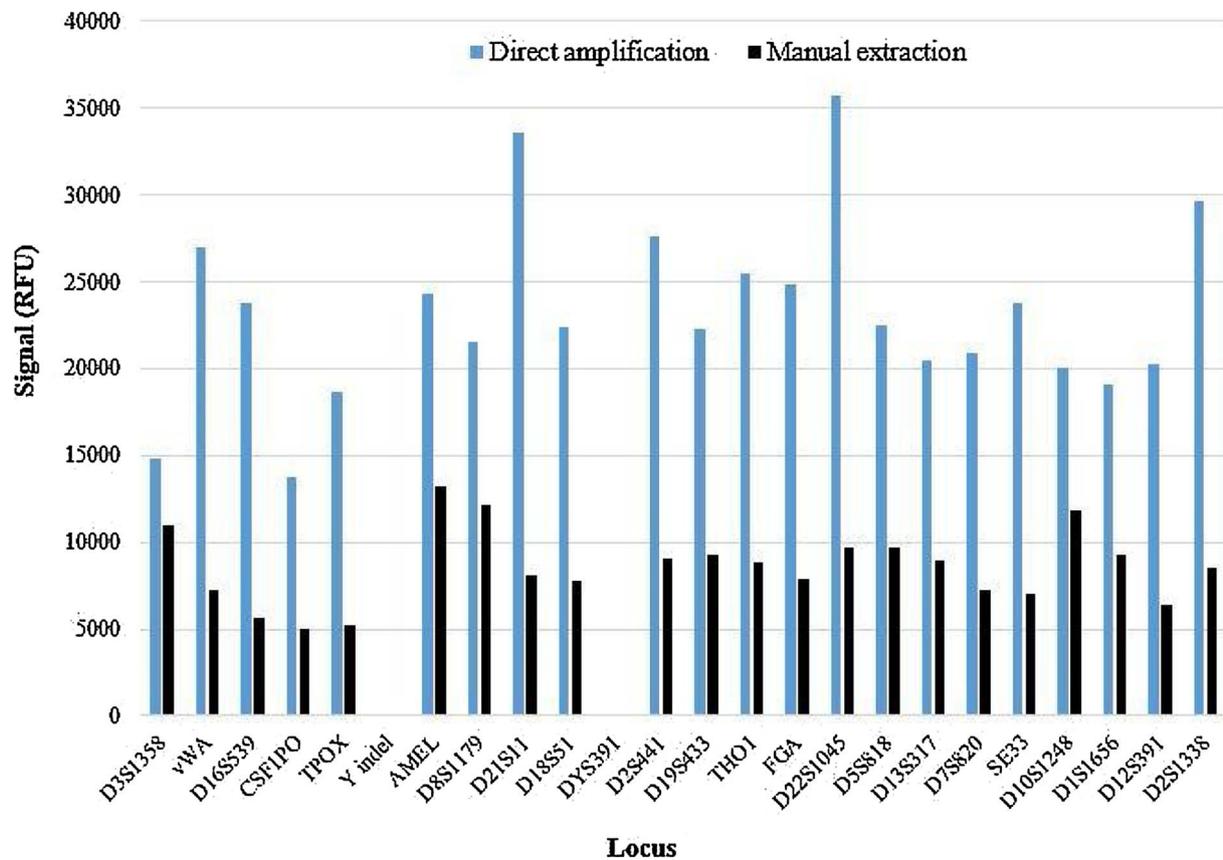
Fig. 3. Comparison of average signal (RFU) per locus from DNA in 5% saliva stains after direct amplification using microFLOQ® swabs (n = 30) and GlobalFiler™ Express versus manual extraction of 4N6 FLOQSwabs® (n = 30) using the QIAamp® DNA Investigator Kit and amplification of 1 ng DNA with the GlobalFiler™ kit. All saliva samples were from female subjects. Standard deviation (SD) values are provided in Supplementary Table 7.

There may be ways to mitigate inhibition by using additives purposefully designed to make the PCR more robust. Future studies should pursue reagents such as Prep-N-Go™ Buffer (Thermo Fisher Scientific) to determine if STR results from direct amplification of buccal swabs can be enhanced.

3.5.1. Comparison of direct amplification of human saliva stains to the traditional (manual extraction) workflow

An additional sample set of 5% saliva stains (n = 30) and 1% saliva stains (n = 30) were compared with traditional collection and extraction methods and typed using 1 ng input DNA. Higher signal (RFU) was obtained for all loci with direct amplification (Figs. 3 and 4). All saliva samples were from female subjects and therefore should not yield data for the Y indel and DYS391 loci. Supplementary Tables 7 and 8 show the locus-by-locus signal and total average signal (with SD) for each sample with both methods for 5% saliva stains and 1% saliva stains, respectively. The same explanations for the larger SDs with direct amplification of bloodstains apply to the saliva stain results.

