Touch DNA in forensic science: The use of laboratory-created eccrine fingerprints to quantify DNA loss

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ARTICLE INFO

ABSTRACT

Touch samples typically contain a limited quantity of DNA, which can be further reduced during collection and analysis. It is not clear, however, at which point(s) the majority of the DNA is lost because there is not a reliable positive control to track the quantity of DNA through the analysis procedures. To take the first step in bridging this gap, we established a set of laboratory-created eccrine, or mock, fingerprints containing known quantities of DNA. Next, we defined a set of process controls to quantify loss at key fail points in the collection/extraction procedures, analyzing a total of 1200 mock fingerprints deposited on four different surfaces. We quantified DNA loss to the surface, the swab and at extraction, completing the evaluation with ANOVA. With better understanding of DNA yields and the mechanisms of loss, targeted process improvements will bring touch DNA samples into even more routine use with standardized, optimized procedures.

1. Introduction

The Locard Exchange Principle holds that, with contact between two items, there will be an exchange of material [1]: a concept central to the science of fingerprints. The fingers act as vectors of transmission, transferring sweat and oil as ridge detail that is the conventional information-bearing component of the exchange [2]. For the last two decades, however, forensic scientists have recognized the added capability of extracting "DNA fingerprints from fingerprints," [3] that is, the sweat and oil exchange contains a second information-bearing component in the DNA-containing cells that support genetic profiling: this is the science of touch DNA.

Touch samples contain DNA, not attributed to a particular body fluid, that is deposited when an object is handled or touched. The origins of the human DNA in a touch sample have not been definitively elucidated, but many lines of evidence indicate that they are likely to include shed corneocytes [4], endogenous or transferred nucleated epithelial cells [2,5–8], fragmented cells and nuclei [9–11], and cell-free DNA [12–14]. There is broad inter- and intra-individual variation in the quality and quantity of DNA contained in a touch sample, and it can vary based on disparate factors including activity of donor, sex, age, substrate, temperature and humidity [6,15–22]. Therefore, it is difficult to define a true fingerprint as the positive control for the collection and analysis of touch samples. To take the first steps in bridging this gap, the aims of the work described here were: (1) establish a method to generate standardized, laboratory-created control fingerprints containing a known quantity of DNA; (2) quantify loss at key fail points during collection and analysis, providing an empirical basis for the optimization of touch sample methodology; and (3) alter one of the variables (surface) to demonstrate the application of the mock fingerprint method.

Efforts have been made to standardize the deposition of the biological components in previous touch DNA experiments, but none have established a definitive positive control. In earlier studies, donors deposited touch samples by contacting sterile tubes, glass plates or another volunteer's hand for a defined period of time, e.g. 3, 10 or 60 s [15,21,23–26]. Other experimental protocols called for volunteers to rub their hands over a substrate or wear an article of clothing for a prescribed period of time and number of occasions [27,28]. However, because there is such great inter- and intra-person variability and even a single individual can be either a good or a bad shedder depending on his/her specific

circumstances at the time of sampling [15], the DNA deposited is not consistent.

Approaches using non-epidermal cell types as a DNA source have been tested as well. Researchers suggested that, since individuals don't carry a consistent amount of cellular material in their fingerprints over time, it was not useful to standardize sampling with multiple collections from the same donor [29]. Other approaches have included the use of known volumes of other body fluids such as blood or saliva [6], or naked, quantified DNA. Such approaches have been employed by multiple groups [20,30–32], but a disadvantage is that these controls have a significantly different biology than touch samples. Differences in cell wall, size and nature of cells from those routinely deposited in true touch DNA cases could limit the applicability and extrapolation of results using these controls from research to crime lab casework.

A different strategy for the standardization of touch DNA controls involved producing the biological material by cell culture. Feine et al. [29] cultured skin-derived human dermal fibroblasts. DNA quantification was indirect; serial dilutions of cell suspensions were extracted and quantified. The quantification values were used to calculate the amount of DNA contained in the cell suspension, and the appropriate volumes were deposited on glass slides for collection by tape-lifting or swabbing. Actively dividing connective tissue cells, the DNA content of which has not been evaluated directly, may not be ideal for use as a control for a touch sample, however.

A challenge in defining a positive control for the collection and analysis of touch DNA lies in the nature of the sample itself − it is a complicated mixture of components that can vary over time [4,33]. In the work described here, we reduced this complexity by defining the basic components of an eccrine touch sample to include human diploid cells in an inorganic solution [34,35]. This simplification established a baseline set of conditions under which we could evaluate the effects of a simple eccrine background on DNA collection and loss. In future experiments, we can independently evaluate the effects of specific variables, such as the inclusion of

fatty acids [36] or different sources of DNA, e.g. microbial or cell-free [12,13,37], in an effort to develop a better understanding of the optimal collection and analysis techniques.

The touch sample positive control described here is a laboratory-created, or mock, eccrine fingerprint. The general preparation scheme is: (1) prepare a suspension of human diploid cells; (2) count the number of cells per microliter; (3) use #cells/µl to calculate the volume of cell suspension containing the target quantity of DNA, e.g. add 2.85 ng to a mock fingerprint (475 cells x 0.006 ng/cell = 2.85 ng DNA). If the count was 110 cells/µl, then 4.3 µl of suspension contained 475 cells, or 2.85 ng DNA; (4) combine the target suspension volume with an inorganic fingerprint solution; (5) deposit the mock fingerprint on a surface; (6) collect and analyze.

Through creation of a consistent reproducible mock fingerprint, the variability of human true fingerprints can be eliminated, thereby enabling improved evaluation of other variables on
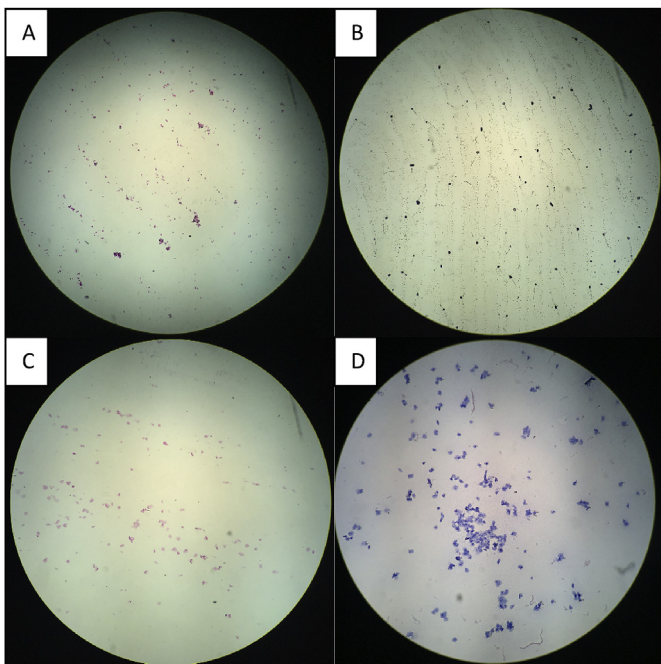

Fig. 2. Cell Clumping, Buccal Epithelial Cells: (A) in PBS, stained with hematoxylin/eosin; (B) in PBS, stained with trypan blue; (C) Accumax suspension of cells, stained with hematoxylin/eosin; (D) Accumax suspension of cells stained with trypan blue. Viewed on a brightfield microscope, 100X.

Table 1
Cell Counting. Five different cell suspensions were used to confirm counting reproducibility. The eighteen counts from the hemocytometer (H1 − H5) or nine counts from the Luna™ automated counter (L1 − L5), were averaged and the standard deviations calculated.

	Suspension	Mean (cells/µl)	SD
Hemocytometer	H1	210	28
	H2	229	30
	H3	87	26
	H4	152	33
	H5	196	25
Luna™ Automated Cell Counter	L1	194	6
	L2	83	9
	L3	90	5
	L4	126	11
	L5	83	4

Fig. 1. Selection of Diploid Cells for Mock Fingerprints. Cells from true fingerprints were stained with (A) hematoxylin/eosin or (B) trypan blue. For comparison, buccal epithelial cells were stained with (C) hematoxylin/eosin or; (D) trypan blue. Cells were viewed by brightfield microscopy, 40X.
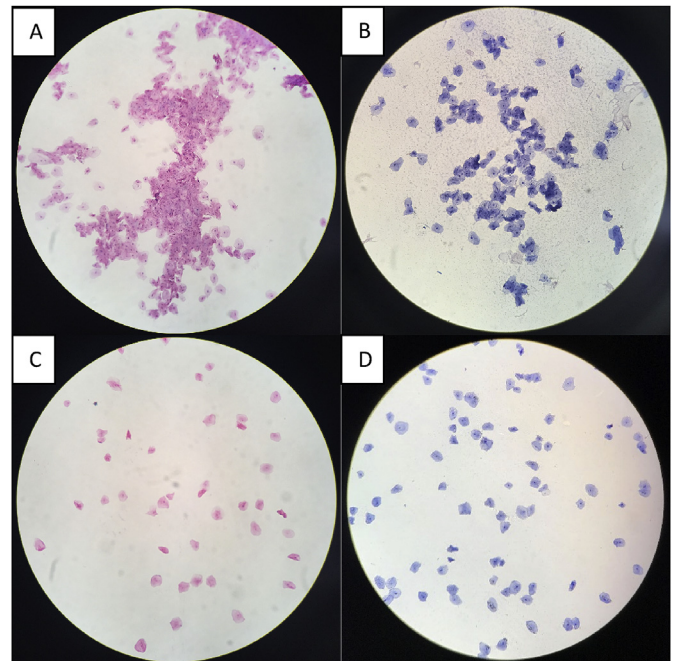
recovery of touch DNA.

