Technical Note

Exploring the Potential of a Wet-Vacuum Collection System for DNA Recovery

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Abstract: Traditional biological collection methods are compared to a wet-vacuum system through the collection of different volumes of blood on tile, denim, and carpet. The wet-vacuum technique was able to recover sufficient amounts of blood for Kastle-Meyer presumptive testing. Although it was possible to detect blood after wet-vacuum collection, swabbing resulted in the highest rate of positive results for the presumptive test.

The DNA yields and detection limits that were obtained when collecting from tile were similar between methods, suggesting they are equivalent in their ability to collect DNA from nonporous surfaces. When the techniques were tested on mock case surfaces, wet-vacuum collection resulted in higher DNA yields than either the double swab or taping methods. However, STR profiles that were obtained from these mock surfaces exhibited extraneous alleles at many loci, suggesting that these higher yields were the result of collecting DNA already present on the substrate.

The wet-vacuum collection efficacy was further tested by examining yields that were obtained when semen and blood were collected from tile, denim, carpet, and brick. Results show that the technique was successful in collecting DNA from all surfaces, although the yield from brick varied widely and was low compared to the other substrates. Of the 16 low-volume samples collected from brick, 8 resulted in no detectable DNA.

Tests that examined the wet-vacuum technique's propensity to spread sample were also performed and demonstrated that DNA was detected up to 4 inches from the collection site, suggesting caution must be taken if collecting biological evidence that is in the vicinity of another probative sample.

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Introduction

The goal of evidence collection is to gather and preserve biological material. There are several methods commonly used for gathering cellular material: cutting, swabbing (double or single swab method), taping, and scraping [1–4]. Cutting generally consists of cutting a small section of material with scissors or a scalpel and placing it in a vial with appropriate buffer for downstream analysis. Although cutting is a convenient way of processing samples, there are a number of issues associated with it. First, the cutting must be small enough to be readily processed. Additionally, during presumptive or DNA procedures, the substrate is usually soaked in buffer, which can introduce the co-elution of known PCR inhibitors. Although new commercially available amplification kits designed to minimize the effects of inhibition exist, these amplification kits are not the complete answer. The optimal recourse would be to apply a sampling method designed to dislodge cells from the substrate while minimizing the co-elution of inhibitors. Both the single and double swab techniques accomplish this.

In the single swab method, a sterile cotton swab is moistened with water or buffer to facilitate the gathering of material from the item of interest. With the double swab method, one swab is moistened and then rubbed across the substrate, and then the second swab is kept dry and rubbed over the same area, and both swabs are processed together. Although this method minimizes the co-elution of inhibitors, the area that can be processed is relatively small. Another concern related to swabbing is that of cell elution off of the swab [5].

A less common collection technique is the taping method. With this method, adhesive tape is pressed on the surface and then pulled away from the substrate. Although this method can cover larger areas than the cutting and swabbing methods, the surface area that can be processed is still relatively small because the tape loses some of its ability to collect biological material with every placement [6].

The scraping method is one that involves the use of a razor or blade or similar tool. The tool is scraped along the article to remove surface particles and cells. A receptacle or clean paper is placed under the item to catch the dislodged material. The advantage of this type of collection is that large surface areas may be sampled. However, the likelihood of retrieving all material from the receptacle is small, especially if it is of a porous nature. In work by Stouder et al., previously worn

garments were scraped and the scrapings were transferred to a pill box. The pill box was then swabbed with a moistened swab, again introducing the issues associated with swabbing [3]. Also, by swabbing the pill box, an extra transfer step is introduced, increasing the chance of contamination.

Although the aforementioned techniques have been a mainstay of forensic analysis, each of the methods has significant drawbacks related to it, which include, but are not limited to, the lack of surface area that may be processed, possible co-elution of PCR inhibitors, and non-optimized elution of cells from the substrate into solution. A technique designed to optimize biological collection from items of interest, particularly large items, is necessary and not currently available for forensic use. One technique recently introduced to the field is a wet-vacuum system. This system consists of a vacuum, handset-collection device, sample bottle, and sterile buffer. The buffer is aspirated onto the stain and the vacuum simultaneously collects the buffer and any cellular material on the surface. Previous work in the field of pathogen testing [7] suggests a wet-vacuum system may have a place in the evidence collection lexicon, and an evaluation of the feasibility of use for DNA collection purposes is therefore necessary.