3.6. Alternate strategies to reduce inhibition during direct amplification

One of the advantages of direct amplification is that the entire sample collected is available for PCR. However, the effects of inhibitors present within the biological tissue or fluid and from the substrate or environment, as well as from an overloaded DNA sample, must be mitigated in order for the reaction to be successful.

In an attempt to address the inhibition observed with more concentrated blood and saliva stains, it was hypothesized that agitating the microFLOQ® swab head in the PCR mix (rather than adding the entire swab head to the reaction) may dislodge adequate cellular material for successful DNA typing, while minimizing the amount of inhibitor or

limiting the amount of template DNA present during the PCR. Studies with agitation of swabs containing neat blood (n = 10) resulted in only a few alleles for most samples and partial, low signal (≤1000 RFU) profiles for two samples (data not shown). Some possible explanations for these results are that 1) heme effectively elutes during agitation and GlobalFiler™ Express chemistry is unable to overcome its effects, and/or 2) the microFLOQ® swab head retains most of the cellular material after agitation and therefore insufficient DNA was available for typing success. With neat saliva (n = 10), full STR profiles were obtained for all samples, but the same incomplete adenylation and "ski slope" effect observed in earlier experiments were present in all electropherograms. Future studies could focus on using more aggressive agitation or alternate buffers for more effective dislodging of DNA.

To further assess the effectiveness of agitation in eluting cellular material from the microFLOQ® swab head, studies with 5% blood and 1% blood were conducted. These samples were tested to assess the consequence of attempting agitation on samples that are not overloaded but assumed to be so. In these experiments, after agitating each swab in the PCR mix, the remaining swab head (post-agitation) was amplified in a separate reaction tube. Complete, balanced profiles were obtained from all 5% blood samples, both from the portion of the stains (n = 10) agitated into the reaction mix and from the stains (n = 10) remaining on the swab heads post-agitation (data not shown). Although complete profiles were obtained for all 5% bloodstains, signal (RFU) was consistently higher for the post-agitated swab heads, suggesting that the fibers of the microFLOQ® swab heads retain a higher proportion of the cellular material compared to that which elutes off the swab during agitation. Results from the same experiments with 1% blood varied, ranging from partial profiles with low signal to complete typing failure. Given that direct amplification of 1% bloodstains previously resulted in successful typing of 29-out-of-30 samples, results from the latter
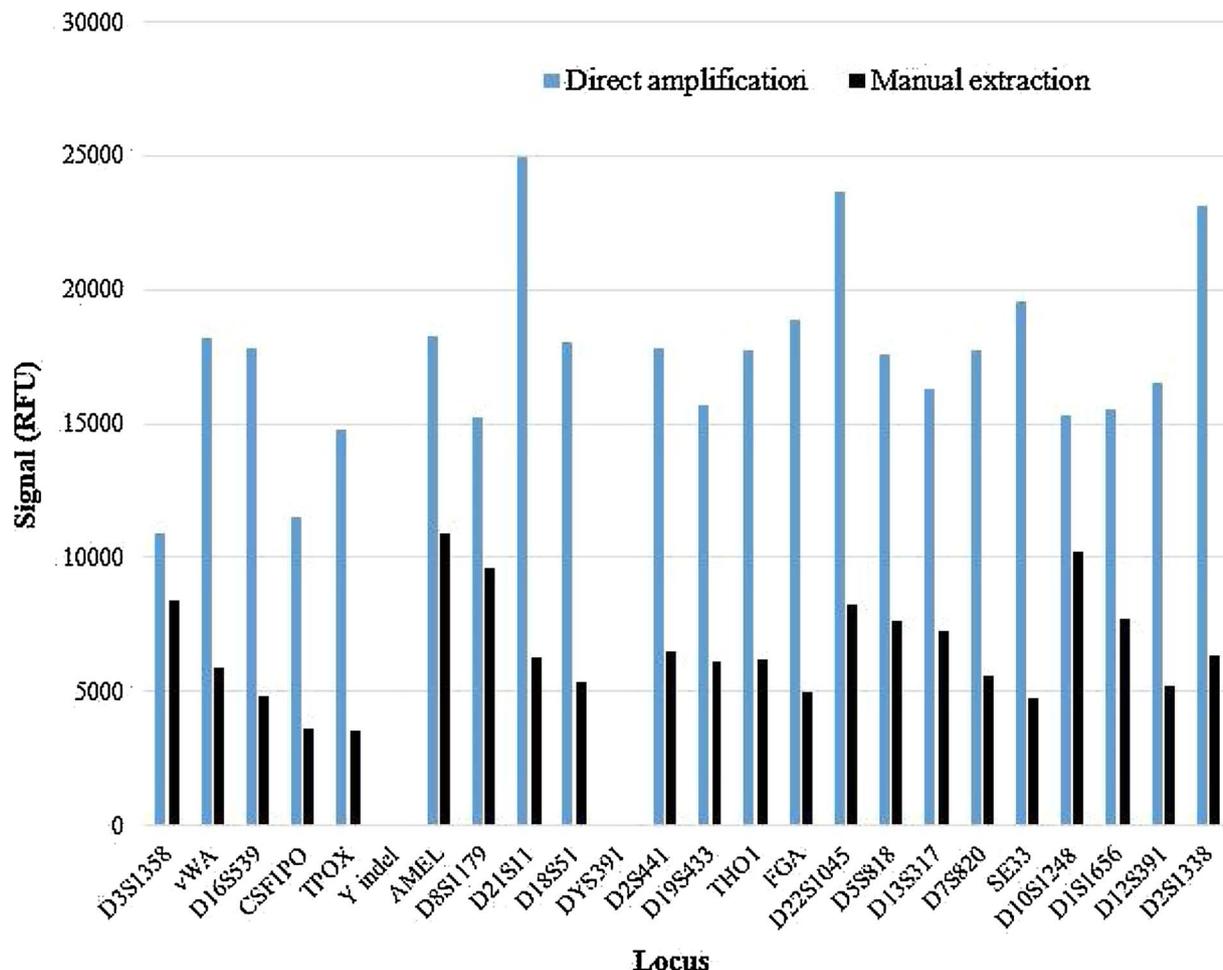
Fig. 4. Comparison of average signal (RFU) per locus from DNA in 1% saliva stains after direct amplification using microFLOQ® swabs (n = 30) and GlobalFiler™ Express versus manual extraction of 4N6 FLOQSwabs® (n = 30) using the QIAamp® DNA Investigator Kit and amplification of 1 ng DNA with the GlobalFiler™ kit. All saliva samples were from female subjects. Standard deviation (SD) values are provided in Supplementary Table 8.

