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PAPER

CRIMINALISTICS

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Comparison of the M-Vac® Wet-Vacuum-Based Collection Method to a Wet-Swabbing Method for DNA Recovery on Diluted Bloodstained Substrates*†‡

ABSTRACT: A wet-vacuum-based collection method with the M-Vac® was compared to a wet-swabbing collection method by examining the recovery of diluted blood on 22 substrates of varying porosity. The wet-vacuum method yielded more total nuclear DNA than wet-swabbing on 18 porous substrates, recovering on average 12 times more DNA. However, both methods yielded comparable amounts of total DNA on two porous and two nonporous substrates. In no instance did wet-swabbing significantly recover more DNA. The wet-vacuum method also successfully collected additional DNA on previously swabbed substrates. Mitochondrial DNA yields were assessed, and outcomes were generally similar to the nuclear DNA outcomes described above. Results demonstrate that wet-vacuuming may serve as an alternative collection method to swabbing on difficult porous substrates and could potentially recover additional DNA on previously swabbed substrates. However, swabbing remains the preferred collection method on substrates with visible stains and/or nonporous surfaces for reasons of convenience, simplicity, and lower cost relative to the wet-vacuum method.

KEYWORDS: wet-vacuum, M-Vac®, wet-swab, blood, DNA collection, DNA extraction, DNA quantification, forensic analysis

The sensitivity of forensic DNA testing has steadily increased and improved over the last 20 years through advances in DNA extraction, detection, and analysis. Yet the routine use of conventional collection methods, for example, swabbing (1–4), cutting (5,6), taping (7–9), means that improvements in sensitivity have been limited to post-collection processing. While these conventional collection techniques are effective for some substrates, they have limited efficacy for large, porous, absorbent, rough, and/or creviced substrates where the DNA may be too diffuse or unavailable for surface sampling. An alternative collection method which utilizes wet-vacuum technology has been developed to optimize DNA recovery from challenging items of interest where DNA may be absorbed within the substrate matrix.

The wet-vacuum-based collection system is designed for recovering DNA from porous substrates (10). The system consists of a vacuum, a hand-held collection device, a sample collection bottle, and sterile solution. It functions by dispensing the sterile solution onto a substrate while simultaneously vacuuming cellular material into the sample collection bottle. The liquid contents of the bottle are then filtered through a 0.45 μM polyethersulfone (PES) membrane in a two-stage filter unit, which traps and concentrates cellular material on the filter. Lastly, the filter is cut from the unit and processed for DNA extraction using common procedures.

There are published (11–14) and other academic research (15–17) studies on the use of a wet-vacuum-based collection system for forensic purposes. In one study, the wet-vacuum method was shown to perform better than double swabbing and taping methods for bloodstain collection on denim and carpet (11). In others, the wet-vacuum approach was more successful at collecting dried saliva from bricks (12) and laminated wood (13) compared to swabbing. It was also demonstrated that DNA quantities recovered with a wet-vacuum were comparable to those recovered with swabbing on nonporous materials, that is, tiles and glass (11,13), as well as human skin before and after showering (16,14). For touch DNA samples on cotton t-shirts, the wet-

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vacuum recovered more DNA than direct fabric cuttings (17). Although the higher yielding wet-vacuum samples provided DNA profiles more consistently than the fabric cuttings, some alleles belonging to individuals outside of the study were observed which increased the degree of mixed profiles (17). Touch DNA samples on bricks were also examined; however, the variability inherent to touch DNA studies limited the author's ability to draw conclusions (12). These previous studies provided some insights as to the performance of wet-vacuum-based collections; however, the variety of substrates tested was limited. Thus, sampling efficiency remains somewhat unclear on many difficult, forensically relevant substrates.