The purpose of this study was threefold. As a result, the study consisted of three phases. The first aim of the study was to quantitatively compare traditional biological collection methods to a wet-vacuum collection system. In this phase, the double swab and taping methods were compared to the wet-vacuum method through the collection of different volumes of blood on sterile and nonsterile surfaces. The sterile surface was a ceramic tile and the nonsterile surfaces were denim and carpet. Twenty-five percent of each bloodstain was subjected to the Kastle-Meyer colorimetric test to evaluate whether wet-vacuum collection samples performed similarly to tape and swab samples during presumptive screening; the remaining 75% of each sample was subjected to DNA extraction and quantification. In addition, one replicate from each collection set was amplified and profiles were generated. The STR profiles were used to determine the minimum number of contributors for each profile and to determine the propensity to collect background DNA. Second, a study designed to establish the potential for the wet-vacuum system to inadvertently spread sample to adjacent surfaces was completed. Lastly, dilution series' of blood and semen were collected from brick, denim, carpet, and tile to confirm the efficacy of wet-vacuum collection on a variety of substrates and body fluids.

Materials and Methods

All aspects of this study were conducted in compliance with ethical standards set forth by the Institutional Review Board. Unless otherwise stated, all reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Phase I: Comparison Between Collection Techniques

One series of blood dilutions (neat, 1:9, 99, and 999) was prepared by mixing the appropriate amount of blood and TE (Tris-EDTA; Ethylenediamine Tetra-Acetic Acid; $1x10^{-4}$ mM). For the double swab and taping collections, a volume of 75 µL of the blood dilution was spotted onto one half of the tile, carpet, or denim. Another 25 µL was spotted onto the other half of the surface to be used in presumptive testing experiments detailed below. The samples were split in 75 and 25 µL portions, based on the presumption that there would be an attempt to conserve as much of the evidence as possible for DNA testing. Thus, the study was designed to mimic procedures in which the sample is exhaustively tested. In such cases as these, it is not uncommon for laboratories to consume a smaller portion of the sample for presumptive testing so the majority of the biological evidence is preserved for downstream DNA testing. For the wet-vacuum collections, the M-Vac (M-Vac Systems, Inc., Sandy, UT) was used and the full volume of blood was spotted onto the tile, collected, and filtered. Each dilution, spotted in triplicate, on each of the surfaces was allowed to dry prior to collection. In conjunction with the collection of blood dilutions, an unstained surface was used as a control. The surface controls were collected and processed through presumptive and DNA testing at the same time and in the same manner as the samples.

The first biological collection technique to be tested was the double swab method. Cutting was excluded as a collection method because it was deemed unlikely that ceramic tile would be cut during collection. A volume of 50 µL of DI-DNA/RNAase free H_2O was pipetted onto a sterile cotton swab. This swab was rotated during collection of the bloodstain. After the use of this wet swab, a dry cotton swab was used to collect residual sample. This second swab was also rotated during collection. The swabs were allowed to dry.

The second collection method was taping. The tape (BVDA Instant Lifters, Haarlem, The Netherlands) was first cut into 8 cm x 2 cm pieces. At 1 cm from each end of the piece of tape, a small slit was made. These 1 cm flaps were used to hold each

piece of tape during collection so as to collect each sample with the full 6 cm x 2 cm tape piece. Before each collection, both sides of the tape pieces were UV irradiated. They were then held on each side using the 1 cm flaps and pressed against the sample 20 times. The tape was then either adhered to a piece of clean filter paper for presumptive testing or placed into a clean weigh boat, covered, and allowed to sit overnight before DNA extraction.

The last collection method tested was wet-vacuuming (M-Vac System, M-Vac Systems Inc., Sandy, UT). Figure 1 shows a schematic of the wet-vacuum system. Prior to sample collection, the sterile collection buffer was placed into the designated pressurization chamber, and the tubing and collection device were attached as per the manufacturer's recommendations [8].

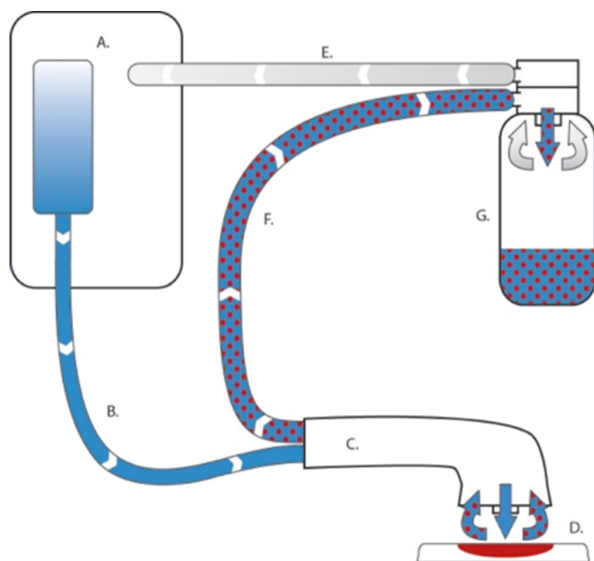

Figure 1

Schematic of the wet-vacuum collection system. The support equipment case (A) delivers pressurized buffer (B) to the collection head (C). Sterile buffer is expelled onto the stained substrate and immediately recovered (D). Negative pressure is applied to the system (E), creating suction that transports sample-containing buffer (F) into the sterile collection bottle (G).

