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This study compared the ability of cotton tip swabs and 4N6FLOQ™Swabs (Life Technologies, Carlsbad, CA) to collect dried bloodstains from various surfaces and recover the DNA transferred onto each swab. This study also examined whether swabs moistened with phosphate buffered saline (PBS) rather than distilled water (dH₂O) provided any advantage in the recovery of DNA. The DNA yield; the quality of the Short Tandem Repeat (STR) profiles generated; and the ease of use were evaluated for the two swabs. The FLOQ™ swabs yielded the most DNA in the majority of the samples collected from each of the surfaces. There was no indication that PBS compared with dH₂O provided any significant advantage in DNA yield and the quality of the STR profiles generated.

THE EVALUATION OF DIFFERENT COLLECTION METHODS FOR THE OPTIMUM
RECOVERY OF DNA FROM BLOODSTAINS ON VARIOUS SURFACES

THESIS

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CHAPTER 1

INTRODUCTION

The purpose of this research was to compare bloodstain collection efficiency and the recovery of DNA from various surfaces with two different collection devices, a standard cotton tip swab (Puritan® Cotton Tipped Applicators (Puritan Medical, Guilford, ME)) and the Copan 4N6FLOQ™ Swab (Life Technologies, Carlsbad, CA) when used in conjunction with distilled water (dH₂O) and phosphate buffered saline (PBS) collection. When bloodstains are found at crime scenes, they are collected, the DNA is extracted, and subsequently analyzed for the source of the contributor. Bloodstains found on different surfaces are generally transferred onto a collection device (swab), which has been moistened with distilled water (dH₂O). The swab is then allowed to air dry, packaged in a paper evidentiary bag, properly labeled, and typically stored at room temperature for further laboratory analysis. Wet bloodstains are generally collected using a dry cotton swab and allowed to air dry prior to placement in an evidentiary paper bag or envelope. Wet bloodstains should never be placed in an airtight container or plastic bag as this may allow bacterial and fungal growth and degrade the evidence sample. Cotton tip swabs, pre-moistened with dH₂O, are currently the standard collection method for dried biological evidence from surfaces found at a crime scene (1). One factor that can affect the ability to obtain a DNA profile from a bloodstain is the quantity of the DNA recovered. In cases where trace amounts of biological samples are present, efficient recovery of the evidence becomes crucial in order to obtain a complete DNA profile. PCR amplification kits have been optimized to obtain a complete profile when between 0.5 and 1.0 ng of DNA is added to amplification reaction. While this input amount of DNA is recommended by kit manufacturers,

full profiles have been obtained in samples that contain as little as 200 pg of DNA. However, full profiles are often not observed with less input amounts of DNA. In a forensic setting, where trace amounts of DNA are present, sample loss resulting from the collection method could be the difference between obtaining a full or partial DNA profile (2).

A problem that occurs with the use of cotton swabs for the recovery of trace amounts of biological evidence is that DNA can become trapped and entangled within the cotton fibers (3, 4, 5, 6). DNA has been shown by electron micrographs to become physically entangled within the fibers of cotton paper or cotton swabs illustrated in Figure 1.

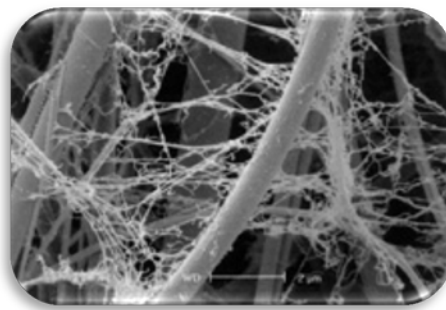


Figure 1: Double stranded DNA captured in the fibers of the FTA matrix (7).
DNA Extraction from FTA® Cards Using the GenSolve Recovery Kit. 2008. Whatman Inc.

If the DNA is entrapped within the cotton fibers the efficiency of DNA recovery can be significantly diminished (3, 4). During the DNA extraction process, nuclei are broken down and the potential for entrapment can occur which could reduce the efficiency of DNA recovery (1, 5, 6). The head of the cotton swab is illustrated in Figure 2.

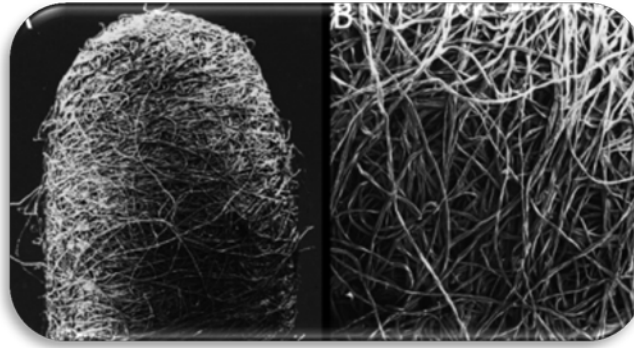


Figure. 2: Scanning Electron Micrograph of the Cotton Swab from Probst A. et al., Validation of a Nylon-Flocked Swab Protocol for Efficient Recovery of Bacterial Spores from Smooth and Rough Surfaces. *Appl. Environ. Microbiol.* August, 2010; vol. 76, **15**; 5148-5158 (8).

Manufacturers of the FLOQ™ have claimed that when these swabs are used for biological evidence collection, the flocked texture allow for more efficient DNA recovery. The texture of the head of the FLOQ™ swab consists of synthetic fibers which are applied perpendicular to the molded plastic shaft. The resulting texture appears as bristles under a microscope as illustrated in Figure 3. The fibers on the flocked swab do not interlock and therefore there is no matrix that could potentially entrap cells, as a result this structure keeps cells near the surface of the swab which can be more easily eluted. The FLOQ™ swab contains an open fiber structure that allows for absorption of the cells and allows them to remain closer to the swab surface. The FLOQ™ swab contains no absorbent core, so it is less likely that the cells will be pulled deep inside the swab. This helps prevent the DNA from becoming entrapped in the fiber bristles and ultimately could result in efficient and optimum DNA recovery from evidentiary stains (4, 5).

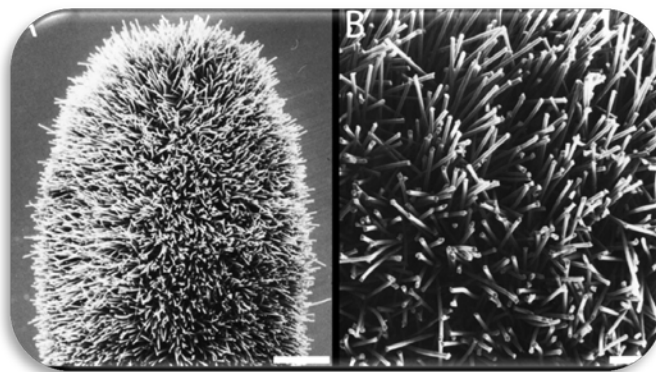


Figure 3: Scanning Electron Micrograph of the FLOQ™ Swab. Probst A. et al., Validation of a Nylon-Flocked Swab Protocol for Efficient Recovery of Bacterial Spores from Smooth and Rough Surfaces. *Appl. Environ. Microbiol.* August, 2010; vol. 76, **15**; 5148-5158 (8).

Limited studies have been conducted comparing cotton and FLOQ™ swabs in forensic casework. One recent study comparing the DNA recovery capabilities of the cotton and flocked swabs from dry saliva stains showed that the cotton swab gave significantly higher yields of DNA from dried saliva stains (1). However, this study conflicted with other studies that compared the recovery of DNA in sexual assault casework and other research which showed that flocked swabs performed better when compared to other types of swabs (4, 5).

Over the years, cotton swabs have been the standard method for biological evidence collection at crime scenes. Law enforcement agencies have used cotton swabs because they are a reliable collection device that is low in cost, easy to store, and are amenable for high-throughput processing (1, 3). In addition, a cotton swab is easy to use and with minimal training the collection can be done efficiently (2). However, improper collection and processing techniques can minimize optimal results with respect to Short Tandem Repeat (STR) amplification and DNA genotyping (9).

The manufacturer of the FLOQ™ swab claims that the open fiber structure was designed for the optimum collection of cells in a forensic setting. The short nylon fiber strands attached to the molded plastic contain a hydrophilic layer of fibers that aids in the efficient collection and release of the biological material (5). Rather than fibers wrapped around the stick, the FLOQ™ nylon fibers are attached perpendicular to the shaft of the swab, reducing the likelihood of DNA entrapment. The swabs are individually packaged and are certified to be DNase, RNase, human DNA-free, and contain no PCR inhibitors (6). A study was conducted in the evaluation of cotton and flocked swabs designed to mimic sperm collection from vaginal swabs in sexual assault evidence casework (4). This study showed that sperm cells eluted from nylon flocked swabs had a greater number of intact nuclei compared to cotton swabs from which sperm cells were damaged and few intact nuclei were observed. Both types of swabs were evaluated post-extraction to determine the amount of DNA retained within the swabs. This study found that the cotton swab retained more DNA within the swab than compared to the flocked swab. In another study that compared flocked and rayon swabs, the flocked swab was proven to release the DNA more readily than the rayon swab where the DNA appeared to be wrapped in the rayon matrix (5). The study stated that the flocked swab yielded greater amounts of DNA possibly related to the slower absorption of DNA into the swab due to the fiber matrix and nylon core. The average DNA yield was shown to be higher from the nylon flocked swab in both experiments (4, 5). In forensic cases, where the collection and evaluation of trace amounts of evidence is crucial, the nylon FLOQ™ swab has been shown to improve the recovery of DNA collection compared to a cotton swab.

Another factor that can affect the efficiency of DNA recovery is the quality of the cells collected. A common practice for the recovery of dried bloodstains is to moisten the swab and

rehydrate the stain in order to transfer the dried material to the swab. In the process of collecting dried biological samples, dH₂O is routinely used to rehydrate the sample for collection (2).

Water can cause a cell to lyse and the resulting DNA from that cell can become tangled and entrapped within the fibers of the swab reducing the amount of DNA recovered during extraction (1, 3, 4, 5, 6, 9). Through the process of osmosis, water moves through the phospholipid bilayer of a cell by water channel proteins to facilitate the influx of water in and out of the cell in order to maintain homeostasis. Deionized water is a hypotonic solution where the concentration of solutes inside of the cell is less than that of the cytosol owing to a high concentration of water outside of the cell. The resulting high concentration of water outside of the cell causes water to rush into the cell which will swell and consequently lyse. An isotonic solution, where the salt concentration inside the cell is the same as the outside, will maintain a neutral osmotic pressure, so that the structure of the cells remain intact (10). The utilization of phosphate buffered saline (PBS) solution in place of dH₂O to rehydrate the sample during collection, could aid in maintaining the integrity of cell and the nuclear membrane. It is possible that by maintaining the integrity of the nucleus, the entrapment of DNA during the collection process could be minimized, and could result in the increased recovery of DNA.

The surface on which blood is deposited can affect the ability for efficient collection of a sample. In this study, blood was applied to several surfaces in order to evaluate the collection performance of the cotton and the FLOQ™ swabs. The surfaces were glass, wood, and marble. These three surfaces are representative of typical crime scenes from where bloodstains can be collected. Glass is a smooth, non-porous, uniform surface from which bloodstains will readily transfer to a swab with little sample loss attributed to the surface. The surface of wood is often porous and absorbent. Wood that has not been treated or stained would be expected to have a

lower level of DNA recovery based on its absorbent nature. Bloodstains recovered from wood may be expected to have a lower DNA yield when compared to glass and marble. The surface of the marble tile chosen for this study was irregular and porous. Bloodstains on the irregular and porous surface of marble could make it more difficult to recover from the pores and the grooves in the tile. These three different surfaces present different challenges that could result in variations of DNA yield in the recovery of dried bloodstains.

To assess the suitability of each swab type to recover bloodstains for forensic DNA casework, four experiments were conducted. The four experiments were designed to test the effectiveness of each swab type when moistened with either dH₂O or PBS prior to sample collection, and then to evaluate not only the quantitative recovery of the DNA but the overall quality of the DNA profiles produced. All four experiments were done consecutively, and all the samples processed and analyzed in triplicate. This project aims to compare the use of cotton swabs and nylon FLOQTM swabs in collection and recovery of bloodstains from three different surfaces; glass, marble, and wood. In addition to determining the swab collection efficiency, we also compared the use of dH₂O or a PBS for sample collection. The choice of reagent for hydrating the swabs may be important in order to preserve the integrity of the cells within the evidentiary sample. The types of swabs will also be assessed by evaluating their application in forensic casework relative to the ease of use, sample processing, and cost effectiveness. The ultimate goal of this study was to determine the most efficient and effective method for recovering DNA from bloodstains.

CHAPTER 2

MATERIALS AND METHODS

Sample Collection

Blood samples from two individuals were collected using an UNTHSC IRB approved protocol (210-132). Blood was drawn from two individuals by venipuncture, with all identifying information removed. One yellow top tube (BD Vacutainer®) with acid citrate dextrose as a preservative was collected for this project (Becton, Dickinson and Company, Franklin Lakes, New Jersey). Blood was taken from each individual at one time to ensure that the white blood cell count would remain the same throughout the course of the experiment. Once the blood was collected, the blood was directly extracted, the dry and pre-moistened control swabs were made, and all three surfaces were simultaneously spotted to reduce sampling variability.

Cotton and 4N6FLOQSwabs™

For this study, the Puritan® Cotton Tipped Applicators (Puritan Medical, Guilford, ME) and the Copan 4N6FLOQSwabs™ (Life Technologies, Carlsbad, CA) were used. Both types of swabs are provided by the manufacturer in individual sterile packaging. After the samples were collected, each swab was labeled with a unique identification number and allowed to air dry overnight in a laminar flow hood.

