

TECHNICAL NOTE**CRIMINALISTICS**

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The Influence of Swabbing Solutions on DNA Recovery from Touch Samples^{*,†}

ABSTRACT: There has been minimal research into how to best obtain DNA from touch samples. Many forensic laboratories simply moisten a swab with water and use it for collecting cells/DNA from evidentiary samples. However, this and other methods have not been objectively studied in order to maximize DNA yields. In this study, fingerprints were collected using swabs moistened with water or laboratory or commercially available detergents, including sodium dodecyl sulfate (SDS), Triton X-100, Tween 20, Formula 409[®], and Simple Green[®]. Prints were swabbed, DNA isolated using an organic extraction, yields quantified, and relative yields compared. In all cases, the detergent-based swabbing solutions outperformed water, with SDS and Triton X-100 producing significant increases in yield. Short tandem repeat profiles were consistent with the individuals that placed them. Subsequent analysis of SDS concentrations for collecting touch DNA demonstrated an increase in DNA yield with increasing SDS concentration, with an optimal concentration of approximately 2%.

KEYWORDS: forensic science, DNA typing/collection, touch DNA, low copy number DNA, sodium dodecyl sulfate (SDS), double swabbing, quantifier, short tandem repeats

Optimizing DNA yields is of prime importance to crime laboratories, particularly when dealing with touch samples where limited quantities of DNA may be left behind (1). The number of cells transferred to touched objects is highly variable, and often results in less than 300 picograms of DNA (2). Most forensic short tandem repeat (STR) kits call for 0.5–1.0 nanograms of DNA (about 200 cells) for full profiles to be generated (3), thus it is crucial to maximize DNA recovery from handled objects.

Forensic laboratories generally collect cells from surfaces using a swab, often moistened with sterile water, which may be followed by a second, dry swab (4) to retrieve liquid and cells left behind. This strategy has been used on touch and other low copy forensic samples (5,6); however, it is not known whether water is an optimal or even particularly good medium for cell recovery (e.g., [7]). Given this, it is possible that swabs moistened with a solution expressly designed to loosen and solubilize cells may increase cell/DNA yields. The research presented here was designed to examine this question, and to determine how to maximize DNA yields from swabbed touch samples. Fingerprints were deposited by volunteers, which were collected using swabs moistened with water, common laboratory detergents, or

commercial cleaners designed to remove oily, biological residue. DNA yields were quantified, and STR typing was conducted on a subset of samples to confirm DNA origin. The variability in DNA yields among volunteers and fingers used to deposit prints were also analyzed. Finally, optimal concentrations of the most effective swabbing solution were examined.

Materials and Methods

Solution and Surface Preparation

Lab coat, sleeves, facemask, and gloves were worn during all pre-amplification procedures. Swabbing solutions (Table 1) were made in concentrations used in typical laboratory applications or according to manufacturers' instructions. Solutions were passed through a 0.22- μ m filter and irradiated, along with consumables, in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 10 min (approximately 5 joules/cm²). Glass microscope slides (1" \times 3") were soaked in 10% commercial bleach for 1 h, rinsed with filtered 18.2 M Ω water, air dried, and UV irradiated for 5 min (approximately 2.5 joules/cm²) on each side.

Cell Recovery

Volunteers applied prints to slides daily over 6 days using the index, middle, and ring fingers of each hand, so that cells from all fingers were collected equivalently using each swabbing solution. DNA was extracted the same day prints were deposited. Four different volunteers were used for each study, and the Michigan State University Institutional Review Board sanctioned all testing. Swabs (25–806 2PC; Puritan Medical Products Co., Guilford, ME) were

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*Presented in part at the 62nd Annual Meeting of the American Academy of Forensic Sciences, February 22–27, 2010, in Seattle, WA.

[†]Co-author S. Thomasma was funded, in part, by Howard Hughes Medical Institute grant 52006304 through the Precollege and Undergraduate Science Education Program.