2. Materials and methods

2.1. Fingerprint solution

The 1X fingerprint solution contained the major inorganic components identified in earlier studies: 0.197 M urea, 0.195 M NaCl, 0.0866 M KCl, 0.0678 M lactic acid in 98% sterile water [34,35].

2.2. Cell suspension

Samples from human subjects were collected with informed consent using the University of Illinois at Chicago protocol (2016_0431). Buccal epithelial cells were collected from multiple donors over the course of the study by swabbing the inside cheek with a sterile cotton-tipped swab (Puritan, Guilford, ME). The cotton tips were removed from the shaft to 500 μl of 1X Accumax™ Cell Dissociation Solution (Innovative Cell Technologies, San Diego, CA and incubated for 15 min at room temperature. The swab was removed from the tube and an additional 500 μl Accumax™ was added before a second 15-min incubation at room temperature.

2.3. Improved Neubauer hemocytometer

A cover slip was placed on the hemocytometer and 10 μl of the homogeneous suspension pipetted under the slip. The cells in the nine squares were counted and concentration determined: (Total # of cells/9 squares) x (1 square/0.1 μl) = cells/μl. Two different aliquots from each suspension were used for counting, for a total of 18
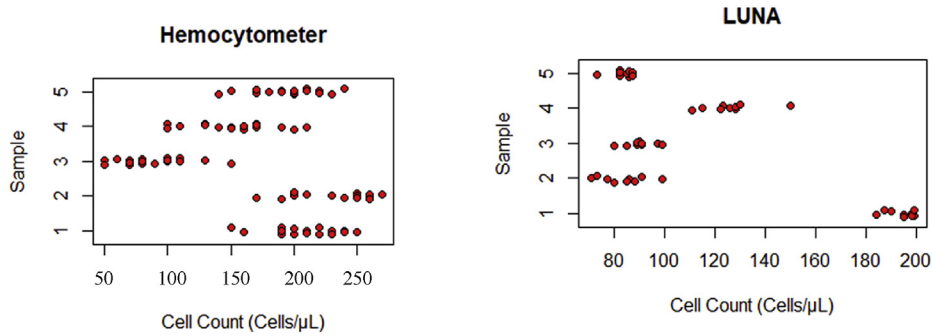
Fig. 3. Clustering of Cell Counts. Eighteen (hemocytometer) and nine (LUNA) replicate counts were taken from each of five cell suspensions. The #cells/μl for each replicate count were plotted to observe clustering of the values.

Fig. 4. Clustering of MFP DNA recoveries. Twenty replicate mock FP for each of the process controls were evaluated for each value on the standard curve. The nanograms of DNA recovered were plotted for each control to observe sample clustering.

data points.

2.4. LUNA™ Automated Cell Counter (logos biosystems, South Korea)

Cells were counted according to the manufacturer's no-dye protocol. Ten microliters of the cell suspension were added to the counting slid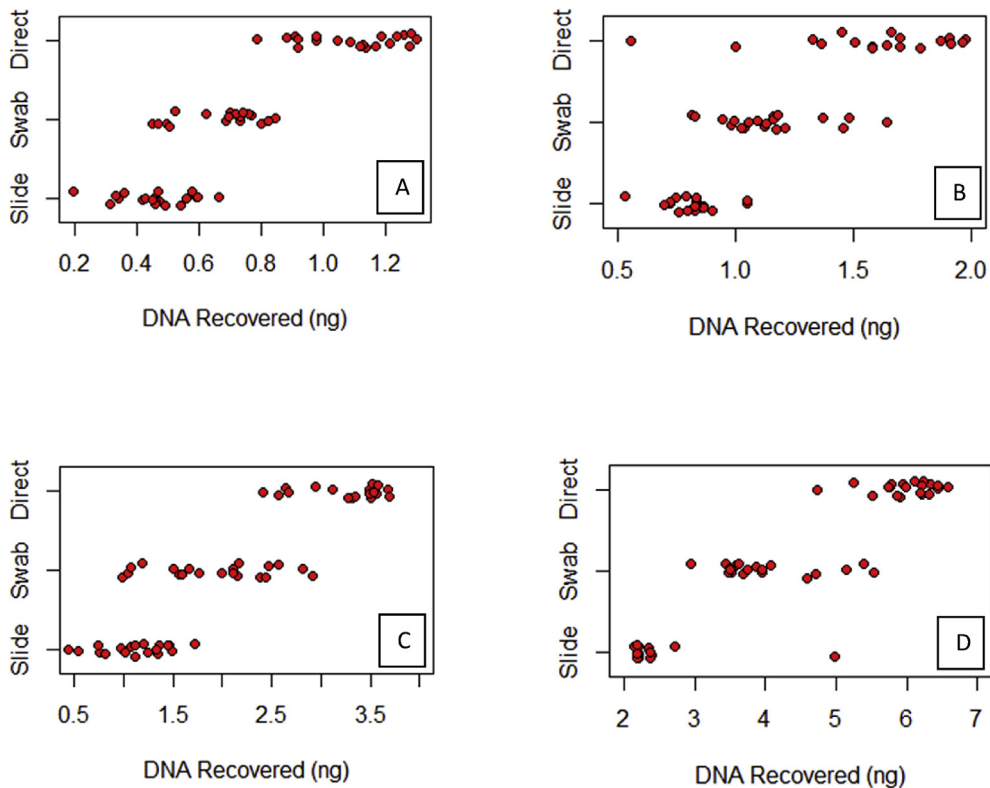e and placed into the cell counter. The operating parameters were: Dilution 1; Noise Reduction 6; Live detection sensitivity 2; Roundness 40; Min. cell size 5; Max cell size 60; De-clustering level Medium. From a single suspension, three aliquots of 10 μl each were counted three times each, for a total of 9 data points.

2.5. Laboratory- created eccrine (mock) fingerprints

Each counting technique was considered individually. The eighteen (hemocytometer) or the nine (LUNA™) cell counts were averaged separately. The LUNA™ cell counts were used for the generation of the data presented here. The mean number of cells per microliter of suspension was converted to volume of suspension required to deliver a specific quantity of DNA (1 epithelial cell = 6 pg DNA). The volume was brought to 18 μl in 1X PBS and combined with 2 μl of 10X Fingerprint Solution. The entire 20 μl volume was pipetted on a surface over an area of approximately 3.63 cm^2 and allowed to dry at room temperature (~1 h). To collect the mock fingerprint, a sterile, cotton-tipped swab was wetted with 20 μl 2% SDS and the area swabbed completely [38]. A total of 1200 mock fingerprints were generated and analyzed - 300 for each of the four surfaces (glass slide, steering wheel, drug baggie, brass door plate).

2.6. Surfaces: decontamination

A total of four surfaces were included in this study. Glass microscope slides (Fisher Scientific, Norcross GA) were decontaminated by placing the slides in a beaker filled with a 10% bleach solution for at least 10 min, then rinsed in water for at least 5 min. The slides were then cleaned with 70% ethanol and air dried. Once they were completely dry, each slide was individually wrapped in foil, set out in a single layer, and sterilized in an autoclave.

The other three surfaces were: (1) a Nissan Altima OEM Steering Wheel (1-stopautoparts, https://www.ebay.com/str/1stopautoparts), (2) drug baggie: 2020 Original Mini Ziplock 2.5 mil Plastic Bags, 2 in x 2 in (5.1 cm × 5.1 cm), Reclosable baggies (Dollar Sign$), (The Baggie Store.com, https://thebaggiestore.com/products/2020-original-mini-ziplock-2-5mil-plastic-bags-2-x-2-reclosable-baggies-dollar-sign), and 3) Brass Door Plate: Rockwood 70A.4 Brass Standard Push Plate, Four Beveled Edges, 12 in (30.5 cm) Height x 3 in (7.6 cm) Width x 0.050 in (0.127 cm) Thick, Satin Clear Coated Finish. (Rockwood, https://www.amazon.com/stores/node/9506259011?_encoding=UTF8&field-lbr_brands_browse-bin=Rockwood&ref_=bl_dp_s_web_9506259011).

For decontamination, these objects were placed in a biological safety cabinet and washed with RNase Away followed by 70% ethanol. Once dry, the surfaces were cleaned with sterile NanoPure water and allowed to air dry.

2.7. ATR FTIR

Fourier transform infrared spectroscopy with attenuated total reflectance was used to confirm that the drug baggies used in this study were similar to baggies collected as evidence by the Illinois State Police Department (Supplementary Fig. 1). A FSC-C Nicolet iS10 FTIR with an ATR diamond crystal collected spectra from 4000 cm^{-1} to 450 cm^{-1} with resolution set to 4 cm^{-1}. A sample was compressed against the diamond cell and the spectrum was collected with a total of 36 scans.

