Improving Results from Touch DNA Evidence with Optimized Direct PCR Methods

Technical Summary
Bode Technology
December 31, 2022

Report submitted to the National Institute of Justice
Grant # 2019-DU-BX-0009
Project Period: 01/01/2020 to 12/31/2022
Award Amount: $453,146

Abigail S. Bathrick, Bode Technology
Anna C. Salmonsen, Bode Technology
Jonathan M. Davoren, Bode Technology

Principal Investigator:
Jonathan M. Davoren
Bode Technology
10430 Furnace Rd, Suite 107
Lorton, VA 22079
Jonathan.Davoren@bodetech.com

This project was supported by Award No. 2019-DU-BX-0009, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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Project Summary

Goals and Objectives

Direct polymerase chain reaction (PCR) is a DNA processing method in which a sample is added directly to an amplification reaction without prior purification or quantification and has been identified as a method that may improve genotyping data from low-yield touch DNA samples. FBI Quality Assurance Standard (QAS) 9.4 requires laboratories in the United States to quantify the amount of human DNA in unknown forensic samples prior to amplification of nuclear DNA [1]. The Organization of Scientific Area Committees for Forensic Science (OSAC) has identified the need to reevaluate this standard in relation to evidentiary sample types known to yield low amounts of DNA. Additionally, the 2018 Forensic Science Technology Working Group (TWG) operational requirements have identified the need for optimization of DNA evidence collection techniques that maximize DNA recovery from the collection substrate and comprehensive studies that provide practical data about touch evidence [2].

The goal of this study was to generate data in support of a reevaluation of QAS 9.4 and the aforementioned TWG operational requirements by evaluating the following: (1) direct PCR-compatible collection methods in conjunction with touch samples on a variety of substrates, (2) direct PCR results from the GlobalFiler and PowerPlex Fusion 6C STR amplification systems, (3) the efficacy of direct PCR when processing swabs that were stored after sample collection, and (4) the efficacy of direct PCR on samples that were re-swabbed after initial analysis was completed. The project was performed in two phases. Phase I examined direct PCR-compatible collection methods in conjunction with mock touch DNA evidence samples on a variety of substrates. The objective of this phase was to determine the best sample collection method(s) to be used on touch DNA evidence items prior to direct PCR processing. Phase II examined direct PCR in conjunction with touch DNA samples that were collected using the optimum method(s) identified in Phase I and stored at room temperature for up to six months after collection. Touch DNA samples that were resampled after initial direct PCR and standard processing were also studied. The objectives of Phase II were to demonstrate whether direct PCR is effective on touch DNA evidence swabs that have been stored prior to DNA processing, generate PowerPlex Fusion 6C direct PCR data for comparison to Phase I GlobalFiler data, and determine whether resampled touch DNA substrates can generate DNA profiles after initial direct PCR analysis has been completed.

Research Questions

PHASE I

The following research questions were examined in Phase I of this study:

- What effect does direct PCR processing have on GlobalFiler STR profile quality for touch DNA samples collected from various substrates with different collection methods (i.e., cotton swabs, microFLOQ direct swabs, and Whatman non-indicating FTA paper moistened with water or 0.1% Triton X-100 or left dry) when compared to standard processing?
- What effects do the aforementioned collection methods have on GlobalFiler STR profile quality when used to collect touch DNA from various substrates?
- What effects do swab types (i.e., cotton, microFLOQ, and FTA paper) have on GlobalFiler STR profile quality when used to collect touch DNA from various substrates?
- What effects do moistening agents (i.e., water, 0.1% Triton X, and dry) have on GlobalFiler STR profile quality when used to collect touch DNA from various substrates?

PHASE II

The following research questions were examined in Phase II of this study:

- What effects do GlobalFiler and PowerPlex Fusion 6C STR amplification systems have on STR profile quality for touch DNA samples when collected from various substrates with the aforementioned collection methods and processed with standard and direct PCR methods?
- What effect does storing touch DNA samples collected with the aforementioned methods for up to 6 months prior to processing with standard and direct PCR methods have on PowerPlex Fusion 6C STR profile quality?
- What effect does resampling touch DNA on previously sampled substrates have on PowerPlex Fusion 6C STR profile quality?

Summary of Project Design and Methods

PHASE I

Twelve substrates were examined in this study: 100% cotton denim shirt, wool blend shirt (87% merino and 13% nylon), 100% merino wool shirt, 100% polyester shirt, plastic microscope slides, metal tools, vinyl shutter samples, plastic handgun grips, 9 mm brass bullets/fired cartridge casings, foam cups, miniature concrete bricks, and unfinished wood tool handles (Figure 1, Table 1). All nonporous substrates except the bullets were submerged in a 10% bleach solution for at least 10 min, rinsed with water, and dried at room temperature overnight. To indicate where the items should be held and sampled, 2.5 cm x 1.6 cm areas were outlined on each item, excluding the fabrics and bullets. All items were then UV decontaminated. Substrate negative controls were collected from each substrate in triplicate. Approximately 5 mm^2 cuttings were taken from the fabrics, and samples were collected from the remaining substrates with Puritan cotton swabs moistened with 7 µL sterile water. All substrate control samples were extracted with the Qiagen EZ1® DNA Investigator® Kit using 500 µL lysis volumes and eluting into 50 µL TE buffer. Samples were quantified with Quantifiler® Trio DNA Quantification Kit (Thermo Fisher) in 11 µL reactions to verify that no exogenous DNA was present before the items were distributed to donors.

Eluted buccal cell samples from one donor were prepared by spotting a volume of cellular suspension equivalent to 1.0 ng DNA onto plastic microscope slides (VWR, Radnor, PA) for a total of 144 cell spots. Cells were eluted from buccal swabs into 1X PBS buffer and counted with a hemocytometer on a light microscope. The amount of DNA present in the cell suspension was verified by extracting 10 µL of the suspension in triplicate with the EZ1 DNA Investigator Kit and quantifying with Quantifiler Trio. Additionally, one donor deposited 144 fingerprints on plastic microscope slides that were placed on a desktop scale. The middle and index fingers of the donor's dominant hand were used to deposit fingerprints by pressing down for 60 s until a weight of approximately 100 g was reached. The donor's hands were washed 2 h prior to each fingerprint deposition.

Each type of shirt was distributed to one donor, who was asked to wear it for a minimum of 12 h during a day in which they performed a normal level of activity. Non-fabric substrates were distributed to three donors. Donors were instructed to wash their hands 2 h prior to donations and then handle each item with their dominant hand for 60 s. Donors ensured all 2.5 cm x 1.6 cm areas were contacted. After handling, bullets were loaded individually into the gun chamber (Glock 19-Gen 3) and were fired by a single individual wearing nitrile gloves. Fired cartridge casings were collected and individually packaged into coin envelopes by the same individual. All bullets handled by one donor were fired and collected prior to firing samples handled by a different donor.

The gun was not cleaned prior to or between firings. For each substrate type, collection method, and processing method, eight replicates were prepared by each donor. A total of 3,376 samples were prepared (Table 2). Nine direct amplification-compatible collection methods were used to collect touch DNA from each handled substrate, excluding the fabrics (Table 3). These collection methods included cotton swabs (Puritan), microFLOQ direct swabs (Copan), and Whatman non-indicating FTA paper (GE Healthcare) that were moistened with DNA-grade sterile water (Thermo Fisher), moistened with 0.1% Triton X-100 (MP Biomedicals), or left dry. Prior to moistening and/or collection, 3.0 mm diameter punches were taken from the cotton swabs, and 1.2 mm diameter punches were taken from the FTA paper. The whole swab head was used for the microFLOQ swabs. Fabric substrates were cut into 2 mm^2 sections.

Samples were processed with two methods: (1) standard processing with DNA extraction, concentration, and quantification prior to amplification and (2) direct PCR. The samples that underwent standard processing were extracted on a VANTAGE liquid handling system (Hamilton®) with the Investigator® STAR™ Lyse&Prep Kit (Qiagen) using 200 µL lysis volumes and eluting into 50 µL TE buffer. Samples were concentrated to 17.5 µL with Microcon® DNA Fast Flow centrifugal filter units (Millipore Sigma). Quantification was performed on the concentrated samples with Quantifiler Trio in 11 µL reactions. For direct PCR, the collected samples were placed in 96-well plates for amplification. Amplification for both the extracted and direct PCR samples was performed with Thermo Fisher's GlobalFiler™ PCR Amplification Kit in 25 µL reactions for 29 cycles on a GeneAmp™ PCR System 9700 (Thermo Fisher). Amplification reactions containing extracted samples targeted 1 ng template DNA. Extracted samples containing less than 1 ng DNA were amplified at the maximum DNA input volume. Capillary electrophoresis (CE) was performed on a 3500xL Genetic Analyzer for Human Identification (Thermo Fisher), and data were analyzed with GeneMapper ID-X® v1.5 (Thermo Fisher) using an analytical threshold (AT) of 125 RFU and a stochastic threshold (ST) of 600 RFU.

STR profile quality was assessed by determining the forensic DNA profile index (FI), percentage of expected loci obtained (% profile), and CODIS eligibility for each sample. A quality score between 0.05 and 10 was also assigned to each profile based on the FI developed by Hedman et al. [4]. The FI provides a quantitative quality score for each DNA profile by taking three factors into consideration: overall peak height, peak height balance within each locus in a profile, and the profile balance across all loci of a profile. A score of 10 indicates a DNA profile of the highest quality, whereas a score of 0.05 indicates a DNA profile of the lowest quality or no profile. Profiles with alleles at a minimum of eight of the original CODIS core loci and match rarities of at least one in ten million were considered CODIS eligible [3].

JMP® Statistical Discovery™ Software v15.2.1 (SAS® Institute Inc., Cary, NC) was used for statistical analysis. Tukey's HSD tests and two-tailed *t* tests were employed as appropriate to perform comparisons between profile quality metrics and various factors including collection method, substrate, and processing method. For all statistical analyses, $\alpha = 0.05$.

PHASE II

Phase II included three studies: a 6-month time study, a comparison of direct PCR performed with PowerPlex Fusion 6C and GlobalFiler, an evaluation of the efficacy of resampling the substrates after standard or direct PCR processing, followed by reprocessing with standard or direct PCR methods, respectively. For the 6-month time study, the collection methods used for each substrate were determined based on their performance with standard and direct PCR processing in Phase I. Collected time study samples were processed immediately (0 months) or stored at room temperature for 3 or 6 months. For the PowerPlex Fusion 6C and GlobalFiler

comparison, the 0-month time study samples for each collection method were used, and an additional set of handled substrates was prepared for cotton swab & water collection if not previously included in the time study. For the resampling study, 0-month time study samples that were collected with the method identified as the best for direct PCR in Phase I were resampled immediately after completing standard or direct PCR processing and reprocessed with standard or direct PCR methods, respectively.

Mock touch DNA evidence samples were prepared on the following substrates as described in Phase I: 100% cotton denim shirt, 100% merino wool shirt, 100% polyester shirt, plastic microscope slides, metal tools, vinyl shutter samples, plastic handgun grips, 9 mm brass bullets/cartridge casings, foam cups, miniature concrete bricks, and unfinished wood tool handles. Each shirt was worn by one donor, and the remaining substrates were distributed to three donors. Eight replicates were prepared for each substrate, donor, collection method, time point, and processing method (Table 4). A total of 2,336 samples were prepared; 2,016 samples were included in the time study, 992 Phase II PowerPlex Fusion 6C samples and 992 Phase I GlobalFiler samples were included in the PowerPlex Fusion 6C and GlobalFiler comparison, and 368 samples were included in the resampling study.

Replicate sets were sampled after handling by donors using the collection methods specified in Table 4. Each collection method was performed as described in Phase I. After collection, the collectors were allowed to dry at room temperature for 24 h before processing the 0-month samples. For the 3-month and 6-month samples, cotton swab punches and microFLOQ swab heads were stored at room temperature in 96-well plates covered with AirPore tape sheets (Qiagen) for 3 or 6 months. The 0-month non-fabric substrates sampled with the methods indicated in the resampling column in Table 4 were retained after collection. Immediately after undergoing standard and direct PCR processing, these substrates were resampled using the indicated collection method(s).

The collected samples were processed at three time points: immediately after collection (0 months), 3 months after collection, and 6 months after collection. At each time point, samples were processed with two methods: (1) standard processing with DNA extraction, concentration, and quantification prior to amplification and (2) direct PCR. Standard processing was performed as specified in Phase I. Amplification for both the extracted and direct PCR Phase II samples was performed with Promega's PowerPlex Fusion 6C System in 25 µL reactions for 29 cycles on a GeneAmp PCR System 9700. CE and data analysis were performed as specified in Phase I using an AT of 100 RFU and an ST of 500 RFU.

STR profile quality was assessed via FI, % profile, and CODIS eligibility as detailed in Phase I. JMP Statistical Discovery Software v15.2.1 was used to perform statistical analysis of the data. Wilcoxon tests were employed as appropriate to perform comparisons between profile quality metrics and various factors including amplification system, collection method, substrate, time point, processing method, and original vs resampled samples. Steel with control tests were also used to compare the 3-month and 6-month time points to the 0-month (control) time points. For all statistical analyses, $\alpha = 0.05$.

Summary of Results

PHASE I

Standard Processing vs Direct PCR

STR profile quality was determined by calculating the mean FI and % profile values for each collection method, processing method, and substrate type; two-tailed *t* tests were used to

compare the standard processing and direct PCR groups for each collection method (Table 5). Electropherograms depicting high (FI ≥ 6), medium (4 ≤ FI < 6), and low (FI < 4) quality STR profiles are presented in Figure 2.

For most of the examined substrates and collection methods, the direct PCR and standard processing methods produced comparable DNA profile results; however, some substrate and collection method combinations produced significantly higher quality profiles when the processing methods were compared. No statistically significant differences in FI were found between processing methods for any collection method used for plastic slides (fingerprints), foam cups, and polyester fabric. Direct PCR processing resulted in significantly higher quality FI values when the following collection methods were used: microFLOQ swabs & water on handgun grips (p=0.038); dry microFLOQ swabs for buccal cells on plastic slides (p=0.020), metal tools (p=0.014), and wood tool handles (p<0.001); FTA & water on metal tools (p=0.011); and FTA & Triton X on metal tools (p<0.001). Direct PCR processing resulted in significantly higher % profiles when the following collection methods were used: microFLOQ swabs & water on metal tools (p=0.025) and handgun grips (p=0.012); dry microFLOQ swabs for buccal cells on plastic slides (p=0.031), metal tools (p=0.015), and wood tool handles (p<0.001); FTA & water on metal tools (p=0.001) and foam cups (p=0.046); dry cotton swabs on fingerprints on plastic slides (p=0.002); FTA & Triton X on metal tools (p=0.004); cotton swabs & Triton X on handgun grips (p=0.001); dry FTA on handgun grips (p=0.018); and microFLOQ swabs & Triton X on foam cups (p=0.018). Regardless of collection method, direct PCR was not a successful processing method for samples collected from concrete bricks, cartridge casings, denim fabric, wool blend fabric, and 100% wool.