Distilled H₂O and PBS Solution

UltraPure™ distilled water (dH₂O) (Life Technologies, Carlsbad, CA) was used to moisten the swabs. This product is promoted as being DNase, RNase, and Protease activity free

and certified for use in the laboratory. The PBS solution contained 0.1 M phosphate buffered saline at pH 7.4 (Sigma Corporation, St Louis, MO). Both dH₂O and PBS were stored at room temperature. The volume used to moisten each swab was 50 µl.

PrepFiler® *Express*TM and AutoMate *Express*TM Forensic DNA Extraction System

All samples in this study were extracted using the PrepFiler® *Express*TM kit in conjunction with the AutoMate *Express*TM instrument, illustrated in Figure 4. The PrepFiler® lysis solution was prepared by mixing 500 µl of PrepFiler® lysis buffer and 5 µl of 1.0 M DTT (Promega, Madison, WI) solution per each sample. The DTT was prepared by adding 771.2 mg of DTT in 100 ml of dH₂O. The PrepFiler® LySepTM column, illustrated in Figure 5, was attached to the PrepFiler® sample tubes and both were labeled with the corresponding sample identification number. The LySepTM column was assembled by placing the filter basket component of the LySepTM column to the top of the sample collection tube. The head of the swab was cut from the stick and placed directly to the PrepFiler® LySepTM column prior to the addition of the PrepFiler® lysis solution. To each column, 500 µl of freshly prepared PrepFiler® lysis solution (495 µl PrepFiler® lysis buffer and 5 µl of 1.0 DTT) were added to cover the swabs. Each swab was then incubated in an Eppendorf Thermomixer® (Hamburg, Germany) at 70°C for 40 minutes while continuously mixing at 750 rpm. Then, the samples were placed into a microcentrifuge and spun at 10,000 x g for two minutes to transfer to lysate from the LySepTM column into the sample tube. The LySepTM column was then detached and discarded from the sample collection tube. The sample tubes were placed into the sample tube rack of the AutoMate *Express*TM. The tips were then placed into T2 section of the tip and tube rack, and the PrepFiler® elution tubes were placed into the section of the E on the rack. Once the samples were placed on the sample rack, the PrepFiler® cartridges were placed into the AutoMate

*Express*TM cartridge rack. Once the racks were securely placed on the instrument, the PrepFiler® *Express*TM for the PrepFiler® lysis buffer DNA isolation protocol was selected. The samples were extracted and eluted in PrepFiler® elution buffer in 50 µl into elution tubes which were labeled with the unique sample identification number.



Figure 4: The AutoMate *Express*TM Forensic DNA Extraction System.

Picture Courtesy of Jonathan King

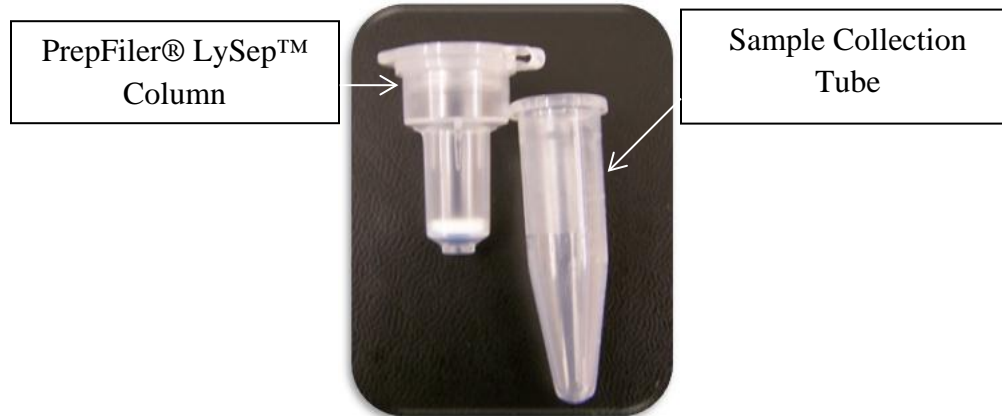


Figure 5: The PrepFiler® LySep™ Column and Sample Collection Tube.

Picture Courtesy of Jonathan King

Bloodstains

In order to mimic the recovery of DNA from dried bloodstains at crime scenes, 5 μ l of blood from two individuals were spotted onto different surfaces. Three surfaces were chosen to evaluate the collection efficiency of both swabs using dH₂O or PBS for collection: glass, marble, and wood. Three inch by two inch glass microscope slides were used for the glass surface (Baxter Scientific Products, Waukegan, IL). The wood surface consisted of birch cut into 4" x 4" pieces. Tumbled marble tile was used for the marble surface (PGM Products, Barrington, NJ). The surfaces were sectioned and the corresponding sample that was to be collected was labeled with a unique identification number. Blood (5 μ l) was spotted onto a surface I using a pipette and allowed to air dry overnight in a laminar flow hood before sample collection for a total of 72 samples. The surfaces are shown in Figure 6.

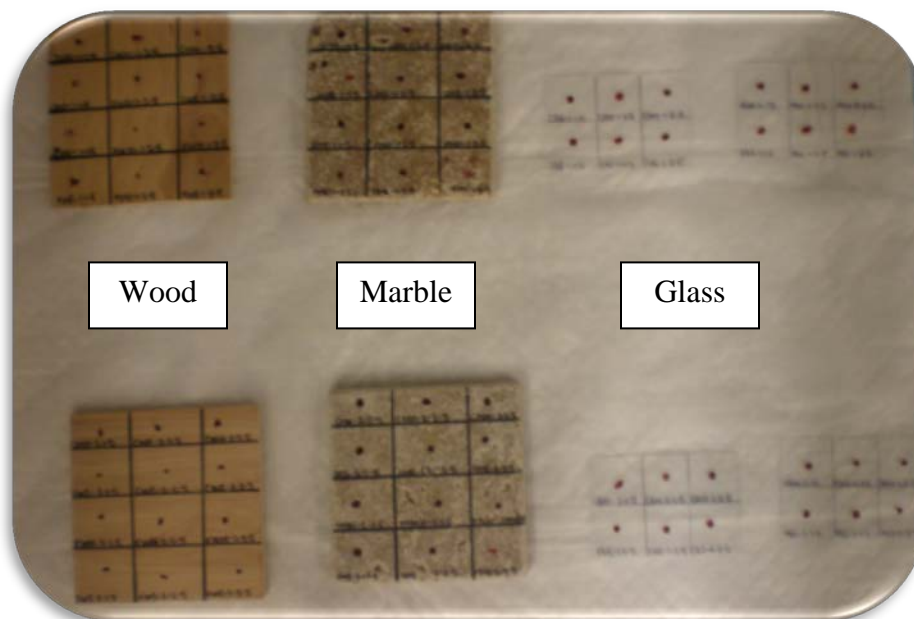


Figure 6. Surface Stains: Five microliters (5 μ l) blood from Individuals 1 and 2 were spotted onto the wood, marble, and glass surfaces.

Direct Blood Samples Control

This experiment was designed to determine overall DNA yield in 5 μ l of blood when added directly to the LySep™ column. Liquid blood of 5 μ l from each subject were directly added to the PrepFiler® LySep™ column in replicates of three for a total of 6 samples into the lysis buffer using PrepFiler® *Express*™ kit in conjunction with the AutoMate *Express*™ instrument (Life Technologies). DNA samples were then quantified using Real-Time PCR using the Quantifiler® Human DNA Quantification Kit (Life Technologies).

Swab Controls

The purpose of the following sets of experiments was to establish swab controls to assess the efficiency of the recovery of DNA from the cotton tipped and the FLOQ™ swab. In one experiment, 5 μ l of blood were added to each of the two swabs previously moistened with 50 μ l

of distilled dH₂O or PBS. The swabs were labeled, air dried overnight, the DNA was extracted, and then quantified using Real-Time PCR. The same method was used in the second set of swab controls; however, blood from each individual was added to a dry swab which was not previously moistened with dH₂O or PBS.

Blood Added to Dry Cotton and FLOQ™ Swabs

This experiment was designed to determine the efficiency of sample release for both types of swabs. Five µl of blood from each subject were directly added to each swab in replicates of three for a total of 12 samples and extracted using the PrepFiler® *Express*™ kit on the AutoMate *Express*™ instrument according to the manufacturer's protocol. The swabs were allowed to dry overnight in a laminar flow hood before the process of extraction.

Blood Added Directly to Cotton and FLOQ™ Swabs Pre-moistened with dH₂O or PBS

This experiment was designed to determine the DNA yield and recovery of each type of swab moistened with dH₂O or PBS. Five µl of blood from each subject were directly added to the cotton or FLOQ™ swabs pre-moistened either with 50 µl dH₂O or PBS. Each sample set was extracted in replicates of three for a total of 24 samples. The samples were allowed to dry overnight in a laminar flow hood before the process of extraction.

Surface Sample Collection

Samples were collected by adding 50 µl of dH₂O or PBS solution directly to both the cotton and FLOQ™ swab using a pipette and rotating the swab to distribute the liquid evenly. To ensure uniform and unbiased swabbing, the surfaces and the stains were swabbed fifteen times, and then the swab was rotated 180 degrees and swabbed fifteen additional times for a total

of thirty strokes per surface stain for a total of 72 samples. Once the samples were collected, the swabs were labeled and allowed to air dry overnight in a laminar flow hood before extraction.

Extraction of Surface Stains

All extractions were performed in replicates of three for each sample type to ensure the reproducibility of the results. After the samples were allowed to dry overnight, the DNA was then extracted using the PrepFiler® *Express*TM kit using the AutoMate *Express*TM instrument according to the protocol described above. All of the surface samples were extracted on the same day to reduce sampling bias. The extracted samples were eluted in 50 µl of PrepFiler® elution buffer.

DNA Quantification

The quantity of DNA in each replicate was determined by the Quantifiler® kit on the ABI 7500 Real-Time PCR System (Life Technologies). The samples were processed using an in-house developed reduced reaction volume protocol for a total reaction volume of 10 µl for a total of 114 samples. Nine tubes were labeled STD 1, STD 2, STD 3, STD 4, STD 5, STD 6, STD 7, STD 8 and NTC (No Template Control). The standards were diluted in TE⁻⁴ buffer. The TE⁻⁴ buffer (with 0.1 mM EDTA) was made by adding 10 ml of Tris-HCl and 40 µl of 0.5 M EDTA to 900 ml of dH₂O. The Quantifiler® Human DNA Standard (200 ng/µl) was vortexed and 10 µl of stock was added to 30 µl of TE⁻⁴ buffer for a final volume of 40 µl. Then, 20 µl of the TE⁻⁴ buffer were added to the remainder of the STD 2-8 tubes and the NTC and 10 µl of the volume from STD 1 was added to STD 2 and the standards were serially diluted by 10 µl in sequential order and no DNA template was added to the NTC which contained only 20 µl of TE⁻⁴ buffer. The Quantifiler® Human Primer Mix and the Quantifiler® PCR Reaction Mix were

vortexed and added into a master mix tube and then vortexed again. Then, 9.2 µl of PCR master mix were transferred into each reaction well and 0.8 µl of the standard, sample, NTC, or 9947A positive control were transferred into the appropriate designated wells for a final volume of 10 µl. The 9947A positive control was run in duplicate using a 0.1 ng/µl and 1.0 ng/µl sample. The reaction plate was then covered and centrifuged at 3000 g for 30 seconds. The samples were then run on the 7500 Real-Time PCR System. The amplification parameters were: initially heated at 95°C for 10 min for 1 cycle; then each cycle was denatured at 95°C for 15 seconds, extended at 60°C for 1 minute for 40 cycles.

STR Amplification

DNA samples were amplified with the AmpF ℓ STR Identifiler® Plus PCR Amplification Kit (Life Technologies). A representative sample from each of the triplicate samples was normalized to a concentration of 0.1 ng/µl in TE⁻⁴ buffer so that 1 ng of DNA was added to the STR amplification reaction. Ten µl (1 ng) of each DNA sample were added to 15 µl of master mix for a final reaction volume of 25 µl in AmpF ℓ STR reaction to a 0.2 ml MicroAmp® tube. The reaction volume consisted of AmpF ℓ STR Identifiler® Plus PCR Reaction Mix and AmpF ℓ STR Identifiler® Plus Primer Mix. The master mix was prepared by adding 10 µl of Identifiler® Plus Primer Mix to 5 µl of Primer Mix per each sample. The positive control DNA 9947A and a negative control consisting of TE⁻⁴ buffer (20 µl) was added to 15 µl of the reaction mix in 10 µl in a MicroAmp® tube. The samples were briefly centrifuged and placed on an AB 9700 Thermal Cycler (Life Technologies). The samples were heated to 95°C for 11 minutes, cycles of 94°C for 1 minute, 59°C for 1 minute, 72°C for 1 minute for a total of 28 cycles, then

the samples were held at 60°C for 60 minutes and then held for 4°C until the samples were ready for further processing.

STR Data Analysis

The PCR products were separated and detected on the AB 3130*xl* Genetic Analyzer™ (Life Technologies) according to the manufacturer's guidelines. A total of 34 samples were injected for 10 seconds at 3 kilovolts and separated using POP-4™ polymer (Life Technologies) using a 2100 second run time and the HIDFragmentAnalysis36_POP4 Module (Life Technologies). Once samples were run through the 3130*xl*, the samples were evaluated using GeneMapper® ID v3.2.1 software (Life Technologies). The detection and interpretation threshold were both set at 50 relative fluorescent units (RFU). Spectral artifacts such as pull-up and stutter were removed from the electropherogram data before it was presented for this research.