2.8. Isolation and purification of DNA

DNA was extracted from samples using a standard phenol:-chloroform method [39]. Briefly, the cotton tip was removed from a swab and incubated overnight at 56 °C in 400 μl DNA extraction buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.0, 25 mM EDTA, 0.5% SDS, 0.1 mg/ml Proteinase K). The swab was removed to a Spin-X filter (Corning, Tewksbury, MA) and the tube centrifuged. Four hundred microliters of 25:24:1 phenol/chloroform/isoamyl alcohol (Fisher, Scientific, Norcross, GA), were added, and the phases were separated in a Phase Lock Gel Tube (2 ml, heavy, Eppendorf, Boulder, CO) according to the manufacturer's protocol. DNA was precipitated for at least 1 h in 1 ml (2.5 vol) absolute ethanol at −20 °C and pelleted by centrifugation. The pellet was washed twice with 1 ml (2.5 vol) 70% ethanol and dried in a 56 °C incubator (~10−20 min). The DNA was re-solubilized in 30 μl sterile water by overnight incubation in a 56 °C water bath (12−18 h).

2.9. Quantification

Human DNA was quantified by Alu-specific real-time PCR [40].

Table 2
DNA Loss: Glass Slides. Twenty replicate mock FP standard curves were produced for each of the three process controls (slide, swab, direct). DNA recovered was quantified by real-time PCR, and the values from a twenty replicate set were used to calculate the mean DNA recovery. Subtracting that value from total quantity deposited gave the mean DNA loss, to provide the average percent DNA loss as follows: ((mean DNA loss/total DNA deposited)*100).

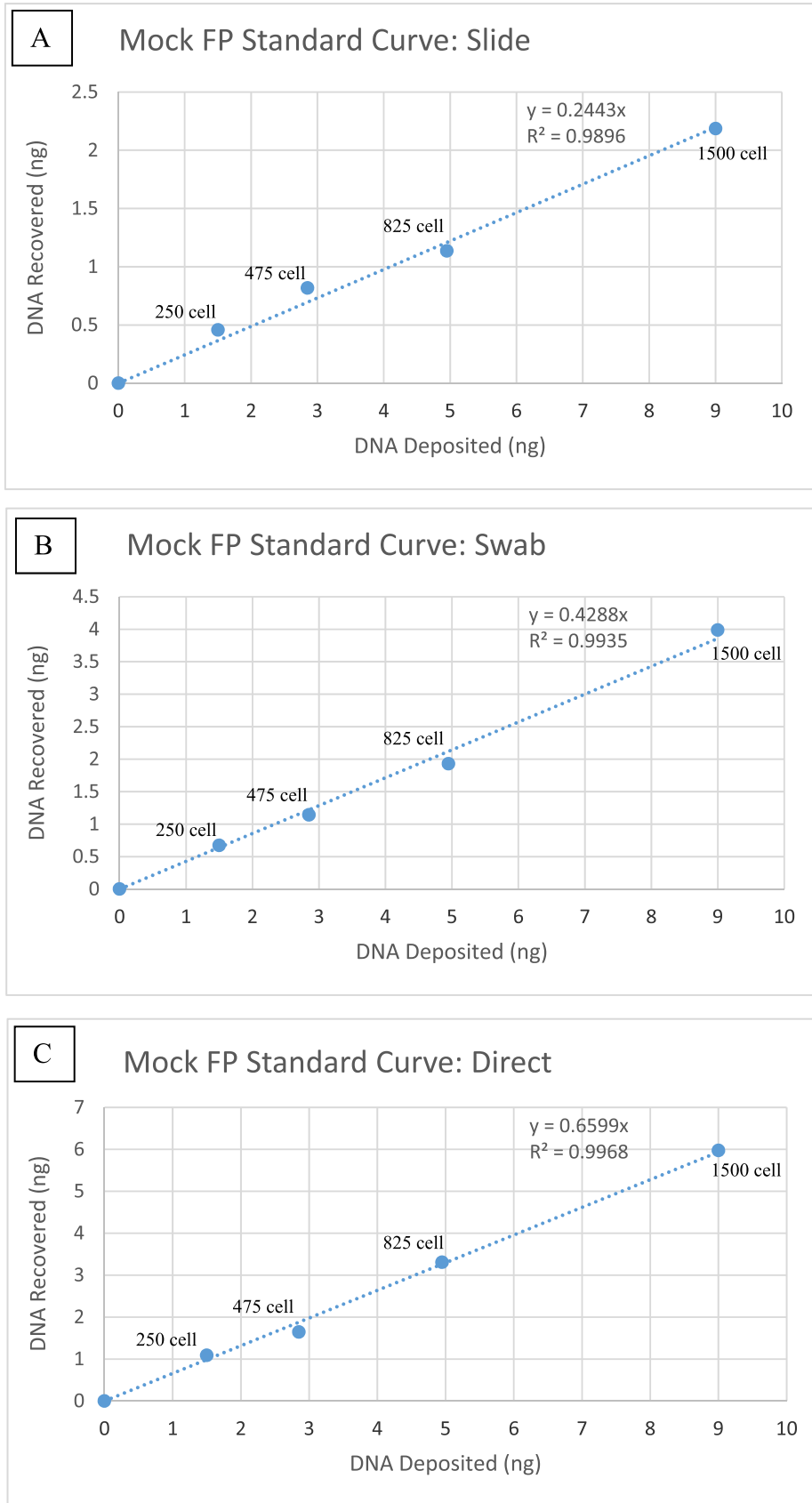| DNA Deposited	Process Control	Mean DNA Recovery (ng)	SD	Mean DNA Loss (ng)	Mean % DNA Loss
250 cells: 1.50 ng	Slide	0.46	0.11	1.04	69
	Swab	0.67	0.12	0.83	55
	Direct	1.09	0.15	0.41	27
475 cells: 2.85 ng	Slide	0.82	0.11	2.03	71
	Swab	1.14	0.21	1.71	60
	Direct	1.64	0.39	1.21	42
825 cells: 4.95 ng	Slide	1.14	0.34	3.81	77
	Swab	1.93	0.59	3.02	61
	Direct	3.31	0.46	1.64	33
1500 cells: 9.00 ng	Slide	2.19	0.80	6.81	76
	Swab	3.99	0.71	5.01	56
	Direct	5.97	0.44	3.03	34

Fig. 5. Process Controls, Glass Slides. The quantities of DNA recovered from twenty replicates of the five mock FPs for each process control were averaged and displayed as standard curves: (A) cells deposited on a slide; (B) cells pipetted on to a swab; and (C) cells added directly to the lysis buffer. Cell counts are indicated above each point.

Ten microliter reactions were prepared containing: 2 μl of purified DNA, 2.6 μl of nuclease-free water, 5 μl of 2X iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, California), and 0.4 μM each primer (forward-GTCAGGAGATCGAGACCATCCC; reverse-TCCT GCCTCAGCCTCCCAAG) (Sigma Aldrich, St. Louis, MO). The standard curve ranged from 0.0077 ng/μl to 16.7 ng/μl with a total of eight data points and was generated using a human genomic DNA standard (Bioline USA Inc., Taunton, MA). The cycling conditions

Table 3

DNA Loss: Key Fail Points. The total percent DNA lost from glass slides was quantified by the surface process control. Loss resulting from the extraction procedure was the value from the direct process control. The quantity of DNA left on the surface was calculated as (percent loss: surface) − (percent loss: swab), and the amount retained on the swab was (percent loss: swab) − (percent loss: direct).

	# cells	DNA (ng)	Total % Loss	% Loss at extraction	% Retained on surface	% Retained on swab
Slide	250	1.50	69	27	14	28
	475	2.85	71	42	11	18
	825	4.95	77	32	17	28
	1500	9.00	76	34	20	22
	Average		73	34	16	65
	SD		3.9	6.2	3.9	4.9

Table 4

Steering Wheel/Drug Baggie/Brass Door Plate: DNA Loss. The quantity of DNA recovered from twenty replicate mock FP standard curves for each of the three process controls (surface, swab, direct) for each surface was determined by real-time PCR and used to calculate mean and standard deviation. Percent DNA recovered was calculated as: ((mean DNA loss/total DNA deposited)*100).

DNA Deposited	Process Control	Mean DNA Recovery (ng)	SD	Mean DNA Loss (ng)	% DNA Loss
Steering Wheel					
250 cells: 1.50 ng	Surface	0.35	0.05	1.15	77
	Swab	0.62	0.05	0.88	59
	Direct	1.05	0.06	0.45	30
475 cells: 2.85 ng	Surface	0.69	0.08	2.16	76
	Swab	1.24	0.12	1.61	56
	Direct	1.92	0.08	0.93	32
825 cells: 4.95 ng	Surface	1.16	0.15	3.79	76
	Swab	1.90	0.07	3.05	62
	Direct	3.43	0.30	1.52	31
1500 cells: 9.00 ng	Surface	1.85	0.20	7.15	79
	Swab	3.78	0.33	5.22	58
	Direct	6.37	0.49	2.63	29
Drug Baggie					
250 cells: 1.50 ng	Surface	0.68	0.08	0.82	55
	Swab	0.69	0.06	0.81	54
	Direct	1.05	0.08	0.45	30
475 cells: 2.85 ng	Surface	1.25	0.10	1.60	56
	Swab	1.27	0.16	1.58	56
	Direct	1.95	0.16	0.90	32
825 cells: 4.95 ng	Surface	2.21	0.26	2.74	55
	Swab	2.29	0.38	2.66	54
	Direct	3.32	0.24	1.63	33
1500 cells: 9.00 ng	Surface	3.70	0.37	5.30	59
	Swab	3.82	0.32	5.18	58
	Direct	6.06	0.29	2.94	33
Brass Door Plate					
250 cells: 1.50 ng	Surface	0.62	0.08	0.88	59
	Swab	0.71	0.07	0.79	53
	Direct	1.07	0.11	0.43	29
475 cells: 2.85 ng	Surface	1.10	0.14	1.75	61
	Swab	1.27	0.18	1.58	56
	Direct	1.98	0.20	0.87	30
825 cells: 4.95 ng	Surface	1.95	0.29	3.00	61
	Swab	2.26	0.29	2.69	54
	Direct	3.20	0.24	1.75	35
1500 cells: 9.00 ng	Surface	3.34	0.91	5.66	63
	Swab	3.95	0.31	5.05	56
	Direct	5.64	0.57	3.36	37

were: 95 °C for 2 min; 35 cycles of 95 °C for 15 s, 68 °C for 1 min. A melt curve was generated from 65 °C to 95 °C to confirm the single PCR product.