Received 26 July 2011; and in revised form 16 Jan. 2012; accepted 21 Jan. 2012.

TABLE 1—Preparations of swabbing solutions were made in the following concentrations.

Solution	Concentration	Supplier
Triton X-100	0.1% v/v	Promega (Madison, WI)
Tween 20	0.1% v/v	Promega
SDS	0.1, 0.5, 1, 2, 5% w/v	Fisher Scientific (Pittsburg, PA)
Digestion buffer*	0.1% SDS	Fisher Scientific
Formula 409 [®]	Neat	The Clorox Company (Oakland, CA)
Simple Green [®]	3% v/v	Sunshine Makers Inc. (Huntington Beach, CA)

*Also contained 20 mM Tris pH 7.5 (Invitrogen, Carlsbad, CA) and 50 mM EDTA (Acrös Organics, Pittsburg, PA).

moistened with 120 μ L of the swabbing solution and thoroughly passed over a print, rolling the swab to maximize transfer potential. A dry swab was then applied to the same area to absorb residual liquid. Controls for every group of prints processed included swabbing a slide with 120 μ L of water prior to laying down prints, a reagent blank containing an unused swab, and a combined swabbing solution blank prepared by applying 20 μ L of each solution to a swab. Swabs were then processed as detailed below. Based on the results of these experiments, various sodium dodecyl sulfate (SDS) concentrations were then examined (Table 1) using the same methods.

DNA Extraction

Entire cotton tips of paired wet/dry swabs were combined into a microcentrifuge tube containing 400 μ L of digestion buffer (20 mM Tris pH 7.5, 50 mM EDTA, 0.1% SDS) and 5 μ L of proteinase K (20 mg/mL) directly following print swabbing. Tubes were vortexed and incubated at 55°C for 2–3 h. The swabs were transferred to a spin basket and centrifuged at 20,800 $\times g$ for 3 min. All liquid was combined and swabs were discarded. An equal volume of phenol was added, and the tubes were vortexed and centrifuged at 20,800 $\times g$ for 5 min. The aqueous layer was transferred to a new tube, an equal volume of chloroform was added, and the tube was vortexed and centrifuged at 20,800 $\times g$ for 5 min. The aqueous layer was transferred to a Microcon[®] YM-30 spin column (Millipore Corporation, Billerica, MA), and centrifuged at 14,000 $\times g$ for 12 min. The retentate was washed twice with 300 μ L of TE (10 mM Tris, 1 mM EDTA, pH 7.5). DNA was collected by adding 20 μ L of TE, inverting the column into a new tube, and centrifuging for 3 min at 1000 $\times g$. DNA was stored at –20°C. In subsequent experiments examining other SDS concentrations, discontinued Microcon[®] columns were replaced with Amicon[®] columns (Millipore), which had the same filter type and molecular weight cutoff. Procedures detailed for Microcon[®] columns were followed except that centrifugation times for the wash steps were 10 min, and DNA was recovered by adding 10 μ L of TE and inverting the column into a new tube and centrifuging for 2 min. This resulted in a final extract volume for both column types of approximately 30 μ L.

DNA Quantification

DNA yields were quantified using a Quantifiler[™] Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) and an iQ[™]5 Real-time PCR Detection System (Bio-Rad,

Hercules, CA). Reactions were carried out in 0.2-mL dome-capped tubes in 15 μ L volumes consisting of 7.5 μ L of Quantifiler[™] PCR Reaction Mix, 6.3 μ L of Quantifiler[™] Human Primer Mix, and 1.2 μ L of sample, control, or standard DNA. Standards (50 ng/ μ L to 0.023 ng/ μ L) were generated according to the manufacturer's instructions and were run in duplicate. Thermal cycling parameters were set as prescribed by the manufacturer (10 min incubation at 95°C followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C).