CHAPTER 3

RESULTS

DNA Extraction Controls

Blood Directly Added to LySep™ Column Prior to Extraction

Five μl of blood were directly added to a LySep™ Column containing 500 μl of PrepFiler® lysis solution. The samples were incubated under standard conditions, centrifuged, and the lysate was loaded onto the AutoMate *Express*™ instrument. Once the samples were loaded onto the instrument, the DNA from the samples was extracted, and the DNA was eluted in 50 μl of PrepFiler® elution buffer. The extracted samples were then quantified. Samples from the two donors were analyzed in triplicate. The concentration and total DNA recovered are shown in Table 1. The average recovery of DNA from 5 μl of blood for individual 1 was 102.5 ng with a standard deviation of ± 15.5 ng; and for individual 2 the average recovery of DNA from 5 μl of blood were 152 ng with a standard deviation of ± 29.5 ng. The white blood cell count was not available for the two individuals who donated blood; therefore it was not possible to calculate the theoretical DNA yield that would be expected from 5 μl of blood. The recovery of DNA can be compared from the quantification results of the direct 5 μl blood extraction of the two donors. The yield between the two types of swabs between each other can also be compared, whether the swabs were pre-moistened with dH_2O or PBS, and the surface (glass, wood, or marble) on which the 5 μl blood stains were prepared.

Blood Added Directly to the LySep™ Column	Individual 1 ng/μl	Individual 1 Total DNA ng	Individual 2 ng/μl	Individual 2 Total DNA Ng
Replicate 1	2.27	114	2.37	119
Replicate 2	2.19	110	3.24	162
Replicate 3	1.69	84.5	3.50	175
Average	2.05	103	3.04	152
SD	0.31	15.5	0.59	29.5

Table 1. Blood Added Directly to the LySep™ Column: Five (5) μl of blood added directly to the LySep™ column from Individual 1 and 2. Samples were eluted in 50 μl.

Blood Directly Added to Dry Cotton Tipped Swabs or to Dry FLOQ™ Swabs

Five (5) μl aliquots of blood were applied directly to both dry cotton and dry FLOQ™ swabs, and then allowed to air dry overnight. The swab heads were cut and placed in separate LySep™ columns which contained 500 μl of the PrepFiler® lysis solution. The samples were incubated under recommended conditions, centrifuged, and the lysates were loaded onto the AutoMate *Express*™ instrument. After approximately 30 minutes, the DNA was eluted in 50 μl of PrepFiler® elution buffer, and quantified as described in the materials and methods. Five μl blood samples from the two donors were analyzed in triplicate for both the cotton and the FLOQ™ swab. The data showed that there was a substantial loss of recoverable DNA when the blood was applied directly to either a dry cotton tipped swab or FLOQ™ swab. The amount of DNA recovered was only 10 to 25 percent of the DNA obtained when the blood was added directly to the LySep™ columns and extracted (data compared from Table 1 and Table 2). There was no significant difference between the amounts of DNA recovered from either type of swab. The loss was considerable from both.

Blood Directly Added to Cotton or to FLOQ™ Swabs Pre-Moistened with dH₂O or PBS

From the results of blood added directly to pre-moistened cotton or FLOQ™ swabs, there does not appear to be any advantages if the swabs are moistened with PBS as compared to dH₂O, prior to the addition of the blood. In fact, it appeared that the swabs pre-moistened with dH₂O gave better yields of DNA. This might be predicted if the lysing of the cells is immediate with dH₂O collection and would allow for the DNA to remain close to the outside of the swab head. The yield of DNA was substantially reduced compared to the direct extraction from liquid blood. The results also indicated that the FLOQ™ swabs pre-moistened with either dH₂O or PBS gave consistently higher yields of DNA as compared to the pre-moistened cotton tipped swabs.

Blood Added Directly to Swabs					
Collection Method					
Individual	Replicate	Swab Type	Dry ng/μl	dH₂O ng/μl	PBS ng/μl
Individual 1 Replicate 1 Cotton			0.17	0.20	0.15
Individual 1 Replicate 2 Cotton			0.17	0.51	0.31
Individual 1 Replicate 3 Cotton			0.27	0.54	0.22
Individual 1 Cotton Average			0.20	0.42	0.23
Individual 1 Cotton SD			0.05	0.19	0.01
Individual 1 Replicate 1 FLOQ™			0.26	0.97	0.43
Individual 1 Replicate 2 FLOQ™			0.71	0.74	0.69
Individual 1 Replicate 3 FLOQ™			0.48	0.30	0.58
Individual 1 FLOQ™ Average			0.48	0.67	0.57
Individual 1 FLOQ™ SD			0.23	0.34	0.13
Individual 2 Replicate 1 Cotton			0.93	0.25	0.12
Individual 2 Replicate 2 Cotton			0.67	0.50	0.14
Individual 2 Replicate 3 Cotton			0.54	0.47	0.36
Individual 2 Cotton Average			0.72	0.40	0.21
Individual 2 Cotton SD			0.20	0.14	0.13
Individual 2 Replicate 1 FLOQ™			0.55	1.27	1.00
Individual 2 Replicate 2 FLOQ™			0.62	0.73	0.91
Individual 2 Replicate 3 FLOQ™			0.55	0.64	0.86
Individual 2 FLOQ™ Average			0.57	0.88	0.92
Individual 2 SD			0.04	0.34	0.07
Individual 1 Average			0.32	0.54	0.40
Individual 1 SD			0.19	0.25	0.20
Individual 2 Average			0.64	0.64	0.39
Individual 2 SD			0.13	0.32	0.22

Table 2. Blood Added Directly to Swabs: Five μl of blood added directly to a dry cotton and FLOQ™ swab. Five μl of blood added directly to a cotton and a FLOQ™ swab pre-moistened with 50 μl of dH₂O or PBS. Samples were eluted in 50 μl of PrepFiler® elution buffer. Standard Deviation (SD).

Bloodstains Prepared on Different Surfaces

The overall collection efficiencies of both the cotton and the FLOQ™ swabs were evaluated by spotting 5 µl of blood on 3 different surfaces: glass; marble; and wood. The samples were spotted in triplicate for each individual on all three surfaces, for collection with the two different swabs moistened with dH₂O or PBS. A total of twelve 5 µl blood spots were prepared for each individual per each of the three surfaces. A total of 72 bloodstains were prepared across the three surfaces. The samples were then collected by both the cotton and FLOQ™ swabs and extracted using the PrepFiler® *Express*™ kit on the AutoMate *Express*™ instrument. Each DNA extract was then quantified using Real-Time PCR with the Quantifiler® kit. The quantification results were then compared for each surface, with the cotton or the FLOQ™ swab, and whether the swabs were moistened with either dH₂O or PBS prior to collection. The results of the samples are represented in the following tables. The collection of blood from the glass surface is illustrated in Table 3, the wood surface is represented in Table 4, and the marble surface is represented in Table 5. The cumulative data for the surface samples is represented in Table 6.

Bloodstains Prepared on Glass

The glass surface is non-absorbent and the majority of the replicates had DNA yields of 50 percent or more as compared to amount of DNA obtained from the direct blood extraction. The FLOQ™ swab averaged 80 percent more DNA yield than the cotton swabs from Individual 1 and 30 percent more for Individual 2. For the bloodstains prepared on glass, the average yield of DNA from swabs moistened with PBS were 10 percent higher than swabs moistened with dH₂O for Individual 1 and 25 percent higher for Individual 2 (Table 3).

Bloodstains Prepared on Wood

The recovery of DNA from the bloodstains prepared on wood was approximately 50 percent less than either the glass or marble surfaces. The low yield of DNA can most likely be attributed to the porous nature of wood, and its ability to absorb and trap the blood cells below the surface. The DNA yield from the bloodstains on the wood was only 30 percent of the DNA yield as compared to the direct blood extraction. The average DNA yield from the FLOQ™ swabs was between 30 and 40 percent more than the DNA yield from the cotton swabs from Individual 1 and 2. The average DNA yield, using swabs moistened with dH₂O or PBS showed no advantage of using one method over the other (Table 4).

Bloodstains Prepared on Marble

The average DNA recovery from the marble surface was between 50 and 60 percent of the amount of the DNA obtained from the direct blood extraction. The average standard deviation in DNA yield from the marble surface was higher, when compared to the other two surfaces. This could potentially be attributed to the irregular marble surface. The average DNA recovery with the FLOQ™ swabs was 30 percent more DNA yield than the cotton swabs for Individual 1 excluding the replicates with PBS and 40 percent more for Individual 2. The DNA recovery with swabs moistened with either dH₂O or PBS showed that dH₂O gave higher yields of DNA than PBS with 20 percent more DNA from Individual 1 and 15 percent for Individual 2 (Table 5).

Glass Surface									
Collection Method									
Cotton and FLOQ™ Swab Collection					dH ₂ O and PBS Collection				
Individual	Replicate	Hydration Method	Cotton ng/μl	FLOQ™ ng/μl	Individual	Replicate	Swab Type	dH ₂ O ng/μl	PBS ng/μl
Individual 1 Replicate 1 dH ₂ O			0.72	1.34	Individual 1 Replicate 1 Cotton			0.72	0.80
Individual 1 Replicate 2 dH ₂ O			1.08	1.25	Individual 1 Replicate 2 Cotton			1.08	0.82
Individual 1 Replicate 3 dH ₂ O			0.62	1.80	Individual 1 Replicate 3 Cotton			0.62	1.03
Individual 1 dH ₂ O Average			0.81	1.46	Individual 1 Cotton Average			0.81	0.89
Individual 1 dH ₂ O SD			0.24	0.30	Individual 1 Cotton SD			0.24	0.13
Individual 1 Replicate 1 PBS			0.80	1.34	Individual 1 Replicate 1 FLOQ™			1.34	1.34
Individual 1 Replicate 2 PBS			0.82	1.61	Individual 1 Replicate 2 FLOQ™			1.25	1.61
Individual 1 Replicate 3 PBS			1.03	1.82	Individual 1 Replicate 3 FLOQ™			1.80	1.82
Individual 1 PBS Average			0.89	1.59	Individual 1 FLOQ™ Average			1.46	1.59
Individual 1 PBS SD			0.13	0.24	Individual 1 FLOQ™ SD			0.30	0.24
Individual 2 Replicate 1 dH ₂ O			1.55	1.55	Individual 2 Replicate 1 Cotton			1.55	1.66
Individual 2 Replicate 2 dH ₂ O			1.45	2.57	Individual 2 Replicate 2 Cotton			1.45	2.05
Individual 2 Replicate 3 dH ₂ O			1.41	2.26	Individual 2 Replicate 3 Cotton			1.41	2.10
Individual 2 dH ₂ O Average			1.47	2.13	Individual 2 Cotton Average			1.47	1.94
Individual 2 dH ₂ O SD			0.07	0.52	Individual 2 Cotton SD			0.07	0.24
Individual 2 Replicate 1 PBS			1.66	2.47	Individual 2 Replicate 1 FLOQ™			1.55	2.47
Individual 2 Replicate 2 PBS			2.05	2.52	Individual 2 Replicate 2 FLOQ™			2.57	2.52
Individual 2 Replicate 3 PBS			2.10	2.67	Individual 2 Replicate 3 FLOQ™			2.26	2.67
Individual 2 PBS Average			1.94	2.55	Individual 2 FLOQ™ Average			2.13	2.55
Individual 2 PBS SD			0.24	0.10	Individual 2 FLOQ™ SD			0.52	0.10
Individual 1 Average			0.84	1.52	Individual 1 Average			1.09	1.19
Individual 1 SD			0.16	0.23	Individual 1 SD			0.41	0.41
Individual 2 Average			1.67	2.31	Individual 2 Average			1.75	2.20
Individual 2 SD			0.29	0.38	Individual 2 SD			0.47	0.36

Table 3. DNA Yield from 5 μl Bloodstains Collected on the Glass Surface: Bloodstains were collected on the glass surface by the addition of either 50 μl of dH₂O or PBS to the cotton or the FLOQ™ swab. Samples were eluted in 50 μl of PrepFiler® elution buffer. Standard Deviation (SD).

Wood Surface									
Collection Method									
Cotton and FLOQ™ Swab Collection					dH ₂ O and PBS Collection				
Individual	Replicate	Hydration Method	Cotton ng/μl	FLOQ™ ng/μl	Individual	Replicate	Swab Type	dH ₂ O ng/μl	PBS ng/μl
Individual 1 Replicate 1 dH ₂ O			0.45	1.28	Individual 1 Replicate 1 Cotton			0.45	0.41
Individual 1 Replicate 2 dH ₂ O			0.51	0.59	Individual 1 Replicate 2 Cotton			0.51	0.65
Individual 1 Replicate 3 dH ₂ O			0.55	0.67	Individual 1 Replicate 3 Cotton			0.55	0.67
Individual 1 dH ₂ O Average			0.50	0.85	Individual 1 Cotton Average			0.50	0.59
Individual 1 dH ₂ O SD			0.05	0.38	Individual 1 Cotton SD			0.05	0.14
Individual 1 Replicate 1 PBS			0.41	0.51	Individual 1 Replicate 1 FLOQ™			1.28	0.51
Individual 1 Replicate 2 PBS			0.65	0.50	Individual 1 Replicate 2 FLOQ™			0.59	0.50
Individual 1 Replicate 3 PBS			0.67	0.53	Individual 1 Replicate 2 FLOQ™			0.67	0.53
Individual 1 PBS Average			0.58	0.51	Individual 1 FLOQ™ Average			0.65	0.51
Individual 1 PBS SD			0.14	0.01	Individual 1 FLOQ™ SD			0.38	0.01
Individual 2 Replicate 1 dH ₂ O			0.97	0.95	Individual 2 Replicate 1 Cotton			0.97	0.83
Individual 2 Replicate 2 dH ₂ O			0.43	1.11	Individual 2 Replicate 2 Cotton			0.43	1.04
Individual 2 Replicate 3 dH ₂ O			0.84	1.05	Individual 2 Replicate 3 Cotton			0.84	0.89
Individual 2 dH ₂ O Average			0.75	1.04	Individual 2 Cotton Average			0.75	0.92
Individual 2 dH ₂ O SD			0.28	0.08	Individual 2 Cotton SD			0.28	0.11
Individual 2 Replicate 1 PBS			0.83	1.02	Individual 2 Replicate 1 FLOQ™			0.95	1.02
Individual 2 Replicate 2 PBS			1.04	1.04	Individual 2 Replicate 2 FLOQ™			1.11	1.04
Individual 2 Replicate 3 PBS			0.89	1.29	Individual 2 Replicate 3 FLOQ™			1.05	1.29
Individual 2 PBS Average			0.92	1.12	Individual 2 FLOQ™ Average			1.04	1.12
Individual 2 PBS SD			0.11	0.15	Individual 2 FLOQ™ SD			0.08	0.15
Individual 1 Average			0.54	0.70	Individual 1 Average			0.65	0.55
Individual 1 SD			0.10	0.11	Individual 1 SD			0.29	0.09
Individual 2 Average			0.82	1.07	Individual 2 Average			0.87	1.12
Individual 2 SD			0.20	0.11	Individual 2 SD			0.23	0.15

Table 4. DNA Yield from 5 μl Bloodstains Collected on the Wood Surface. Bloodstains were collected on the wood surface by the addition of either 50 μl of dH₂O or PBS to the cotton or FLOQ™ swab. Samples were eluted in 50 μl PrepFiler® elution buffer. Standard Deviation (SD).