Briefly, with the vacuum switch in the off position, the sampling handset of the wet-vacuum system was placed perpendicular to the surface. The vacuum switch was turned on and the buffer switch located on the handset was pushed to the on position. By steadily holding the handset on the surface, the handset was continuously repositioned over the stain such that the entire stain made contact with the buffer. A volume of 100 mL of buffer was used to collect each sample. Once the sample was collected, the buffer switch was turned off while the vacuum remained on and the handset continued to be in contact with the surface for an additional 5 seconds. The vacuum was then switched off and the collection bottle was detached for processing. Following this, the solution in the collection bottles was filtered through Millipore-Durapore 0.45 µm membrane filters. The filter paper was allowed to dry and was then cut into ¼ and ¾ slices for presumptive and DNA processing, respectively. If it is assumed that the cellular material is equally dispersed in the M-Vac collection buffer, and the filtering occurs through the center of the apparatus, then cutting the filter into ¼ and ¾ slices represents 25% and 75% of the available biological material. A negative glassware control was collected using a sterile swab, moistened with DI H_2O. This swab was rubbed across the glassware, focusing on the areas where the DNA may have come into contact. Negative glassware controls all showed expected results (data not shown).

Once collected, each 25 µL dilution was presumptively tested for blood using the Kastle-Meyer colorimetric test. For swabs, the tip of the swab was cut off and placed in a 12-well ceramic spot plate. The reagents (10 µL phenolphthalin followed by 10 µL hydrogen peroxide) were added to the swab tip. For taped samples, Kastle-Meyer reagents were added to the filter paper backing to which the tape was adhered following collection, and allowed to run through to the cellular material on the tape surface. Color change results were observed through the transparent tape. On the Durapore membranes from M-Vac collection and filtration, reagents were added directly to the surface of the ¼ slice of filter paper. Presumptive test results were visually assigned positive and negative values.

When extracting DNA from the swabs, the cotton portions of all swabs were cut using sterile scalpels and placed into 2 mL microcentrifuge tubes. Prior to placing the tape into the tube, each 1 cm flap that was used to hold the tape during collection was removed and discarded. The tape was cut into smaller pieces with sterile scissors and placed in 2 mL microcentrifuge tubes. A volume of 75 µL of the blood dilution was collected for DNA

processing. When using the wet-vacuum technology to collect, a full 100 µL was collected at the same time. The membrane slice representing 75 % of the sample was then cut into small pieces and placed into a 2 mL microcentrifuge tube. After transferring the substrates or whole blood to the tubes, DNA extraction using the QIAamp Investigator extraction protocol (Qiagen, Valencia, CA) ensued as per the recommended protocol [9]. The final DNA extract volume was 20 µL. DNA quantification was performed using the Quantifiler Duo Quantification Kit (Applied Biosystems, Foster City, CA) as per the manufacturer's recommendations [10] using the 7500 Detection System (Applied Biosystems, Foster City, CA). The C_T results were analyzed using a single calibration curve as per methods described elsewhere [11, 12]. Differences in DNA yield were examined by comparing the average and the minimum and maximum yields obtained. If there was overlap between yields, then the collection methods were not considered significantly different. The false negative rates (i.e., the number of samples resulting in nondetection of DNA as per qPCR) were also examined. The replicate from each collection set and the surface controls with the highest DNA yields were subjected to STR analysis. This resulted in 36 DNA profiles and 9 substrate control profiles. Amplification was performed using the PowerPlex 16 HS System (Promega, Madison, WI) and, depending on quantification results, a target of either 0.7 ng or 10 µL of extract was utilized. Capillary electrophoresis was performed using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and a 10-second injection time and 3-kV injection voltage. Fragment analysis was accomplished using the GeneMapper ID-X Software v 1.1.1 (Applied Biosystems, Foster City, CA) and an analytical threshold of 50 RFU. Artifacts, including stutter, minus A, and pull-up, were removed prior to interpretation.

Phase II: Determination of the Wet-Vacuum Collection Range

Tiles containing the equivalent of 100, 10, 1, or 0.1 µL of blood were surrounded on all sides by four sterilized tiles. As shown in Figure 2, the blood sample spotted on the center tile was then collected using the wet-vacuum technique. Following collection, the surrounding four tiles were swabbed using the double swab method at 1-inch increments to collect any sample that may have spread during the wet-vacuum collection process. This was performed in quadruplicate. These swabs were dried and stored until DNA testing (described above) ensued.