STR Analyses

Two or more randomly selected print DNA samples collected using each swabbing solution were amplified with MiniFiler[™] (Applied Biosystems) to confirm DNA origin (final $n = 22$). Likewise, any reagent blank control with detectable DNA based on Quantifiler[™] was also amplified. Reactions were carried out in 15 μ L volumes, consisting of 6.7 μ L of Master Mix, 3.3 μ L Primer Set, 0.5–0.75 ng of DNA template, and water. If that quantity of DNA was not available, the maximum 5 μ L of DNA template was added. Negative controls contained water instead of DNA, and positive controls contained 0.5 ng of control DNA 007 provided in the kit. Thermal cycling parameters were as prescribed by the manufacturer (11 min incubation at 95°C followed by 30 cycles of 20 sec denaturation at 94°C, 2 min annealing at 59°C, and 1 min extension at 72°C, followed by a 45 min final extension at 60°C).

One microliter of amplified DNA or allelic ladder was combined with 24 μ L of deionized formamide and 1.0 μ L of GeneScan 500 LIZ Size Standard (Applied Biosystems). DNAs were separated on an ABI PRISM[®] 310 Genetic Analyzer (Applied Biosystems) with a 36-cm capillary using POP4 polymer (Applied Biosystems). Parameters consisted of an injection period of 3 sec, injection voltage of 15.0 kV, run voltage of 15.0 kV, run temperature of 60°C, and run time of 30 min. Data analysis was conducted using GeneMapper[®] ID v3.2.1 software (Applied Biosystems). Peaks above 50 relative fluorescence units were compared with previously generated profiles of volunteers to confirm the origin of the DNA.

Statistical Analyses

Any outlier in DNA yield was identified via the R Project for Statistical Computing (8) by means of Grubbs' test for one outlier, followed by Grubbs' test for two outliers if a first outlier was identified (this maximum normed residual test detects one outlier at a time using the largest absolute deviation from the same mean as the test statistic; if an outlier is detected, the test is then reiterated following removal of the outlier from the data set until none remain). Differences between DNA yields for individual swabbing media and water were analyzed using an independent samples t -test, and a regression analysis was performed to measure any trend in average yield for the SDS solutions and water. Differences in DNA yields among volunteers or finger used to deposit prints were compared via multiple comparisons analysis of variance and Tukey's HSD using the Statistical Package for Social Sciences (IBM, Armonk, NY), with a p -value less than 0.05 considered significant.

Results

Two hundred ninety-four prints were swabbed and processed in this study (150 examining the different swabbing solutions

and 144 testing SDS concentrations), as were 51 reagent blanks. During cell collection, both the laboratory detergents and commercial solutions wetted the swabbed surface more readily and thoroughly than did water alone.

DNA Yields Using Different Swabbing Solutions

The 25 sets of prints used to assay the different swabbing solutions/water produced 142 usable results (eight were outliers). In these and subsequent experiments, the Quantifiler™ internal positive control showed no indication of PCR inhibition. Overall, DNA yields ranged from 0 to 881 pg/μL. One hundred thirty-one (92.3%) of the samples had DNA yields below 400 pg/μL, 86 (60.6%) of which were below 100 pg/μL. All of the detergent-based swabbing solutions produced average DNA quantities greater than that for water (Fig. 1). Two resulted in significantly higher DNA yields than water (digestion buffer, $p = 0.03$; Triton X-100, $p = 0.04$), while the remainder, although higher, were not significantly so (Formula 409®, $p = 0.07$; Simple Green®, $p = 0.22$; Tween 20, $p = 0.37$).

DNA Yields Among Volunteers and Fingers

The average quantity of DNA obtained from volunteers differed significantly ($p = 0.04$; Fig. 2), although results were highly variable. Fingerprints from volunteer A produced the highest average quantity of DNA (201 pg/μL), and ranged from 0 to 881 pg/μL, while volunteer C had the lowest average yield (90.7 pg/μL), ranging from 8.1 to 682 pg/μL. Fingerprints from volunteers B and D produced intermediate DNA yields, averaging 149 pg/μL and 125 pg/μL, and ranging from 10.8 to 625 pg/μL and 0.0 to 370 pg/μL, respectively.