Marble Surface									
Collection Method									
Cotton and FLOQ™ Swab Collection					dH ₂ O and PBS Collection				
Individual	Replicate	Hydration Method	Cotton ng/μl	FLOQ™ ng/μl	Individual	Replicate	Swab Type	dH ₂ O ng/μl	PBS ng/μl
Individual 1 Replicate 1 dH ₂ O			0.97	1.32	Individual 1 Replicate 1 Cotton			0.97	1.41
Individual 1 Replicate 2 dH ₂ O			0.74	1.26	Individual 1 Replicate 2 Cotton			0.74	1.16
Individual 1 Replicate 3 dH ₂ O			1.35	1.41	Individual 1 Replicate 3 Cotton			1.35	1.30
Individual 1 dH ₂ O Average			1.02	1.33	Individual 1 Cotton Average			1.02	1.29
Individual 1 dH ₂ O SD			0.31	0.08	Individual 1 Cotton SD			0.31	0.13
Individual 1 Replicate 1 PBS			1.41	0.61	Individual 1 Replicate 1 FLOQ™			1.32	0.61
Individual 1 Replicate 2 PBS			1.16	0.34	Individual 1 Replicate 2 FLOQ™			1.26	0.34
Individual 1 Replicate 3 PBS			1.30	0.69	Individual 1 Replicate 2 FLOQ™			1.41	0.69
Individual 1 PBS Average			1.29	0.55	Individual 1 FLOQ™ Average			1.33	0.55
Individual 1 PBS SD			0.13	0.19	Individual 1 FLOQ™ SD			0.08	0.19
Individual 2 Replicate 1 dH ₂ O			1.81	1.87	Individual 2 Replicate 1 Cotton			1.81	1.38
Individual 2 Replicate 2 dH ₂ O			1.12	3.43	Individual 2 Replicate 2 Cotton			1.12	2.00
Individual 2 Replicate 3 dH ₂ O			1.47	2.16	Individual 2 Replicate 3 Cotton			1.47	1.65
Individual 2 dH ₂ O Average			1.47	2.49	Individual 2 Cotton Average			1.47	1.68
Individual 2 dH ₂ O SD			0.35	0.83	Individual 2 Cotton SD			0.35	0.31
Individual 2 Replicate 1 PBS			1.38	2.35	Individual 2 Replicate 1 FLOQ™			1.87	2.35
Individual 2 Replicate 2 PBS			2.00	1.34	Individual 2 Replicate 2 FLOQ™			3.43	1.34
Individual 2 Replicate 3 PBS			1.65	1.67	Individual 2 Replicate 3 FLOQ™			2.16	1.67
Individual 2 PBS Average			1.68	1.79	Individual 2 FLOQ™ Average			2.49	1.79
Individual 2 PBS SD			0.31	0.52	Individual 2 FLOQ™ SD			0.83	0.52
Individual 1 Average			1.13	0.99	Individual 1 Average			1.15	0.97
Individual 1SD			0.24	0.43	Individual 1 SD			0.25	0.42
Individual 2 Average			1.57	2.19	Individual 2 Average			1.98	1.72
Individual 2 SD			0.25	0.68	Individual 2 SD			0.80	0.35

Table 5. DNA Yield from 5 μl Bloodstains Collected on the Marble Surface: Bloodstains were collected on the marble surface by the addition of either 50 μl of dH₂O or PBS to the cotton or FLOQ™ swab. Samples were eluted in 50 μl of PrepFiler® elution buffer. Standard Deviation (SD).

Swab	Hydrating Agent	Replicate	Surface					
			Glass		Marble		Wood	
			Indiv. I	Indiv. II	Indiv. I	Indiv. II	Indiv. I	Indiv. II
Cotton	dH ₂ O	1	0.72	1.55	0.97	1.81	0.45	0.97
		2	1.08	1.45	0.74	1.12	0.51	0.43
		3	0.62	1.41	1.35	1.47	0.55	0.84
	PBS	1	0.80	1.66	1.41	1.38	0.41	0.83
		2	0.82	2.05	1.16	2.00	0.65	1.04
		3	1.03	2.10	1.30	1.65	0.67	0.89
FLOQ™	dH ₂ O	1	1.34	1.55	1.32	1.87	1.28	0.95
		2	1.25	2.57	1.26	3.43	0.59	1.11
		3	1.80	2.26	1.41	2.16	0.67	1.05
	PBS	1	1.34	2.47	0.61	2.35	0.51	1.02
		2	1.61	2.52	0.34	1.34	0.50	1.04
		3	1.82	2.67	0.69	1.67	0.53	1.29

Table 6. Surface Sample Data: The DNA yield from the surfaces (glass, marble, and wood) represented in ng/μl.

Nonparametric Mann-Whitney U Test

The DNA quantification results of the three surfaces (glass, wood, and marble) were evaluated for the cotton and the FLOQ™ as well as the results of dH₂O and PBS from both individuals using the nonparametric Mann-Whitney U test at the 0.05 significance level. The initial data calculations for the cotton and FLOQ™ swab showed that there was no significant difference in the cotton and the FLOQ™ with the p value at 0.07. With the initial data calculations for dH₂O and PBS, the p value also indicated that there was no difference between dH₂O and PBS collection. For the following calculations, the outlier data set from the marble surface with Individual 1 with PBS collection was not included in the statistical calculations. The group statistics and the nonparametric tests were evaluated using SPSS software. The results of the group statistics and the Mann-Whitney test for the cotton and FLOQ™ swab are illustrated in Table 7 and 8.

Concentration			
Cotton and FLOQ™	Mean ng/μl	N	Standard Deviation ng/μl
Cotton	1.11	36	0.48
FLOQ	1.55	33	0.73

Table 7. Group Statistics for the Cotton and FLOQ™ Swab: SPSS Data. The average DNA yield from both individuals and standard deviation from glass, wood, and marble (N=69). The results of this data include the cumulative DNA yields from dH₂O and PBS collection from both individuals.

Hypothesis Test Summary			
Null Hypothesis	Test	Significance	Decision
The concentration distribution is the same across the categories of the cotton and FLOQ™ swab.	Independent Samples Mann-Whitney U Test	0.01	Reject the null hypothesis.
Asymptotic significances are displayed. Significance level is 0.05.			

Table 8. Independent Samples Mann-Whitney U Test of the Cotton and FLOQ™ Swab. SPSS Data. Independent samples Mann-Whitney U test at the 0.05 significance level from both individuals based on the DNA yield (ng/μl) from glass, wood, and marble (N=69). The results of this data include the cumulative DNA yields from dH₂O and PBS collection from both individuals.

The results of the group statistics from the DNA quantification data from the cotton and FLOQ™ swab showed average yield of DNA was 1.11 ng/μl for the cotton swab and 1.55 ng/μl for the FLOQ™ swab. The FLOQ™ swab averaged a higher DNA yield than the cotton tipped swab. The Mann-Whitney U test demonstrated that the differences in DNA yields between both swabs were statistically different at the 0.05 significance level. The FLOQ™ swab gave a 40 percent higher DNA average yield than the cotton tipped swab. The results of the group statistics and the Mann-Whitney U test for the dH₂O and PBS are illustrated in Table 9 and 10.

Concentration			
dH ₂ O and PBS	Mean ng/μl	N	Standard Deviation ng/μl
dH ₂ O	1.28	36	0.64
PBS	1.35	33	0.63

Table 9. Group Statistics for dH₂O and PBS Collection: SPSS Data. The average DNA yield from both individuals and standard deviation from glass, wood, and marble (N=69). The results of this data include the cumulative DNA yields from cotton and FLOQ™ swabs from both individuals.

The results of the group statistics from the quantification data from dH₂O and PBS collection showed the average yield of DNA was 1.28 ng/μl for the dH₂O and 1.35 ng/μl for the PBS. Based on the averages there appeared to be no difference between dH₂O and PBS collection. From the result of the Mann-Whitney U test, the DNA yields between the two collection methods were shown to not be statistically different at the 0.05 significance level.

Hypothesis Test Summary			
Null Hypothesis	Test	Significance	Decision
The distribution of the concentration is the same across the categories of dH ₂ O and PBS.	Independent Samples Mann-Whitney U test	0.61	Retain the null hypothesis.
Asymptotic significances are displayed. The significance level is 0.05.			

Table 9. Independent Samples Mann-Whitney U test of dH₂O and PBS Collection. SPSS Data. Independent samples Mann-Whitney U test at the 0.05 significance level from both individuals based on the DNA yield (ng/μl) from glass, wood, and marble (N=69). The results of this data include the cumulative DNA yields from cotton tipped and FLOQ™ swab from both individuals.

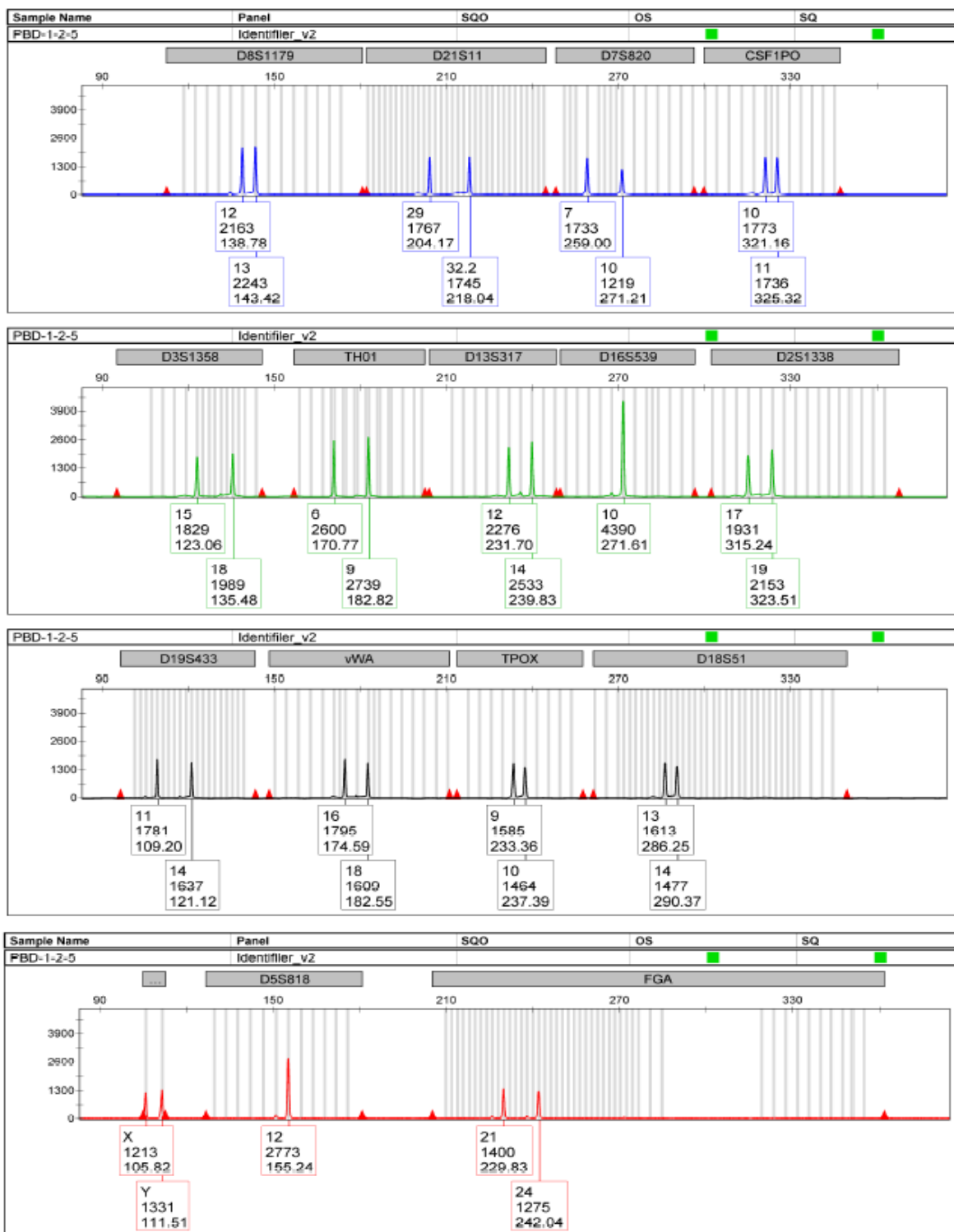


Figure 7: Individual 1 Profile: The complete Identifiler® Plus profile from Individual 1 from adding the blood directly to the PrepFiler® LySep™ column from 1 ng of DNA input.