agitation experiments suggest that for very dilute samples the entire portion of the stain collected by the microFLOQ® swab is better to obtain quality results.

3.7. Direct amplification of touch samples

Previous studies have investigated methods for collection and direct amplification of touch DNA. Templeton et al. [24] compared the performance of foam swabs, cotton swabs, and nylon FLOQSwabs® in collecting trace levels of DNA from fingerprints and found that the latter swabs generated the highest DNA yield. The study was expanded using the FLOQSwabs® to recover touch DNA from a variety of different substrates and then compared the direct amplification approach to the standard (extraction) workflow. Results from these controlled studies support the potential use of the FLOQSwab® system and direct amplification as a method to obtain increased signal from low level (touch) samples as well as to conserve resources and reduce the potential of contamination [25]. Given the promising results obtained using the larger (standard) version of the FLOQSwab®, the utility of the microFLOQ® swab for collection of trace levels of touch DNA was investigated. Four computer keyboards, three door handles, two computer mouses, two cell phones, and a necklace were sampled for analysis.

All computer keyboards were from private desk areas and presumably are used only by the owner(s). Partial, single-source profiles were obtained from three of the computer keyboards, while another keyboard yielded a full single source STR profile when amplified with

GlobalFiler™ Express. These single source profiles were consistent with the user(s) of the keyboards. Given that office keyboards are routinely used throughout the work day but are not cleaned on a regular basis, it would be expected that sufficient touch DNA would be present for genotyping. However, the absence of full profiles from three of the keyboards could simply be due to variation in sampling, particularly the specific key or area that was swabbed for testing.

A computer mouse encounters more sustained contact with a person's hand than a keyboard, the latter of which involves only brief tapping of keys during the course of typing. A full, single source profile was obtained from one of the computer mouses tested, and a second mouse yielded a full profile that was a mixture of two individuals. In the first scenario, the single source profile was consistent with the mouse owner, and the primary user of the second mouse tested was included as a contributor to the DNA mixture. This limited sampling was consistent with the expectation that prolonged contact with a surface will result in greater transfer of DNA and therefore higher chance of success in DNA typing.

Door handles are another type of surface where repetitive, casual contact can result in deposition of touch DNA. Partial, single-source profiles were obtained from two of the door handle swabbings, while another sampled area yielded a mixture of at least three individuals.

Two cell phones were swabbed for this study. A swab of the hand grip area of one cell phone yielded a full, male STR profile that was consistent with the owner of the phone. Three different areas of another cell phone were swabbed separately with microFLOQ® swabs: hand grip area, mouthpiece, and earpiece. A full, single source STR profile was

obtained from the hand grip area (consistent with that phone's owner). No data were obtained from the swabbing of the mouthpiece, and only two alleles were recovered from the earpiece. There likely is prolonged contact by the hand as a user is holding a phone as opposed to the mouthpiece, where direct contact with the phone is not as likely. Possibly the earpiece may not be as good a source of touch DNA as areas where the hand is in contact.