DNA yields from separate fingers also differed significantly ($p = 0.01$; Fig. 2). The highest average amount of DNA was obtained from prints placed by index fingers (199 pg/μL), followed by the middle finger (132 pg/μL) and the ring finger (99.5 pg/μL).

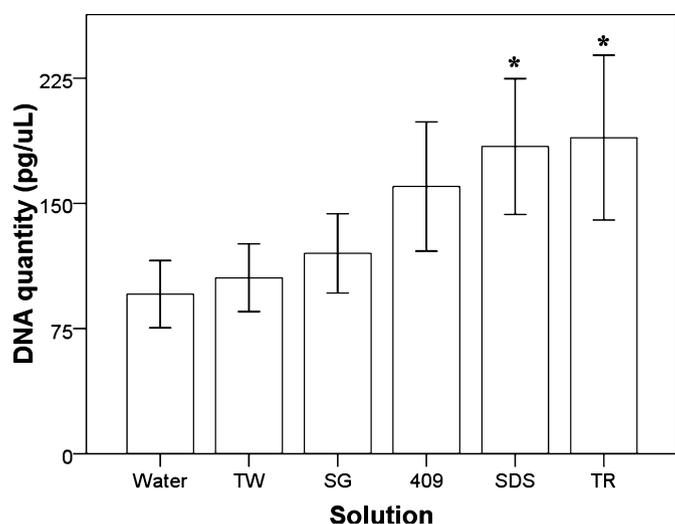


FIG. 1—DNA quantity (pg/μL) recovered from touch samples, sorted by swabbing solutions. Data presented as mean ± SE (TW = Tween 20, SG = Simple Green®, 409 = Formula 409®, SDS = digestion buffer, TR = Triton X-100). Water, TW, SG, and SDS had $n = 23$; 409 and TR had $n = 25$ after outlier removal. * denotes significantly higher yields than water, based on independent samples *t*-test.

DNA Yields Using Different Concentrations of SDS

The 24 sets of prints used to assay increasingly higher concentrations of SDS produced 140 results (four were outliers). In general, the different volunteers in this portion of the study deposited less DNA than those in the first set of experiments, ranging from 0 to 41.3 pg/μL. Figure 3 shows that all SDS solutions resulted in higher average DNA yields than did water alone, with a general trend of greater SDS concentrations yielding more DNA ($R = 0.647$). The highest DNA yields resulted from 2% SDS. No individual water/SDS comparisons were statistically different in this portion of the study (0.1%, $p = 0.29$; 0.5%, $p = 0.46$; 1%, $p = 0.17$; 2%, $p = 0.06$; 5%, $p = 0.11$).

STR Profiles

All MiniFiler™ profiles from fingerprints were consistent with the known profiles of volunteers, although in some