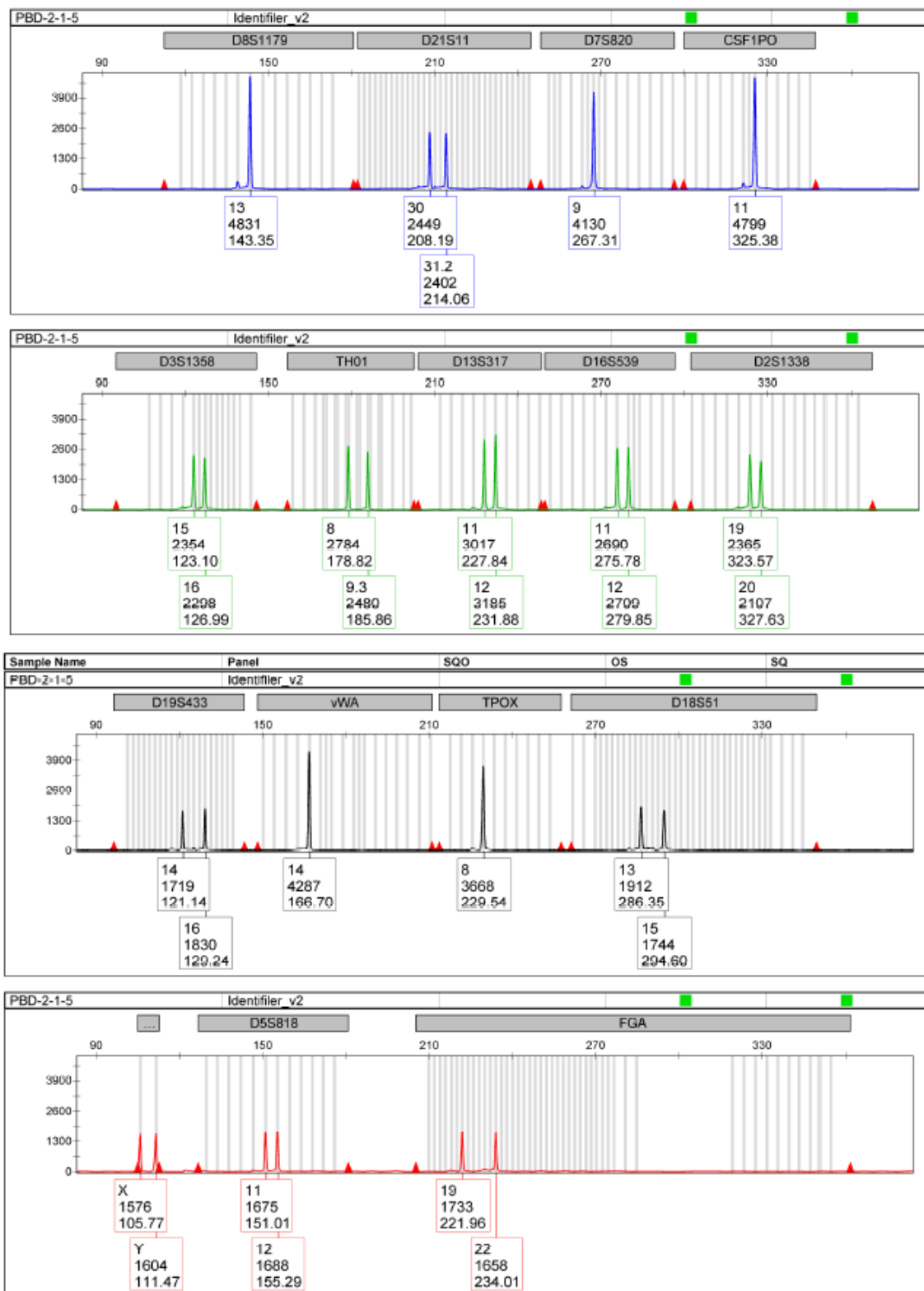


Figure 8. Individual 2 Profile: The complete Identifier® Plus profile from Individual 2 from adding the blood directly to the PrepFiler® LySep™ column from 1 ng of DNA input.

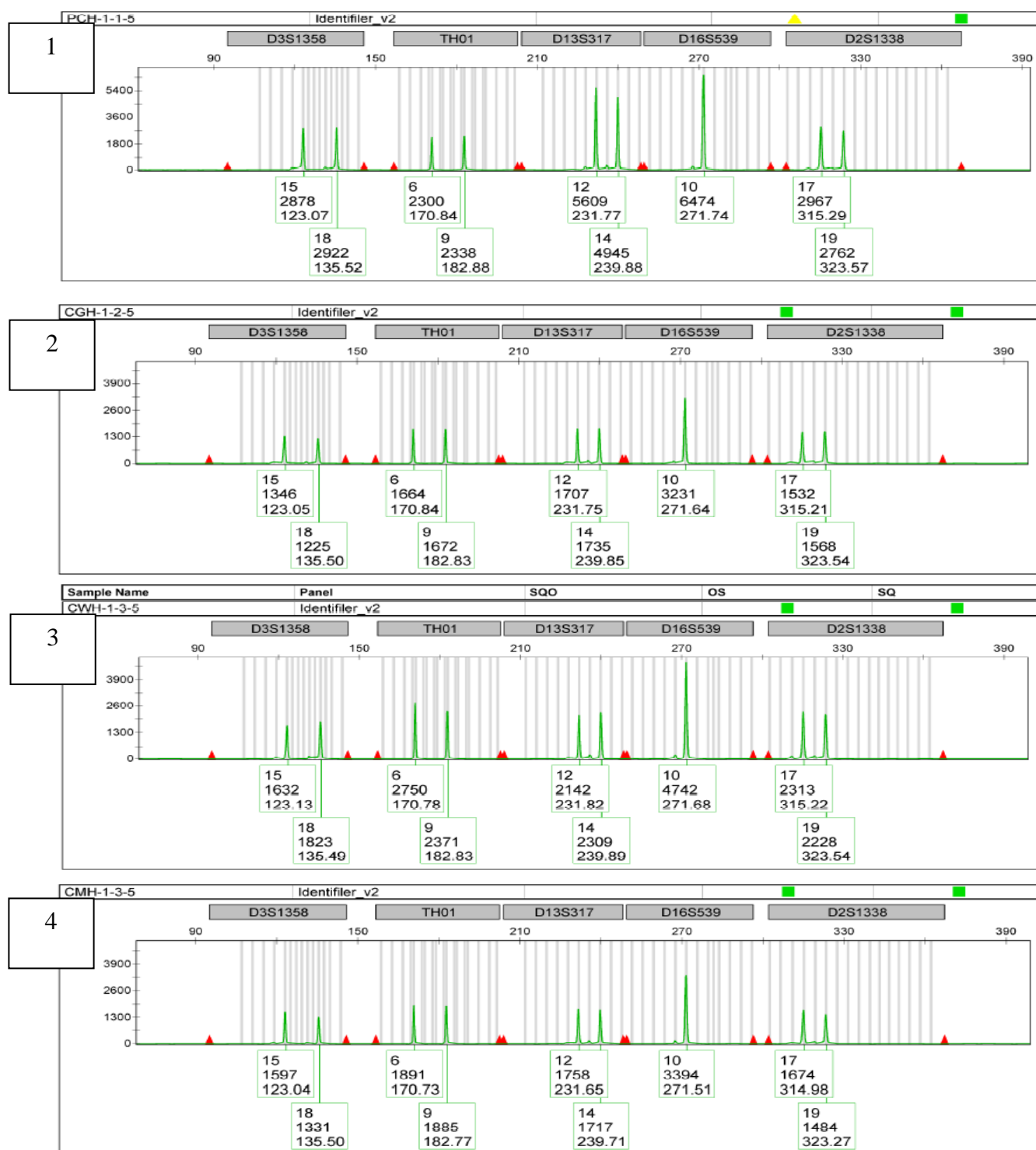


Figure 9. Cotton and dH₂O Collection Profiles from Individual 1: The Identifiler® Plus Profiles from Individual 1 of Loci D3S1358, TH01, D13S317, D2S1338: 1 ng of DNA input in descending order 1) blood added directly to a cotton swab pre-moistened with 50 µl of dH₂O, 2) glass surface, 3) wood surface, and 4) marble surface.

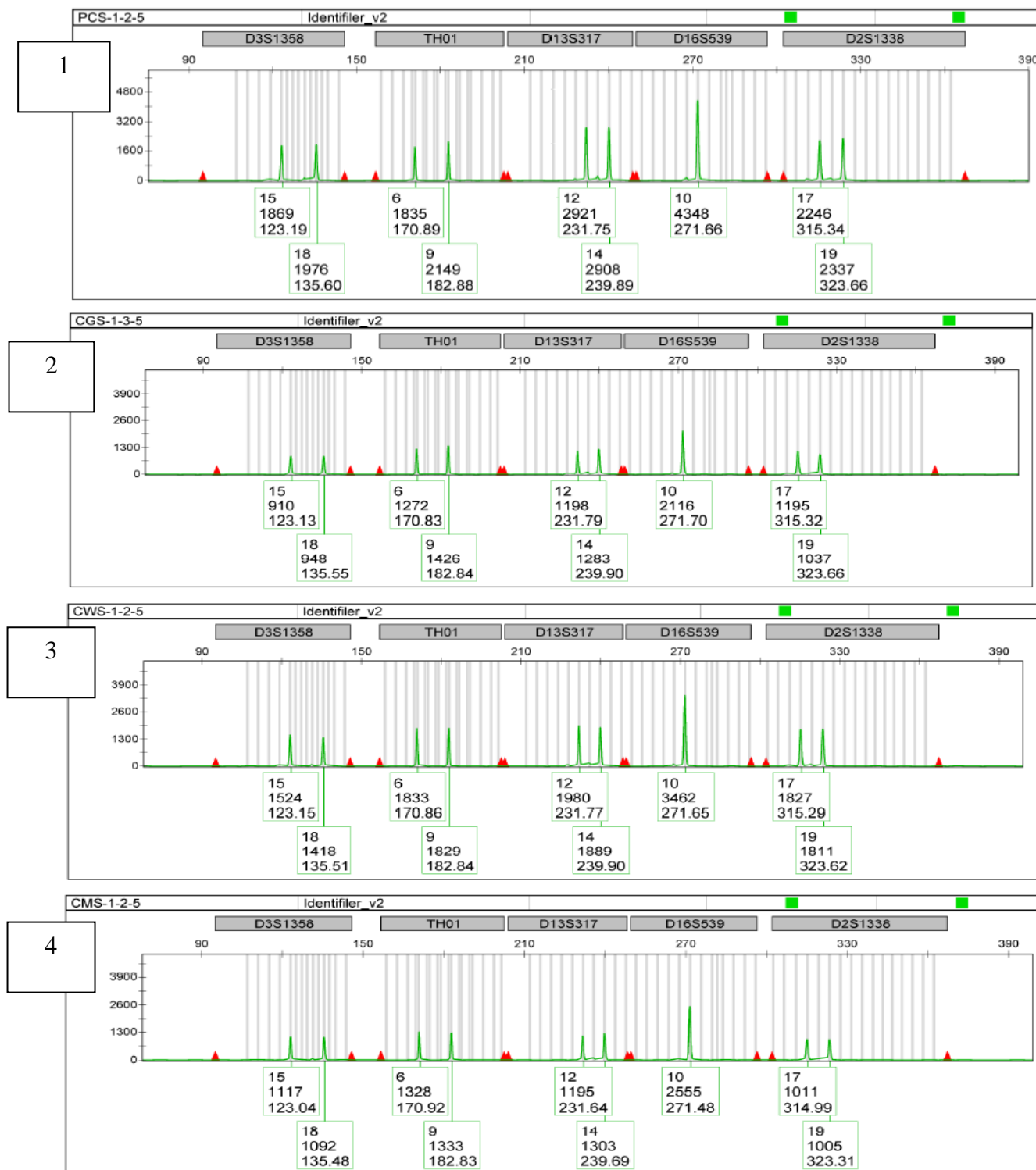


Figure 10. Cotton and PBS Collection Profiles from Individual 1: The Identifiler® Plus Profiles from Individual 1 of Loci D3S1358, TH01, D13S317, D2S1338: 1 ng of DNA input in descending order 1) blood added directly to a cotton swab pre-moistened with 50 µl of PBS, 2) glass surface, 3) wood surface, and 4) marble surface.