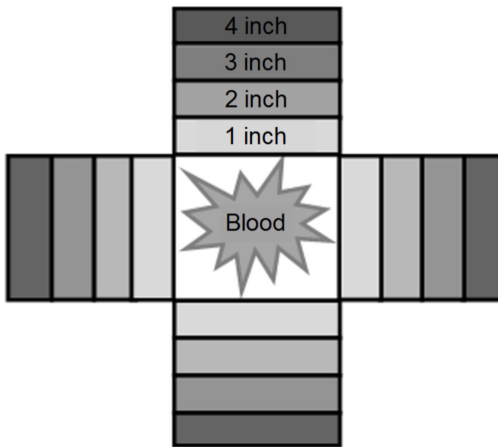

Figure 2

Schematic of the clean tiles arranged around the sample tile containing 100, 10, 1, or 0.1 µL. The shaded areas represent the 1-, 2-, 3-, and 4-inch sample areas that were collected via swab for DNA.

Phase III: Wet-Vacuum Collection of Blood and Semen

Four substrates were chosen to further examine the efficiency of vacuum collection for forensic purposes. These substrates were tile, brick, carpet, and denim. The tile was first cleaned by soaking in a 10% bleach solution for 10 minutes and then wiped with 70% ethanol. The brick was cleaned using water to remove any loose cement fragments and dirt. The carpet and denim were used without additional cleaning. Four volumes of blood and semen were chosen to test the recovery of the vacuum collection. The volumes were 100, 10, 1, and 0.1 µL. The whole blood and semen samples were spotted onto the chosen substrate via pipette and allowed to dry before collection. Each test was conducted in quadruplicate. The wet-vacuum collection occurred as previously described, except the collection volume was 150 mL. Extraction and quantification of the DNA was completed in the same manner as described above.

Results

Phase I: Comparison Between Collection Techniques

To compare the efficacy of each collection method to collect DNA, the average concentration of DNA from tile was determined and compared against the concentrations for whole blood extractions. Table 1 shows the average, minimum, and maximum yields of DNA detected from each surface, for a given volume of blood, using three collection methods: the double swab, taping, and wet-vacuum collection techniques. When 75 µL of whole blood was extracted, the DNA yield ranged from 902 to 1200 ng. This range was slightly lower than the range recovered when the double swab method was utilized to collect the same volume of whole blood (i.e., 1344 to 1611 ng), suggesting that for this volume, the test samples had slightly more cellular material than the whole blood samples. When comparing between methods, Table 1 indicates that all methods had similar collection efficiencies when collecting blood from tile. Except for the 75 µL sample, all collection methods resulted in 50 to 80% lower DNA yield than the whole blood extraction.

Substrate	Collection Method	75 µL	7.5 µL	0.75 µL	0.075 µL
	Whole Blood	1044 (902–1200)	129 (73–211)	10 (9–11)	0.7 (0.5–1.1)
Tile	Double Swab	1507 (1344–1611)	55 (30–92)	3 (2–4)	0.2 (0.1–0.4)
	Tape	995 (781–1317)	26 (20–37)	2 (1–3)	0.4 (0.2–0.6)
	Wet-Vacuum	1321 (1245–1373)	63 (51–82)	4 (4–6)	0.2 (0.06–0.4)
Denim	Double Swab	175 (163–181)	10 (6–14)	0.3 (0.1–0.4)	0.03 (0–0.08)*
	Tape	65 (42–104)	45 (34–57)	2 (1–4)	0.08 (0.05–0.13)
	Wet-Vacuum	1290 (1263–1326)	96 (94–97)	3 (3–3)	0.4 (0.2–0.9)
Carpet	Double Swab	542 (448–630)	23 (10–41)	0.2 (0.1–0.2)	0.03 (0–0.05)
	Tape	174 (148–194)	5 (2–7)	2 (1–4)	0.03 (0–0.05)
	Wet-Vacuum	729 (592–821)	13 (7–16)	2 (1–2)	0.6 (0.4–0.8)

*Two samples resulted in a recovery of 0 ng of DNA

Table 1

Average DNA yield (minimum to maximum) in ng as per qPCR after collection of various volumes of blood with each method from tile, denim, and carpet.

To further compare between methods, the limit of detection (LOD) for each procedure was calculated. The LOD represents the smallest volume of blood that can reliably be detected [13]. To calculate the LOD, a comparison of the volume of whole blood collected to the average cycle threshold (C_T), obtained from qPCR, for each collection method was made. This was followed by an ordinary least squares linear regression to determine the slope, *y*-intercept, and their respective errors. In TaqMan-based qPCR assays, the fluorescence signal is measured at every cycle. The cycle at which the fluorescence crosses a predetermined signal threshold is recorded as the C_T. If there is a large quantity of DNA, the fluorescence will cross the threshold at early cycles; if there is a small quantity of DNA, the fluorescence will cross the threshold at higher cycle numbers. Thus, the C_T is indirectly proportional to the logarithm of the concentration of DNA. Figure 3 shows the resultant best-fit curve for each collection method and its respective regression equation and correlation coefficient.