This study endeavored to expand evaluation of the wet-vacuum system as a possible DNA recovery method for use on multiple challenging substrates. Blood was deposited on 22 substrates in a diluted concentration designed to allow the differences in DNA recovery efficiency to be evaluated. First, DNA recovery from items collected with the wet-vacuum and the wet-vacuum manufacturer's extraction protocol was compared to DNA recovery using a conventional wet-swabbing and an automated magnetic bead-based extraction technique. Second, the wet-vacuum was also used on 10 previously swabbed substrates to recover potentially uncollected DNA. Lastly, efficiency of the collection techniques was assessed by using the same downstream extraction method for both wet-vacuum and wet-swab collections. Total DNA yields obtained from wet-vacuuming and wet-swabbing were quantitatively compared to assess each method's capability to recover DNA on challenging substrates. By overcoming some of the limitations associated with traditional collection techniques for specific substrate types, the wet-vacuum approach may be an effective alternative for forensic examiners when conventional methods yield poor DNA results.

Materials and Methods

Substrate Preparation

The 22 substrates of varying porosity examined in this study included household items (glass, wood countertop, drywall painted with flat, satin, semi-gloss, and gloss paints, carpet padding, and outdoor carpet), construction materials (pressure-treated wood, oak, pine, plywood, brick, hemp rope, nylon rope, cinderblock, and unpainted drywall), and automotive items (carpet, seat cushion insert, seat cushion collar, trunk liner, and trunk mat). Glass, which served as a control substrate, and wood countertop were the only nonporous substrates examined.

Blood was voluntarily obtained from a single human subject with informed consent, and the resultant DNA extracts were quantified but not sequenced or typed in this study. Blood was diluted 1/100 in sterile Butterfield's buffer (0.3 mM monobasic potassium phosphate, pH 7.2, M-Vac® Systems, Inc., Sandy, UT). Substrates were obtained in new condition, except for automotive items, and were wiped with disposable low-lint laboratory wipes to remove dust and/or loose debris then UV irradiated for 15 min before sample application. The automotive carpet and seating were laundered and UV irradiated before sample application.

A 12-multichannel pipet was used to evenly distribute the diluted blood into 12 rows of 10 μL drops in an approximate 100 cm^2 area on most substrates for a total volume of 1.44 mL (14.4 μL of whole blood). The nylon and hemp rope substrates were cut into one-foot segments and spotted with 1.44 mL diluted blood along the horizontal length of the rope. Spotting was performed in triplicate for both the wet-swabbing and wet-vacuum methods on each substrate. The bloodstains were allowed to air dry overnight prior to collection. Reagent blank controls consisting of Butterfield's buffer were prepared for the wet-vacuum and wet-swab methods on all substrates.

Wet-Swab Method

Dried bloodstains were collected using a single wet-swab method with a sterile wooden-stemmed cotton swab (Puritan, Guilford, ME) moistened with 50 μL molecular biology grade (MBG) water. Then, the swab was rubbed and rolled over the spotted area in back-and-forth motions with pressure until the visible stain was transferred. Some substrates, for example, carpet, cinderblock, and drywall, required more wetting for stain retrieval; therefore, an additional 50 μL of MBG water was pipetted onto the swab head. Swab heads were then cut off from the wooden stem using sterile scissors and transferred into Investigator™ Lyse&Spin baskets (Qiagen, Hilden, Germany). DNA extraction was performed according to an automated magnetic bead-based method wherein swab heads were digested in 423 μL of Buffer G2 (Qiagen), 13.5 μL of 20 mg/mL proteinase K, and 13.5 μL of 1 M dithiothreitol (DTT) at 56°C for 1 h with 200 rpm shaking (18). After incubation, the samples were centrifuged at 16K × *g* for 5 min. The baskets containing the swab heads were discarded and the lysates were processed on the EZ1 Advanced XL (Qiagen) using the large volume protocol and eluted in 50 μL of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

Wet-Vacuum Method

The M-Vac® (M-Vac® Systems, Inc.), consisting of a Support Equipment Case (SEC) 100, a sampling device with collection bottle, Butterfield's buffer, and plastic tubing, was used for wet-vacuum collections per the manufacturer's instructions (19). Collection consisted of applying the vacuum force and spray dispersal while repeatedly moving the hand-held sampling device in forward and backward motions over the entire spotted area (12 rows × 12 columns of 10 μL spots – 1440 μL of 1/100 diluted blood in total – over an approximate 100 cm^2 area). The sampling device was also dragged over the spotted area with only the vacuum force applied, that is, solution spray off, to collect the residual liquid remaining on the substrate. A new sampling device was used for each collection. The collection volume was approximately 150 mL of Butterfield's buffer per sample, which permitted three passes of the ~100 cm^2 substrate area with the wet-vacuum collecting about 50 mL for each pass. The dried bloodstains required repeated collection cycles to release the stain from the substrates. Additionally, the technique and volume used here may not be optimal in all scenarios; collection can vary depending on the type of sample and surface it is deposited on.