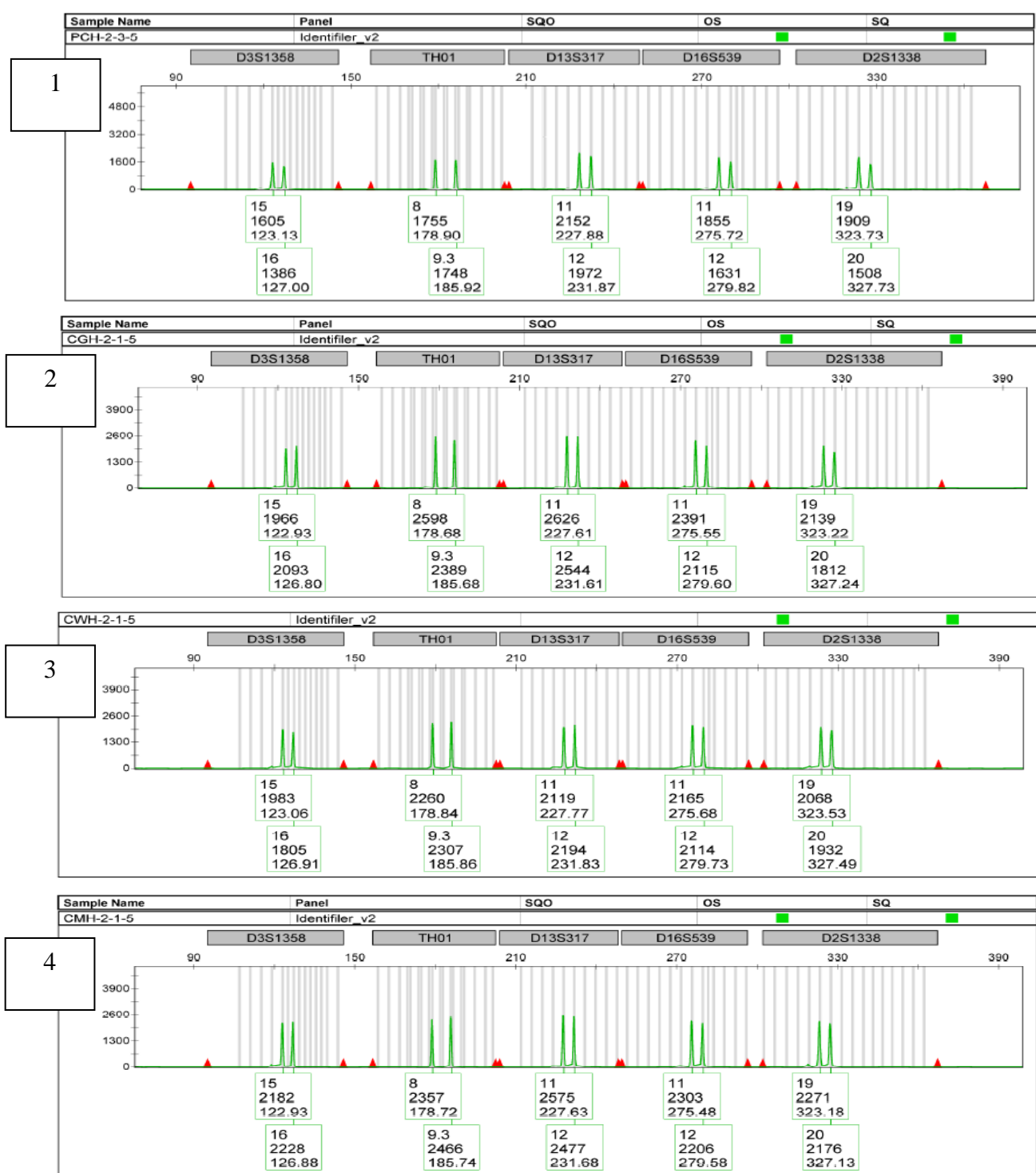


Figure 11. Cotton and dH₂O Collection Profiles from Individual 2: The Identifiler® Plus Profiles from Individual 2 of Loci D3S1358, TH01, D13S317, D2S1338: 1 ng of DNA input in descending order 1) blood added directly to a cotton swab pre-moistened with 50 µl of dH₂O, 2) glass surface, 3) wood surface, and 4) marble surface.

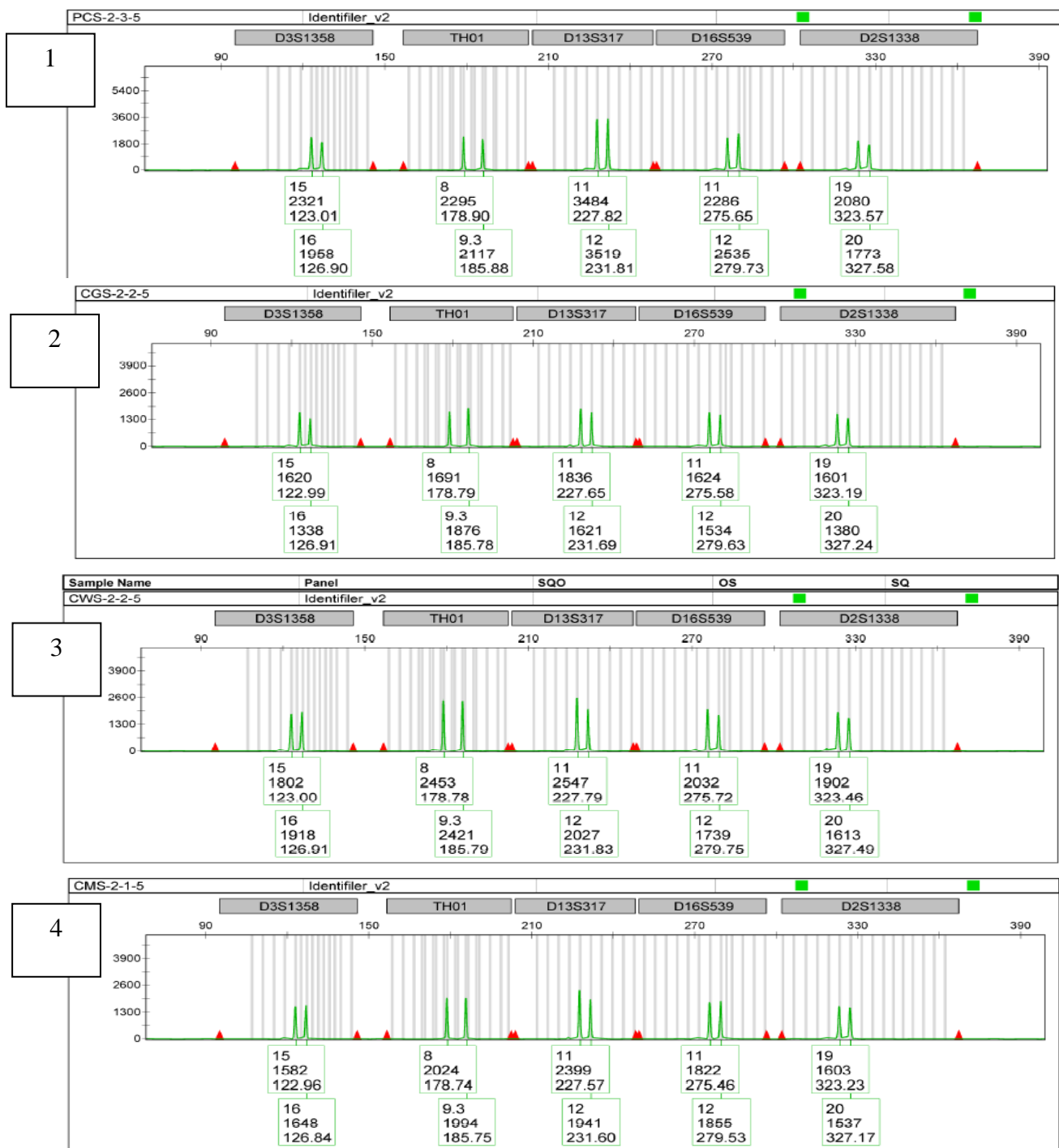


Figure 12. Cotton and PBS Collection Profiles from Individual 2: The Identifier® Plus Profiles from Individual 2 of Loci D3S1358, TH01, D13S317, D2S1338: 1 ng of DNA input in descending order 1) blood added directly to a cotton swab pre-moistened with 50 µl with PBS, 2) glass surface, 3) wood surface, and 4) marble surface.

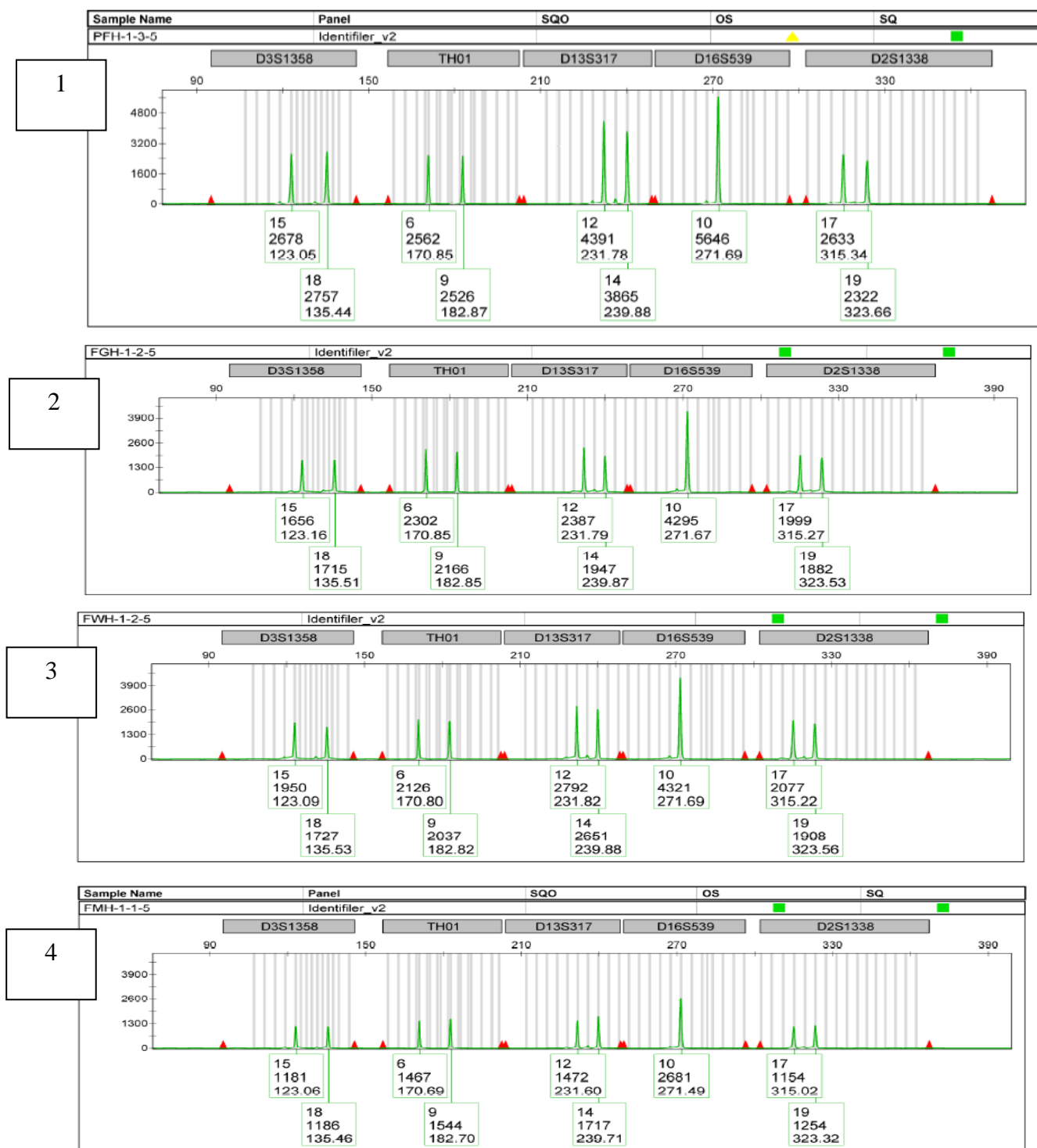


Figure 13. FLOQ™ and dH₂O Collection Profiles from Individual 1: The Identifier® Plus Profiles from Individual 1 of Loci D3S1358, TH01, D13S317, D2S1338: 1 ng of DNA input in descending order 1) blood added directly to a FLOQ™ swab pre-moistened with 50 µl with dH₂O, 2) glass surface, 3) wood surface, and 4) marble surface.

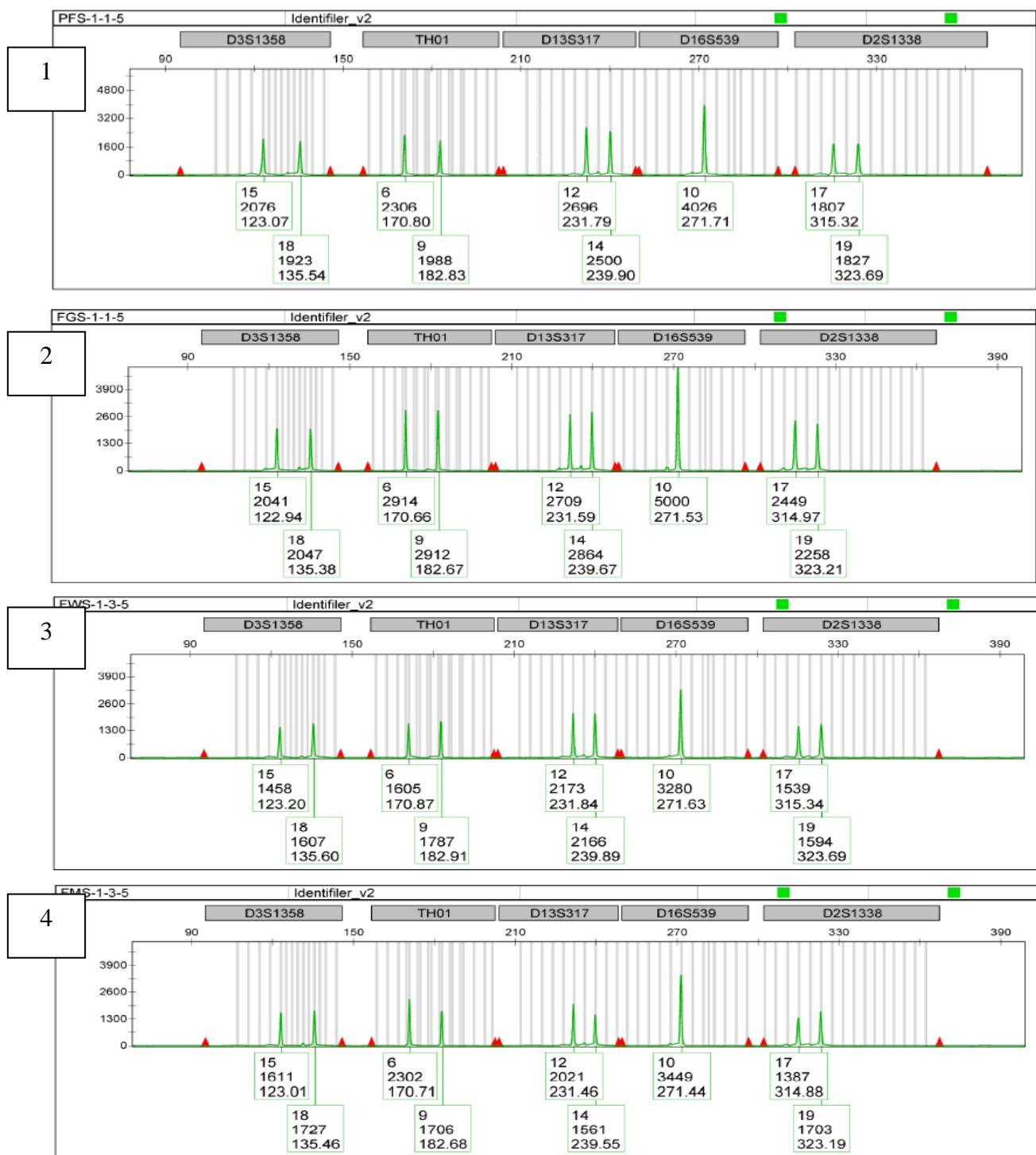


Figure 14. FLOQ™ and PBS Collection Profiles from Individual 1: The Identifier® Plus Profiles from Individual 1 of Loci D3S1358, TH01, D13S317, D2S1338: 1ng of DNA input in descending order 1) blood added directly to a FLOQ™ swab pre-moistened with 50 µl with PBS, 2) glass surface, 3) wood surface, and 4) marble surface.