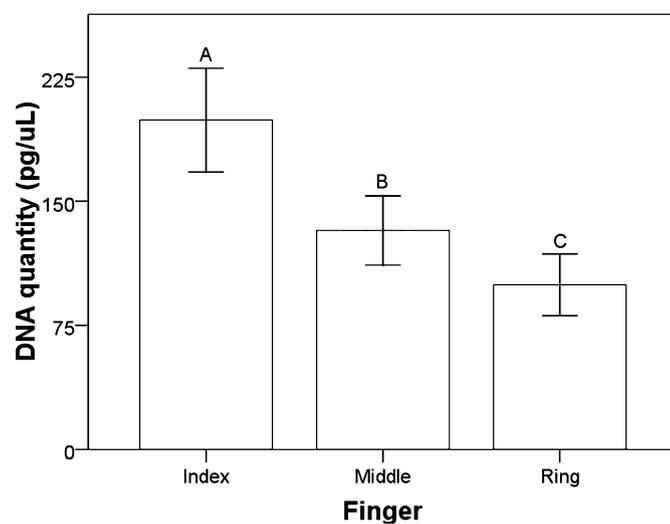
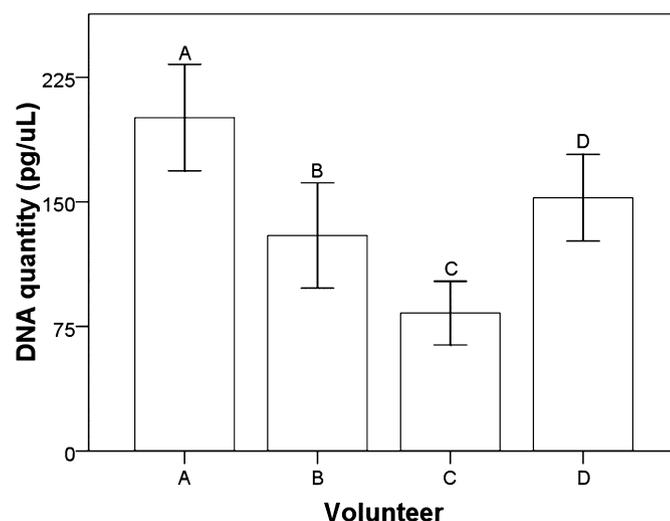


FIG. 2—DNA quantity (pg/μL) recovered from touch samples, sorted by volunteer or finger used to leave print sample. Data presented as mean ± SE. Each letter denotes a significant difference as determined by Tukey's HSD. Volunteer A, $n = 39$; Volunteers B and C, $n = 35$; Volunteer D, $n = 33$. Index and Middle finger, $n = 47$; Ring finger, $n = 48$ following outlier removal.

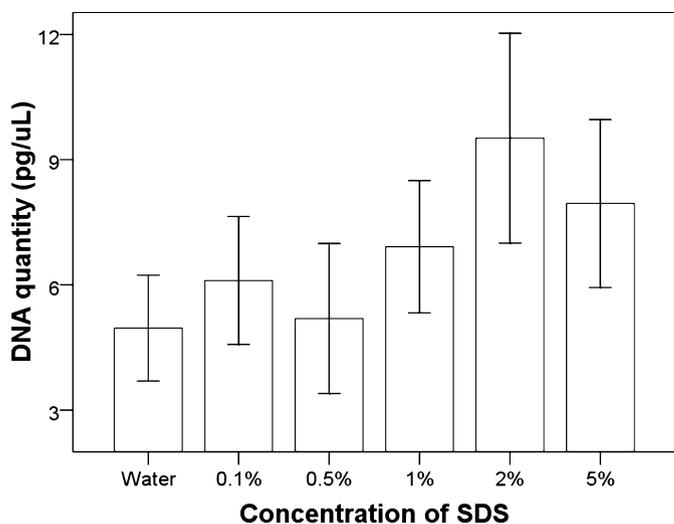


FIG. 3—DNA quantity (pg/uL) recovered from touch samples, sorted by concentration of SDS in solution. All SDS solutions had higher yields than swabbing with water alone, with a 2% SDS solution resulting in the highest DNA yield. Data presented as mean \pm SE. Water, 0.1%, 0.5%, and 5% had $n = 23$; 1% and 2% had $n = 24$ following outlier removal.

instances not all loci produced results. The lowest amount of DNA added to a reaction was 236.5 pg (5 μ L at 47.3 pg/ μ L), while the lowest quantity that produced a full profile was 468.5 pg (93.7 pg/ μ L), however a full range of DNA concentrations was not examined. Nine samples showed minor dropin at one or very few loci, although the peaks were short and were not the same among samples (i.e., there was no indication of contamination). No alleles above threshold occurred in the tested reagent blanks.