The head of the sampling device is flat, and therefore, the spraying action and suction of the wet-vacuum system worked optimally when it was in complete contact with the substrate; indeed, many of the substrates in this study were relatively flat. Because ropes have irregular and rounded surfaces, they were placed inside a sterile plastic tray for wet-vacuum collection. The wet-vacuum was applied to the surface of the ropes and the residual, run-off liquid which was not captured during collection on the surface was retrieved in the tray via suction as well.

The collected buffer solution was concentrated by pouring the liquid contents of the collection bottle through a Nalgene™ Rapid-Flow™ two-stage filter unit with a 0.45 μM PES membrane filter (Thermo Fisher Scientific, Waltham, MA). To maximize the recovery of cellular material, the filtrate was poured back into the collection bottle, swirled to dislodge cells from the bottle walls and poured over the same PES membrane filter once again. The membrane filter, while still damp, was cut into eight strip pieces using a sterile scalpel and transferred into two Investigator® Lyse&Spin baskets (Qiagen) with sterile forceps, four filter strips in each, for efficient digestion. To maximize surface area exposure, the filter strips were stacked and loosely rolled into coils when they were placed inside the baskets.

The filter samples were then extracted following the manufacturer's recommended automated magnetic bead-based method (M-Vac® Systems, Inc., personal communication, Jan. 24, 2018). Briefly, samples were digested in 490 μL of 1:1 diluted Buffer G2 (Qiagen) in MBG water and 10 μL of 20 mg/mL proteinase K at 56°C for 15 min with shaking at 850 rpm. After incubation, samples were centrifuged at 16K × *g* for 5 min to collect the filtered lysate. The spin basket containing the filter strips was removed, and Buffer MTL plus 1 μg/μL carrier RNA (Qiagen) was added. Finally, the samples were purified on the EZ1 Advanced XL (Qiagen) using the large volume protocol and eluted in 50 μL of TE buffer. The eluates from the same filter were then combined into a single tube for a 100 μL total elution volume.

DNA Quantification

Samples were quantified in duplicate using the Quantifiler™ Human Plus (HP) DNA Quantification Kit (Applied Biosystems, Foster City, CA) in accordance with the manufacturer's protocol and analyzed using the small autosomal target concentration. Samples were also quantified using a published and validated real-time quantitative PCR assay for human mitochondrial DNA (mtDNA) (20). Both assays were performed on an ABI 7500 Real-Time PCR System with the HID Real-Time PCR Software (Applied Biosystems). Average total DNA yields from the wet-vacuum method and the wet-swabbing method were compared to approximate relative collection efficiencies. Although mtDNA analysis is not typically performed on surface-deposited DNA, mtDNA was used as another quantitative measure to determine collection efficiency. MtDNA outcomes were generally similar to nuclear DNA (nDNA) outcomes and are therefore presented as Figures S1–S4. Additionally, all reported total DNA yields are detailed in Tables S1–S4.

Wet-Vacuum Recovery Following Wet-Swabbing

Ten of the previously swabbed substrates were subsequently subjected to wet-vacuum collection in an attempt to recover additional DNA. The following substrates were chosen based on relatively low DNA yields obtained via swabbing: pressure-treated wood, pine, brick, automotive seating (cushion collar), automotive carpet, trunk liner, trunk mat, carpet padding, and painted drywall (flat and satin paint). The length of time which passed between the initial swabbing and subsequent wet-vacuuming of these substrates ranged from 2 to 79 days. Wet-vacuum collection, concentration via filtration, extraction, and quantification were performed in the same manner as previously described.