Specifics regarding the methods by which the LOD can be calculated from qPCR have previously been described [14]. When collecting from tile, each method shows similar slopes, suggesting the collection efficiencies for each of the methods is

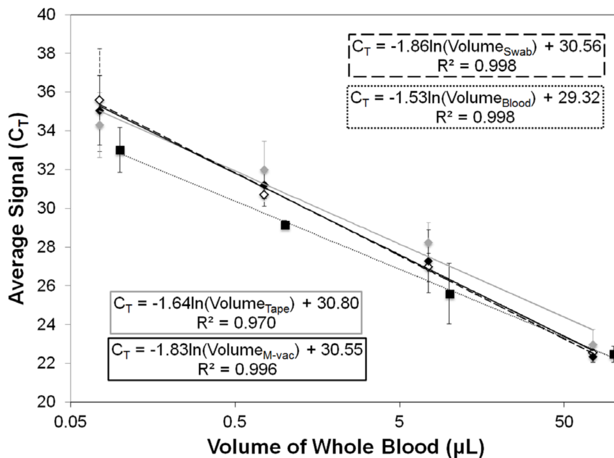

Figure 3

The average C_T obtained from qPCR plotted against the volume of whole blood (µL) collected from tile and its respective equation using the (–♦–) double swab, (–♦–) taping, and (---◊---) wet-vacuum techniques. (·····■·····) represents the signal obtained when whole blood was extracted.

equivalent. When extracting whole blood using Qiagen columns, only 2.4 nL of whole blood was required to obtain reliable detection and supports the common view that DNA profiling is a powerful tool for identity purposes. When collecting from tile, the wet-vacuum system, the double swab method, and taping all resulted in similar LODs of 15, 14, and 13 nL, respectively. The similar LODs support the findings in Table 1 and suggest that each method is equivalent in its ability to collect biological material from tile. These data also indicate that only nanoliters of blood are required for DNA detection, supporting the notion that DNA testing is powerful and that a sufficient amount of genetic information can be garnered from very small stains. In contrast, the DNA yields (Table 1) obtained from mock case surfaces, such as denim or carpet, suggest that the collection method did impact the ability to recover the biological specimen.

Table 1 summarizes the DNA yield that was retrieved from denim for all volumes of blood using each method. When collecting from denim, regardless of volume, the wet-vacuum collection technique resulted in the highest average DNA yields. When examining the range of yields obtained for a given blood volume, the minimum yield from the wet-vacuum system was a

factor of one to eight greater than the maximum yield of either of the other two methods. For the lowest blood volumes (i.e., 0.75 and 0.075 µL), the wet-vacuum and taping methods resulted in consistently greater yields than the double swab method and never resulted in a nondetection of DNA, whereas the double swab technique resulted in two false negatives. The differences between wet-vacuuming and taping are less consistent: only the 7.5 and 0.075 µL samples exhibited improved collection with the wet-vacuuming over taping. Taping never resulted in a yield that was consistently greater than yields obtained with vacuuming.

For the samples collected from carpet, the DNA yields (Table 1) that were obtained between collection methods show that when collecting 75 µL of blood, wet-vacuum and double swab methods collected significantly more DNA than the taping technique. When 7.5 µL of blood was deposited and collected, the double swab method yielded greater DNA levels than taping. The wet-vacuum technique and taping outperformed swabbing when 0.75 µL of blood was deposited and collected, and wet-vacuuming outperformed both methods when the smallest volume of blood (i.e., 0.075 µL) was collected. Furthermore, when 0.075 µL of blood was collected, both the double-swab and taping techniques resulted in one false negative, indicating the absence of detectable DNA.

In summary, of the four volumes tested from denim and the four volumes tested from carpet, all showed that wet-vacuum collection resulted in consistently higher yields of DNA than either the double swab or taping methods. The double swab method exhibited significantly higher yields than taping three times (75 µL from denim and carpet, and 7.5 µL from carpet), and taping consistently outperformed double-swabbing three times (7.5 µL from denim, and 0.75 µL from denim and carpet). Neither the double swab nor taping method was shown to outperform the wet-vacuum technique in any instance, although there were occasions where the differences were indiscernible.