Collection Method Comparison

For each substrate and processing method, Tukey's HSD pairwise comparisons of collection methods (Table 6, Table 7, Table 8, Table 9), swab types (Table 11), and moistening agents (Table 13) were performed for FI and % profile. Two-tailed *t* tests were also used to compare the standard and direct PCR processing methods for each swab type (Table 10) and moistening agent (Table 12). The main findings were as follows:

- In general, touch DNA collection with FTA underperformed when compared to the cotton and microFLOQ swabs.
- MicroFLOQ swabs produced higher FI and % profile values when used on the substrates that were successful with direct PCR, although these differences were not always significant. The microFLOQ swabs were also easier and faster to use for direct PCR. Both the cotton swabs and FTA paper were cut/punched and UV decontaminated prior to DNA collection to ensure they could be fully submerged in 25 µL PCR reactions and would contain all of the collected DNA. Cutting and decontaminating the cotton swabs and FTA paper represented large investments of time and labor, whereas the microFLOQ swabs needed no prior preparation due to their miniature swab heads, which were already sized appropriately for PCR reactions. Additionally, the cotton and FTA cuttings/punches were quite small (≤ 3.0 mm diameter) and were manipulated with forceps during DNA collection and transfer to 96-well plates. This process was found to be finicky and introduced the possibility of dropping or losing the cutting/punch. In comparison, the miniature microFLOQ swab heads are attached to a perforated handle that allowed for easy maneuvering during DNA collection and transfer to 96-well plates.
- When used with direct PCR, no significant differences in FI or % profile were observed between water and triton X as moistening agents.

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- Dry collection efficacy was dependent on the touch DNA substrate. After processing with direct PCR, no significant differences were observed between dry collection and water or Triton X on most substrates; however, dry collection resulted in significantly higher FI values compared to water (p=0.006) and Triton X (p=0.010) on wood tool handles; and significantly higher % profile values compared to water (p=0.004) and Triton X (p=0.002) on wood tool handles and water on handgun grips (p=0.008). Dry collections resulted in significantly lower % profile values compared to Triton X on plastic slides (fingerprints) (p=0.014).

CODIS Eligibility

To determine whether the success of direct PCR profiling was substrate dependent, CODIS-eligible profiles were tallied across all collection methods for each substrate and processing method. Success rates were determined by calculating the percentage of profiles that were CODIS eligible for each processing method (Table 14). Across the substrates for which direct PCR was more successful than standard processing, the direct PCR success rate was 49%, and the standard processing success rate was 36%. Across the substrates for which direct PCR was less successful than standard processing, the direct PCR success rate was 8%, and the standard processing success rate was 30%. The low direct PCR success rates for denim, wool, concrete bricks, and cartridge casings are expected as these substrates contain known PCR inhibitors or are otherwise challenging [5-7]. The low direct PCR success rate for the vinyl shutters was likely due to inhibition caused by a yellow dye that was found on the samples, which demonstrated the effect unexpected inhibitors can have on direct PCR. These results highlight the importance of identifying substrates that can be successfully processed with direct PCR and indicate that, when implemented strategically, direct PCR results in more CODIS-eligible profiles than standard processing methods.

Foreign Alleles

All DNA profiles were examined to determine whether they matched the expected donor profiles. Profiles containing foreign alleles were divided into four categories:

1. Indirect transfer (known source): profile contained one or more foreign alleles that could be attributed to a known cohabitant of one donor. Foreign DNA was likely introduced prior to lab processing.
2. Contamination (known source): profile contained one or more foreign alleles that could be attributed to a known source that was likely introduced during lab processing.
3. Unknown source ≤6 alleles: profile contained six or fewer foreign alleles that could not be attributed to a known source. It could not be determined when the foreign DNA was introduced to the sample.
4. Unknown source ≥7 alleles: profile contained seven or more foreign alleles that could not be attributed to a known source. It could not be determined when the foreign DNA was introduced to the sample.

Foreign alleles were observed in 74 of the standard processing profiles (4%) and 99 of the direct PCR profiles (6%) (Table 24). Of these profiles, 80% contained three or fewer foreign alleles. The foreign alleles observed in this study highlight the sensitivity of both standard processing and direct PCR. The standard processing profiles contained more foreign alleles that were attributed to contamination, which could be due to the additional handling required for standard processing (e.g., tube transfers). The direct PCR profiles contained more foreign alleles attributed to indirect transfer, which could be due to the increased sensitivity of the direct PCR method. Indirect transfer occurs when DNA from an individual is deposited on an item via one or more intermediary transfer

steps [8]. In this study, foreign DNA was transferred from a cohabitating member of one donor's household to the donor and subsequently to the items handled by the donor. When processing evidentiary touch DNA samples, practitioners should be aware that mixture deconvolution may be needed.

Collection Methods Selected for Phase II

Based on the Phase I data, up to three collections methods were selected for use with each substrate in Phase II: a commonly used control method (cotton swabs & water); the optimal collection method for standard processing; and the optimal collection method for direct PCR. In some instances, one method was selected as optimal for both standard and direct PCR processing. Optimal collection methods were selected by identifying the method(s) that produced significantly higher quality STR profiles than other collection method(s) as measured by FI. When two or more methods produced FI values that were significantly higher than other collection methods, the method that produced the highest mean FI was selected. Refer to Table 4 for the collection methods selected for each substrate.

PHASE II

GlobalFiler vs PowerPlex Fusion 6C

STR profile qualities were determined by examining the median FI and % profile values for each collection method, processing method, substrate type, and amplification system. Wilcoxon tests were used to compare the GlobalFiler and PowerPlex Fusion 6C results for each collection and processing method (Table 15, Table 16) and the standard and direct PCR results for each collection method and amplification system (Table 17, Table 18). The main findings were as follows:

- Direct PCR with PowerPlex Fusion 6C and GlobalFiler was successful for many substrates without using specialized amplification kits or modifying validated amplification SOPs.
- GlobalFiler performed better than PowerPlex Fusion 6C when amplifying samples collected with microFLOQ swabs from several substrates. Significantly higher GlobalFiler FI and % profile values were obtained for samples collected with microFLOQ swabs from plastic slides (buccal cells), handgun grips, and wood tool handles when compared to direct PCR with PowerPlex Fusion 6C. Significantly higher GlobalFiler % profile values were obtained for samples collected with microFLOQ swabs from foam cups. Refer to Table 16 for *p*-values.
- The highest quality direct PCR profiles were obtained with GlobalFiler using the following collection method and substrate combinations: microFLOQ swabs & water on plastic slides (buccal cells), polyester cuttings, dry microFLOQ swabs on metal tools, cotton swabs & water on handgun grips, and dry microFLOQ swabs on wood tool handles.
- PowerPlex Fusion 6C performed better than GlobalFiler when amplifying samples collected with cotton swabs from several substrates. Significantly higher PowerPlex Fusion 6C FI and % profile values were obtained for samples collected with cotton swabs from vinyl shutters. Significantly higher FI values were obtained for samples collected with cotton swabs from plastic slides (fingerprints) and wood handles. Refer to Table 16 for *p*-values.
- Direct PCR with PowerPlex Fusion 6C produced significantly higher FI values for 100% wool cuttings and significantly higher FI and % profiles for denim cutting, indicating it may have greater resistance to some PCR inhibitors when compared to GlobalFiler. Refer to Table 16 for *p*-values.

- The highest quality direct PCR profiles were obtained with PowerPlex Fusion 6C using the following collection method and substrate combinations: cotton swabs & water on plastic slides (fingerprints), denim cuttings, 100% wool cuttings, cotton swabs & water on vinyl shutters, and cotton swabs & water on foam cups.
- Direct PCR of concrete bricks and fired cartridge casings was not successful with either amplification system.
- Regardless of the collection and processing methods used, all touch DNA samples contained highly variable quantities of DNA.

Time Study

STR profile qualities for the time study samples were determined by examining the median FI (Table 19) and % profile values (Table 20) for each collection method, processing method, substrate type, and time point. Steel with Control tests were used to compare the 3-month and 6-month times points to the 0-month time point (control). Wilcoxon tests were used to compare standard processing and direct PCR results within each time point. The main findings were as follows:

- Direct PCR results were comparable to or better than standard processing for all substrates and time points, excluding 100% wool at 6 months, concrete bricks, and cartridge casings.
- Direct PCR results from swabs and cuttings stored for 3 and 6 months did not significantly differ from those processed immediately, excluding dry microFLOQ swabs on plastic slides (fingerprints) at 6 months, dry cotton swabs on metal tools at 6 months, cotton swabs & water on handgun grips at 6 months, denim cuttings at 3 and 6 months, and polyester cuttings at 6 months. Refer to Table 19 and Table 20 for *p*-values.

Resampling Study

STR profile qualities for the resampling study were determined by examining the median FI (Table 21) and % profile values (Table 22) for each collection method, substrate, and processing method for samples processed directly following the first collection and samples that were resampled after initial analysis. Wilcoxon tests were used to compare results obtained after the first collection and resampling.

Resampling was not successful for most substrates. When compared to the results from the initial direct PCR analysis, resampling produced significantly lower median FI and % profile values for buccal cells and fingerprints on plastic slides and metal tools; significantly lower FI values were obtained for vinyl shutters. Resampling the handgun grip, cartridge casing, foam cup, and concrete brick samples resulted in very low median FI and % profile values that were comparable to those obtained after initial direct PCR analysis. Refer to Table 21 and Table 22 for *p*-values.

Wood tool handles were the only substrate for which resampling was moderately successful. For these samples, the median FI and % profile values obtained after initial direct PCR analysis were 3.89 and 78.1%, respectively. After resampling, the median FI and % profiling values were 2.43 and 62.5%, respectively. The unfinished wood may retain more DNA due to its porous, textured surface.

CODIS Eligibility

CODIS-eligible profiles were tallied across all collection methods for each substrate, processing method, and time point. CODIS-eligible profiles resulting from the resampling method were also tallied. Success rates were determined (Table 23). Direct PCR was as or more successful than standard processing for fingerprints on plastic slides, denim, polyester, metal tools, vinyl

shutters, handgun grips, foam cups, and wood tool handles at all time points and buccal cells on plastic slides at 3 and 6 months. Resampling was more successful on samples were processed with direct PCR compared to those that had been processed with standard methods on metal tools, vinyl shutters, handgun grips, and wood tool handles. Resampling was less successful on samples that were processed with direct PCR compared to those that had been processed with standard methods for plastic slides (buccal cells) and cartridge casings at 0 months, 100% wool at 6 months, and concrete bricks at all time points.

Foreign Alleles

All DNA profiles were examined to determine whether they matched the expected donor profiles. Profiles containing foreign alleles were divided into the four categories described in Phase I. Foreign alleles were observed in 24 of the standard processing profiles (2%) and 106 of the direct PCR profiles (8%) (Table 25). Of these profiles, 76% contained three or fewer foreign alleles. Similar to phase I, the phase II standard processing profiles contained more foreign alleles that were attributed to contamination, and the direct PCR profiles contained more foreign alleles attributed to indirect transfer. The direct PCR profiles also contained more foreign alleles that could not be attributed to a known source. These alleles could be due to indirect transfer from unknown individuals that interacted with the donors prior to donation (e.g., delivery drivers, family members, etc.).

Applicability to Criminal Justice

Currently, FBI QAS 9.4 requires all unknown forensic samples to undergo human-specific DNA quantification prior to amplification of STR loci [1]; however, OSAC has identified the need to reevaluate this standard in relation to evidentiary samples known to yield low amounts of DNA, specifically to allow direct PCR of these samples. Direct PCR has been identified as a method that may improve the generation of genotyping data from low-yield DNA samples. By circumventing the extraction, quantification, and concentration processes, maximum quantities of DNA can be targeted, laboratory personnel error and exogenous DNA contamination may be minimized, and overall sample processing time and cost could be reduced. Direct PCR of appropriate crime scene samples may provide a savings of 3–4 hours of hands-on time for laboratory personnel [9] and at least 25% in reagent costs [10], allowing for faster turnaround times and increased laboratory throughput. Reduced processing times and costs may also prompt laboratories that do not currently process touch DNA evidence for budgetary reasons to reconsider their capacity for including touch DNA capabilities.

The results from this study may assist the Scientific Working Group on DNA Analysis Methods (SWGDAM) in their reevaluation of QAS 9.4 by bolstering the argument for direct PCR of evidentiary samples and supporting modification of the guidelines requiring quantification. Most of the Phase I touch DNA samples did not benefit from quantification. Only 0.6% of the Phase I direct PCR samples were overblown, and only one of those samples needed to be diluted and reinjected to obtain a useable profile. Of the Phase I standard processing samples, 99% were consumed after quant and 0.5% had quant values less than 1 pg/µl but produced CODIS-eligible profiles. Quantification was really only useful for 12 standard processing samples that had enough DNA extract remaining for re-amplification.

While this study will not be the sole catalyst for changes to the FBI QAS, such work lays a strong foundation for further research, particularly when considered with the existing direct PCR literature [11-14]. If the FBI QAS quantification requirement is modified, this study will provide laboratories with a useful roadmap for proceeding with their own direct PCR evaluations and

internal validations. The results of this study may aid laboratories in assessing the suitability of their current sample collection methods, determining touch DNA samples that are likely to benefit from direct PCR, evaluating the strengths and weaknesses of the expanded STR amplification kits, and selecting the amplification kit that best meets the laboratory's needs. The study's results may also facilitate improvements to sample collection and submission guidelines and current touch DNA processing techniques.

Ultimately, dissemination of the results of this study may lead to increased touch DNA profiling success rates, increased laboratory throughput and productivity, decreased turnaround times, and lowered processing costs. Data on these topics will be of direct benefit to crime laboratories, crime scene investigators, and prosecutors; improve case resolution; support the identification of repeat offenders; and help victims and victims' families.

Tables & Figures

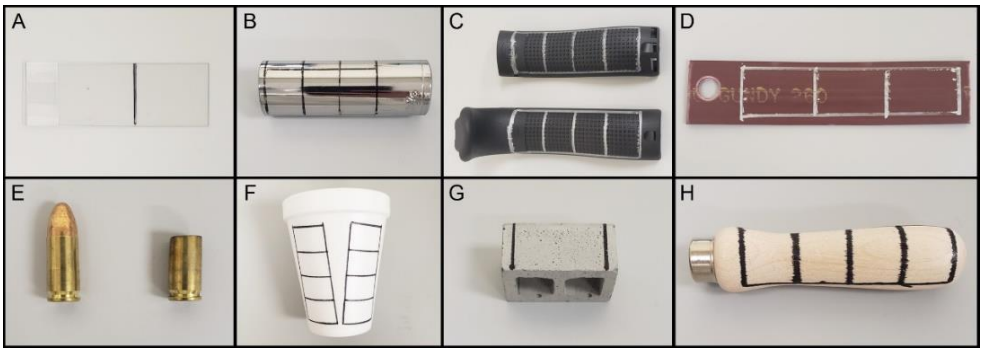

Figure 1: Non-fabric substrates prepared for donor handling: (A) plastic microscope slide, (B) metal tool, (C) handgun grip, (D) vinyl shutter sample, (E) brass bullet and fired cartridge casing, (F) foam cup, (G) miniature concrete brick, and (H) wood tool handle.