Collection Efficiency

To isolate cellular collection efficiency (while holding DNA isolation efficiency constant), diluted 1/100 bloodstains on glass were collected with the wet-vacuum and wet-swabbing techniques in triplicate and were extracted using the same automated magnetic bead-based extraction protocol previously described under the wet-swab method (18). Quantification was performed as previously described for nDNA recovery.

Statistical Analysis

Unpaired, two-tailed *t* tests, assuming equal or unequal variance as determined by *F* tests, were carried out to determine significant differences between wet-vacuum or wet-swab data sets at a 0.05 significance level.

Results

Comparing Total DNA Yields from Wet-Vacuum and Wet-Swab Methods

The average total DNA yields obtained with a wet-vacuum on 22 substrates were compared to those recovered with a wet-swab method. Figures 1–3 show the average total nDNA yields for the two methods from household items, construction materials, and automotive items, respectively. There was no indication of PCR inhibition or degradation in any of the samples according to the qPCR results and no reagent blank yielded DNA as expected (data not shown).

Of the 20 porous substrates, the wet-vacuum method resulted in consistently greater nDNA yields than the wet-swab method on all but two surfaces, that is, cinderblock and unpainted drywall. In total, wet-vacuuming recovered more nDNA on 18 porous substrates compared to wet-swabbing, eight of which were significantly greater. Additionally, the amount of DNA recovered with the wet-vacuum was generally several-fold greater, ranging from 3× to 66× on the household items, 2×–28× on the construction materials, and 10×–47× on the automotive items. Overall, the wet-vacuum yielded an average of 12 times more nDNA compared to the wet-swab. Average mtDNA yields were 17 times greater overall for wet-vacuuming than for wet-swabbing (Figures S1–S3).

Wet-swabbing did not recover significantly more DNA on any substrate. However, both methods yielded comparable DNA amounts on two nonporous (glass and wood countertop) and two porous substrates (unpainted drywall and cinderblock). Yields from both methods on the unpainted drywall and cinderblock were generally lower than the yields obtained from other porous substrates.

Wet-Vacuum Recovery Following Wet-Swabbing

Average nDNA yields from the wet-vacuum method only, the wet-swab method only, as well as the wet-vacuum following the wet-swab method were compared for 10 of the originally tested substrates (Fig. 4). For nine substrates, the wet-vacuum recovered additional DNA that was, at minimum, equivalent to the initial swabbing, and maximally 46-fold more. For three substrates, the wet-vacuum recovered significantly greater yields than the initial swabbing. The satin-painted drywall was the only substrate where the wet-vacuum after wet-swab DNA yields were lower than the initial swabbing. Altogether, wet-vacuuming

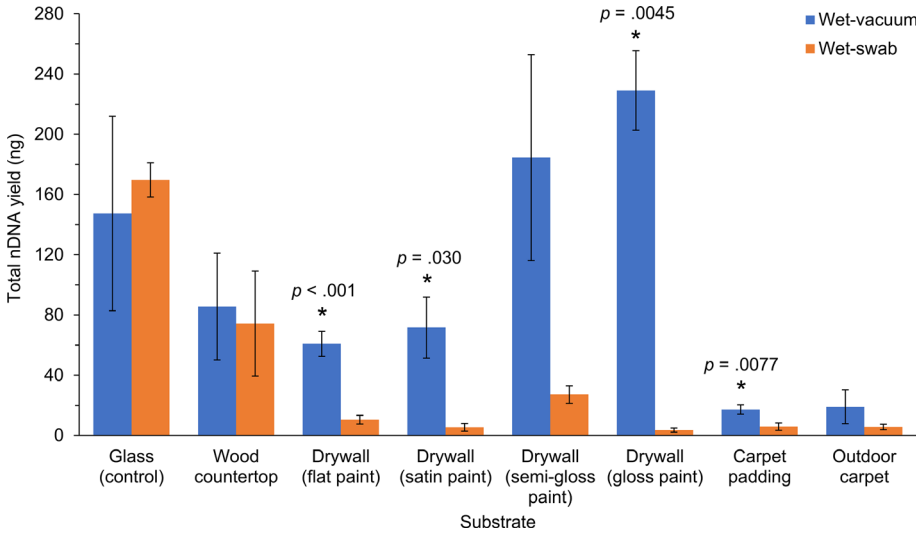

FIG. 1—*Average total nDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto household items. Error bars represent the standard deviation of three replicates. Asterisks indicate significantly greater mean yields; the* p*-values are reported above. [Color figure can be viewed at wileyonlinelibrary.com]*