2.10. Visualization of cells

Trypan Blue. Buccal epithelial cells were suspended in either 1X PBS (Fisher Scientific) or 1X Accumax™ (Innovative Cell Technologies, Inc, San Diego CA). Ten microliters of the cell suspension were added to an equal volume of 0.4% dye. After incubation for 3 min at room temperature, the entire volume was pipetted onto a clean glass slide and allowed to dry under a biosafety hood for 1 h. The slides were heat-fixed for 1.5 min at 105 °C. After cooling, the slides were mounted in Permount™, allowed to dry overnight before visualization under a bright field microscope.

True fingerprints were deposited on clean glass slides. Prior to donation, volunteers carried on with their normal daily activities, but refrained from handwashing for at least an hour. Prints were collected from the thumb, index, and middle fingers. Donors placed their fingers on the glass slides and rolled them from side-to-side for 10 s. The slide was stained with 10 µl of 0.4% trypan blue and visualized immediately under the bright field microscope with 40X or 100X magnification.

Hematoxylin & Eosin (H & E). Mock and true fingerprints were dried in a biosafety hood for 1 h and heat-fixed for 1.5 min at 105 °C. After cooling, the slides were placed in slide holders (LabScientific, Highlands, NJ) and incubated in staining chambers (LabScientific, Highlands, NJ): (a) Gill 2 Hematoxylin (3 min in staining chamber); (b) 1 min rinse under running tap water; (c) brief rinse with 1% acid alcohol (3% HCl, 95% ethanol) (Fisher Scientific); (d) 1 min rinse under running tap water; (e) Scott's Tap Water Substitute 45s in (staining chamber); (f) 1 min rinse under running tap water; (g) Eosin Y, alcoholic 10s in staining chamber; (h) 95% ethanol (1 min in staining chamber); (i) 100% ethanol (1 min in staining chamber); (j) Xylene (1 min in staining chamber). The slides were mounted in Permount™ and dried overnight at room temperature before visualization under a bright field microscope.

2.11. DNA amplification and genetic analysis

Purified DNA was profiled using the AmpℓSTR® Identifiler ® Plus PCR Amplification Kit (ThermoFisher Scientific, Waltham, MA), according to the manufacturer's protocol. PCR product was analyzed using the 3130 Genetic Analyzer (ThermoFisher Scientific). One microliter of PCR product was combined with 0.5 µl GS LIZ 600 Lane Standard and 9.5 µl deionized formamide. Samples were electrophoresed on the 3130 Genetic Analyzer with the following run parameters: G5 dye set; Oven Temperature: 60 °C; Polymer_Fill_Volume: 6500 steps; Current Stability: 5 µA; PreRun Voltage: 15 kV; Pre Run Time: 180 s; InjectionVoltage: 1.2 kV; Injection Time: 10 s; Voltage Number of Steps: 40 nk; Voltage Step Interval 15 s; Data Delay Time: 1 s; Run Voltage: 15 kV; Run Time 1500 s.

3. Results and discussion

3.1. Selection of human diploid cells

The outer layer of the hand's epidermis comprises tightly packed, keratinized, squamous epithelial cells that can be deposited as components of touch DNA [41—43]. These characteristics were considered when identifying the type of human diploid cells to include as the DNA source in the laboratory-created eccrine fingerprint, or mock fingerprint (MFP). Both isolated white blood cells and palmar scrapings were evaluated and discarded as the cell source. The white blood cell was too dissimilar to the epidermal epithelial cell, and palmar scrapings contained only a sparse population of anucleate cells.

The squamous epithelial cells lining the mucosa of the buccal cavity were similar to palmar cells but retained intact nuclei. To evaluate their use, cell suspensions were prepared from both true and mock fingerprints and deposited on glass slides. The cells were stained using both hematoxylin/eosin (H&E) and trypan blue and were visualized by bright field microscopy. The H&E stained true fingerprint contained flattened, irregularly-shaped, and mostly anucleate cells (Fig. 1A). In the dye exclusion test, the majority of
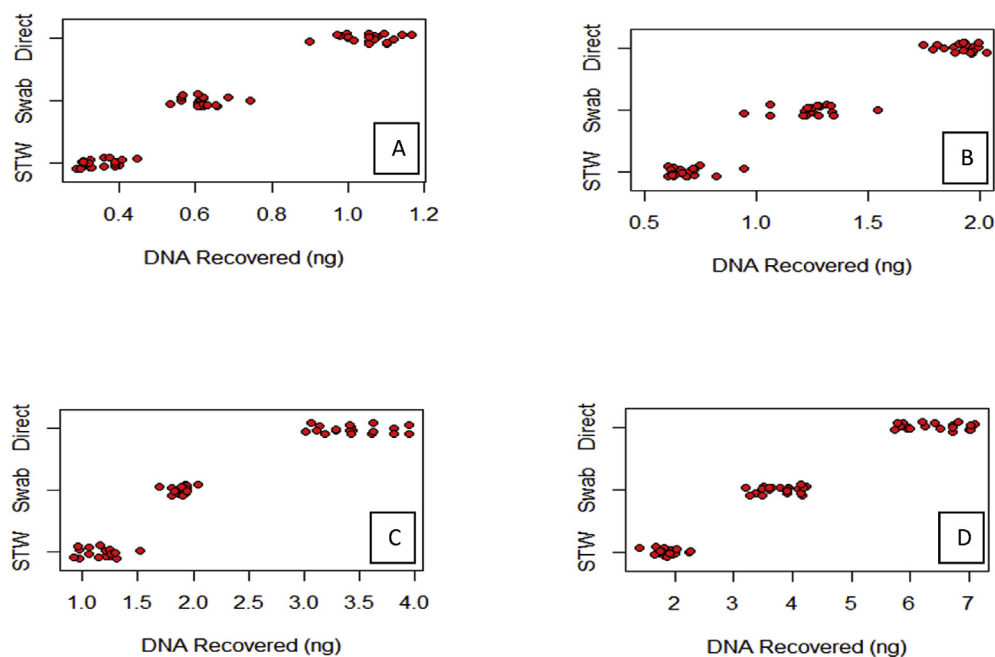