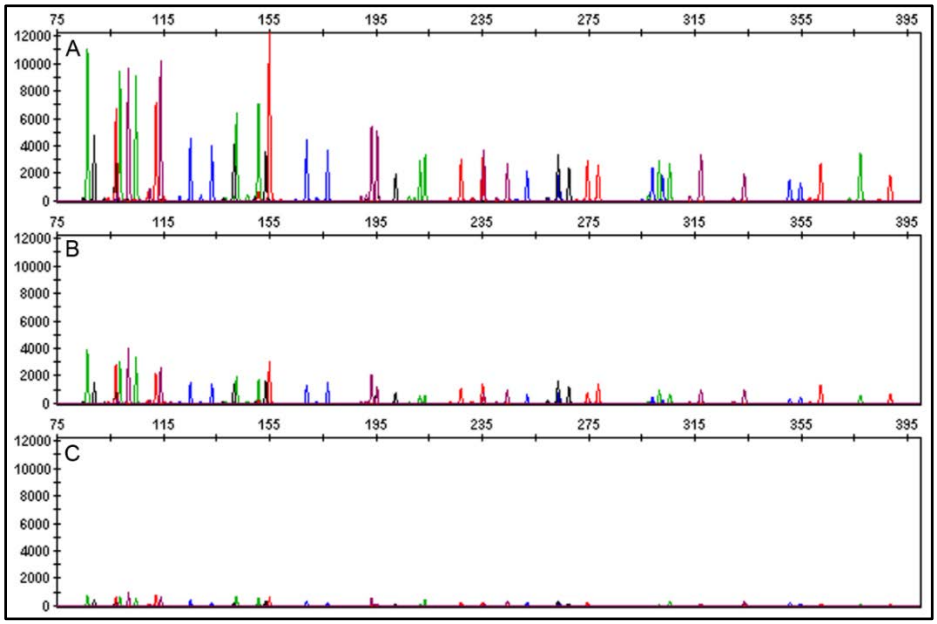

Figure 2: A) A full GlobalFiler profile (100% profile obtained) was generated with direct PCR for a touch DNA sample collected from a metal tool with a microFLOQ swab & water. This sample had a high FI ranking of 6.58 and an average peak height value of 4300 RFU. (B) A full GlobalFiler profile (100% profile obtained) was generated with direct PCR for a touch DNA sample collected from a metal tool with a microFLOQ swab & Triton X. The sample had

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a medium FI ranking of 5.43 and an average peak height value of 1433 RFU. (C) A partial GlobalFiler profile (73.9% profile obtained) was generated with direct PCR for a touch DNA sample collected from a metal tool with a dry microFLOQ swab. This sample had a low FI ranking of 2.37 and an average peak height value of 383 RFU.

Table 1: Substrate product information

Touch Substrate	Brand (Vendor)	Product Number	Item Information
Plastic Microscope Slides	VWR	470014-584	Polyvinyl chloride
100% Cotton Denim Shirt	Amazon Essentials	B07HL3KDWS	Slim-fit long-sleeve denim shirt, medium blue, 100% cotton
Wool Blend Shirt	Smartwool®	SW015255	Merino 150 baselayer long sleeve, light marlin blue, 87% merino wool, 13% nylon
100% Wool Shirt	Icebreaker®	104471	Merino 175 every day long-sleeve crew thermal top, snow, 100% merino wool
100% Polyester Shirt	Hanes®	O9308	Sport™ Cool DRI® Performance long-sleeve t-shirt, fresh berry, 100% polyester
Metal Tools	Husky (Home Depot)	202934342	½" drive ¾" 12-point SAE deep socket; chrome alloy steel
Vinyl Shutter Samples	Decorative Shutters	N/A	Legends vinyl shutter color samples
Handgun Grips	Glock Store	SKM 30818	Backstrap Set G17, G22, G31, G34, G35, G37 (Gen 4 & Gen 5 Only); black
Cartridge Casings	American Eagle	UPC 029465062477	9 mm Luger, brass rounds
Foam Cups	DART (Amazon)	B01N0WXYLK	White foam cup, 10 oz
Concrete Bricks	Acacia Grove (Amazon)	B07T4HN3T4	Mini cinder blocks, 1/12 scale
Wood Tool Handles	Mercer Industries (Amazon)	B01MYBH12Y	Wood file handle, 4-6"

Table 2: Phase I samples

Sample Type	Substrate	Donors	Collection Methods	Processing Methods	Replicates	Total Samples
1 ng buccal cells	Microscope slide	1	9	2	8	144
Fingerprint	Microscope slide	1	9	2	8	144
Worn	100% denim	1	1	2	8	16
	Wool blend	1	1	2	8	16
	100% wool	1	1	2	8	16
	100% polyester	1	1	2	8	16
Touch	Metal tool	3	9	2	8	432
	Vinyl shutter	3	9	2	8	432
	Handgun grip	3	9	2	8	432
	Cartridge casing	3	9	2	8	432
	Foam cup	3	9	2	8	432
	Concrete brick	3	9	2	8	432
	Wood tool handle	3	9	2	8	432
					TOTAL	**3,376**

Table 3: Touch DNA sample collection methods

Collection Substrate	Size (mm^2)	Collection Method	Moistening Agent
Puritan® cotton swab	3.0	Swabbing	3 µL Sterile H_2O 3 µL 0.1% Triton X None - dry
Copan microFLOQ® swab	1.2-2.0*	Swabbing	1 µL Sterile H_2O 1 µL 0.1% Triton X None - dry
GE Healthcare Whatman™ non-indicating FTA paper	1.2	Rubbing/scraping	1 µL Sterile H_2O 1 µL 0.1% Triton X None - dry

* Size of swab head varied slightly between swabs

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Table 4: Phase II samples

Sample Type	Substrate	Collection Method	Donors	Time Points*	Processing Methods	Replicates	Samples per Study: Time Study	GF vs 6C		Resampling
1 ng buccal cells	Microscope slide	microFLOQ & water‡	1	3	2	8	48	16	16	16
		cotton & water†	1	1	2	8	N/A	16	16	N/A
Fingerprints	Microscope slide	dry microFLOQ§	1	3	2	8	48	16	16	N/A
		microFLOQ & Triton X¶	1	3	2	8	48	16	16	16
		cotton & water†	1	1	2	8	N/A	16	16	N/A
Worn	100% denim	cutting	1	3	2	8	48	16	16	N/A
	100% wool	cutting	1	3	2	8	48	16	16	N/A
	100% polyester	cutting	1	3	2	8	48	16	16	N/A
Touch	Metal tool	dry cotton§	3	3	2	8	144	48	48	N/A
		dry microFLOQ¶	3	3	2	8	144	48	48	48
		cotton & water†	3	1	2	8	N/A	48	48	N/A
	Vinyl shutter	dry cotton§	3	3	2	8	144	48	48	N/A
		microFLOQ & water¶	3	3	2	8	144	48	48	48
		cotton & water†	3	1	2	8	N/A	48	48	N/A
	Handgun grip	microFLOQ & water‡	3	3	2	8	144	48	48	48
		cotton & water†	3	3	2	8	144	48	48	N/A
	Cartridge casing	microFLOQ & Triton X‡	3	3	2	8	144	48	48	48
		cotton & water†	3	1	2	8	N/A	48	48	N/A
	Foam cup	dry microFLOQ‡	3	3	2	8	144	48	48	48
		cotton & water†	3	1	2	8	N/A	48	48	N/A
	Concrete brick	cotton & Triton X§	3	3	2	8	144	48	48	N/A
		microFLOQ & Triton X¶	3	3	2	8	144	48	48	48
		cotton & water†	3	1	2	8	N/A	48	48	N/A
	Wood handle	dry cotton§	3	3	2	8	144	48	48	N/A
		dry microFLOQ¶	3	3	2	8	144	48	48	48
		cotton & water†	3	1	2	8	N/A	48	48	N/A
						TOTAL	2,016	1,984**		368

*When one time point is listed, samples were processed immediately at 0 months. GF vs 6C and resampling study samples were only processed at 0 months.

†Control collection method, commonly used for collection of touch DNA evidence samples.

‡Optimal collection method for both standard and direct PCR processing based on Phase I data.

§Optimal collection method for standard processing based on Phase I data.

¶Optimal collection method for direct PCR processing based on Phase I data.

**GlobalFiler samples (n=992) were processed in Phase I, and PowerPlex Fusion 6C samples (n=992) were processed in Phase II.

Table 5: Phase I mean FI, mean % profile, and standard deviation values for each substrate, collection method, and processing method and two-tailed *t* test comparisons of standard processing and direct PCR

Substrate	Collection Method	Mean FI: Standard	Mean FI: Direct PCR	*p*-value	Mean % Profile: Standard	Mean % Profile: Direct PCR	*p*-value
Plastic slide Buccal cells	cotton & water	3.79 ± 3.58	3.44 ± 1.87	0.809	54.5±49.0	86.6±11.4	0.092
	cotton & Triton X	6.35 ± 2.29	3.13 ± 1.86	0.008*	94.0±16.9	83.2±14.6	0.193
	dry cotton	4.38 ± 2.37	6.00 ± 0.66	0.085	70.2±43.5	97.7±3.8	0.096
	microFLOQ & water	7.09 ± 0.27	6.81 ± 0.31	0.077	100.0±0.0	100.0±0.0	1.000
	microFLOQ & Triton X	6.03 ± 2.43	6.43 ± 0.67	0.659	93.2±18.4	99.1±2.4	0.378
	dry microFLOQ	2.48 ± 1.69	5.21 ± 2.41	0.020*	57.7±29.3	87.8±20.1	0.031*
	FTA & water	6.00 ± 2.44	6.73 ± 0.27	0.412	90.3±25.5	99.4±1.1	0.331
	FTA & Triton X	5.40 ± 2.81	6.69 ± 0.28	0.219	79.5±38.8	98.9±1.2	0.181
	dry FTA	3.47 ± 2.89	4.35 ± 2.47	0.523	64.2±42.3	83.5±19.2	0.260
Plastic slide Fingerprints	cotton & water	0.56 ± 0.73	0.05 ± 0.00	0.069	19.9±23.4	25.0±20.0	0.646
	cotton & Triton X	0.27 ± 0.62	0.05 ± 0.00	0.334	9.9±10.5	15.3±9.9	0.307
	dry cotton	0.68 ± 1.23	1.35 ± 1.74	0.386	15.6±24.2	58.5±21.9	0.002*
	microFLOQ & water	0.43 ± 0.71	0.05 ± 0.00	0.155	14.8±18.4	11.1±8.6	0.616
	microFLOQ & Triton X	0.27 ± 0.62	1.41 ± 1.92	0.133	14.5±12.3	15.6±32.5	0.928
	dry microFLOQ	0.87 ± 2.31	0.18 ± 0.36	0.417	21.6±33.9	19.9±17.6	0.902
	FTA & water	0.49 ± 0.81	0.90 ± 0.91	0.359	5.1±5.0	10.5±20.3	0.478
	FTA & Triton X	0.27 ± 0.62	0.27 ± 0.62	1.000	11.9±12.8	6.3±7.8	0.304
	dry FTA	0.49 ± 0.81	0.05 ± 0.00	0.149	7.4±9.0	13.1±10.8	0.271