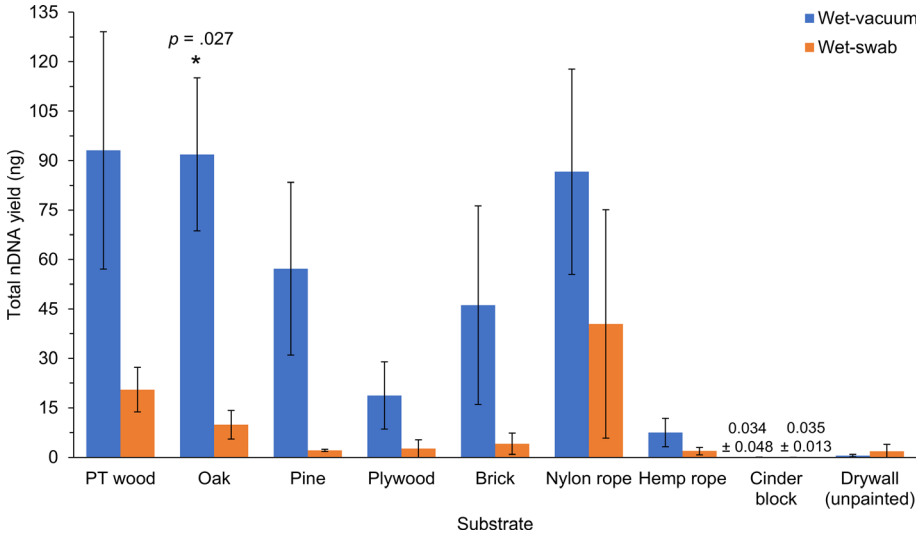

FIG. 2—*Average total nDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto construction materials. The average yields from both methods were very low for cinderblock; therefore, those quantities (mean ± SD, ng) are reported within the figure. Error bars represent the standard deviation of three replicates. The asterisk indicates a significantly greater mean yield; the* p*-value is reported above. PT wood, pressure-treated wood. [Color figure can be viewed at wileyonlinelibrary.com]*

after swabbing yielded an average of 10 times more nDNA and nine times more mtDNA (Figure S4) as compared to the initial wet-swabbing. These results demonstrated that considerable DNA remained in or on these substrates after wet-swab collection.

Collection Efficiency

Results from evaluating the different collection techniques on glass with the same DNA isolation protocol showed a modest increase in DNA yields for the wet-vacuum samples, 189 ± 33 ng (mean ± SD), compared to the wet-swab samples, 149 ± 33 ng; however, the difference was not significant (Figure S5). Thus, the overall increase in DNA yields from wet-vacuuming compared to wet-swabbing on the other substrates tested may be attributed to the collection technique - to include recovery from the membrane or swab - and that the differences between the two extraction methods were negligible.

Discussion

The efficiency of DNA collection methods is largely dependent on the physical characteristics of the substrate being sampled. The size, absorbency, irregular shape, and coarse nature of a particular substrate can present challenges to traditional DNA collection methods, such as swabbing of the surface or direct cuttings of small areas. The data presented in this study have shown that a wet-vacuum-based collection method not only has the ability to successfully recover biological material from a variety of challenging porous substrate types, but also often exhibits improved performance over a conventional swabbing method. Furthermore, the wet-vacuum technique not only