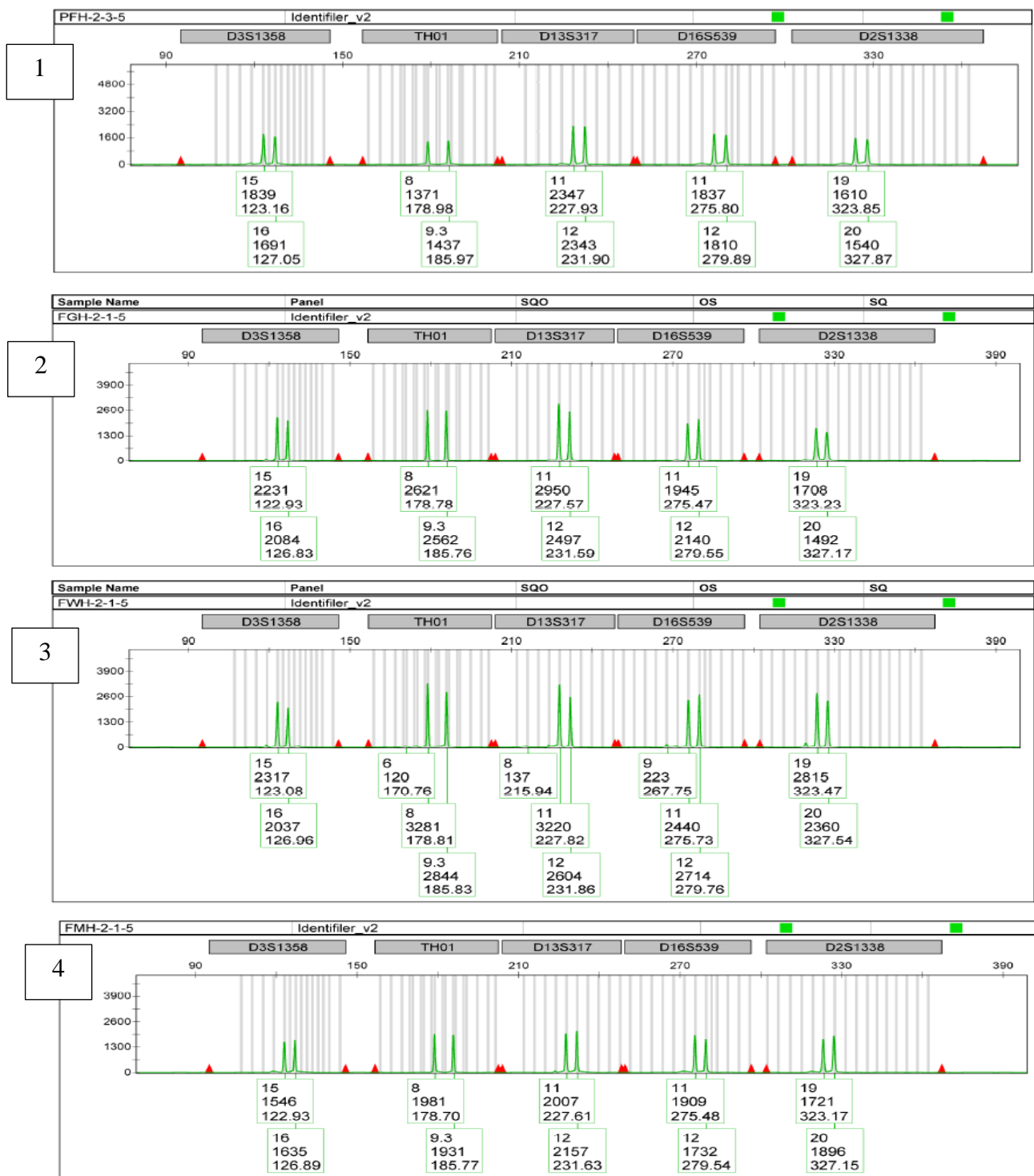


Figure 15. FLOQ™ and dH₂O Collection Profiles from Individual 2: The Identifier® Plus Profiles from Individual 2 of Loci D3S1358, TH01, D13S317, D2S1338: In descending order 1) blood added directly to a FLOQ™ swab pre-moistened with 50 µl with dH₂O, 2) glass surface, 3) wood surface, and 4) marble surface.

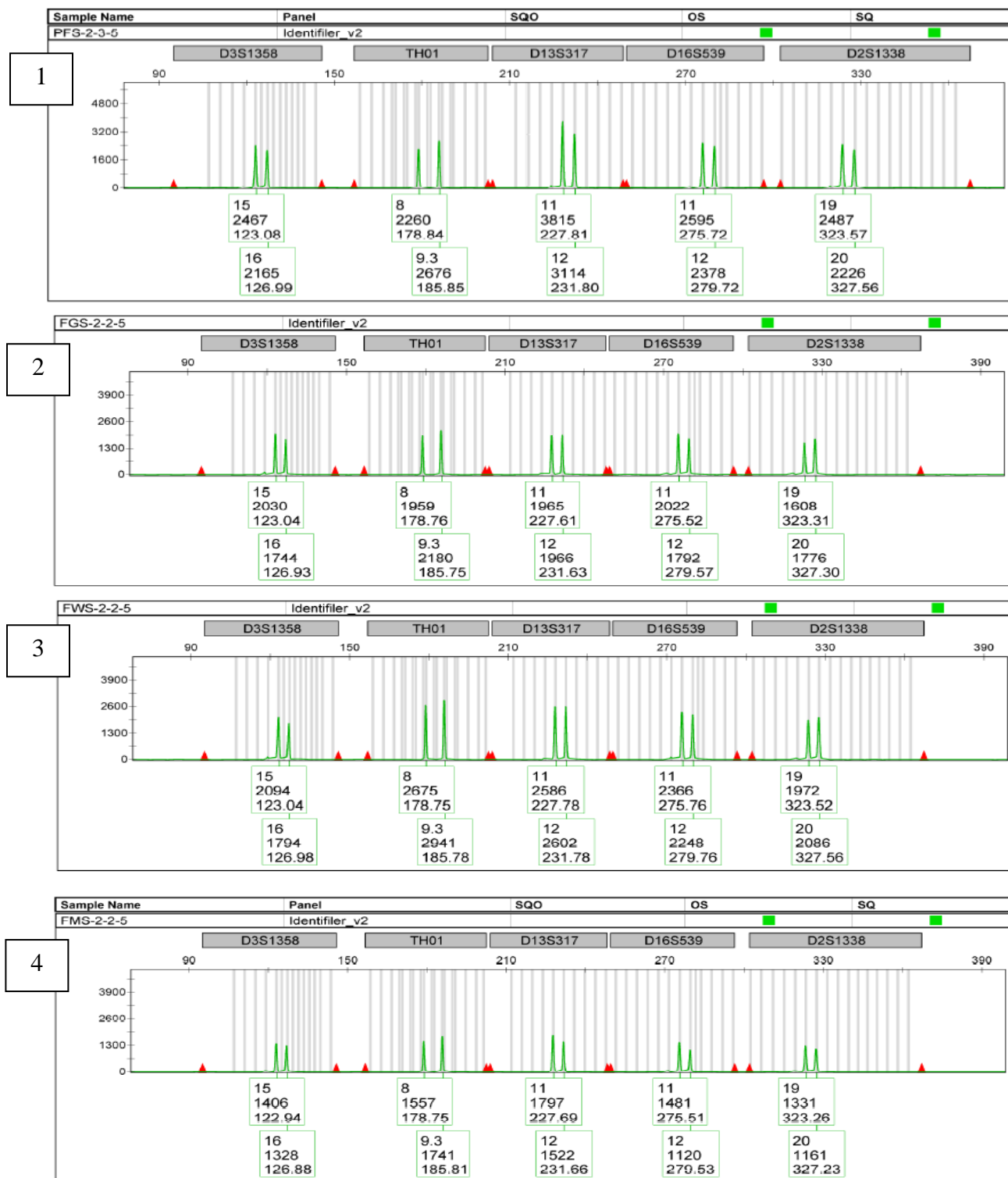


Figure 16. FLOQ™ and PBS Collection Profiles from Individual 2: The Identifier® Plus Profiles of Loci D3S1358, TH01, D13S317, D2S1338: 1 ng of DNA input in descending order 1) blood added directly to a FLOQ™ swab pre-moistened with 50 µl with PBS, 2) glass surface, 3) wood surface. and 4) marble surface.

STR Profiles Using DNA Recovered from Dried Bloodstains on the Different Surfaces

DNA samples (1 ng) extracted from bloodstains from all three surfaces, representing each of the four collection methods (Cotton swabs with dH₂O, Cotton swabs with PBS, FLOQ™ swabs with dH₂O, and FLOQ™ swabs with PBS) were amplified using the AmpF ℓ STR Identifiler® Plus kit. The profiles generated from the DNA obtained from each individual, on the three different surfaces, with the four different collections were compared to the profiles amplified with DNA obtained from the blood directly added to the LySep™ column. The quality of the DNA profiles was evaluated based upon the total number of reportable alleles, peak heights, peak height ratios, and the presence of allelic dropout. The results of the cotton swab peak height ratios for the electropherogram data are represented in Table 11 and the FLOQ™ swab data are represented in Table 12.

The peak heights of the alleles are measured in relative fluorescence units (RFUs). A relative fluorescent unit is a measurement that is determined by the fluorescence given off by an allele fragment during the data collection process of DNA genotyping. The number of reportable alleles is defined by the alleles within the loci analyzed where the peak heights were above a certain detection threshold. For the purpose of this study, the detection threshold was set at 50 RFUs. The peak height ratios are determined by the ratio of the heterozygote peak heights within the each of the loci examined. Allelic dropout is observed when the peak heights of alleles do not meet or exceed the detection threshold and are therefore not called by the software.

Peak Height Ratio	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539
Cotton ALL Channels-PHR	0.93	0.91	0.92	0.90	0.92	0.96	0.92	0.93
Standard Deviation	0.05	0.06	0.06	0.08	0.05	0.05	0.06	0.05
Peak Height Ratio	D2S1338	D19S433	vWA	TPOX	D18S51	Amelogenin	D5S818	FGA
Cotton ALL Channels-PHR	0.92	0.91	0.94	0.93	0.93	0.91	0.79	0.93
Standard Deviation	0.06	0.05	0.04	0.03	0.05	0.04	0.36	0.06

Table 11. Cotton Swab Peak Height Ratios.

The data the cotton and the FLOQ™ swab showed similar peak height ratios. Notably, for the cotton swab, the locus D5S818 showed some decrease in the peak height ratio. The imbalance was caused by two samples from the same individual but otherwise the surface and the hydration method were different.

Peak Height Ratio	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539
FLOQ™ ALL Channels-PHR	0.90	0.93	0.89	0.90	0.93	0.92	0.90	0.88
Standard Deviation	0.06	0.05	0.09	0.05	0.05	0.07	0.08	0.07
Peak Height Ratio	D2S1338	D19S433	vWA	TPOX	D18S51	Amelogenin	D5S818	FGA
FLOQ™ ALL Channels-PHR	0.90	0.92	0.95	0.92	0.90	0.91	0.87	0.94
Standard Deviation	0.04	0.06	0.03	0.03	0.09	0.05	0.07	0.06

Table 12. FLOQ™ Swab Peak Height Ratios.

The results of the data in the electropherograms indicate that both types of swabs were capable of producing a full DNA profile. The same conclusion can be drawn in the case of using dH₂O and PBS solution. The heterozygote peaks of the profiles were uniform compared to the sister peaks across of the loci represented the both profiles. The peak heights of the heterozygote peaks were approximately 50 percent of the homozygote peak in Individual 1. There was no evidence of degradation in any of the profiles and no evidence of inhibition from the surfaces, swabs, or the use of dH₂O or PBS.

CHAPTER 4

DISCUSSION

The purpose of this research was to compare bloodstain collection efficiency and the recovery of DNA from various surfaces with two different collection devices, a standard cotton tip swab and the Copan 4N6FLOQ™ Swab when used in conjunction with dH₂O and PBS.

The total DNA recovery for each individual from 5 µl of blood was determined from addition of blood added directly to the LySep™ column which was run with the AutoMate *Express*™ instrument. From Table 1 the average recovery of DNA from 5 µl of blood was 2.05 ng/µl for Individual 1 and 3.04 ng/µl for Individual 2. From the dry and moistened swabs the average recovery of DNA was between 15 and 25 percent. The loss of DNA can be explained that when the blood was added to dry or wet swabs, the blood cells and nuclear membranes broke down and the DNA became trapped within the head of the swab. As a result, during the extraction process, the DNA that became entangled in the head of the swab cannot be effectively released during the lysis step of the extraction.