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Denim	cutting	6.20 ± 0.79	0.05 ± 0.00	<0.001*	98.9±2.3	0.0±0.0	<0.001*
Wool blend	cutting	6.91 ± 0.62	0.57 ± 0.98	<0.001*	100.0±0.0	40.6±25.6	<0.001*
100% wool	cutting	7.29 ± 0.29	0.05 ± 0.00	<0.001*	100.0±0.0	37.2±15.6	<0.001*
Polyester	cutting	2.23 ± 2.87	3.42 ± 2.82	0.471	45.5±39.7	71.6±25.8	0.141
Metal tool	cotton & water	1.60 ± 2.17	2.33 ± 2.75	0.312	39.5±35.2	58.2±34.9	0.071
	cotton & Triton X	1.57 ± 2.16	2.21 ± 2.25	0.320	44.6±34.6	61.3±31.9	0.089
	dry cotton	4.06 ± 2.87	4.22 ± 1.75	0.815	73.3±31.3	84.1±26.7	0.204
	microFLOQ & water	2.24 ± 2.71	3.38 ± 2.57	0.142	51.5±40.2	74.1±25.7	0.025*
	microFLOQ & Triton X	3.62 ± 3.03	3.80 ± 2.92	0.836	70.4±33.8	72.0±35.0	0.876
	dry microFLOQ	2.89 ± 2.19	4.46 ± 2.09	0.014*	69.6±24.5	86.3±21.2	0.015*
	FTA & water	0.81 ± 1.26	2.40 ± 2.65	0.011*	21.7±24.6	53.0±37.1	0.001*
	FTA & Triton X	1.53 ± 2.07	4.11 ± 2.48	<0.001*	47.4±31.0	75.5±33.6	0.004*
	dry FTA	3.49 ± 3.01	1.31 ± 2.07	0.005*	66.5±39.7	50.0±31.2	0.116
Vinyl shutter	cotton & water	1.15 ± 1.40	0.12 ± 0.36	0.001*	35.0±7.1	31.3±15.4	0.662
	cotton & Triton X	1.42 ± 2.06	0.28 ± 0.59	0.013*	38.6±7.9	20.1±22.2	0.024*
	dry cotton	2.18 ± 2.47	0.44 ± 0.68	0.001*	44.2±9.0	28.7±23.4	0.193
	microFLOQ & water	1.50 ± 2.18	1.58 ± 2.27	0.899	40.3±8.2	42.7±37.8	0.349
	microFLOQ & Triton X	1.17 ± 1.89	1.25 ± 2.05	0.878	38.0±7.8	29.2±34.2	0.802
	dry microFLOQ	1.21 ± 2.12	1.40 ± 2.07	0.755	37.5±7.7	42.6±34.6	0.268
	FTA & water	1.70 ± 2.42	1.35 ± 1.80	0.566	46.9±9.6	34.3±38.2	0.914
	FTA & Triton X	1.30 ± 1.64	1.35 ± 2.17	0.936	37.6±7.7	39.2±37.5	0.555
	dry FTA	0.79 ± 1.58	0.38 ± 0.92	0.285	31.1±6.4	22.7±27.5	0.983
Handgun grip	cotton & water	2.40 ± 2.98	3.41 ± 2.85	0.239	46.2±40.3	76.5±29.6	0.005*
	cotton & Triton X	0.70 ± 1.40	1.16 ± 1.76	0.318	29.0±26.3	55.9±23.2	0.001*
	dry cotton	1.81 ± 2.82	2.49 ± 2.79	0.406	34.8±39.7	52.1±41.3	0.148
	microFLOQ & water	2.11 ± 2.81	3.90 ± 2.99	0.038*	45.4±42.6	73.4±30.4	0.012*
	microFLOQ & Triton X	2.33 ± 2.87	3.24 ± 3.23	0.309	38.0±46.1	52.4±44.9	0.280
	dry microFLOQ	2.05 ± 2.56	2.96 ± 3.20	0.282	37.0±43.7	54.5±42.0	0.163
	FTA & water	1.62 ± 2.26	2.38 ± 3.20	0.346	29.5±39.9	42.5±42.4	0.280
	FTA & Triton X	2.07 ± 2.70	2.96 ± 3.07	0.292	37.1±44.7	56.0±42.9	0.142
	dry FTA	0.55 ± 1.14	1.08 ± 2.20	0.300	6.8±19.7	26.9±34.9	0.018*
Cartridge casing	cotton & water	0.38 ± 1.29	0.05 ± 0.00	0.218	6.5±22.8	0.0±0.0	0.167
	cotton & Triton X	0.29 ± 0.64	0.05 ± 0.00	0.078	5.6±14.5	0.0±0.0	0.063
	dry cotton	0.27 ± 0.59	0.05 ± 0.00	0.076	2.1±8.0	0.1±0.4	0.223
	microFLOQ & water	0.92 ± 1.89	0.13 ± 0.39	0.051	14.9±29.7	0.5±1.8	0.022*
	microFLOQ & Triton X	0.99 ± 1.99	0.20 ± 0.49	0.065	17.2±32.3	0.5±1.3	0.015*
	dry microFLOQ	0.41 ± 1.12	0.20 ± 0.49	0.401	9.4±21.5	0.3±1.0	0.043*
	FTA & water	0.12 ± 0.36	0.05 ± 0.00	0.323	0.1±0.5	0.1±0.4	0.975
	FTA & Triton X	0.36 ± 0.73	0.05 ± 0.00	0.039*	6.5±17.3	0.0±0.0	0.072
	dry FTA	0.45 ± 1.03	0.05 ± 0.00	0.062	4.2±17.6	0.0±0.0	0.252
Foam cup	cotton & water	1.03 ± 1.79	0.35 ± 0.80	0.094	15.8±30.2	30.3±23.1	0.067
	cotton & Triton X	0.78 ± 1.45	0.40 ± 0.80	0.264	18.3±26.4	26.1±22.3	0.278
	dry cotton	1.12 ± 1.82	1.08 ± 1.73	0.930	29.4±34.8	27.7±30.4	0.856
	microFLOQ & water	0.99 ± 1.74	1.11 ± 1.60	0.798	12.1±27.9	24.3±31.2	0.160
	microFLOQ & Triton X	0.20 ± 0.50	0.50 ± 0.93	0.157	5.0±8.0	17.2±23.1	0.018*
	dry microFLOQ	1.72 ± 2.35	1.59 ± 2.36	0.849	32.5±41.0	33.9±39.1	0.909
	FTA & water	0.56 ± 1.21	1.46 ± 2.35	0.101	9.1±21.2	27.2±38.0	0.046*
	FTA & Triton X	0.56 ± 0.81	0.34 ± 0.67	0.318	8.0±11.0	10.6±14.0	0.481
	dry FTA	0.12 ± 0.36	0.10 ± 0.24	0.785	1.0±2.7	4.5±13.4	0.214
Concrete brick	cotton & water	2.80 ± 2.55	0.05 ± 0.00	<0.001*	65.6±33.4	0.0±0.0	<0.001*
	cotton & Triton X	3.35 ± 2.95	0.05 ± 0.00	<0.001*	66.7±37.0	0.0±0.0	<0.001*
	dry cotton	1.82 ± 2.55	0.05 ± 0.00	0.001*	42.2±40.1	0.0±0.0	<0.001*
	microFLOQ & water	3.05 ± 2.67	0.05 ± 0.00	<0.001*	62.5±39.5	0.0±0.0	<0.001*
	microFLOQ & Triton X	2.95 ± 2.62	0.05 ± 0.00	<0.001*	64.6±34.6	0.0±0.0	<0.001*
	dry microFLOQ	0.63 ± 1.62	0.05 ± 0.00	0.085	26.9±27.9	0.0±0.0	<0.001*
	FTA & water	0.80 ± 2.03	0.05 ± 0.00	0.077	20.7±32.4	0.0±0.0	0.003*
	FTA & Triton X	0.80 ± 1.80	0.05 ± 0.00	0.047*	27.2±29.9	0.0±0.0	<0.001*
	dry FTA	0.65 ± 0.98	0.05 ± 0.00	0.004*	19.2±24.8	0.0±0.0	<0.001*
Wood handle	cotton & water	2.54 ± 2.70	1.82 ± 2.14	0.314	52.8±40.7	53.1±36.6	0.978
	cotton & Triton X	2.53 ± 3.31	2.01 ± 2.48	0.540	51.0±39.3	50.8±37.7	0.988
	dry cotton	2.88 ± 2.74	2.67 ± 2.33	0.780	58.8±38.2	69.2±29.3	0.294
	microFLOQ & water	1.71 ± 2.64	2.74 ± 3.00	0.211	42.4±34.1	61.1±33.2	0.060
	microFLOQ & Triton X	1.30 ± 2.18	2.31 ± 2.72	0.162	37.7±35.5	54.5±37.4	0.117
	dry microFLOQ	2.28 ± 2.69	5.22 ± 2.14	<0.001*	46.0±41.0	89.3±22.7	<0.001*
	FTA & water	0.35 ± 1.14	0.76 ± 1.49	0.298	9.6±21.2	18.5±27.4	0.219
	FTA & Triton X	0.50 ± 0.87	1.18 ± 1.85	0.109	13.1±24.8	22.6±34.9	0.279
	dry FTA	1.49 ± 2.04	1.32 ± 2.18	0.790	29.8±39.4	35.8±36.1	0.587

*$p < 0.05$ (two-tailed t test)

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Table 6: Phase I FI value Tukey HSD pairwise comparisons of all collection methods within the standard processing group

Collection Methods Compared		Buccal		Metal	Vinyl	Handgun	Cartridge	Foam	Concrete	Wood
Method 1	Method 2	Cells	Fingerprints	Tool	Shutter	Grip	Casing	Cup	Brick	Handle
cotton & water	dry FTA	1.000	1.000	0.165	0.999	0.194	1.000	0.454	0.034*	0.840
cotton & Triton X	cotton & water	0.496	1.000	1.000	1.000	0.299	1.000	1.000	0.996	1.000
cotton & Triton X	FTA & water	1.000	1.000	0.978	1.000	0.933	1.000	1.000	0.004*	0.045*
cotton & Triton X	dry FTA	0.336	1.000	0.147	0.974	1.000	1.000	0.829	0.002*	0.847
dry cotton	cotton & water	1.000	1.000	0.018*	0.695	0.996	1.000	1.000	0.858	1.000
dry cotton	cotton & Triton X	0.803	0.998	0.015*	0.927	0.825	1.000	0.997	0.329	1.000
dry cotton	microFLOQ & water	0.421	1.000	0.206	0.961	1.000	0.635	1.000	0.630	0.743
dry cotton	FTA & water	0.924	1.000	<0.001*	0.996	1.000	1.000	0.925	0.829	0.009*
dry cotton	FTA & Triton X	0.996	0.998	0.013*	0.846	1.000	1.000	0.925	0.831	0.018*
dry cotton	dry FTA	0.998	1.000	0.997	0.286	0.699	1.000	0.321	0.698	0.529
microFLOQ & water	cotton & water	0.177	1.000	0.993	1.000	1.000	0.830	1.000	1.000	0.953
microFLOQ & water	cotton & Triton X	1.000	1.000	0.989	1.000	0.565	0.669	1.000	1.000	0.956
microFLOQ & water	microFLOQ & Triton X	0.994	1.000	0.574	1.000	1.000	1.000	0.644	1.000	1.000
microFLOQ & water	dry microFLOQ	0.011*	0.996	0.992	1.000	1.000	0.869	0.739	0.009*	0.996
microFLOQ & water	FTA & water	0.993	1.000	0.530	1.000	0.999	0.351	0.985	0.020*	0.565
microFLOQ & water	FTA & Triton X	0.905	1.000	0.986	1.000	1.000	0.807	0.986	0.021*	0.706
microFLOQ & water	dry FTA	0.100	1.000	0.704	0.948	0.419	0.915	0.525	0.010*	1.000
microFLOQ & Triton X	cotton & water	0.669	1.000	0.104	1.000	1.000	0.706	0.571	1.000	0.678
microFLOQ & Triton X	cotton & Triton X	1.000	1.000	0.091	1.000	0.357	0.523	0.904	1.000	0.688
microFLOQ & Triton X	dry cotton	0.916	0.998	1.000	0.713	0.998	0.488	0.427	0.737	0.348
microFLOQ & Triton X	FTA & water	1.000	1.000	0.003*	0.991	0.986	0.236	0.995	0.034*	0.905
microFLOQ & Triton X	FTA & Triton X	1.000	1.000	0.081	1.000	1.000	0.678	0.995	0.034*	0.962
microFLOQ & Triton X	dry FTA	0.497	1.000	1.000	0.999	0.239	0.824	1.000	0.017*	1.000
dry microFLOQ	cotton & water	0.978	1.000	0.668	1.000	1.000	1.000	0.800	0.031^	1.000
dry microFLOQ	cotton & Triton X	0.060	0.970	0.633	1.000	0.623	1.000	0.417	0.002^	1.000
dry microFLOQ	dry cotton	0.832	1.000	0.775	0.756	1.000	1.000	0.899	0.677	0.995
dry microFLOQ	microFLOQ & Triton X	0.114	0.970	0.982	1.000	1.000	0.757	0.013*	0.015^	0.883
dry microFLOQ	FTA & water	0.121	0.999	0.085	0.995	1.000	0.996	0.149	1.000	0.118
dry microFLOQ	FTA & Triton X	0.319	0.970	0.601	1.000	1.000	1.000	0.150	1.000	0.191
dry microFLOQ	dry FTA	0.997	0.999	0.995	0.998	0.475	1.000	0.007*	1.000	0.964
FTA & water	cotton & water	0.686	1.000	0.971	0.989	0.975	0.998	0.972	0.064	0.043^
FTA & water	FTA & Triton X	1.000	1.000	0.984	0.999	0.999	0.999	1.000	1.000	1.000
FTA & Triton X	cotton & water	0.925	1.000	1.000	1.000	1.000	1.000	0.973	0.065	0.078
FTA & Triton X	cotton & Triton X	0.997	1.000	1.000	1.000	0.597	1.000	1.000	0.004^	0.081
FTA & Triton X	dry FTA	0.818	1.000	0.132	0.993	0.450	1.000	0.983	1.000	0.879
dry FTA	FTA & water	0.515	1.000	0.006*	0.813	0.853	0.990	0.983	1.000	0.774

*Mean FI from Method 1 was significantly higher than Method 2 ($p < 0.05$)
*Mean FI from Method 2 was significantly higher than Method 1 ($p < 0.05$)

Table 7: Phase I FI value Tukey HSD pairwise comparisons of all collection methods within the direct PCR group

Collection Methods Compared		Buccal		Metal	Vinyl	Handgun	Cartridge	Foam	Concrete	Wood
Method 1	Method 2	Cells	Fingerprints	Tool	Shutter	Grip	Casing	Cup	Brick	Handle
cotton & water	dry FTA	0.948	1.000	0.868	1.000	0.114	1.000	1.000	NA	0.998
cotton & Triton X	cotton & water	1.000	1.000	1.000	1.000	0.146	1.000	1.000	NA	1.000
cotton & Triton X	FTA & water	<0.001^	0.688	1.000	0.354	0.862	1.000	0.235	NA	0.624
cotton & Triton X	dry FTA	0.778	1.000	0.934	1.000	1.000	1.000	0.998	NA	0.983
dry cotton	cotton & water	0.028*	0.151	0.155	0.999	0.972	1.000	0.729	NA	0.936
dry cotton	cotton & Triton X	0.008*	0.151	0.100	1.000	0.796	1.000	0.808	NA	0.986
dry cotton	microFLOQ & water	0.973	0.151	0.956	0.256	0.740	0.983	1.000	NA	1.000
dry cotton	FTA & water	0.986	0.988	0.191	0.574	1.000	1.000	0.992	NA	0.098
dry cotton	FTA & Triton X	0.990	0.365	1.000	0.577	1.000	1.000	0.724	NA	0.376
dry cotton	dry FTA	0.416	0.151	0.002*	1.000	0.734	1.000	0.338	NA	0.525
microFLOQ & water	cotton & water	0.001*	1.000	0.854	0.049*	1.000	0.983	0.676	NA	0.902
microFLOQ & water	cotton & Triton X	<0.001*	1.000	0.757	0.123	0.029*	0.983	0.761	NA	0.973
microFLOQ & water	microFLOQ & Triton X	1.000	0.116	1.000	0.999	0.997	0.994	0.882	NA	0.999
microFLOQ & water	dry microFLOQ	0.453	1.000	0.829	1.000	0.967	0.994	0.970	NA	0.007^
microFLOQ & water	FTA & water	1.000	0.688	0.894	1.000	0.654	0.983	0.996	NA	0.074
microFLOQ & water	FTA & Triton X	1.000	1.000	0.981	1.000	0.968	0.983	0.671	NA	0.313
microFLOQ & water	dry FTA	0.040*	1.000	0.079	0.200	0.021*	0.983	0.291	NA	0.453
microFLOQ & Triton X	cotton & water	0.005*	0.116	0.472	0.271	1.000	0.617	1.000	NA	0.998
microFLOQ & Triton X	cotton & Triton X	0.001*	0.116	0.354	0.486	0.228	0.617	1.000	NA	1.000
microFLOQ & Triton X	dry cotton	1.000	1.000	1.000	0.711	0.992	0.617	0.913	NA	1.000
microFLOQ & Triton X	FTA & water	1.000	0.975	0.537	1.000	0.982	0.617	0.365	NA	0.320
microFLOQ & Triton X	FTA & Triton X	1.000	0.299	1.000	1.000	1.000	0.617	1.000	NA	0.739
microFLOQ & Triton X	dry FTA	0.138	0.116	0.013*	0.632	0.183	0.617	0.989	NA	0.860
dry microFLOQ	cotton & water	0.314	1.000	0.063	0.141	1.000	0.617	0.087	NA	<0.001*
dry microFLOQ	cotton & Triton X	0.137	1.000	0.037*	0.295	0.421	0.617	0.121	NA	<0.001*