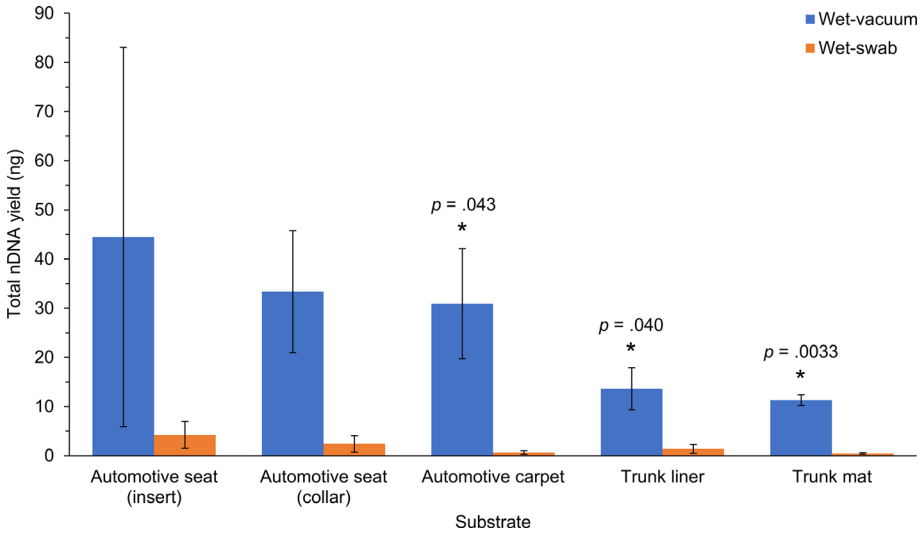

FIG. 3—*Average total nDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto automotive items. Error bars represent the standard deviation of three replicates. Asterisks indicate significantly greater mean yields; the* p*-values are reported above. [Color figure can be viewed at wileyonlinelibrary.com]*

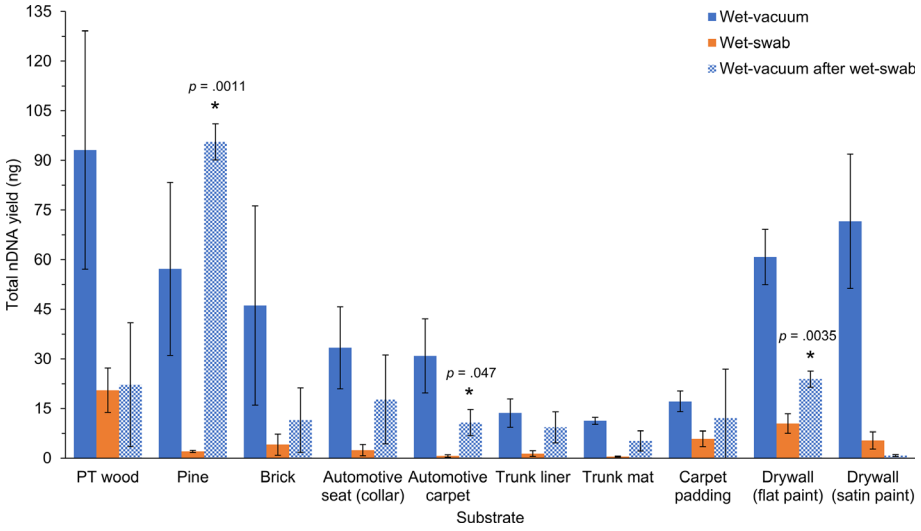

FIG. 4—*Comparison of total nDNA yields recovered from the wet-vacuum, wet-swab, or wet-vacuum after wet-swab collection methods. Error bars represent the standard deviation of three replicates. Asterisks indicate significantly greater mean yields from the wet-swab versus wet-vacuum after wet-swab methods; the* p*-values are reported above. [Color figure can be viewed at wileyonlinelibrary.com]*

recovered additional DNA from previously swabbed substrates, but it also frequently recovered more DNA than the initial swabbing.

There were two instances during this study where efficacies of both collection methods were greatly influenced by the physical characteristics of substrates. Unpainted drywall, an absorbent material, negatively affected DNA yields of the wet-vacuum more so than the wet-swab, possibly because it required substantially more liquid for collection. Also, the abrasive surface of cinderblock—the most rough and porous material tested—caused the cotton head of the swab to fray or break off. When wet-vacuumed, the solution passed through the pores/holes of the cinderblock quicker than the vacuum's capability to suction it back up. Therefore, and not surprisingly, substrate characteristics should be considered when deciding upon an appropriate collection technique.