Fig. 6. Mock Fingerprints: Steering Wheel (STW). The twenty replicates of each mock FP process control were plotted against the nanograms of DNA recovered after extraction: (A) 250 cell, 1.5 ng; (B) 475 cell, 2.85 ng; (C) 825 cell, 4.95 ng; and D) 1500 cell, 9.00 ng.
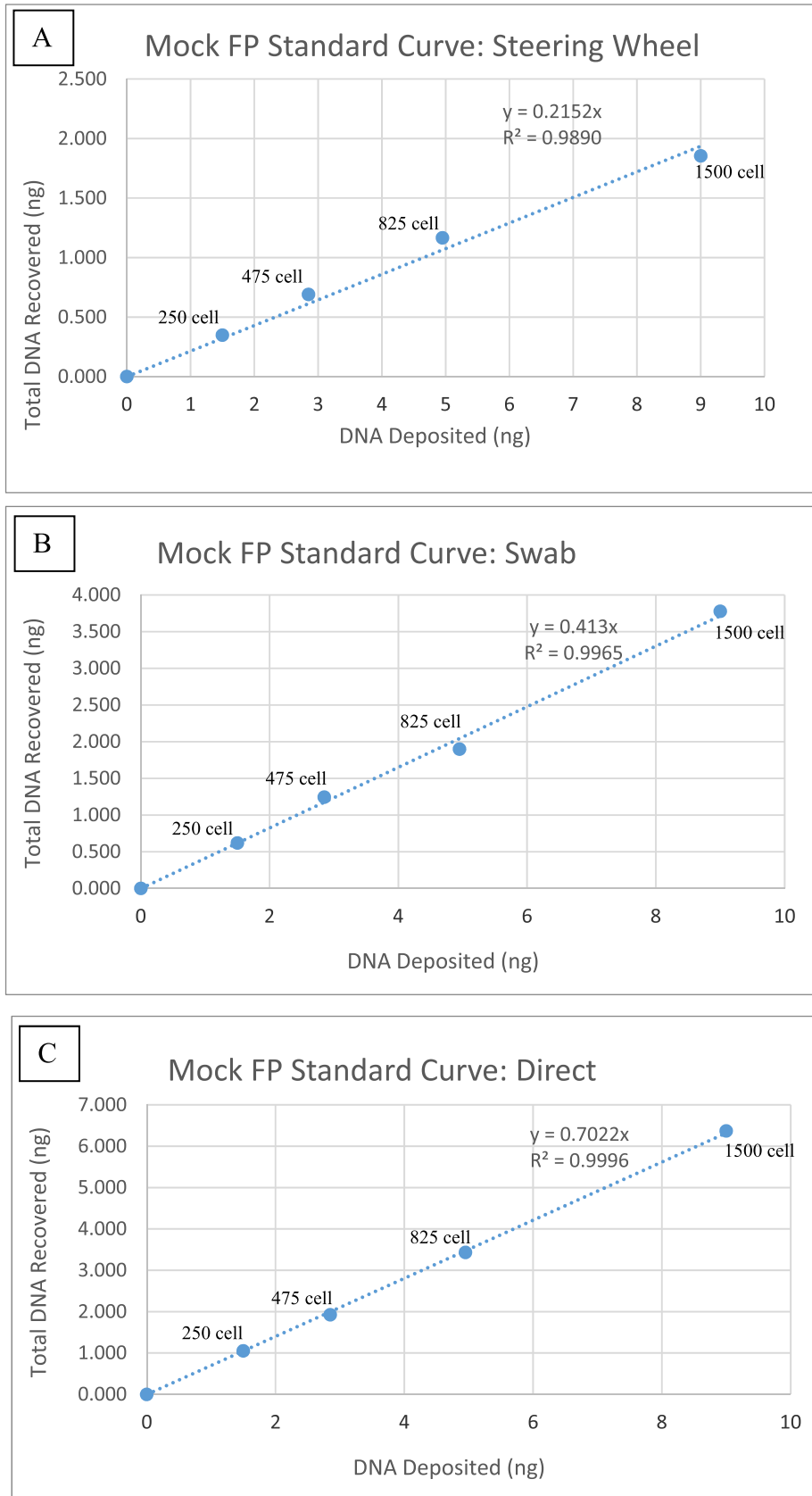
Fig. 7. Process Controls, Steering Wheel. The quantities of DNA recovered from twenty replicates of the five mock FPs for each process control were averaged and displayed as standard curves: (A) cells deposited on a steering wheel; (B) cells pipetted on to a swab; and (C) cells added directly to the lysis buffer. Cell counts are indicated above each point.
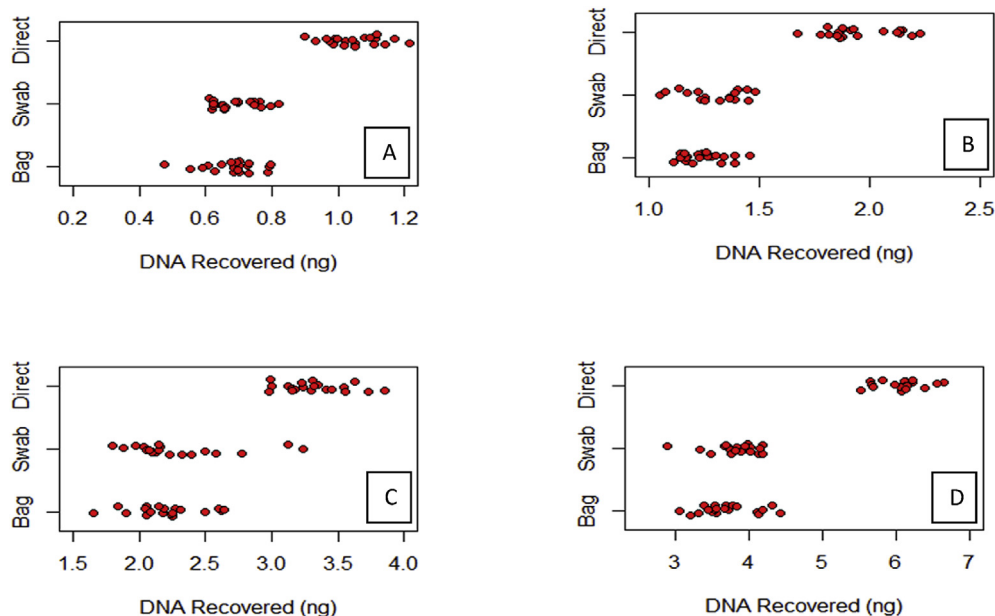
Fig. 8. Mock Fingerprints: Drug Baggies. The twenty replicates of each mock FP process control were plotted against the nanograms of DNA recovered after extraction: (A) 250 cell, 1.5 ng; (B) 475 cell, 2.85 ng; (C) 825 cell, 4.95 ng; and (D) 1500 cell, 9.00 ng.

these cells took up trypan blue (Fig. 1B). The buccal cells in the MFPs had a similar morphology but retained their nuclei (Fig. 1C) and gave comparable results in the dye exclusion test (Fig. 1D). As a result of these similarities, buccal squamous epithelial cells were selected as the DNA source in the MFPs.

3.2. Laboratory-created (mock) eccrine fingerprints

Accurate cell counts were critical for the conversion of #cells/μl to ng DNA/μl. However, cells suspended in water or PBS formed large aggregates, or clumps, making accurate counting impossible (Fig. 2 A&B). Anti-clumping was achieved by suspending cells in Accumax™ Cell Dissociation Solution [44], a reagent optimized to replace trypsin and collagenase treatments in applications such as tissue dissociation, cell counting, and the dissolution of cell clumps (Fig. 2C&D).

To confirm the success of the anti-clumping protocol, reproducibility of the cell counting techniques were evaluated. Counts were completed for each of five different suspensions using either a manual (improved Neubauer hemocytometer) or an automated method (LUNA™ Automated Cell Counter). The eighteen (hemocytometer) or nine (LUNA™) replicate counts for each of the five suspensions were averaged and the standard deviations calculated (Table 1). Complete supporting data is provided as Supplementary Table 1 A&B. The averaged cell count values from each suspension differed, which was to be expected due to biological variation, but data points from a single suspension clustered around the mean, as demonstrated by strip chart (Fig. 3).

With the reproducibility of both manual and automated cell counting established, the #cells/μl of suspension could be used to calculate the volume containing the target DNA quantity, e.g. add 9 ng DNA to a mock fingerprint (1500 cells x 0.006 ng/cell = 9 ng DNA). If the cell count was 168 cells/μl, then 8.9 μl of suspension contained 1500 cells, or 9 ng DNA, which were added to the Fingerprint Solution to produce a MFP.

The complete MFP procedure was: (1) generate a suspension of buccal epithelial cells in Accumax™; (2) count the # cells/μl and convert to ng DNA/μl; (3) add the volume of cell suspension

containing the target DNA quantity to Fingerprint Solution; (4) pipet the MFP onto a substrate, air-dry and collect by swabbing; and (5) extract the DNA.

In the present study, we optimized mock eccrine fingerprints containing directly quantified amounts of DNA sourced from stratified squamous epithelial cells. Recent published studies [41–43] and our own work (Figs. 1 and 2) have demonstrated that nucleated cells can be visualized in deposited fingerprints. The number of nucleated cells can be directly and reliably equated to DNA content for the purpose of tracking loss during collection/extraction. Using these results as a baseline for comparison, we can selectively alter, add and/or remove various other organic and inorganic components of touch samples to evaluate the specific effects on DNA loss and provide an empirical rationale for targeted process improvements.

3.3. Tracking DNA loss

A careful evaluation of the steps of collection and extraction lead to the identification of three major bottlenecks, or key fail points, at which the DNA population in a sample could be reduced significantly: (1) remaining on the substrate; (2) retained on the swab; or (3) lost during the manipulations of DNA extraction. Three process controls were established to monitor loss: (a) depositing the MFP on a surface (surface sample); (b) pipetting the MFP onto a swab (swab sample); and (c) adding the MFP directly to the lysis buffer (direct sample). During protocol development and optimization, MFPs containing cells numbering from 0 to 8000 (0−48.0 ng DNA) were evaluated. Ultimately, we refined this to a five-point range encompassing the typical quantities of DNA recovered from true fingerprints in our hands; this could be extended to include higher numbers as method sensitivity increases with targeted process improvements. Each of the five points was defined by the number of cells, which translated to the nanograms of DNA deposited, and was given a unique identifier: (a) 0 cells, 0.00 ng - MFP1; (b) 250 cells, 1.50 ng − MFP2; (c) 475 cells, 2.85 ng − MFP3; (d) 825 cells, 4.95 ng − MFP4; and 1500 cells, 9.00 ng − MFP5.