To examine whether the discrepancy between the LODs obtained from sterile tile and the DNA recoveries obtained from the mock surfaces is exclusively due to the ability of the wet-vacuum technique to more efficiently collect the source DNA from these items, STR profiles were obtained. Figure 4 displays the blue STR loci of the 0.075 µL sample collected from carpet via double swab, taping, and wet-vacuuming. Some of the DNA profiles indicate the presence of multiple contributors, whereas other profiles are incomplete. No indication of PCR inhibition was detected, as evidenced by the peak height balance between loci. Additionally, the average C_Ts of the IPCs (internal PCR controls) for all samples ranged from 29.3 (± 0.1) to 29.6 (± 0.3), indicating the absence of inhibition.

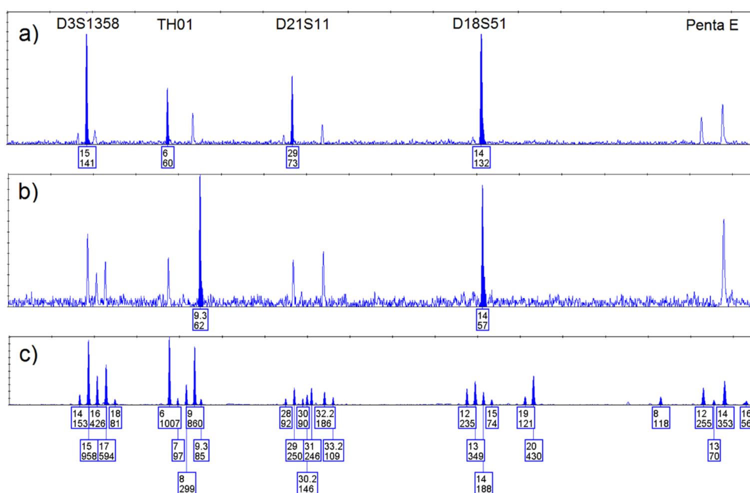

Figure 4

Representative electropherograms from the blue-labeled loci obtained after amplification with PowerPlex 16 HS. In this example, 0.075 µL was collected from carpet using (a) double swab, (b) taping, and (c) wet-vacuum methods.

Kastle-Meyer presumptive testing results are depicted in Table 2, and Table 3 depicts the minimum number of contributors determined from the STR profiles. The minimum number of contributors for the substrate controls is also provided. The presence of background DNA is observed on the denim and carpet when taping and wet-vacuuming is utilized as the collection method.

Collection Method	Substrate	25 µL	2.5 µL	0.25 µL	0.025 µL	0.0025 µL	0.00025 µL
Swab	Tile	+	+	+	+	+	-
	Denim	+	+	-	-	-	-
	Carpet	+	+	+	-	-	-
Tape	Tile	+	+	+	+	-	-
	Denim	+	+*	-	-	-	-
	Carpet	+	+	+*	-	-	-
Wet-Vacuum	Tile	+	+	+	-	-	-
	Denim	+	+	-	-	-	-
	Carpet	+	+	+	-	-	-

Table 2

Each volume was tested in triplicate. (+) indicates a positive result on all three replicates; (–) indicates no visible color change reaction on any of the three replicates; (*) indicates that only one of three replicates yielded a positive result at this blood volume.

Collection Method	Substrate	75 µL	7.5 µL	0.75 µL	0.075 µL	Substrate Control
Whole Blood Extractions	N/A	1	1	1	1	N/A
Double Swab	Tile	1	1	1	1	0
	Denim	1	1	1	1^{4}	0
	Carpet	1	1	1	1^{14}	0
Tape	Tile	1	1	1	1	0
	Denim	1	1	1	2	1
	Carpet	1	1	1	1^{7}	0
Wet-Vacuum	Tile	1	1	1	1	0
	Denim	1	1	1	3	1
	Carpet	1	1	3	4	3

Table 3

Minimum number of contributors determined from STR profiles examined from samples of 0.075–75 µL blood collected using various methods. If there were instances of drop-out, then the number of alleles detected is superscripted.

Phase II: Determination of the Wet-Vacuum Collection Range

Another aspect to consider with wet-vacuum collection is that the system sprays the collection buffer from the center of the handset with force. Therefore, it was of interest to determine whether this force is significant enough to propel cellular material from the collection area to surrounding areas. The surface chosen for this experiment was tile because it was hypothesized that this hard, nonporous surface was expected to have the highest potential for buffer propulsion to surrounding areas.

The DNA yield (ng) found at the four distances (i.e., 1 to 4 inches from the collection area) was determined, and the qPCR results are shown in Table 4. The 100 µL blood sample resulted in the highest DNA levels collected from the surrounding areas, whereas the other three volumes resulted in similar concentrations to one another. There was no correlation between distance and concentration of extraneous DNA collected. That is, similar levels of DNA were found at 1 inch and 4 inches away from the collection area.