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dry microFLOQ	dry cotton	0.979	0.259	1.000	0.504	1.000	0.617	0.954	NA	0.005*
dry microFLOQ	microFLOQ & Triton X	0.780	0.206	0.990	1.000	1.000	1.000	0.209	NA	0.001*
dry microFLOQ	FTA & water	0.522	0.839	0.081	1.000	0.999	0.617	1.000	NA	<0.001*
dry microFLOQ	FTA & Triton X	0.562	1.000	1.000	1.000	1.000	0.617	0.085	NA	<0.001*
dry microFLOQ	dry FTA	0.963	1.000	<0.001*	0.424	0.355	0.617	0.015*	NA	<0.001*
FTA & water	cotton & water	0.001*	0.688	1.000	0.178	0.946	1.000	0.177	NA	0.800
FTA & water	FTA & Triton X	1.000	0.919	0.264	1.000	0.999	1.000	0.173	NA	0.999
FTA & Triton X	cotton & water	0.002*	1.000	0.219	0.180	1.000	1.000	1.000	NA	0.988
FTA & Triton X	cotton & Triton X	<0.001*	1.000	0.146	0.356	0.417	1.000	1.000	NA	0.944
FTA & Triton X	dry FTA	0.061	1.000	0.003*	0.495	0.351	1.000	1.000	NA	1.000
dry FTA	FTA & water	0.053	0.688	0.823	0.492	0.811	1.000	0.038^	NA	0.995

* Mean FI from Method 1 was significantly higher than Method 2 ($p < 0.05$)
^ Mean FI from Method 2 was significantly higher than Method 1 ($p < 0.05$)

Table 8: Phase I % profile Tukey HSD pairwise comparisons of all collection methods within the standard processing group

Collection Methods Compared		Buccal		Metal	Vinyl	Handgun	Cartridge	Foam	Concrete	Wood
Method 1	Method 2	Cells	Fingerprints	Tool	Shutter	Grip	Casing	Cup	Brick	Handle
cotton & water	dry FTA	1.000	0.917	0.117	0.979	0.017*	1.000	0.551	<0.001*	0.382
cotton & Triton X	cotton & water	0.301	0.978	1.000	1.000	0.842	1.000	1.000	1.000	1.000
cotton & Triton X	FTA & water	1.000	1.000	0.294	0.998	1.000	0.991	0.944	<0.001*	0.003*
cotton & Triton X	dry FTA	0.672	1.000	0.357	0.777	0.566	1.000	0.326	<0.001*	0.503
dry cotton	cotton & water	0.989	1.000	0.015*	0.999	0.985	0.998	0.654	0.287	1.000
dry cotton	cotton & Triton X	0.873	1.000	0.074	1.000	1.000	1.000	0.855	0.226	0.998
dry cotton	microFLOQ & water	0.672	1.000	0.365	0.993	0.991	0.436	0.322	0.484	0.804
dry cotton	FTA & water	0.947	0.969	<0.001*	0.996	1.000	1.000	0.138	0.398	<0.001*
dry cotton	FTA & Triton X	1.000	1.000	0.154	0.996	1.000	0.998	0.096	0.832	0.001*
dry cotton	dry FTA	1.000	0.993	0.999	0.734	0.245	1.000	0.005*	0.303	0.114
microFLOQ & water	cotton & water	0.147	1.000	0.943	1.000	1.000	0.892	1.000	1.000	0.984
microFLOQ & water	cotton & Triton X	1.000	1.000	0.998	0.996	0.877	0.823	0.995	1.000	0.996
microFLOQ & water	microFLOQ & Triton X	1.000	1.000	0.566	1.000	0.999	1.000	0.989	1.000	1.000
microFLOQ & water	dry microFLOQ	0.218	0.998	0.622	1.000	0.998	0.991	0.133	0.009*	1.000
microFLOQ & water	FTA & water	1.000	0.981	0.053	1.000	0.895	0.238	1.000	0.001*	0.043*
microFLOQ & water	FTA & Triton X	0.943	1.000	1.000	1.000	0.998	0.890	1.000	0.010*	0.105
microFLOQ & water	dry FTA	0.432	0.997	0.826	0.996	0.021*	0.673	0.858	<0.001*	0.950
microFLOQ & Triton X	cotton & water	0.329	1.000	0.038*	1.000	0.998	0.682	0.873	1.000	0.865
microFLOQ & Triton X	cotton & Triton X	1.000	1.000	0.156	0.995	0.997	0.578	0.681	1.000	0.932
microFLOQ & Triton X	dry cotton	0.894	1.000	1.000	0.992	1.000	0.215	0.031^	0.346	0.503
microFLOQ & Triton X	FTA & water	1.000	0.984	<0.001*	1.000	0.998	0.098	1.000	<0.001*	0.144
microFLOQ & Triton X	FTA & Triton X	0.996	1.000	0.290	1.000	1.000	0.679	1.000	0.005*	0.290
microFLOQ & Triton X	dry FTA	0.705	0.998	1.000	0.997	0.131	0.408	1.000	<0.001*	0.998
dry microFLOQ	cotton & water	1.000	1.000	0.049*	1.000	0.996	1.000	0.370	0.003^	0.999
dry microFLOQ	cotton & Triton X	0.410	0.943	0.187	0.991	0.999	0.999	0.601	0.002^	1.000
dry microFLOQ	dry cotton	0.998	0.999	1.000	0.986	1.000	0.947	1.000	0.815	0.944
dry microFLOQ	microFLOQ & Triton X	0.443	0.998	1.000	1.000	1.000	0.927	0.008*	0.004^	0.997
dry microFLOQ	FTA & water	0.557	0.708	<0.001*	1.000	0.999	0.817	0.045*	0.999	0.014*
dry microFLOQ	FTA & Triton X	0.918	0.981	0.336	1.000	1.000	1.000	0.029*	1.000	0.041*
dry microFLOQ	dry FTA	1.000	0.844	1.000	0.999	0.162	0.993	0.001*	0.997	0.817
FTA & water	cotton & water	0.432	0.813	0.642	1.000	0.864	0.975	0.992	<0.001^	0.001^
FTA & water	FTA & Triton X	0.999	0.998	0.159	1.000	0.999	0.976	1.000	0.999	1.000
FTA & Triton X	cotton & water	0.841	0.995	0.996	1.000	0.996	1.000	0.980	0.003^	0.004^
FTA & Triton X	cotton & Triton X	0.993	1.000	1.000	0.998	0.999	1.000	0.899	0.002^	0.009^
FTA & Triton X	dry FTA	0.990	1.000	0.553	0.994	0.160	1.000	0.990	0.996	0.786
dry FTA	FTA & water	0.806	1.000	<0.001*	0.993	0.534	0.999	0.976	1.000	0.570

* Mean % profile from Method 1 was significantly higher than Method 2 ($p < 0.05$)
^ Mean % profile from Method 2 was significantly higher than Method 1 ($p < 0.05$)

Table 9: Phase I % profile Tukey HSD pairwise comparisons of all collection methods within the direct PCR group

Collection Methods Compared		Buccal		Metal	Vinyl	Handgun	Cartridge	Foam	Concrete	Wood
Method 1	Method 2	Cells	Fingerprints	Tool	Shutter	Grip	Casing	Cup	Brick	Handle
cotton & water	dry FTA	1.000	0.926	0.992	0.988	<0.001*	1.000	0.036*	-	0.674
cotton & Triton X	cotton & water	1.000	0.978	1.000	0.944	0.618	1.000	1.000	-	1.000
cotton & Triton X	FTA & water	0.114	1.000	0.991	0.814	0.947	1.000	1.000	-	0.025*
cotton & Triton X	dry FTA	1.000	1.000	0.942	1.000	0.161	1.000	0.150	-	0.822
dry cotton	cotton & water	0.570	0.014*	0.101	1.000	0.376	1.000	1.000	-	0.758
dry cotton	cotton & Triton X	0.218	<0.001*	0.221	0.989	1.000	1.000	1.000	-	0.597
dry cotton	microFLOQ & water	1.000	<0.001*	0.971	0.825	0.568	0.852	1.000	-	0.995
dry cotton	FTA & water	1.000	<0.001*	0.019*	1.000	0.994	1.000	1.000	-	<0.001*
dry cotton	FTA & Triton X	1.000	<0.001*	0.989	0.963	1.000	1.000	0.438	-	<0.001*
dry cotton	dry FTA	0.241	<0.001*	0.006*	0.999	0.333	1.000	0.091	-	0.0168*
microFLOQ & water	cotton & water	0.317	0.841	0.709	0.939	1.000	0.627	0.998	-	0.996
microFLOQ & water	cotton & Triton X	0.090	1.000	0.890	0.229	0.798	0.627	1.000	-	0.977

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microFLOQ & water	microFLOQ & Triton X	1.000	1.000	1.000	0.851	0.587	1.000	0.994	-	0.999
microFLOQ & water	dry microFLOQ	0.437	0.988	0.912	1.000	0.722	0.998	0.954	-	0.085
microFLOQ & water	FTA & water	1.000	1.000	0.322	0.991	0.108	0.852	1.000	-	0.001*
microFLOQ & water	FTA & Triton X	1.000	1.000	1.000	1.000	0.800	0.627	0.730	-	0.003*
microFLOQ & water	dry FTA	0.102	1.000	0.163	0.385	0.001*	0.627	0.246	-	0.175
microFLOQ & Triton X	cotton & water	0.405	0.982	0.841	1.000	0.394	0.627	0.777	-	1.000
microFLOQ & Triton X	cotton & Triton X	0.128	1.000	0.958	0.985	1.000	0.627	0.972	-	1.000
microFLOQ & Triton X	dry cotton	1.000	0.001^	0.915	1.000	1.000	0.852	0.925	-	0.837
microFLOQ & Triton X	FTA & water	1.000	1.000	0.469	1.000	0.992	0.852	0.942	-	0.007*
microFLOQ & Triton X	FTA & Triton X	1.000	0.982	1.000	0.972	1.000	0.627	0.996	-	0.028*
microFLOQ & Triton X	dry FTA	0.143	1.000	0.266	0.998	0.317	0.627	0.805	-	0.577
dry microFLOQ	cotton & water	1.000	1.000	0.053	0.943	0.529	0.969	1.000	-	0.006*
dry microFLOQ	cotton & Triton X	0.996	1.000	0.129	0.237	1.000	0.969	0.987	-	0.003*
dry microFLOQ	dry cotton	0.703	0.002^	1.000	0.833	1.000	0.998	0.997	-	0.480
dry microFLOQ	microFLOQ & Triton X	0.536	1.000	0.810	0.858	1.000	0.998	0.480	-	0.011*
dry microFLOQ	FTA & water	0.503	0.982	0.008*	0.992	0.973	0.998	0.996	-	<0.001*
dry microFLOQ	FTA & Triton X	0.570	0.855	0.956	1.000	1.000	0.969	0.087	-	<0.001*
dry microFLOQ	dry FTA	0.998	0.998	0.003*	0.395	0.214	0.969	0.009*	-	<0.001*
FTA & water	cotton & water	0.375	0.809	1.000	1.000	0.051	1.000	1.000	-	0.011*
FTA & water	FTA & Triton X	1.000	1.000	0.239	1.000	0.947	1.000	0.477	-	1.000
FTA & Triton X	cotton & water	0.437	0.515	0.604	0.994	0.620	1.000	0.244	-	0.043*
FTA & Triton X	cotton & Triton X	0.143	0.985	0.817	0.459	1.000	1.000	0.578	-	0.086
FTA & Triton X	dry FTA	0.160	0.998	0.113	0.653	0.160	1.000	0.998	-	0.907
dry FTA	FTA & water	0.128	1.000	1.000	0.930	0.879	1.000	0.105	-	0.678

* Mean % profile from Method 1 was significantly higher than Method 2 ($p < 0.05$)
^ Mean % profile from Method 2 was significantly higher than Method 1 ($p < 0.05$)

Table 10: Phase I mean FI, mean % profile, and standard deviation values for each swab type, substrate, and processing method and two-tailed *t* test comparisons of standard processing and direct PCR

		Mean FI			Mean % Profile		
Substrate	**Swab Type**	**Standard**	**Direct PCR**	***p*-value**	**Standard**	**Direct PCR**	***p*-value**
Plastic slide Buccal cells	cotton	4.84±2.91	4.19±1.99	0.367	72.9±40.8	89.2±12.2	0.068
	microFLOQ	5.20±2.60	6.15±1.56	0.131	83.6±26.9	95.6±12.6	0.053
	FTA paper	4.96±2.82	5.92±1.79	0.163	78.0±36.4	93.9±13.0	0.050
Plastic slide Fingerprints	cotton	0.502±0.878	0.48±1.15	0.950	15.2±19.9	33.0±25.6	0.010*
	microFLOQ	0.521±1.400	0.54±1.25	0.952	17.0±22.6	15.5±21.3	0.824
	FTA paper	0.415±0.727	0.41±0.71	0.962	8.1±9.5	9.9±13.7	0.600
Metal tool	cotton	2.41±2.66	2.92±2.44	0.232	52.5±36.5	67.9±33.0	0.009*
	microFLOQ	2.92±2.69	3.88±2.55	0.029*	63.9±34.1	77.4±28.2	0.010*
	FTA paper	1.94±2.48	2.60±2.64	0.124	45.2±36.9	59.5±35.5	0.019*
Vinyl shutter	cotton	1.58±2.04	0.28±0.57	<0.001*	39.5±39.0	26.7±20.9	0.016*
	microFLOQ	1.29±2.04	1.41±2.10	0.729	31.6±38.1	38.2±35.6	0.287
	FTA paper	1.26±1.93	1.03±1.75	0.439	29.5±38.8	32.1±35.0	0.681
Handgun grip	cotton	1.64±2.57	2.35±2.65	0.102	36.7±36.3	61.5±33.6	<0.001*
	microFLOQ	2.16±2.71	2.27±3.13	0.015*	40.1±43.7	60.1±40.2	0.005*
	FTA paper	1.41±2.20	2.14±2.93	0.094	24.5±38.2	41.8±41.4	0.010*
Cartridge casing	cotton	0.31±0.89	0.056±0.00	0.014*	4.76±16.2	0.0±0.3	0.014*
	microFLOQ	0.77±1.71	0.17±0.46	0.005*	13.9±28.0	0.4±1.4	<0.001*
	FTA paper	0.31±0.76	0.05±0.00	0.004*	3.6±14.3	0.0±0.3	0.037*
Foam cup	cotton	0.98±1.68	0.61±1.22	0.132	21.2±30.8	28.0±25.2	0.146
	microFLOQ	0.97±1.80	1.07±1.76	0.735	16.5±30.9	25.1±32.1	0.104
	FTA paper	0.41±0.88	0.64±1.52	0.289	6.0±14.1	14.1±26.1	0.022*
Concrete brick	cotton	2.66±2.73	0.05±0.00	<0.001*	58.2±38.1	0.0±0.0	<0.001*
	microFLOQ	2.21±2.58	0.05±0.00	<0.001*	51.3±38.1	0.0±0.0	<0.001*
	FTA paper	0.75±1.64	0.05±0.00	<0.001*	22.3±29.0	0.0±0.0	<0.001*
Wood handle	cotton	2.65±2.89	2.17±2.32	0.272	54.2±39.0	57.2±35.2	0.571
	microFLOQ	1.76±2.51	3.42±2.91	<0.001*	42.0±36.6	68.3±34.8	<0.001*
	FTA paper	0.78±1.50	1.09±1.85	0.277	17.5±30.5	25.6±33.4	0.130