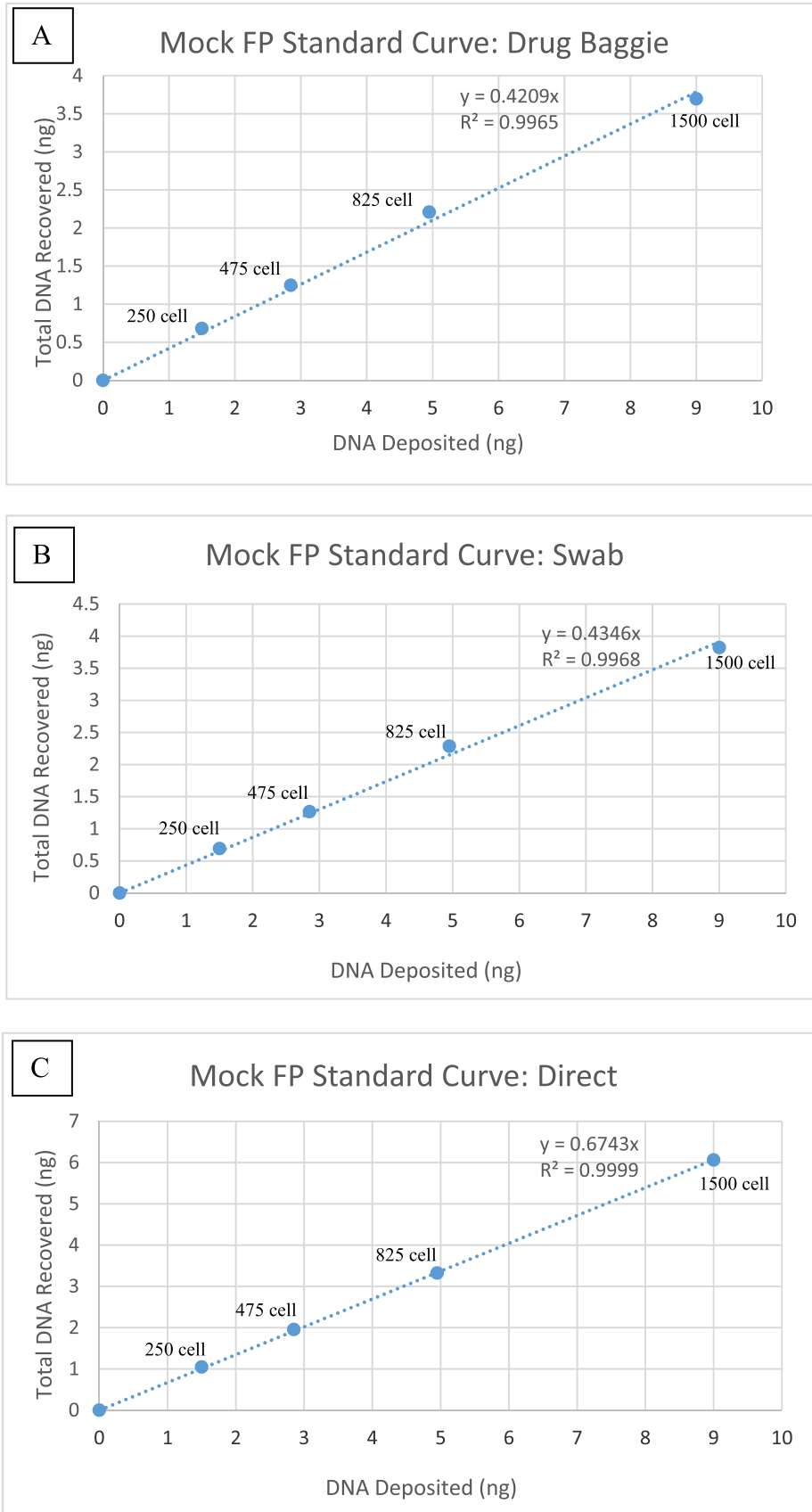Cells for the MFPs were drawn from five single-source

Fig. 9. Process Controls, Drug Baggies. The quantities of DNA recovered from twenty replicates of the five mock FPs for each process control were averaged and displayed as standard curves: (A) cells deposited on drug baggies; (B) cells pipetted on to a swab; and (C) cells added directly to the lysis buffer. Cell counts are indicated above each point.

suspensions donated by one male and two female volunteers over the course of five days. Twenty replicates were generated for each of the five points, MFP1 through MFP5, and deposited on glass slides to produce a five-point, one-hundred sample set for each process control (surface, swab and direct). Negative controls including a clean swab, a swab of a clean slide, and a reagents-only sample were completely free from DNA in all experiments. The sets of MFPs were collected with cotton-tipped swabs wetted with 2% SDS. The DNA was extracted using a standard phenol:chloroform protocol and quantified by real-time PCR. Strip charts showed the twenty-point data sets for each of the process controls clustering around their respective means (Fig. 4).

There was no DNA recovered from any of the zero-cell samples, and they were omitted from further analyses. We averaged the DNA extracted from the twenty replicates within each of the remaining sets to calculate the mean DNA recovery (Table 2, complete data provided in Supplementary Table 2). Subtracting that value from total DNA deposited gave us the mean DNA loss, to provide the average percent DNA loss as follows: ((mean DNA loss/total DNA deposited)*100) (Table 2). For the surface samples, the average DNA loss ranged from 69 to 77% (mean 73%, SD 3.6). The range for the swab samples was 55–61% (mean 59%, SD 2.9), and for the direct samples, 27–42% (mean 34%, SD 6.2). Further strengthening confidence in the data, plotting the data points as a standard curve of DNA recovered vs DNA deposited (Fig. 5) confirmed linearity, with R^2 values averaging 0.9933 (0.9896-surface; 0.9935-swab; 0.9968-direct).

We used the mean DNA loss values for the three process controls to track the loss at each of the key fail points. The slide control accounted for DNA loss over the complete collection and extraction procedures, or total percent loss. The swab sample represented the quantity of DNA remaining on the swab plus the material that was lost during extraction. Loss solely due to the extraction procedure was defined by the direct sample. Therefore, the quantity of DNA remaining on the slide was calculated as: (surface) − (swab), and the amount retained of the swab was: (swab) − (direct). A summary of the data is included in Table 3.

An average of 73% (SD 3.9) of the total DNA deposited was lost during the complete collection and extraction procedures, which is generally in agreement with the published literature [45–47]. The mean DNA remaining on the slide after mock FP collection was 16% (SD 3.9), and an average of 24% (SD 4.9) of the DNA was retained on the swab. It was not clear at this point, however, whether these losses resulted from properties of the collection device or characteristics of the surface. Finally, an average of 34% (SD 6.2) of the DNA was lost during extraction. We chose the organic phenol:chloroform extraction because it has long been considered a gold standard [48,49], but it remains possible that the use of protocol enhancements, such as the inclusion of glycogen or carrier RNA [50–52], or of different extraction technologies would have resulted in a lower DNA loss and should be explored. Using the collection and extraction methods in this study, these results indicate that the majority of the DNA in a touch sample is lost during collection and analysis, reducing the value and utility of such evidence in an operational crime laboratory.

Together, the losses to the surface and the swab account for more than half of the total. Therefore, we began to further differentiate these key fail points by altering the surface variable. We selected three items, representative of surfaces where biological evidence might be deposited at a crime scene, to include further testing - a (Nissan Altima) steering wheel, drug baggies, and a brass door plate (Supplementary Fig. 2). Using the baseline protocol developed with the glass slides, we evaluated DNA loss with these surfaces at the key fail points. Twenty replicate mock FPs for of the five points comprising the standard curve (0.00, 1.50, 2.85, 4.95, 9.00 ng DNA) were deposited as the three process controls (surface, swab,
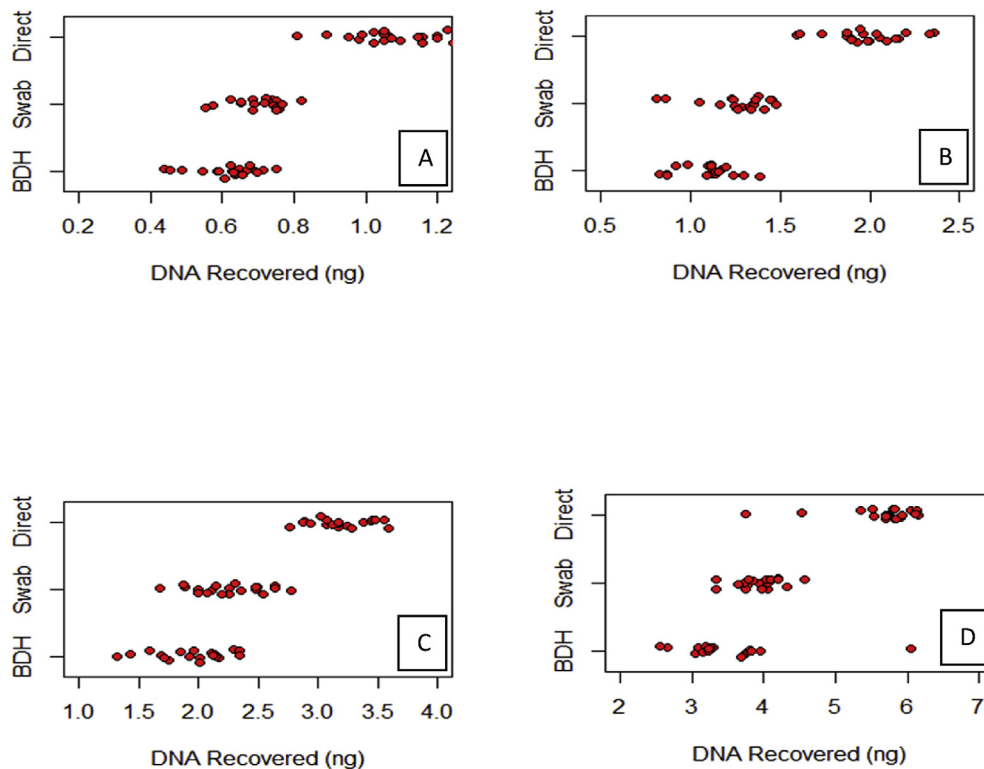