For other difficult or porous substrates, the use of a wet-vacuum technique may be advantageous compared to a traditional wet-swab collection method. First, the amount of biological material that is collected is potentially increased due to the force and agitation of collection buffer which is applied to the substrate when using a wet-vacuum system. The wet-vacuum technique could allow for better retrieval of DNA hidden within fibers or crevasses of absorbent and/or porous items that a swab would otherwise leave behind. In addition, while cuttings can address cells trapped within porous materials, they are limited to smaller areas with visible staining, whereas the wet-vacuum method is not constrained as such. The latter may enable large substrates to be more efficiently processed compared to swabbing or cutting, particularly when biological material is diffuse or not isolated to a specific area of the substrate.

From a practical standpoint, swabbing is more convenient, simple, time-efficient, and inexpensive relative to the wet-vacuum method, and can be very effective in collection from visible stains or areas of repeated handling/contact. Swabbing and other traditional collection techniques should remain the preferred

collection method on surfaces with visible stains since DNA quantities may be relatively abundant, whereas the wet-vacuum collection and processing method would require greater effort and cost. The estimated start-up cost for the wet-vacuum system ranges from US$43,000–45,000, while the cost per sample is about US $90 (M-Vac® Systems, Inc., personal communication, Apr. 25, 2019) compared to less than US$15 for the wet-swab method. Additionally, even when a stain is not visible, these results suggest that swabbing may yet be most appropriate and efficient for flat, smooth, or nonporous surfaces. For example, both wet-swab and wet-vacuum techniques had similar collection efficiencies when collecting blood on glass and wood countertop substrates.

The increased level of collection efficiency over larger areas offered by the wet-vacuum collection approach may allow diffuse or diluted staining to be more efficiently collected, which also makes case scenario consideration of paramount importance. While wet-vacuuming methods have the advantage of expanding the area of sampling, it can also potentially recover more DNA, which may unnecessarily increase the complexity of the DNA mixtures obtained and negate the probative nature of a more targeted sampling. As always, care must be taken when selecting items and areas for sampling when used in a forensic context.

This study demonstrated that a wet-vacuum-based collection system is capable of collecting diluted blood on multiple types of challenging substrates, often with increased collection efficiency over traditional swabbing techniques. While blood was chosen for this project specifically for convenience, homogeneity, and reproducibility, the results described here likely serve as a proxy for other cell types or body fluids. Although this study focused on substrates that may absorb biological material, the wet-vacuum method, owing to its high efficiency of collection, might also be suitable for collection of touch DNA.

While this study focused on efficacy of the wet-vacuum collection method on multiple substrates, there is room for further studies to improve this method and expand its forensic applications. As observed by Vickar *et al*., cell-free fragments of DNA can be lost during the wet-vacuum filtration step (12). Indeed, the 0.45 μM PES membrane has very low DNA-binding properties and filters solely by particle size. Therefore, while whole cells are captured on the membrane, smaller cell fragments and/or cell-free DNA may easily pass through, suggesting the need to optimize their retention. The wet-vacuum method might also be successful for recovering water-insoluble explosive residue as well as trace evidence, for example, hair, fibers, soil, polymer particles, etc., with appropriate extraction procedure modifications. Additional research and modifications in these areas are warranted in order to optimize the wet-vacuum method for forensic use.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Average total mtDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto household items.

Figure S2. Average total mtDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto construction materials.

Figure S3. Average total mtDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto automotive items.

Figure S4. Comparison of total mtDNA yields recovered from the wet-vacuum, wet-swab, or wet-vacuum after wet-swab collection methods.

Figure S5. Comparison of wet-vacuum and wet-swab collection efficiencies.

Table S1. Average total nDNA and mtDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto household items.

Table S2. Average total nDNA and mtDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto construction materials.

Table S3. Average total nDNA and mtDNA yields recovered with the wet-vacuum or wet-swab methods for 1/100 bloodstains applied onto automotive items.

Table S4. Average total nDNA and mtDNA yields recovered with the wet-vacuum after wet-swab method.