Both the dry and pre-moistened swabs gave substantially lower DNA yields than the bloodstains collected from the three surfaces. The surface stains gave an average of 60 percent greater DNA yield than compared to the blood added directly to the both dry and pre-moistened swabs. This can be explained by the use of liquid blood added directly to the swab head versus the collection of a dried bloodstain. In the case of the dried bloodstain collection from the surfaces, blood cells lysed on the surface where it was deposited. The contents of the dried bloodstain were transferred onto the head of the swab. The flakes of blood that were picked up

by the swab remained on the surface of the swab head. Once the swab head was cut and placed into the LySep™ column, the lysis buffer was added. During the incubation step, the dried blood flakes dissolved into the lysis buffer and the DNA was released into the lysis solution prior to the purification on the AutoMate *Express*™ instrument. In contrast, when blood was added directly to the swab head, the DNA became entrapped in the fibers of the head, and was not effectively released into the lysis buffer. Evidence of dried blood flakes are illustrated in Figure 17.



Figure 17. Dried Blood Flakes: On the left are flakes of dried blood on a cotton swab and on the right are dried blood flakes from the glass surface.

Two factors should be taken into consideration when evaluating the efficiency of DNA recovery between different swabs. The first factor that must be considered is if the swab has the ability to efficiently collect the dried bloodstain and the associated DNA. The second factor, is whether the swab can efficiently release the DNA that has been transferred from the sample. A swab may be effective at collecting the DNA from a dried bloodstain; however, if the DNA cannot be efficiently released during the extraction process then DNA recovery is diminished.

Both types of swabs in this experiment produced a sufficient amount DNA recovered. The recovery of DNA was 50 percent for both the glass and marble surfaces. The wood surface

averaged 25 percent of DNA recovery from both swabs. From the wood surface the loss of recoverable DNA was substantial; this can potentially be attributed to the absorbent texture of wood. The collection of the blood from the three surfaces was relatively easy despite the varying textures. Both types of swabs provided sufficient DNA to yield full profiles from the bloodstains collected from all three surfaces.

The average yield of DNA from the cotton and FLOQ™ swab from both individuals across the three surfaces showed that the average DNA yield of the FLOQ™ swab was higher than the cotton (1.11 ng/μl for the cotton tipped swab and 1.55 ng/μl for the FLOQ™ swab). The Mann-Whitney U test indicated that the average increase in DNA yield of the FLOQ™ over the cotton swab was statistically different based on the 0.05 significance level.

From the results of the STR amplification, both the cotton and the FLOQ™ swabs, moistened with either dH₂O or PBS, produced high quality profiles from both individuals. No evidence of degradation was present in the STR profiles, as illustrated by the allele peak heights across the loci. No evidence of allelic dropout was observed in any of the samples. These results were what would be expected with fresh blood samples and based on the 1 ng of DNA input in the STR amplification reaction. From Individual 1, on the wood surface of the FLOQ™ swab and PBS collection, additional peaks were observed, which indicated the possibility of another profile other than the blood donor in three of the loci. The results of the reagent blanks and negative controls revealed no evidence of laboratory contamination. The profiles from the surface of the wood could be attributed to the processing of wood; it was possible that the DNA contribution of these additional peaks may have occurred during the manufacturing process or subsequently the handling of the wood before it entered the laboratory.

The FLOQ™ swab was designed to include an easy break point close to the swab head. The molded plastic of the FLOQ™ swab and the break point in the shaft allowed for the swab head to be easily snapped. The break point also made it easy to determine where the swab should be cut so that it could fit into the LySep™ column. The sticks of both the cotton and the FLOQ™ swabs were easily snapped. However, the cotton swab wooden stick allowed for more vigorous swabbing than that of the FLOQ™ swab which tended to bend during the process of sample collection.

A factor that cannot be overlooked in the choice of a swab and hydrating agent is the cost of the materials. The FLOQ™ swab was ten times greater in cost than the cotton swab (\$0.10 for the cotton swab and \$1.00 for the FLOQ™ swab) (11). The difference in cost is notable with laboratories that process a high number of samples. Alternatively, the cost of additional sample processing when the nuclear DNA obtained from a biological sample is insufficient to produce an identifiable profile, additional testing of the sample may be required. The additional cost of reagents and time required by the lab to further process samples which yielded insufficient DNA would justify the use of the FLOQ™ swab over the cheaper option of the cotton swab.

A visual inspection of the surfaces before and after sample collection showed no significant differences between the two types of swabs shown in Figure 18. Similarly, a visual inspection of the cotton and the FLOQ™ swab after the process of extraction showed no indication of blood left behind on either of the swabs.

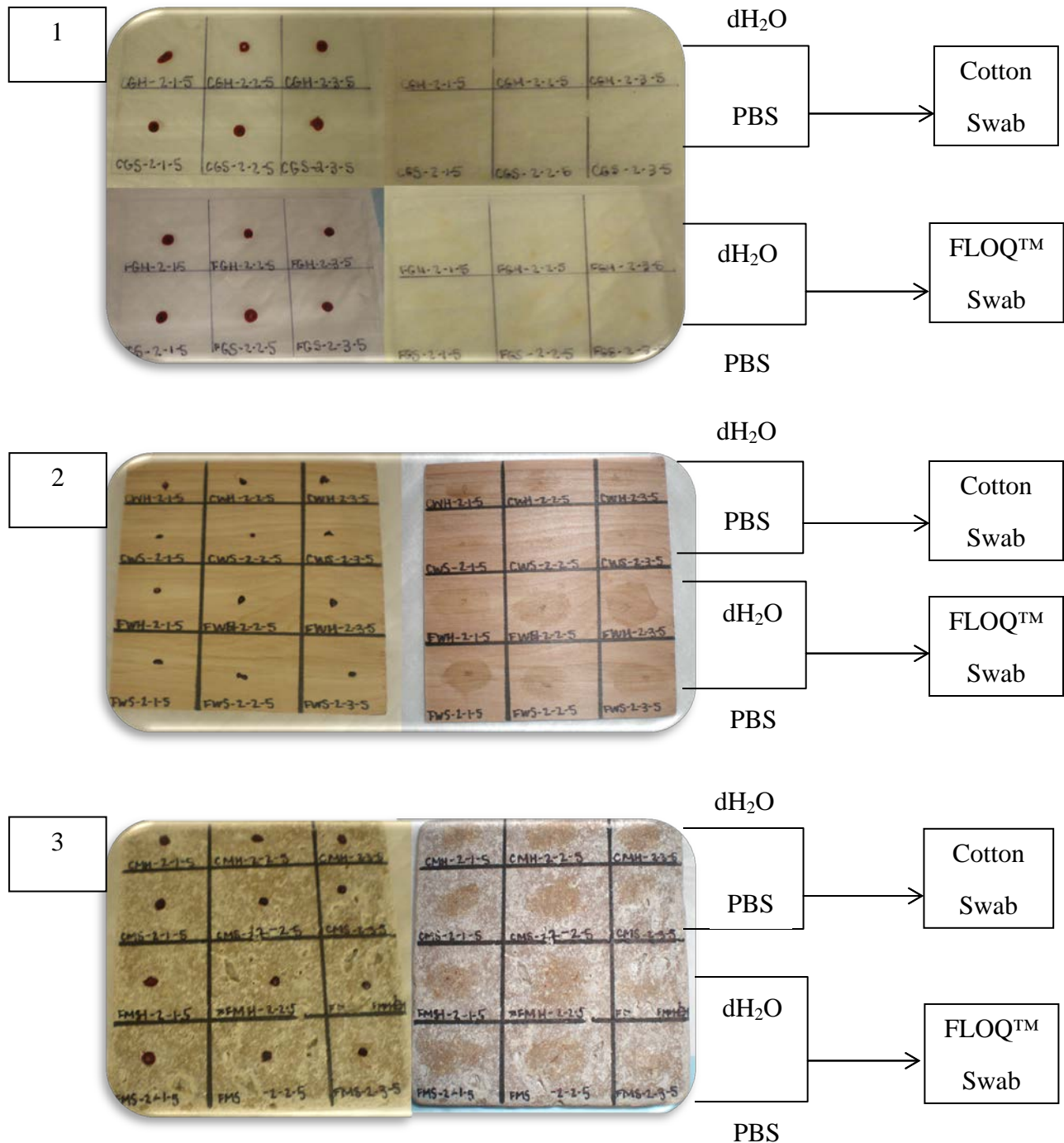


Figure 18: Before and After Pictures of the Surfaces: On the left, 5 μ l of blood added in descending order to 1) glass, 2) wood, and 3) marble surface. On the right, the surfaces after sample collection using the cotton and the FLOQ™ swab with dH₂O or PBS. The first two rows of each surface represent a replicate that was collected using a cotton swab. Rows three and four on each surface represent a replicate that was collected using the FLOQ™ swab. Rows 1 and 3 were collected using dH₂O and rows 2 and 4 were collected using PBS solution. The columns represent the replicates.

There was no evidence to suggest, based on the results of the quantification or in the quality of the profiles obtained, that the use of dH₂O versus PBS for evidence collection had an advantage over the other. The yield of DNA from blood added directly to both the pre-moistened cotton and the FLOQ™ swabs, the use of PBS to moisten the swabs gave an average of 30 percent less DNA yield than the use of dH₂O. However, the average results of dH₂O and PBS collection across the three surfaces are similar (1.28 ng/μl for dH₂O and 1.35 ng/μl for PBS). The results of the Mann-Whitney U test indicated that there is no significant difference in the use of dH₂O and PBS for sample collection. For the use in crime scene evidence collection, single use ampoules of dH₂O or PBS can be easily carried in a kit. Single use ampoules are routinely used by crime scene investigators. On average, a sterile saline ampoule costs twice as much as a sterile dH₂O ampoule (11). Since there was no distinguishable difference in the average DNA yield between dH₂O and PBS collection, the use of dH₂O to moisten swabs prior to the collection of bloodstains, the logical choice.

CHAPTER 5

CONCLUSION

In conclusion, the results obtained in these experiments indicated that both types of swabs were capable of recovering sufficient DNA to produce complete STR profiles. The FLOQ™ swab consistently averaged higher DNA yields than the cotton in all of the replicates with a few exceptions. Based on the data generated, the average yield of the FLOQ™ swab was shown to be statistically different than the cotton tipped swab. The use of dH₂O or PBS collection showed no statistically significant difference in the yield of DNA. Although the FLOQ™ swabs consistently provided greater DNA yields, the results of the electropherogram data showed no difference in the quality of the profiles produced from 1 ng of DNA input. There was no evidence of inhibition or degradation from the DNA recovered from either of the swabs, the hydrating agent, or any of the surfaces.

The data appeared to indicate that DNA yields are consistently lower when blood is added to directly to swabs. The conclusion drawn from the experimental control of blood added directly to swabs emphasize that DNA from cells lysing on a swab become entangled within the fibers of a swab head. Once cells lysed and nuclear membrane broke down, the surface upon which the blood was deposited greatly affected the ability of recovering the DNA.

The average recovery of DNA from the FLOQ™ swab was 40 percent greater than cotton swab across the glass, wood, and marble surfaces. The average yield of DNA for the cotton swab was 1.11 ng/μl and 1.55 ng/μl for the FLOQ™ swab. The results of the Mann-Whitney U test indicated that the increase in DNA yields of the FLOQ™ swab were significant at the 0.05 significance level, excluding the marble surface replicates with Individual 1 and PBS collection. The data showed the average yield of DNA from dH₂O was 1.28 ng/μl and 1.35 ng/μl for PBS

collection. The results of the Mann-Whitney U test, which compared the swabs moistened with either dH₂O or PBS collection, showed that there was no significant difference between the two hydration agents.

A few limitations in this study were: the small replicate size; the number of donors used; and the inherent difficulty in working with whole blood. Based upon these results, for future studies the number of replicates and the number of donors could be increased. By increasing the replicate size, outliers within data sets become less significant and would have a lower potential of skewing the data. The standard deviation could potentially be decreased with a larger number of replicates. The two donors in this study were both males. In addition to more donors, blood from both males and females should be collected in future studies. Initial attempts were made to collect blood without any anticoagulant; however, the blood was difficult to pipette because it began to rapidly clot from the time that it was drawn. Due to the inconsistency in some of the replicates and the viscous nature of blood, the variability could potentially be reduced by diluting the blood in PBS solution before spotting the surfaces. However, for the purpose of this research, the decision was made not to dilute the blood in PBS in order to mimic crime scene stains as closely as possible. This research could be further expanded upon by the comparison of the cotton and the FLOQ™ swab with touch DNA samples or aged samples that have been subjected to harsh environmental conditions. The bloodstains could be prepared by exposing them to different temperatures, humidity, varying light sources, or other factors that would replicate harsh environmental conditions. The bloodstains could also be collected at intervals of time in an attempt to determine if blood collected at different time spans has any effect on the quantity or quality of the DNA profiles produced.

Distilled H₂O, is the standard means of transferring evidentiary samples to swabs. Distilled H₂O collection, was less expensive compared to the cost of PBS. In cases where the size of the bloodstain appears to be substantial, the cotton swab moistened with dH₂O was the most cost effective choice. However, in the majority of the replicates, the FLOQ™ swab consistently averaged higher DNA yields over the cotton tipped swab (between 30 and 40 percent greater). From the results of the experimental data, the FLOQ™ swab was the better choice to optimize the maximum collection of DNA from bloodstains. Based upon the evidence obtained in this study, the next step in the evaluation of nylon 4N6FLOQSwab™ should be for smaller bloodstain samples that appear to be low in quantity or possibly degraded in an attempt to maximize DNA evidence collection.

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