Volume of Blood Deposited on Center Tile	DNA (ng) at 1"	DNA (ng) at 2"	DNA (ng) at 3"	DNA (ng) at 4"
100 µL	0.02	ND	ND	ND
	ND	ND	ND	ND
	0.34	0.32	0.02	ND
	ND	ND	ND	0.22
10 µL	ND	ND	ND	ND
	0.12	ND	ND	ND
	ND	ND	ND	ND
	0.04	ND	ND	ND
1 µL	ND	ND	0.06	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	0.02	ND	ND	ND
0.1 µL	ND	ND	ND	ND
	ND	0.12	ND	ND
	0.002	ND	ND	ND
	ND	ND	0.02	ND

Table 4

Yield of DNA detected via qPCR after swabbing the tiles surrounding the area of interest. ND = not detected.

Phase III: Wet-Vacuum Collection of Blood and Semen

In another set of experiments, blood and semen were spotted onto four new substrates (denim, carpet, tile, and brick) at decreasing volumes (100, 10, 1, and 0.1 µL). These samples were then collected with the wet-vacuum technique, concentrated via filtration, extracted, and the DNA was quantified. Data in Table 5 show the average DNA yield, the minimum and the maximum yield resulting from wet-vacuum collection from multiple substrates. Similar to the results from Phase I, denim and carpet were the two substrates that resulted in detectable DNA in the substrate controls. This level of DNA from the substrate controls ranged from 0.012 to 0.02 ng. Brick has traditionally been considered a difficult substrate from which to collect samples. It was therefore of interest to test the collection capability of the wet-vacuum technique on this substrate. The wet-vacuum collection technique was able to recover DNA from brick when 100, 10, and l µL of blood was deposited. However, there was one false negative when 1 µL of blood was deposited on brick. All samples showed insufficient DNA yield when 0.1 µL of blood was deposited on brick. These results, in general, are consistently lower than the results obtained from tile, carpet, and denim, indicating that collection efficiency is highly dependent on substrate and less so on volume of fluid or fluid type. Results from the collection of semen from the tile, denim, and carpet show the same trend. In general, the range of DNA yields obtained from collecting semen from brick using wet-vacuum collection was large. For example, when collecting 100 µL of semen from brick, the final yields were 5, 10, 1084, and 1203 ng, showing a 3-order of magnitude difference between the minimum and maximum yields. The large discrepancy between samples on brick was the result of depositing the semen on different sides of the brick, where one side was glossy, indicating a type of coating. The side that was coated gave rise to higher yields and better collection capability, again indicating that the substrate type or surface treatment has a significant impact on the ability to efficiently collect biological evidence. It should be noted that neither taping nor swabbing of the brick was deemed practically feasible because the brick caused fraying of the cotton swab or loss of adhesiveness of the tape on contact.

Substrate	Body Fluid	100 µL	10 µL	1 µL	0.1 µL
Tile	Blood	1173 (1091-1298)	61 (43–69)	8 (7–10)	0.2 (0.1–0.2)
	Semen	2132 (1715–2378)	193 (151–236)	11 (2–24)	2 (1–3)
Denim	Blood	308 (264–361)	23 (22–24)	3 (2–5)	0.3 (0.2–0.6)
	Semen	2015 (1556–2361)	221 (202–231)	20 (13–23)	3 (2–3)
Carpet	Blood	225 (176–259)	36 (14–51)	8 (6–8)	2 (1–4)
	Semen	1635 (1099–2297)	190 (142–223)	15 (9–20)	2 (0–3)
Brick	Blood	3 (1–8)	0.2 (0.1–0.3)	0.1 (0–0.3)	0 (0–0)
	Semen	574 (5–1203)	0.3 (0.1–0.6)	2 (0–4)	0.02 (0–0.05)*

*Two samples resulted in a recovery of 0 ng of DNA

Table 5

Average DNA yield (minimum to maximum) in ng as per qPCR after wet-vacuum collection of various volumes of blood and semen from tile, denim, carpet, and brick.

Discussion

For genetic analysis purposes, the results of Phase I of this study indicate that there are instances in which the collection method has an impact on increasing the chances of obtaining larger quantities of genetic material from a substrate. For porous or fibrous surfaces, or in instances where low, inconsistent, or negative presumptive results are observed, there is a better chance of recovering more genetic material with the wet-vacuum technique, such as in the case of the low-volume samples collected from denim and carpet.