Discussion

The goal of the research presented here was to systematically determine whether detergent-based swabbing solution(s) significantly increase DNA yields from touch samples when compared to swabs moistened with water, as are often utilized in U.S. crime laboratories. Multiple solutions were tested, including commercially produced ones that are specifically designed to remove biological stains like fingerprints from surfaces, with the thought that these have been optimized to do so [in fact, Formula 409[®] was the 409th formulation tested for that purpose (<http://www.formula409.com/>)]. In all instances, detergent-based swabbing solutions led to greater DNA recovery from prints than did water, two of which (Triton X-100 and SDS) were significantly greater. Such detergents are amphiphilic in nature (allow solubility in both water and nonpolar solvents), thus the organic molecules that make up cells, including fats, lipids, and proteins, become suspended in solution. Water itself does not have these properties and therefore is presumably less effective in solubilizing cellular components. It seems logical that detergents, as well as aiding in cell lysis during DNA extraction (9–11), cause the elements of a fingerprint to become suspended in the aqueous solution, enhancing cellular recovery during swabbing.

SDS was more effective than water in cell/DNA recovery and has long been employed in forensic laboratories for DNA isolation; therefore, it was of interest to see whether concentrations higher than the 0.1% resulted in even greater DNA yields. A general (although not statistically significant) trend existed in

which higher SDS concentrations produced more DNA, with optimum concentrations in the 1–5% range. However, when SDS concentrations are high, there is a greater chance of it precipitating; therefore, a 1 or 2% solution is likely optimal for swabbing purposes. We have not observed any negative impacts of SDS swabbing even after prolonged storage (data not shown; in the current study, swabs were processed immediately), although it is possible that the introduction of SDS could interfere with DNA recovery when using certain commercial DNA extraction kits, thus a detergent other than SDS may be more conducive to successful DNA isolation in those instances. Further, the research presented here was only conducted on fingerprints deposited on glass slides so as to reduce variables, although results can likely be extrapolated to other surfaces as well.

The tendency both among and within volunteers (Fig. 2) to deposit epithelial cells, as assayed by DNA yields, differed substantially, which is consistent with other lines of research (e.g., [12,13]). In spite of this variability, at least one finger from every set of prints produced quantifiable levels of DNA. Interestingly, the volunteer who deposited the highest amounts of DNA in the swabbing solution study produced no DNA in some prints, while all prints from the lowest depositor produced some DNA. Interindividual variability became even more apparent in the test of different SDS concentrations, as all volunteers deposited far less DNA on average than those in the swabbing solution study, yet they too produced measurable DNA from at least one print in every set. The overall lower levels of DNA from volunteers in the SDS concentration study likely caused the lack of significance between SDS and water, although again, a trend existed in which higher SDS concentrations resulted in greater DNA yields.

Finally, there was also a significant difference in DNA yields based on which finger was used to leave a print, with index fingers depositing more DNA than middle fingers, which in turn produced more than ring fingers. This seems somewhat counter-intuitive in that index fingers are likely used more than the other fingers and thus loose cells would presumably be rubbed away prior to print deposition. Instead, it may be that the index finger experiences faster turnover of dermal tissue owing to heavier use, producing fingerprints with higher quantities of cells/DNA than the middle or ring finger. It is also possible that the index finger is used more often to touch one's face or other parts of the body, resulting in more loose cells being present.

Conclusion

DNA profiling is one of the most well-established and reliable forms of forensic identification, and the potential to generate a DNA profile from a touched item means that maximizing DNA yield when swabbing such evidence is crucial. The findings detailed here show that inclusion of a detergent in the swabbing solution can significantly increase DNA yields from fingerprints when compared with wetting swabs with water alone, as is currently done in many forensic laboratories. Likewise, the detergent used can influence DNA yields, where common laboratory reagents, most notably SDS and Triton X-100, produce the best results. Solutions containing SDS are regularly employed by forensic biologists, thus a transition to a 2% SDS solution, which provided the best results here, should be relatively straightforward and noncontroversial, and could directly enhance the likelihood of successfully generating a DNA profile from touch samples.

Acknowledgments

The authors thank the members of the Michigan State University Forensic Science Program for their help and support.

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