*$p < 0.05$ (two-tailed *t* test)

Table 11: Phase I FI and % profile Tukey HSD pairwise comparisons of swab types for each substrate and processing method

	Swab Types Compared		**FI *p*-value**		**% Profile *p*-value**	
Substrate	**Swab 1**	**Swab 2**	**Standard**	**Direct PCR**	**Standard**	**Direct PCR**
Plastic slide Buccal cells	cotton	microFLOQ	0.895	0.001^	0.546	0.187
	microFLOQ	FTA paper	0.950	0.898	0.847	0.886
	FTA paper	cotton	0.989	0.004*	0.870	0.399
Plastic slide Fingerprints	cotton	microFLOQ	0.998	0.979	0.938	0.014*
	microFLOQ	FTA paper	0.995	0.893	0.223	0.622
	FTA paper	cotton	0.955	0.965	0.383	0.001^

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Metal tool	cotton	microFLOQ	0.472	0.062	0.139	0.181
	microFLOQ	FTA paper	0.068	0.008*	0.006*	0.003*
	FTA paper	cotton	0.537	0.739	0.445	0.266
Vinyl shutter	cotton	microFLOQ	0.657	<0.001^	0.443	0.074
	microFLOQ	FTA paper	0.996	0.326	0.944	0.469
	FTA paper	cotton	0.606	0.017*	0.272	0.564
Handgun grip	cotton	microFLOQ	0.422	0.095	0.86	0.975
	microFLOQ	FTA paper	0.174	0.033*	0.048*	0.013*
	FTA paper	cotton	0.853	0.900	0.154	0.007^
Cartridge casing	cotton	microFLOQ	0.057	0.015^	0.022^	0.024^
	microFLOQ	FTA paper	0.057	0.015*	0.008*	0.024*
	FTA paper	cotton	1.000	1.000	0.937	1.000
Foam cup	cotton	microFLOQ	0.999	0.169	0.545	0.808
	microFLOQ	FTA paper	0.074	0.204	0.048*	0.050
	FTA paper	cotton	0.065	0.994	0.002^	0.009^
Concrete brick	cotton	microFLOQ	0.496	N/A	0.476	N/A
	microFLOQ	FTA paper	0.001*	N/A	<0.001*	N/A
	FTA paper	cotton	<0.001^	N/A	<0.001^	N/A
Wood handle	cotton	microFLOQ	0.068	0.005^	0.101	0.158
	microFLOQ	FTA paper	0.036*	<0.001*	<0.001*	<0.001*
	FTA paper	cotton	<0.001^	0.020^	<0.001^	<0.001^

* Mean from Method 1 was significantly higher than Method 2 ($p < 0.05$)
^ Mean from Method 2 was significantly higher than Method 1 ($p < 0.05$)

Table 12: Phase I mean FI, mean % profile, and standard deviation values for each moistening agent, substrate, and processing method and two-tailed *t* test comparisons of standard processing and direct PCR.

		Mean FI			Mean % Profile		
Substrate	**Moistening Agent**	**Standard**	**Direct PCR**	***p*-value**	**Standard**	**Direct PCR**	***p*-value**
Plastic slide Buccal cells	Water	5.63±2.77	5.66±1.92	0.961	81.6±36.4	95.4±8.9	0.079
	Triton-X	5.93±2.44	5.42±1.99	0.429	88.9±26.3	93.8±11.2	0.413
	None	3.45±2.40	5.19±2.06	0.010*	64.0±37.6	89.7±16.6	0.004*
Plastic slide Fingerprints	Water	0.49±0.72	0.33±0.65	0.423	13.3±17.8	15.5±17.8	0.660
	Triton-X	0.27±0.59	0.58±1.27	0.289	12.1±11.5	12.4±19.7	0.952
	None	0.68±1.52	0.53±1.25	0.699	14.9±24.3	30.5±26.3	0.038*
Metal tool	Water	1.55±2.19	2.70±2.66	0.005*	37.6±35.7	61.7±33.7	<0.001*
	Triton-X	2.24±2.62	3.37±2.67	0.011*	54.1±34.7	69.6±33.6	0.008*
	None	3.48±2.72	3.33±2.42	0.730	69.8±32.1	73.4±31.2	0.491
Vinyl shutter	Water	1.45±2.03	1.02±1.78	0.175	33.3±40.4	36.1±32.2	0.639
	Triton-X	1.30±1.85	0.96±1.80	0.273	35.3±37.8	29.5±32.5	0.323
	None	1.39±2.14	0.74±1.42	0.033*	32.0±38.3	31.3±29.7	0.907
Handgun grip	Water	2.04±2.68	3.23±3.04	0.014*	40.4±41.1	64.1±37.5	<0.001*
	Triton-X	1.70±2.49	2.45±2.88	0.095	34.7±39.7	54.8±37.8	0.002*
	None	1.47±2.36	2.18±2.84	0.106	26.2±38.1	44.5±41.0	0.006*
Cartridge casing	Water	0.47±1.36	0.08±0.22	0.016*	7.2±22.2	0.2±1.1	0.008*
	Triton-X	0.55±1.30	0.10±0.29	0.005*	9.8±23.1	0.2±0.8	0.001*
	None	0.38±0.93	0.10±0.29	0.017*	5.2±16.7	0.1±0.6	0.011*
Foam cup	Water	0.86±1.59	0.97±1.75	0.683	12.3±26.5	27.3±31.0	0.002*
	Triton-X	0.51±1.02	0.42±0.80	0.525	10.4±17.9	18.0±20.9	0.022*
	None	0.99±1.83	0.92±1.78	0.825	21.0±33.8	22.0±31.9	0.850
Concrete brick	Water	2.22±2.60	0.05±0.00	<0.001*	49.6±40.4	0.0±0.0	<0.001*
	Triton-X	2.37±2.71	0.05±0.00	<0.001*	52.8±38.1	0.0±0.0	<0.001*
	None	1.03±1.89	0.05±0.00	<0.001*	29.4±32.6	0.0±0.0	<0.001*
Wood handle	Water	1.53±2.42	1.77±2.41	0.552	35.0±37.5	44.2±37.2	0.138
	Triton-X	1.44±2.46	1.83±2.39	0.336	33.9±36.9	42.6±38.9	0.168
	None	2.22±2.54	3.07±2.72	0.053	44.9±40.8	64.8±36.9	0.003*

*$p < 0.05$ (two-tailed *t* test)

Table 13: Phase I FI and % profile Tukey HSD pairwise comparisons of moistening agents for each substrate and processing method

	Moistening Agents Compared		**FI *p*-value**		**% Profile *p*-value**	
Substrate	**Agent 1**	**Agent 2**	**Standard**	**Direct PCR**	**Standard**	**Direct PCR**
Plastic slide Buccal cells	Water	Triton-X	0.911	0.905	0.737	0.899
	Triton-X	None	0.003*	0.915	0.034*	0.509
	None	Water	0.011^	0.688	0.176	0.273
Plastic slide Fingerprints	Water	Triton-X	0.734	0.706	0.976	0.871
	Triton-X	None	0.361	0.985	0.866	0.014^
	None	Water	0.808	0.802	0.952	0.050
Metal tool	Water	Triton-X	0.230	0.270	0.011^	0.323

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	Triton-X	None	0.010^	0.995	0.018^	0.759
	None	Water	<0.001*	0.317	<0.001*	0.085
Vinyl shutter	Water	Triton-X	0.888	0.978	0.946	0.419
	Triton-X	None	0.957	0.705	0.864	0.936
	None	Water	0.982	0.578	0.979	0.631
Handgun grip	Water	Triton-X	0.693	0.252	0.667	0.317
	Triton-X	None	0.844	0.835	0.405	0.253
	None	Water	0.357	0.080	0.084	0.008^
Cartridge casing	Water	Triton-X	0.929	0.874	0.735	0.975
	Triton-X	None	0.673	1.000	0.391	0.975
	None	Water	0.880	0.873	0.841	0.903
Foam cup	Water	Triton-X	0.359	0.072	0.908	0.122
	Triton-X	None	0.149	0.115	0.050	0.665
	None	Water	0.870	0.976	0.130	0.510
Concrete brick	Water	Triton-X	0.928	N/A	0.859	N/A
	Triton-X	None	0.003*	N/A	0.001*	N/A
	None	Water	0.011^	N/A	0.004^	N/A
Wood handle	Water	Triton-X	0.973	0.989	0.985	0.965
	Triton-X	None	0.147	0.010^	0.202	0.002^
	None	Water	0.224	0.006*	0.271	0.004*

* Mean from Method 1 was significantly higher than Method 2 ($p < 0.05$)
^ Mean from Method 2 was significantly higher than Method 1 ($p < 0.05$)

Table 14: Percentage of Phase I profiles that were CODIS eligible

CODIS Eligibility Success Rates (%)		
Substrate	**Standard**	**Direct PCR**
Plastic slide buccal cells	82	97
Plastic slide fingerprints	7	14
Denim	100	0
Wool blend	100	25
100% wool	100	0
Polyester	63	88
Metal tool	56	69
Vinyl shutter	35	25
Handgun grip	33	54
Cartridge casing	6	0
Foam cup	13	19
Concrete brick	44	0
Wood handle	37	50

Table 15: Phase II standard processing FI and % profile means, medians, and standard deviations for each collection method, substrate, and amplification system and Wilcoxon test comparisons of GlobalFiler and PowerPlex Fusion 6C

		Mean FI ± SD		Median FI			Mean % Profile ± SD		Median % Profile		
Substrate	**Collection Method**	**GF**	**6C**	**GF**	**6C**	***p*-value**	**GF**	**6C**	**GF**	**6C**	***p*-value**
Plastic slide Buccal cells	Cotton + water	3.79±3.58	3.13±1.79	4.29	3.37	0.833	54.5±49.0	72.9±20.3	59.0	75.0	0.831
	MicroFLOQ + water	7.09±0.27	2.88±1.23	7.13	2.66	0.001*	100.0±0.0	69.3±15.2	100.0	67.0	<0.001*
Plastic slide Fingerprints	Cotton + water	0.56±0.73	1.44±1.18	0.05	1.72	0.100	20.0±23.5	24.6±29.8	11.5	6.0	0.875
	MicroFLOQ + Triton X	0.27±0.62	0.47±0.77	0.05	0.05	0.644	14.6±12.4	6.0±6.7	12.5	3.0	0.139
	Dry microFLOQ	0.87±2.31	0.28±0.58	0.05	0.05	0.644	21.6±34.0	14.0±14.7	8.0	9.5	0.832
Denim	Cutting	6.20±0.79	0.05±0.00	6.33	0.05	<0.001*	98.9±2.47	7.0±5.2	100.0	8.0	0.001*
Wool	Cutting	7.29±0.29	2.57±2.47	7.24	1.63	0.001*	100.0±0.0	52.6±37.1	100.0	44.0	0.001*
Polyester	Cutting	2.23±2.87	0.26±0.59	0.93	0.05	0.074	45.5±39.7	9.0±7.1	41.0	7.0	0.051
Metal tool	Cotton + water	1.60±2.17	1.48±2.04	0.05	0.05	0.838	39.5±35.2	29.2±39.2	33.5	4.0	0.051
	Dry cotton	4.06±2.87	1.34±1.79	4.36	0.67	0.001*	73.3±31.3	27.7±32.1	86.0	12.5	<0.001*
	Dry microFLOQ	2.89±2.19	2.95±2.51	3.39	2.86	0.868	69.5±24.5	60.9±35.9	76.0	67.0	0.563
Vinyl shutter	Cotton + water	1.15±1.40	0.82±1.40	0.51	0.05	0.137	34.8±35.0	25.0±32.2	22.5	6.0	0.197
	Dry cotton	2.18±2.47	1.34±1.93	1.05	0.05	0.204	42.2±44.3	31.3±37.7	20.5	11.0	0.377
	MicroFLOQ + water	1.50±2.18	1.34±1.75	0.05	0.05	0.918	32.0±40.3	27.7±37.2	10.5	2.0	0.577
Handgun grip	Cotton + water	2.40±2.98	2.03±1.88	0.05	1.95	0.740	46.1±40.3	46.3±33.4	32.0	51.0	0.836
	MicroFLOQ + water	2.11±2.81	0.55±0.81	0.05	0.05	0.086	45.3±42.6	8.3±14.1	42.0	2.0	0.012*
Cartridge casing	Cotton + water	0.38±1.29	0.39±1.35	0.05	0.05	1.000	6.5±23.0	7.5±21.2	0.0	0.0	0.086
	MicroFLOQ + Triton X	0.99±1.99	0.19±0.47	0.05	0.05	0.086	17.2±32.3	1.0±3.2	1.0	0.0	0.005*
Foam cup	Cotton + water	1.03±1.79	0.47±0.74	0.05	0.05	0.218	15.8±30.2	2.3±5.3	4.5	1.0	0.012*
	Dry microFLOQ	1.72±2.35	0.31±0.60	0.05	0.05	0.018*	32.5±41.0	4.2±11.6	8.0	0.0	0.003*
Concrete brick	Cotton + water	2.80±2.55	1.84±2.10	2.99	0.98	0.188	65.5±33.4	41.7±36.8	75.0	28.0	0.011*
	Cotton + Triton X	3.35±2.95	2.84±2.38	4.71	3.03	0.586	66.7±37.0	61.1±34.8	91.0	67.5	0.567
	MicroFLOQ + Triton X	2.95±2.62	2.76±2.40	2.08	2.55	0.859	64.7±34.5	53.6±39.9	64.5	59.5	0.188

	Cotton + water	2.54±2.70	3.02±3.06	1.62	1.84	0.360	52.8±40.7	53.8±41.5	43.0	55.0	0.851
Wood handle	Dry cotton	2.88±2.74	3.13±3.34	2.36	1.72	0.783	58.8±38.1	51.2±43.3	63.5	34.0	0.770
	Dry microFLOQ	2.28±2.69	2.75±2.53	0.69	1.72	0.399	45.9±41.0	55.5±38.6	41.0	47.0	0.449

*p<0.05 (Wilcoxon test)

Table 16: Phase II direct PCR FI and % profile means, medians, and standard deviations for each collection method, substrate, and amplification system and Wilcoxon test comparisons of GlobalFiler and PowerPlex Fusion 6C