In instances where the presumptive result is unambiguously positive or the stain is visible, the wet-vacuum technique is unnecessarily complex. For example, both the wet-vacuum and double swabbing techniques were able to recover similar total quantities of DNA, ample for subsequent genetic analysis, from most blood dilutions on tile. Even in cases where wet-vacuuming is expected to result in higher yields of DNA, STR results suggest that consideration of which collection technique to use should take into account whether the surface is expected to contain large levels of interfering background DNA. The presence of multiple contributors, both in the low-volume and surface control samples, explicates the apparent discrepancy between LOD and yield. That is, the fibrous, nonsterile substrates bore significant background DNA that the wet-vacuum method was

able to collect, suggesting that the wet-vacuum technique is efficient in collecting biological material from these types of surfaces, so much so, that when collecting from porous surfaces in high-traffic areas, the STR profiles may result in complex DNA mixture profiles. Therefore, if a stain is visible, and on the surface of the substrate, taping or swabbing may be the preferred method of collection. In contrast, if the stain is suspected to contain a low concentration of cells, contained in a rough surface and in a low-traffic area, wet-vacuuming may be ideal. Also, the area surrounding the collection site needs to be considered. Until it is demonstrated that wet-vacuuming on fibrous or porous surfaces does not result in sample dispersion to adjacent areas, or until the handset is redesigned to alleviate sample dispersion, collection with wet-vacuum technology should not be performed in the vicinity of another sample that has probative value. This is particularly true if adjacent stains are suspected to originate from different contributors. The potential for sample spread also suggests that evidence collection using wet-vacuum systems may need to be performed in designated areas, where independent samples are not exposed to the buffer from the collection site. Further, if there are two adjacent probative stains, one would have to be collected using traditional techniques (i.e., swabbing, taping, cutting) and the subsequent collection could be performed using any method.

Conclusion

The wet-vacuum collection technique is a potentially useful tool in forensic casework environments. Data indicate this technique may be most valuable for collection of low-level biological evidence in low-traffic areas. If wet-vacuum technology is to be utilized, then probative evidence in the vicinity of the stain which is to be vacuumed should be collected first.

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References

1. Sweet, D.; Lorente, M.; Lorente, J. A.; Valenzuela, A.; Villanueva, E. An Improved Method to Recover Saliva from Human Skin: The Double Swab Technique. *J. For. Sci*. **1997**, *42* (2), 320–322.
2. Li, R. C.; Harris, H. A. Using Hydrophilic Adhesive Tape for Collection of Evidence for Forensic DNA Analysis. *J. For. Sci*. **2003**, *48* (6), 1318–1321.
3. Stouder, S. L.; Reubush, K. J.; Hobson, D. L.; Smith, J. L. Trace Evidence Scrapings: A Valuable Source of DNA? *For. Sci. Comm*. **2001**, *3* (4), 1–6.
4. Mulligan, C. M.; Kaufman, S. R.; Quarino, L. The Utility of Polyester and Cotton as Swabbing Substrates for the Removal of Cellular Material from Surfaces. *J. For. Sci*. **2011**, *56* (2), 485–490.
5. Voorhees, J. C.; Ferrance, J. P.; Launders, J. P. Enhanced Elution of Sperm from Cotton Swabs Via Enzymatic Digestion for Rape Kit Analysis. *J. For. Sci*. **2006**, *51* (3), 574–579.
6. Thornton, K. K. A Comparison of Sampling Methods for the Collection of Cellular Material from Clothing. Master of Science, Boston University School of Medicine, Boston, 2009.
7. Bradley, B.; Saddler, F. Comparisons of Meat Carcass Surface Bacterial Collection Efficiencies Utilizing a Novel Wet-vacuum Microbial Sampler and the Sponge Method. Presented at 54th Annual Reciprocal Meat Conference, Indianapolis, IN, 2001.
8. Microbial-Vac Systems, Inc. SEC 100 User Guide. Microbial-Vac Systems, Inc., Sandy, UT, 2010.
9. *QIAamp DNA Investigator Handbook*. QIAGEN, Hilden, Germany. April 2010.
10. Life Technologies. *Quantifiler Duo DNA Quantification Kit User's Manual*; Life Technologies Corporation: Carlsbad, CA, 2008.
11. Grgicak, C. M.; Urban, Z. M.; Cotton, R. W. Investigation of Reproducibility and Error Associated with qPCR Methods using Quantifiler Duo DNA Quantification Kit. *J. For. Sci*. **2010**, *55* (5), 1331–1339.
12. Grgicak, C. Weighted Regression Template. http://www.bumc.bu.edu/biomedforensic/faculty-and-staff/faculty/grgicak/tools/ (Accessed June 21, 2013).
13. Winefordner, J. D.; Long, G. L. Limits of Detection: A Closer Look at the IUPAC Definition. *Anal. Chem*. **1983**, *55* (7), 712A–724A.

14. Oechsle, C. S.; Haddad, S.; Sgueglia, J. B.; Grgicak, C. M. Screening Biological Stains with qPCR versus Lateral Flow Immunochromatographic Test Strips: A Quantitative Comparison using Analytical Figures of Merit. *J. For. Sci*. **2014**, *59* (1), 199–207.