Fig. 10. Mock Fingerprints: Brass Door Plate. The twenty replicates of each mock FP process control were plotted against the nanograms of DNA recovered after extraction: (A) 250 cell, 1.5 ng; (B) 475 cell, 2.85 ng; (C) 825 cell, 4.95 ng; and (D) 1500 cell, 9.00 ng.
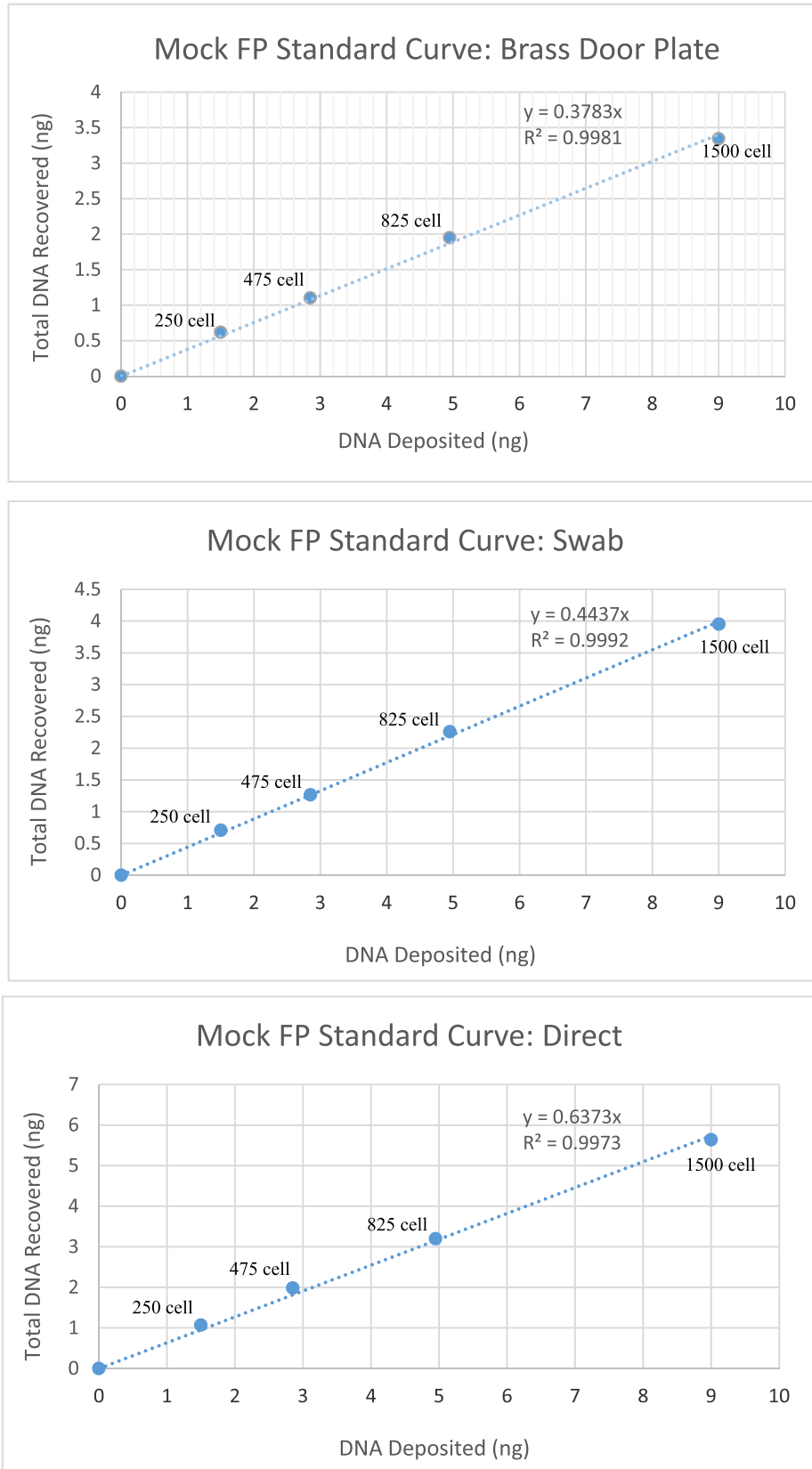
Fig. 11. Process Controls, Brass Door Plate (BDH). The quantities of DNA recovered from twenty replicates of the five mock FPs for each process control were averaged and displayed as standard curves: (A) cells deposited on a brass door plate; (B) cells pipetted on to a swab; and (C) cells added directly to the lysis buffer. Cell counts are indicated above each point.

swab and direct), resulting in one-hundred sample standard curves for each new surface. DNA recovered from the samples was quantified by real-time PCR, and the values averaged to calculate mean DNA recovered (Table 4, complete data is available in Supplementary Table 3). From these values, we calculated the mean

DNA loss per MFP set (DNA deposited minus DNA recovered) and mean percent DNA loss ((mean DNA loss/DNA deposited)*100) (Table 4). Next, we calculated the average percent DNA loss from the four positive mock fingerprints - MFP2 through MFP5 - for each process control on each surface.

Steering Wheel. Strip charts were generated from the twenty-point data sets deposited on the steering wheel, on the swab and into the lysis buffer, showing the values for DNA recovered clustering around the mean (Fig. 6). Plotting the averaged data points as a standard curve of total DNA recovered vs DNA deposited confirmed the linearity of the data (Fig. 7), with R^2 values averaging 0.9950 (0.9965-surface; 0.9890-swab; 0.9996-direct). The averaged percent DNA loss across MFP2 through MFP5 within each of the three process control sets was relatively constant; 77% (SD 1.4) for the surface samples, 59% (SD 2.2) for the swab samples, and 31% (SD 1.4) for the direct samples.

Drug Baggie. Strip charts of the DNA recovered from MFPs that were deposited along the zip top closure, on the swab and directly into lysis buffer confirmed the clustering of the values (Fig. 8). A standard curve of total DNA recovered vs total DNA deposited confirmed linearity (Fig. 9); R^2 values averaged 0.9977 (0.9999-surface; 0.9968-swab; 0.9965-direct). We averaged the percent DNA loss for MFP2 through MFP5 for each of the process controls, finding it to be relatively constant: 56% (SD 1.9) for the surface samples, 55% (SD 1.7) for the swab samples, and 32% (SD 1.2) for the direct samples.

Brass Door Plate. The DNA recovered from MFPs deposited on the brass door plate, on the swab and into the lysis buffer displayed as clustered strip charts (Fig. 10). The standard curves of total DNA recovered vs total DNA deposited showed the data fitting closely to

Table 5
DNA Loss: Key Fail Points. Total percent loss from the steering wheel, drug baggies, and brass door plate was evaluated using the surface process control. Loss resulting from the extraction procedure was quantified with the direct process control. The quantity of DNA left on the surface was calculated as (percent loss: surface) − (percent loss: swab), and the amount retained on the swab was (percent loss: swab) − (percent loss: direct).

	# cells	DNA (ng)	Total % Loss	% Loss at extraction	% Retained on surface	% Retained on swab
Steering Wheel	250	1.50	77	30	18	29
	475	2.85	76	32	19	24
	825	4.95	76	31	15	31
	1500	9.00	79	29	21	29
	Average		77	31	18	28
	SD		1.4	1.3	2.5	3.0
Drug Baggie	250	1.50	55	30	1	24
	475	2.85	56	32	1	24
	825	4.95	55	33	2	21
	1500	9.00	59	33	1	25
	Average		56	32	1	24
	SD		1.9	1.4	0.5	1.7
Brass Door Plate	250	1.50	59	29	6	24
	475	2.85	61	30	6	26
	825	4.95	61	35	6	19
	1500	9.00	63	37	7	19
	Average		61	33	6	22
	SD		1.6	3.9	0.5	3.2

Table 6
ANOVA with Post-Hoc Tukey HSD Test. Four treatments were defined: slide, steering wheel, drug baggie, brass door plate. Within each treatment, pairwise comparisons using the averaged percent DNA loss for MFP2 − MFP5 were completed by ANOVA with Post-Hoc Tukey HSD Tests.