		Mean FI ± SD		Median FI			Mean % Profile ± SD		Median % Profile		
Substrate	**Collection Method**	**GF**	**6C**	**GF**	**6C**	***p*-value**	**GF**	**6C**	**GF**	**6C**	***p*-value**
Plastic slide Buccal cells	Cotton + water	3.44±1.87	1.62±1.31	3.43	1.53	0.059	86.8±11.4	57.6±17.7	87.5	56.0	0.006*
	MicroFLOQ + water	6.81±0.31	4.30±1.72	6.76	4.75	0.001*	100.0±0.0	82.9±19.0	100.0	89.5	<0.001*
Plastic slide Fingerprints	Cotton + water	0.05±0.00	1.77±1.33	0.05	1.91	0.004*	25.0±20.0	47.8±29.0	19.5	58.5	0.065
	MicroFLOQ + Triton X	1.41±1.92	0.96±0.88	0.93	1.09	0.742	15.5±32.4	20.0±21.0	3.5	13.5	0.458
	Dry microFLOQ	0.18±0.36	0.66±1.37	0.05	0.05	0.538	19.9±17.6	32.0±27.9	12.5	30.0	0.527
Denim	Cutting	0.05±0.00	1.80±1.14	0.05	1.64	0.001*	0.0±.0.0	51.1±26.1	0.0	58.0	<0.001*
Wool	Cutting	0.05±0.00	1.51±2.17	0.05	0.77	0.011*	37.3±15.7	38.9±33.7	33.0	42.0	1.000
Polyester	Cutting	3.42±2.82	2.39±2.09	3.42	1.96	0.598	71.6±25.8	58.3±25.6	76.0	54.0	0.344
Metal tool	Cotton + water	2.33±2.75	1.93±2.24	0.40	1.58	0.879	58.2±34.8	47.9±34.0	54.5	44.5	0.306
	Dry cotton	4.22±1.75	2.06±2.09	4.48	1.66	0.001*	84.1±26.7	58.2±29.1	92.0	56.0	0.001*
	Dry microFLOQ	4.46±2.09	3.21±2.40	4.76	3.75	0.057	86.3±21.2	62.8±38.6	99.0	79.0	0.016*
Vinyl shutter	Cotton + water	0.12±0.36	2.41±2.32	0.05	2.38	<0.001*	31.4±15.4	65.3±30.0	35.50	74.50	<0.001*
	Dry cotton	0.44±0.68	1.88±1.84	0.05	1.63	0.002*	28.7±23.4	52.1±34.9	31.5	55.0	0.013*
	MicroFLOQ + water	1.58±2.27	1.96±2.03	0.05	1.72	0.294	42.8±37.9	42.3±39.7	36.5	33.0	0.967
Handgun grip	Cotton + water	3.41±2.85	2.22±1.70	4.08	1.72	0.237	76.5±29.6	63.3±24.4	97.0	64.5	0.055
	MicroFLOQ + water	3.90±2.99	1.59±2.31	3.72	0.05	0.002*	73.3±30.5	38.3±38.5	80.5	25.0	0.001*
Cartridge casing	Cotton + water	0.05±0.00	0.05±0.00	0.05	0.05	1.000	0.0±0.0	0.0±0.0	0.0	0.0	1.000
	MicroFLOQ + Triton X	0.20±0.49	0.05±0.00	0.05	0.05	0.153	0.4±1.18	0.0±0.0	0.0	0.0	0.077
Foam cup	Cotton + water	0.35±0.80	0.39±0.72	0.05	0.05	0.635	30.4±23.1	25.2±18.6	25.0	22.0	0.522
	Dry microFLOQ	1.59±2.36	0.99±1.86	0.05	0.05	0.257	33.8±39.1	12.9±30.3	13.0	0.0	0.007*
Concrete brick	Cotton + water	0.05±0.00	0.05±0.00	0.05	0.05	1.000	0.0±0.0	0.2±0.6	0.0	0.0	0.153
	Cotton + Triton X	0.05±0.00	0.26±0.56	0.05	0.05	0.077	0.0±0.0	0.4±1.2	0.0	0.0	0.077
	MicroFLOQ + Triton X	0.05±0.00	0.05±0.00	0.05	0.05	1.000	0.0±0.0	0.2±0.8	0.0	0.0	0.317
Wood handle	Cotton + water	1.82±2.14	3.97±3.29	1.19	3.21	0.014*	53.1±36.6	69.6±32.1	55.5	76.0	0.085
	Dry cotton	2.67±2.33	4.61±3.17	3.20	4.89	0.019*	69.2±29.2	78.2±26.0	74.0	94.0	0.245
	Dry microFLOQ	5.22±2.14	3.41±2.80	5.93	3.89	0.047*	89.3±22.7	67.4±34.8	100.0	78.0	0.047*

*p<0.05 (Wilcoxon test)

Table 17: Phase II GlobalFiler FI and % profile means, medians, and standard deviation for each collection method, substrate, and processing method and Wilcoxon test comparisons of standard processing and direct PCR

		Mean FI ± SD		Median FI			Mean % Profile ± SD		Median % Profile		
Substrate	**Collection Method**	**Standard**	**Direct**	**Standard**	**Direct**	***p*-value**	**Standard**	**Direct**	**Standard**	**Direct**	***p*-value**
Plastic slide Buccal cells	Cotton + water	3.79±3.58	3.44±1.87	4.29	3.43	0.833	54.5±49.0	86.8±11.4	59.0	87.5	0.831
	MicroFLOQ + water	7.09±0.27	6.81±0.31	7.13	6.76	0.093	100.0±0.0	100.0±0.0	100.0	100.0	1.000
Plastic slide Fingerprints	Cotton + water	0.56±0.73	0.05±0.00	0.05	0.05	0.065	20.0±23.5	25.0±20.0	11.5	19.5	0.597
	MicroFLOQ + Triton X	0.27±0.62	1.41±1.92	0.05	0.93	0.074	14.5±12.4	15.5±32.4	12.5	3.5	0.290
	Dry microFLOQ	0.86±2.31	0.18±0.36	0.05	0.05	0.927	21.6±34.0	19.9±17.6	8.0	12.5	0.368
Denim	Cutting	6.20±0.79	0.05±0.00	6.33	0.05	<0.001*	98.9±2.5	0.0±0.0	100.0	0.0	<0.001*
Wool	Cutting	7.29±0.29	0.05±0.00	7.24	0.05	<0.001*	100.0±0.0	37.3±15.7	100.0	33.0	<0.001*
Polyester	Cutting	2.23±2.87	3.42±2.82	0.93	3.42	0.332	45.5±39.7	71.6±25.8	41.0	76.0	0.204
Metal tool	Cotton + water	1.60±2.17	2.33±2.75	0.05	0.40	0.410	39.5±35.2	58.2±34.8	33.5	54.5	0.047*
	Dry cotton	4.06±2.87	4.22±1.75	4.36	4.48	0.828	73.3±31.3	84.1±26.7	86.0	92.0	0.261
	Dry microFLOQ	2.89±2.19	4.46±2.09	3.39	4.76	0.010*	69.5±24.5	86.3±21.2	76.0	99.0	0.006*
Vinyl shutter	Cotton + water	1.15±1.40	0.12±0.36	0.51	0.05	<0.001*	34.8±35.0	31.4±15.4	22.5	35.5	0.718
	Dry cotton	2.47±0.50	0.44±0.68	1.05	0.05	0.015*	42.2±44.3	28.7±23.4	20.5	31.5	0.796
	MicroFLOQ + water	1.50±2.18	1.58±2.27	0.05	0.05	0.744	32.0±40.3	42.8±37.9	10.5	36.5	0.316
Handgun grip	Cotton + water	2.40±2.98	3.41±2.85	0.05	4.08	0.204	46.1±40.3	76.5±29.6	32.0	97.0	0.005*
	MicroFLOQ + water	2.11±2.81	3.90±2.99	0.05	0.94	0.015*	45.3±42.6	73.3±30.5	42.0	80.5	0.011*
Cartridge casing	Cotton + water	0.38±1.29	0.05±0.00	0.05	0.05	0.153	6.5±23.0	0.0±0.0	0.0	0.0	0.020*
	MicroFLOQ + Triton X	0.99±1.99	0.20±0.49	0.05	0.05	0.105	17.2±32.3	0.4±1.2	1.0	0.0	0.003*
Foam cup	Cotton + water	1.03±1.79	0.35±0.80	0.05	0.05	0.452	15.8±30.2	30.4±23.1	4.5	25.0	0.002*
	Dry microFLOQ	1.72±2.35	1.59±2.36	0.05	0.05	0.890	32.5±41.0	33.8±39.1	8.0	13.0	0.594
Concrete brick	Cotton + water	2.80±2.55	0.05±0.00	2.99	0.05	<0.001*	65.5±33.4	0.0±0.0	75.0	0.0	<0.001*
	Cotton + Triton X	3.35±2.95	0.05±0.00	4.71	0.05	<0.001*	66.7±37.0	0.0±0.0	91.0	0.0	<0.001*
	MicroFLOQ + Triton X	2.95±2.62	0.05±0.00	2.08	0.05	<0.001*	64.7±34.5	0.0±0.0	64.5	0.0	<0.001*
Wood handle	Cotton + water	2.54±2.70	1.82±2.14	1.62	0.05	0.441	52.8±40.7	53.1±36.6	43.0	55.5	0.901

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	Dry cotton	2.88±2.74	2.67±2.33	2.36	3.20	0.753	58.8±38.1	69.2±29.2	63.5	74.0	0.424
	Dry microFLOQ	2.28±2.69	5.22±2.14	0.69	5.93	0.001*	45.9±41.0	89.3±22.7	41.0	100.0	<0.001*

*p<0.05 (Wilcoxon test)

Table 18: Phase II PowerPlex Fusion 6C FI and % profile means, medians, and standard deviations for each collection method, substrate, and processing method and Wilcoxon test comparisons of standard processing and direct PCR

		Mean FI ± SD		Median FI			Mean % Profile ± SD		Median % Profile		
Substrate	**Collection Method**	**Standard**	**Direct**	**Standard**	**Direct**	***p*-value**	**Standard**	**Direct**	**Standard**	**Direct**	***p*-value**
Plastic slide Buccal cells	Cotton + water	3.13±1.79	1.62±1.31	3.37	1.53	0.074	72.9±20.3	57.6±17.7	75.0	56.0	0.155
	MicroFLOQ + water	2.88±1.23	4.30±1.72	2.66	4.75	0.072	69.3±15.2	82.9±19.0	67.0	89.5	0.072
Plastic slide Fingerprints	Cotton + water	1.44±1.18	1.77±1.33	1.72	1.91	0.459	24.6±29.8	47.8±29.0	6.0	58.5	0.155
	MicroFLOQ + Triton X	0.47±0.77	0.96±0.88	0.05	1.09	0.247	6.0±6.7	20.0±21.0	3.0	13.5	0.222
	Dry microFLOQ	0.28±0.58	0.66±1.37	0.05	0.05	0.890	14.0±14.7	32.0±27.9	9.5	30.5	0.188
Denim	Cutting	0.05±0.00	1.80±1.14	0.05	1.64	0.002*	7.0±5.2	51.1±26.1	8.0	58.0	0.005*
Wool	Cutting	2.57±2.47	1.51±2.17	1.63	0.77	0.315	52.6±37.1	38.9±33.7	44.0	42.0	0.527
Polyester	Cutting	0.26±0.59	2.39±2.09	0.05	1.96	0.006*	9.0±7.1	58.3±25.6	7.0	54.0	0.001*
Metal tool	Cotton + water	1.48±2.04	1.93±2.24	0.05	1.56	0.402	29.2±39.2	47.9±34.0	4.9	44.5	0.018*
	Dry cotton	1.34±1.79	2.06±2.09	0.67	1.66	0.253	27.7±32.1	58.2±29.1	12.5	56.0	0.002*
	Dry microFLOQ	2.93±2.49	3.21±2.40	2.86	3.75	0.708	60.9±35.9	62.8±38.6	67.0	79.0	0.748
Vinyl shutter	Cotton + water	0.82±1.40	2.37±2.24	0.05	2.38	0.003*	25.0±32.2	65.3±30.0	6.0	74.5	<0.001*
	Dry cotton	1.34±1.93	1.88±1.84	0.05	1.63	0.162	31.3±37.7	52.1±34.9	11.0	55.0	0.022*
	MicroFLOQ + water	1.34±1.75	1.97±2.03	0.05	1.72	0.229	27.7±37.2	42.3±39.7	2.0	33.0	0.084
Handgun grip	Cotton + water	2.03±1.88	2.22±1.70	1.95	1.72	0.569	46.3±33.4	63.3±24.4	51.0	64.5	0.083
	MicroFLOQ + water	0.55±0.81	1.59±2.31	0.05	0.05	0.197	8.3±14.1	38.3±37.5	2.0	25.0	0.001*
Cartridge casing	Cotton + water	0.38±1.35	0.05±0.00	0.05	0.05	0.153	7.5±21.2	0.0±0.0	0.0	0.0	<0.001*
	MicroFLOQ + Triton X	0.19±0.47	0.05±0.00	0.05	0.05	0.153	1.0±3.2	0.0±0.0	0.0	0.0	0.077
Foam cup	Cotton + water	0.47±0.74	0.39±0.72	0.05	0.05	0.654	3.0±5.3	25.2±18.6	1.0	22.0	<0.001*
	Dry microFLOQ	0.31±0.60	0.99±1.86	0.05	0.05	0.229	4.2±11.6	12.9±30.3	0.0	0.0	0.184
Concrete brick	Cotton + water	1.84±2.10	0.05±0.00	0.98	0.05	<0.001*	41.7±36.8	0.7±0.6	28.0	0.0	<0.001*
	Cotton + Triton X	2.84±2.38	0.26±0.56	3.03	0.05	<0.001*	61.1±34.8	0.4±1.2	67.5	0.0	<0.001*
	MicroFLOQ + Triton X	2.76±2.40	0.05±0.00	2.55	0.05	<0.001*	53.6±39.9	0.2±0.8	59.5	0.0	<0.001*
Wood handle	Cotton + water	3.02±3.06	3.97±3.29	1.84	1.30	0.191	53.8±41.5	69.6±32.1	55.0	76.0	0.211
	Dry cotton	3.13±3.34	4.74±3.19	1.72	5.05	0.059	51.2±43.3	78.2±26.0	34.0	94.0	0.049*
	Dry microFLOQ	2.75±2.53	3.41±2.80	1.72	3.89	0.429	55.5±38.6	67.4±34.8	47.0	78.0	0.251

*p<0.05 (Wilcoxon test)

Table 19: Phase II PowerPlex 6C FI medians for each collection method, substrate, time point, and processing method; Steel with Control test comparisons of 3- and 6-month time points with the 0-month time point (control); and Wilcoxon test comparisons of standard processing and direct PCR.