A swabbing of a necklace worn daily by a female subject resulted in a mixture of two individuals. The DNA mixture was consistent with the female subject and her boyfriend.

Results from this limited set of touch samples support that sufficient cellular material can be retrieved using the miniaturized microFLOQ® swab head to obtain complete DNA profiles from a variety of surfaces. However, the amount of material present and the type of surface will impact the likelihood of success, as is expected for collection of any evidence by swabbing.

4. Conclusion

Samples collected with microFLOQ® swabs and direct amplification of DNA with GlobalFiler™ Express appears to allow for low quantities of DNA to be typed in a facile and expeditious manner. It was hypothesized that the swab design and presentation of the sample on the surface of the swab head would create an environment such that the sample essentially would be concentrated and accessible for amplification. Thus, smaller amounts of sample could be used for testing compared with traditional extraction-based DNA typing protocols. The results support that subsampling small portions of stains yields higher signal compared to full consumption of the same stains using the standard casework approach and 1 ng input DNA.

Several studies have documented challenges with PCR amplification of blood samples without first performing DNA isolation [26–30]. Whole human blood contains a number of known inhibitors, including heme [31], anticoagulants [32], bile salts [33], and immunoglobulin G [34]. These blood constituents affect PCR by suppressing the activity of DNA polymerases. A variety of approaches have been used to address inhibition, including addition of bovine serum albumin [35], enhancement of PCR buffers with adjuvants [26,36–42], incorporating buffers with a higher pH (pH 9.1–9.8) into PCRs [27], alteration of standard thermal cycling parameters [28], and use of inhibition-resistant forms of Taq polymerase [29]. It is possible that similar measures could be taken to diminish the effects of inhibitors on GlobalFiler™ Express chemistry. Further studies are needed. Additionally, other commercially available kits may be more resilient for direct amplification of DNA in forensic samples and should be tested.

The results of this study potentially may have important implications for analysis of low quantity and/or degraded samples that plague forensic casework. The purification step in the traditional workflow increases chances of sample contamination and results in loss of DNA. With direct amplification, no sample loss occurs and more template is available during amplification. Therefore, a better means for obtaining more complete profiles could be possible with direct amplification compared with traditional methods. Moreover, direct amplification of a sample on a microFLOQ® swab consumes a very small portion of the stain, preserving valuable evidence for re-analysis or additional testing.

With these features it may be worthwhile to consider an alternate workflow in which subsampling is performed first on all stains, and if the results are acceptable, no additional testing is performed. This approach would preserve precious sample for additional forensic analyses. If the results are limited or inconclusive, then the entire stain can be collected and extracted using traditional methods. One criticism of the direct amplification approach is that there is no quantification step. Quantification allows for optimum sample input to prevent overloading of a PCR and can assist in the identification of samples that may contain PCR inhibitors. The latter information can indicate whether additional cleanup steps are needed prior to targeted PCR or could suggest

selection of an alternate assay. Collectively, the information obtained from the quantification step helps ensure that a profile with the highest possible quality is obtained. However, the quantification step itself requires consumption of limited volumes of extracted DNA, involves further manipulation of the sample, and adds time and cost to the assay. The studies herein show that with the microFLOQ® direct amplification approach the extraction and quantification steps can be eliminated, reducing sample manipulation and the amount of time and labor required for processing. Additionally, the quantity or volume of sample used for testing is so minimal that consumption is not a real concern. Essentially the entire sample still is available if subsequent collection/extraction is deemed necessary.

Another benefit of the microFLOQ® swab relates to the small surface area of the swab head. It may provide a better way to collect samples in areas that are difficult to access, such as seams of mechanical or electronic devices and cracks in flooring. Future studies will 1) evaluate the benefit of this alternate workflow in terms of time, cost, and sample consumption; 2) further validate this direct amplification method for collection of samples deposited on porous surfaces, such as textiles, and 3) determine if other direct amplification kits may be more refractory to the effects of inhibition.

This research represents an initial proof-of-concept study and the results demonstrate that direct amplification of bloodstain and saliva stain samples collected with the microFLOQ® swabs can produce comparable or better results than the traditional extraction workflow. To optimize this alternate approach further studies should be conducted regarding cycle numbers and PCR additives. Additionally, when the method is deemed ready for implementation, it would be worthwhile to perform a comparative analysis by first sampling with the microFLOQ® swab followed by direct amplification versus the standard method on the same samples to determine the cost benefit of this approach.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2017.10.010.

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