| Total % Loss			
Treatment Pairs	Tukey HSD Statistic	Tukey HSD p-value	Tukey HSD Inference
slide vs steering wheel	3.1164	0.1773828	insignificant
slide vs drug baggie	14.1279	0.0010053	p < 0.01
slide vs door plate	10.1804	0.0010053	p < 0.01
steering wheel vs drug baggie	17.2443	0.0010053	p < 0.01
steering wheel vs door plate	13.2968	0.0010053	p < 0.01
drug baggie vs door plate	3.9475	0.067887	insignificant
% Loss at Extraction			
Treatment Pairs	Tukey HSD Statistic	Tukey HSD p-value	Tukey HSD Inference
slide vs steering wheel	1.7144	0.6215408	insignificant
slide vs drug baggie	0.9231	0.8999947	insignificant
slide vs door plate	0.5275	0.8999947	insignificant
steering wheel vs drug baggie	0.7913	0.8999947	insignificant
steering wheel vs door plate	1.1869	0.8203717	insignificant
drug baggie vs door plate	0.3956	0.8999947	insignificant
% Retained on Surface			
Treatment Pairs	Tukey HSD Statistic	Tukey HSD p-value	Tukey HSD Inference
slide vs steering wheel	2.3586	0.3816024	insignificant
slide vs drug baggie	12.221	0.0010053	p < 0.01
slide vs door plate	7.9336	0.0010053	p < 0.01
steering wheel vs drug baggie	14.5807	0.0010053	p < 0.01
steering wheel vs door plate	10.2923	0.0010053	p < 0.01
drug baggie vs door plate	4.2885	0.0448168	p < 0.05
% Retained on Swab			
Treatment Pairs	Tukey HSD Statistic	Tukey HSD p-value	Tukey HSD Inference
slide vs steering wheel	2.502	0.3335667	insignificant
slide vs drug baggie	0.2944	0.8999947	insignificant
slide vs door plate	1.3246	0.7684717	insignificant
steering wheel vs drug baggie	2.7963	0.2493041	insignificant
steering wheel vs door plate	3.8266	0.0784855	insignificant
drug baggie vs door plate	1.0302	0.8794199	insignificant
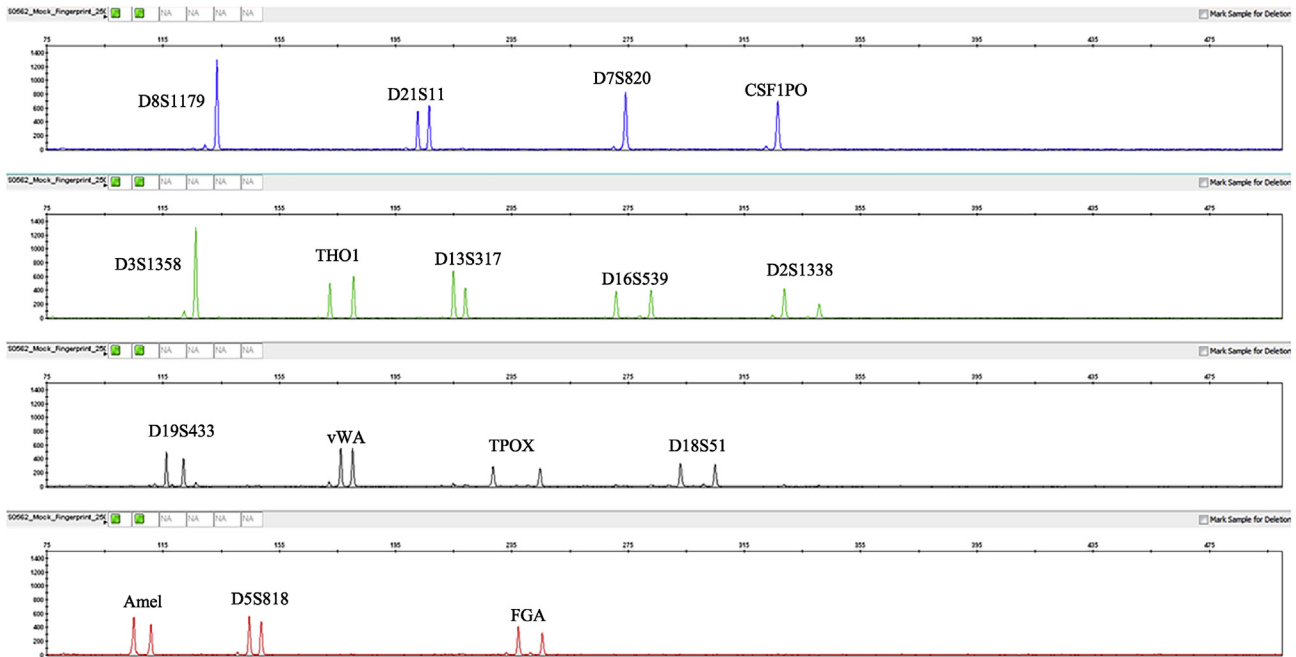
Fig. 12. Samples from each of the process control sets were tested using the AmpFℓSTR® Identifiler® Plus PCR Kit. The 250 cell, slide control sample is representative of the results.

the regression line (Fig. 11), with an average R^2 value of 0.9982 (0.9992-surface; 0.9973-swab; 0.9981-direct). For each of the process controls, the average percent loss for MFP2 − MFP5 was, again, relatively constant at 61% surface (SD 1.7), 55% swab (SD 1.4) and 33% direct (SD 4.1).

We tracked the mean DNA loss at each of the key fail points using the values collected for the process controls; they were: total percent loss (surface sample), percent loss at extraction (direct sample), percent retained on surface (surface minus swab), and percent retained on swab (swab minus direct). A summary of the data is included in Table 5. The major differences were detected in the mean percentages of DNA retained on the surfaces and total DNA lost. The drug baggies and the door plate retained between 3 and 18 times less DNA than the slide and steering wheel, and the total loss decreased by approximately 30%. To provide a further statistical evaluation of the data, we defined four treatments − slide, steering wheel, drug baggie, brass door plate. Within each treatment, we used the averaged percent loss for MFP2 − MFP5, and completed pairwise comparisons by ANOVA with post-Hoc Tukey HSD test (Table 6).

Variance was insignificant for both the % retained on the swab and % lost at extraction for all pairwise comparisons. This was not unexpected because the "swab" and "direct" procedures were not altered between sample sets. There were two treatment pairs with no statistically significant differences in total % loss: slide/steering wheel and baggie/door plate. The total percent DNA retained on the surface was not variable within the slide/steering wheel group, but was for the baggie/door plate. Significant variance in the total % loss was observed between: (1) slide and baggie; (2) slide and door plate; (3) steering wheel and baggie; and (4) steering wheel and door plate. The pairwise comparisons also showed significant differences in the % retained on the surface between: (1) slide and baggie; (2) slide and door plate; (3) steering wheel and baggie; (4) steering wheel and door plate; and (5) baggie and door plate.

Although the relative porosity and finish of the slide and the steering wheel were dissimilar, there was insignificant variance between their total % loss and the % retained on the surfaces. A closer look at the composition of the slides provided a likely explanation for the results. The majority of commercial glass, including microscope slides, is soda-lime glass. Typical components are: Na_2O (soda, 12−16 w/t%), CaO (lime, 10−15 w/t%), and SiO_2 (silica, 70−75 w/t%) [53].

There was an insignificant variance between the total % losses from the baggie and the door plate. This was consistent with the nature of the materials; the baggies were made of polyethylene - waterproof and resistant to condensation - and the door plate was satin clear-coated brass, optimized to protect the surface and inhibit corrosion. The difference between the average percent retained on the individual surfaces was statistically significant, with 1% retention on the baggie and 6% on the door plate, but these numbers were in sharp contrast to those resulting from the slide and steering wheel.

As a final test, samples from each of the process control groups were amplified using the AmpFℓSTR® Identifiler® Plus PCR Kit to confirm that the mock fingerprints supported downstream DNA profiling. Complete profiles were generated for all samples; a representative sample is Fig. 12 (250 cell, surface control).

Collectively, these baseline results suggest that, as a starting point, the exploration of alternative collection devices, additional surfaces and different extraction techniques in future experiments is warranted. This information should help us optimize the collection and extraction protocols for touch DNA, taking into consideration the variables of each scenario.

4. Conclusions

The goal of forensic science is to maximize the value of evidence. Through examining the process flow to demonstrate key fail points, touch DNA will be better utilized to solve and prevent crimes. Published data and reports from operational scientists have indicated that up to 90% of the DNA in a sample can be lost during collection and analysis [46], however other studies average this loss at approximately 39% [47]. In the present study, we calculated percent DNA loss from touch DNA samples ranging from 56 to 77%. Considering any of these values, this means, from an operational stand-point, a vast amount of evidence and the answers it may have

provided have potentially been lost. This is particularly problematic in cases of touch DNA, where sample is limited to begin with. It has been difficult to quantify this loss and evaluate improvements empirically because there has been no positive control since the DNA content in true fingerprints is extremely variable, thus a starting point cannot be established. In this work, we describe efforts to bridge this gap that are rooted in a collaboration between research and operational forensic scientists. This synergy has allowed each group to provide specific expertise to maximize the efforts and produce a valuable tool for future studies as well as enhance applicability to forensic casework.

As a positive control for the collection and analysis of touch DNA, a protocol for laboratory-created eccrine fingerprints containing a known quantity of DNA was developed and optimized. The complexity of a touch sample was reduced to establish a baseline set of conditions against which other variables, such as the addition of fatty acids or the effects of different collection devices and extraction procedures, can be independently evaluated in future studies. The three process controls allowed us to quantify DNA loss at key points in collection and analysis. Creation of a robust fingerprint standard curve enabled elimination of the large range of variation seen between individuals. In future experiments, this will enable the range of DNA deposited at a crime scene by sloughers and shedders to be framed, so that variables which impact the capture of DNA can be established.

An increase in understanding of the challenges in efficiency of each step in the DNA sample workflow has major ramifications in terms of potential improvement for all biological sample types, including semen, blood and saliva. Improvements in efficiency in obtaining samples from substrates and subsequent extraction will broaden the number of samples that will yield successful profiles, particularly when the starting quantities are low or environmentally challenged, as is the case with touch samples.

With better understanding of DNA yields and the mechanisms of loss, targeted process improvements will bring touch DNA samples into even more routine use with standardized optimized procedures. Depending on substrate type and porosity, it is a challenge to obtain all the available DNA, however these findings demonstrate key points of loss to be targeted by process optimization. This will translate to a wider variety of successful sample types and percentage success on similar case sample types. The results will be an improved achievement of the crime lab mission of maximization of evidentiary value.

Declaration of competing interest

None.

CRediT authorship contribution statement

Jessica Tang: Investigation, Methodology, Validation, Visualization, Writing - original draft. **Jennifer Ostrander:** Formal analysis, Investigation, Validation. **Ray Wickenheiser:** Conceptualization, Methodology, Visualization, Writing - review & editing. **Ashley Hall:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing - review & editing.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsisyn.2019.10.004.

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