		Standard Processing					Direct PCR					Standard vs Direct		
		Median FI			*p*-value		Median FI			*p*-value		*p*-value		
Substrate	**Collection Method**	**0 month**	**3 month**	**6 month**	**0 vs 3 month**	**0 vs 6 month**	**0 month**	**3 month**	**6 month**	**0 vs 3 month**	**0 vs 6 month**	**0 month**	**3 month**	**6 month**
Plastic slide Buccal cells	microFLOQ & water	2.66	3.95	2.55	0.504	0.906	4.75	3.24	2.91	0.266	0.119	0.074	0.600	0.916
Plastic slide Fingerprints	dry microFLOQ	0.05	0.05	0.05	0.289	0.811	0.05	0.05	0.05	0.865	0.289	0.890	0.065	0.317
	microFLOQ & Triton X	0.05	0.05	0.05	0.289	0.289	1.09	0.05	0.05	0.160	0.205	0.247	0.317	0.144
Metal tool	dry cotton	0.67	0.05	0.05	0.126	0.026*	1.66	0.22	0.05	0.437	0.041*	0.253	0.084	0.153
	dry microFLOQ	2.86	0.19	0.75	0.137	0.254	3.75	2.46	3.38	0.982	0.998	0.740	0.116	0.080
Vinyl shutter	dry cotton	0.05	0.05	0.05	0.838	0.021*	1.63	1.80	0.17	0.711	0.193	0.162	0.012†	0.002†
	microFLOQ & water	0.05	0.05	0.05	0.060	0.137	1.72	0.12	0.83	0.778	0.912	0.229	0.013†	0.016†
Handgun grip	microFLOQ & water	0.05	0.05	0.05	0.871	1.000	0.05	0.41	0.05	0.756	0.889	0.197	0.135	0.320
	cotton & water	1.95	0.05	0.05	0.005*	0.023*	1.72	0.63	0.05	0.167	0.001*	0.569	0.010†	0.664
Cartridge casing	microFLOQ & Triton X	0.05	0.05	0.05	0.275	0.275	0.05	0.05	0.05	1.000	1.000	0.153	1.000	1.000
Foam cup	dry microFLOQ	0.05	0.05	0.05	0.075	0.595	0.05	0.05	0.05	0.998	0.277	0.229	0.005†	0.586
Concrete brick	cotton & Triton X	3.03	1.72	1.72	0.344	0.251	0.05	0.05	0.05	0.144	0.144	<0.001‡	<0.001‡	<0.001‡
	microFLOQ & Triton X	2.55	1.64	0.05	0.399	0.003*	0.05	0.05	0.05	0.530	1.000	<0.001‡	<0.001‡	0.005‡
Wood handle	dry cotton	1.72	1.50	2.40	0.871	0.964	4.89	4.58	2.29	0.999	0.186	0.064	0.083	0.690
	dry microFLOQ	1.72	2.01	0.78	0.996	0.437	3.89	4.25	4.72	1.000	0.872	0.429	0.261	0.013†
Denim	cutting	0.05	0.05	0.05	0.289	0.289	1.64	0.05	0.05	0.013*	0.019*	0.001†	0.898	1.000
Wool	cutting	1.63	2.11	3.25	0.878	0.682	0.77	2.42	0.05	0.638	0.055	0.315	0.749	0.002‡
Polyester	cutting	0.05	0.05	0.05	0.898	0.898	1.96	0.37	0.05	0.638	0.226	0.006†	0.228	0.523

*FI at 3 or 6 months significantly lower than 0 months (p<0.05, Steel with Control test)

†Direct PCR FI significantly higher than standard (p<0.05, Wilcoxon test)

‡Direct PCR FI significantly lower than standard (p<0.05, Wilcoxon test)

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Table 20: Phase II PowerPlex 6C % profile medians for each collection method, substrate, time point, and processing method; Steel with Control test comparisons of 3- and 6-month time points with the 0-month time point (control); and Wilcoxon test comparisons of standard processing and direct PCR.

		Standard Processing					Direct PCR					Standard vs Direct		
		Median % profile			*p*-value		Median % profile			*p*-value		*p*-value		
Substrate	Collection Method	0 month	3 month	6 month	0 vs 3 month	0 vs 6 month	0 month	3 month	6 month	0 vs 3 month	0 vs 6 month	0 month	3 month	6 month
Plastic slide Buccal cells	microFLOQ & water	67.0	81.0	64.5	0.752	0.849	89.5	80.0	73.0	0.434	0.146	0.072	0.958	0.674
Plastic slide	dry microFLOQ	9.5	4.0	0.0	0.558	0.163	30.0	18.0	0.0	0.968	0.006*	0.188	0.087	1
Fingerprints	microFLOQ & Triton X	3.0	0.0	0.0	0.145	0.201	13.5	2.0	3.0	0.255	0.461	0.222	0.227	0.247
Metal tool	dry cotton	12.5	9.0	2.0	0.561	0.026*	56.0	35.0	17.0	0.089	0.001*	0.002†	0.007†	0.035†
	dry microFLOQ	67.0	28.0	36.5	0.129	0.272	79.0	66.0	80.5	0.992	0.898	0.748	0.139	0.042†
Vinyl shutter	dry cotton	11.0	7.0	0.0	0.905	0.210	55.0	41.5	30.0	0.861	0.106	0.022†	0.038†	0.002†
	microFLOQ & water	2.0	3.0	0.0	0.420	0.163	33.0	31.5	40.5	0.963	0.975	0.084	0.001†	0.001†
Handgun grip	microFLOQ & water	2.0	4.0	4.0	0.723	0.651	25.0	23.0	2.0	0.983	0.162	0.001†	0.017†	0.793
	cotton & water	51.0	5.0	2.0	0.018*	0.003*	64.5	47.5	22.5	0.199	<0.001*	0.083	<0.001†	0.037†
Cartridge casing	microFLOQ & Triton X	0.0	0.0	0.0	0.144	0.144	0.0	0.0	0.0	1.000	1.000	0.077	1.000	1.000
Foam cup	dry microFLOQ	0.0	0.0	0.0	0.996	0.562	0.0	1.0	0.0	0.834	0.969	0.184	0.061	0.087
Concrete brick	cotton & Triton X	67.5	43.5	45.5	0.075	0.259	0.0	0.0	0.0	0.144	0.144	<0.001‡	<0.001‡	<0.001‡
	microFLOQ & Triton X	59.5	10.0	10.5	0.306	0.009*	0.0	0.0	0.0	0.810	1.000	<0.001‡	<0.001‡	<0.001‡
Wood handle	dry cotton	34.0	37.5	58.5	0.984	0.982	94.0	92.0	64.5	0.977	0.227	0.049†	0.104	0.390
	dry microFLOQ	47.0	59.5	22.0	0.992	0.396	78.0	85.5	94.0	0.815	0.814	0.251	0.407	0.007†
Denim	cutting	8.0	1.0	3.0	0.981	0.998	58.0	25.5	23.0	0.198	0.058	0.005†	0.124	0.223
Wool	cutting	44.0	59.5	77.0	0.992	0.342	42.0	53.0	13.5	0.787	0.280	0.527	0.636	0.004‡
Polyester	cutting	7.0	4.0	3.0	0.748	0.156	54.0	34.5	8.5	0.573	0.025*	0.001†	0.003†	0.069

*% profile at 3 or 6 months significantly lower than 0 months ($p<0.05$, Steel with Control test)

†Direct PCR % profile significantly higher than standard ($p<0.05$, Wilcoxon test)

‡Direct PCR % profile significantly lower than standard ($p<0.05$, Wilcoxon test)

Table 21: Phase II PowerPlex Fusion 6C FI medians for samples processed directly following the first collection and resampled samples and Wilcoxon test comparisons of first collection and resampling FI results.

		Median FI					
		Standard Processing			Direct PCR		
Substrate	Collection Method	First	Resampled	*p*-value	First	Resampled	*p*-value
Plastic slide Buccal cells	microFLOQ & water	2.66	0.88	0.011*	4.75	0.05	<0.001*
Plastic slide Fingerprints	microFLOQ & Triton X	0.05	0.05	0.644	1.09	0.05	0.011*
Metal tool	dry microFLOQ	2.86	0.05	0.001*	3.75	0.05	0.001*
Vinyl shutter	microFLOQ & water	0.05	0.05	0.017*	1.72	0.05	0.006*
Handgun grip	microFLOQ & water	0.05	0.05	0.079	0.05	0.05	0.785
Cartridge casing	microFLOQ & Triton X	0.05	0.05	0.153	0.05	0.05	0.317
Foam cup	dry microFLOQ	0.05	0.05	0.174	0.05	0.05	0.151
Concrete brick	microFLOQ & Triton X	2.55	0.05	0.008*	0.05	0.05	1.000
Wood handle	dry microFLOQ	1.72	0.70	0.074	3.89	2.43	0.402

* $p<0.05$ (Wilcoxon test)

Table 22: Phase II PowerPlex Fusion 6C % profile medians for samples processed directly following the first collection and resampled samples and Wilcoxon test comparisons of first collection and resampling % profile results.

		Median % Profile					
		Standard Processing			Direct PCR		
Substrate	Collection Method	First	Resampled	*p*-value	First	Resampled	*p*-value
Plastic slide Buccal cells	microFLOQ & water	66.7	2.1	0.001*	89.6	2.1	0.001*
Plastic slide Fingerprints	microFLOQ & Triton X	3.1	0.0	0.018*	13.5	0.0	0.021*
Metal tool	dry microFLOQ	66.7	0.0	<0.001*	79.2	9.4	0.001*
Vinyl shutter	microFLOQ & water	2.1	2.1	0.193	33.3	12.0	0.147
Handgun grip	microFLOQ & water	2.1	0.0	0.032*	26.0	5.9	0.112
Cartridge casing	microFLOQ & Triton X	0.0	0.0	0.600	0.0	0.0	0.153
Foam cup	dry microFLOQ	0.0	0.0	0.051	0.0	0.0	0.321
Concrete brick	microFLOQ & Triton X	59.4	15.3	0.029*	0.0	0.0	0.589
Wood handle	dry microFLOQ	46.9	15.1	0.018*	78.1	62.5	0.214

* $p<0.05$ (Wilcoxon test)

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Table 23: Percentage of Phase II profiles that were CODIS eligible

	CODIS Eligibility Success Rates (%)							
	0 month		3 month		6 month		0 month (resampled)	
Substrate	Standard	Direct PCR	Standard	Direct PCR	Standard	Direct PCR	Standard*	Direct PCR†
Plastic slide buccal cells	100	94	100	100	88	100	0	0
Plastic slide fingerprints	21	33	0	19	6	6	0	0
Denim	0	100	13	25	25	13	N/A	N/A
100% wool	63	63	63	75	88	13	N/A	N/A
Polyester	0	75	0	50	0	13	N/A	N/A
Metal tool	49	65	31	52	29	52	17	25
Vinyl shutter	31	60	21	50	6	40	13	21
Handgun grip	33	63	25	52	21	27	4	33
Cartridge casing	2	0	0	0	0	0	0	0
Foam cup	2	19	0	21	4	13	0	8
Concrete brick	64	0	48	0	40	0	38	0
Wood handle	60	81	56	77	50	79	33	63

*After first collection and resampling, samples were processed with standard methods.
†After first collection, samples were processed with direct PCR. After resampling, samples were processed with standard methods.

Table 24: Phase I profiles with foreign alleles

		Phase I profiles with foreign alleles			
		Standard		Direct PCR	
Source	Category	Number	% of total (%)	Number	% of total (%)
Known	Indirect transfer	31	1.8	68	4.0
Known	Contamination	7	0.4	0	0.0
Unknown	≤6 alleles	32	1.9	30	1.8
Unknown	≥7 alleles	4	0.2	1	0.1

Table 25: Phase II profiles with foreign alleles

		Phase II profiles with foreign alleles			
		Standard		Direct PCR	
Source	Category	Number	% of total (%)	Number	% of total (%)
Known	Indirect transfer	11	0.8	62	4.6
Known	Contamination	7	0.5	1	0.1
Unknown	≤6 alleles	6	0.4	41	3.0
Unknown	≥7 alleles	0	0.0	2	0.1

Products

Scholarly Products

- Salmonsen AC. Evaluation of Collection Methods for Direct Amplification of Touch DNA Samples [master's thesis]. Washington, DC: The George Washington University, 2021.
- Bathrick AS, Salmonsen AC, Davoren JM. Improving results from touch DNA evidence with optimized direct PCR methods. (2022) Dryad, Dataset. https://doi.org/10.5061/dryad.mcvdnck4p
- Salmonsen AC, Davoren JM, Bathrick AS. Evaluation of touch DNA collection methods for use with direct PCR amplification. (2022) [Unpublished manuscript].
- Study-level information and a link to the Dryad dataset for NIJ award 2019-DU-BX-0009 have been submitted to the National Archive of Criminal Justice Data (NACJD).

Dissemination Activities

- Salmonsen AC, Bathrick AS, Davoren JM. Evaluation of Collection Methods for Direct Amplification of Touch DNA Samples. Poster presented at 2021 NIJ Forensic Science R&D Symposium. Virtual. February 16, 2021.

- Salmonsen AC. Evaluation of Collection Methods for Direct Amplification of Touch DNA Samples. Oral presentation to The George Washington University FORS 6292 (Graduate Seminar - 25 students, faculty, and thesis committee members). April 26, 2021.
- Salmonsen AC, Bathrick AS, Davoren JM. Evaluation of Collection Methods for Direct Amplification of Touch DNA Samples. Poster presented at the 32nd International Symposium on Human Identification. Orlando, FL. September 15, 2021.
- Salmonsen AC, Evaluation of Collection Methods for Direct PCR Amplification of Touch DNA Samples. ISHI Blog. November 2, 2021. https://www.ishinews.com/evaluation-of-collection-methods-for-direct-pcr-amplification-of-touch-dna-samples/
- Salmonsen AC, Bathrick AS, Davoren JM. Evaluation of Collection Methods for Direct Amplification of Touch DNA Samples. Poster presented at the 74th Annual AAFS Scientific Conference. Seattle, WA. February 23, 2022.
- Salmonsen AC, Bathrick AS, Davoren JM. Comparison of GlobalFiler™ and PowerPlex® Fusion 6C for Direct PCR Amplification of Touch DNA Samples. Poster presented at the 2022 NIJ Forensic Science R&D Symposium. Virtual. March 2, 2022.
- Bathrick AS, Salmonsen AC, Davoren JM. Direct PCR Artifacts Identified in Touch DNA from Fired Cartridge Casings. Poster presented at: 29th Congress of the International Society for Forensic Genetics. Washington, DC. September 2, 2022.
- Bathrick AS, Salmonsen AC, Davoren JM. Evaluation of Direct PCR on Aged Touch DNA Cuttings & Swabs. Poster presented at: 33rd International Symposium on Human Identification. Washington, DC. November 2, 2022.
- Bathrick AS, Salmonsen AC, Davoren JM. Direct PCR of Forensic Evidence: Making the Case to Modify the Quantification Requirement. Oral presentation at: 33rd International Symposium on Human Identification. Washington, DC. November 3, 2022.

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