



RESEARCH ARTICLE

Managing Porous Substrate Wicking in Trace DNA Collection: Impact of Swab Wetting Volume on Fabric Evidence

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ABSTRACT

Trace DNA recovery from clothing remains highly variable and strongly dependent on pre-analytical sampling conditions. While moist swabbing is widely adopted for fabric substrates, the volume of liquid applied to the swab is rarely standardised and has not been systematically evaluated as a mechanistic determinant of recovery efficiency. This study investigated the effect of controlled swab wetting volume on trace DNA recovery and STR profile quality from worn fabric substrates with differing absorbency, and quantitatively linked biological outcomes to physical measurements of fabric absorbency and swab wetness loss.

Garments representing common forensic clothing materials (cotton, cotton/polyester blend, polyester, nylon, denim, and fleece) were worn by volunteers under routine daily activity conditions. Sampling was performed using cotton swabs moistened with 100, 150, or 200 µL of sterile distilled water under a standardised protocol. Fabric absorbency was quantified gravimetrically, and swab wetness loss during sampling was measured to estimate liquid sequestration into the substrate. DNA was extracted, quantified, and STR typed using a single analytical workflow. DNA yield, STR profile completeness, allele counts, and RFU values were evaluated using mixed-effects statistical models.

Increasing wetting volume significantly enhanced trace DNA recovery and STR profile completeness ($p < 0.001$), with the strongest effects observed for highly absorbent fabrics (cotton, denim, and fleece) and smaller effects for low-absorbency materials (nylon and polyester). Fabric absorbency and swab wetness loss were independently associated with DNA yield ($p < 0.01$), supporting a physical model in which textile substrates act as competing liquid sinks that reduce effective wet contact at low wetting volumes. Modest increases in DNA quantity produced disproportionate gains in full STR profile recovery, consistent with threshold behaviour in low-template amplification. No systematic PCR inhibition was observed. These findings demonstrate that swab wetting volume is a critical, substrate-dependent parameter governing trace DNA recovery from fabrics. By linking textile liquid-transport behaviour with biological recovery outcomes, this study provides a mechanistic and predictive framework for fabric sampling and supports the transition from heuristic wet swabbing toward quantitatively controlled, substrate-aware collection protocols. Optimisation of wetting volume represents a simple, low-cost intervention capable of materially improving evidential yield from clothing evidence without changes to consumables or analytical workflows.

Keywords: Forensic science; Forensic genetics; Trace DNA; DNA recovery; Fabric substrates; Cotton swab; Swab wetting volume; Textile absorbency; Sampling optimisation

1 Introduction

Trace DNA represents a critical category of forensic evidence frequently recovered from crime scenes and plays a central role in associating individuals with criminal activities^{1–7}. Unlike biological fluids, trace DNA is typically deposited through casual contact with surfaces such as tools, door handles, and clothing, either through direct handling or through prolonged wear of an item, thereby providing probative information when other biological material is absent^{3,8–10}. In the context of clothing and personal items, trace DNA may originate from the individual who wears or uses the item, as well as from secondary contact with other surfaces or persons. Despite its evidential value, the recovery and interpretation of trace DNA are often constrained by pronounced variability in both the quantity and quality of DNA obtained. Such variability reflects a combination of factors, including the chemical and physical properties of the substrate^{11–14}, exposure to environmental conditions that accelerate degradation or loss^{15–18}, and differences in collection methodology applied at the scene or during laboratory processing^{11,19–22}. Additional sources of variation include the choice of moistening agent and the number of collection passes or lifts applied, both of which can materially influence recovery efficiency and downstream profile quality^{23–30}.

Beyond sampling-related influences, variability is further compounded by differences in extraction and quantification strategies^{2,4,12,31–35}, risks of contamination, and well-documented inter-individual differences in DNA shedding and transfer dynamics^{36–45}. Consequently, selection of an appropriate sampling tool—such as cotton swabs, nylon-flocked swabs, or adhesive tapes—should be guided by the characteristics of the surface being sampled to maximise recovery^{10,11,23,24}. Smooth, non-porous surfaces (e.g., glass and plastic) are generally well suited to swabbing^{11,25}, whereas porous and fibrous substrates, including fabrics, frequently yield improved results when sampled using adhesive tape-lifting approaches^{46–51}.

In recent years, several alternative or hybrid sampling strategies have been proposed to address limitations of conventional methods. These include the combined use of cotton and microFLOQ® swabs within direct amplification workflows, the application of wet-vacuum collection systems for challenging substrates, and chemical formulations designed to improve DNA detachment and recovery^{28,51–53}. The development of these approaches reflects the continued evolution of trace DNA collection practices. Importantly, the wide variability in DNA recovery observed across substrates and environmental contexts has led to increasing recognition that sampling strategies should be adapted to the specific characteristics of the evidence rather than applied uniformly^{54–57}.

Alongside methodological development, there is growing emphasis on flexible investigative approaches that integrate technological advances with responsive evidence-collection strategies to meet contemporary forensic demands^{58,59}. Conventional DNA workflows—

particularly those relying on silica-based extraction—can also suffer from sample loss, a limitation that is especially consequential for low-template and environmentally challenged samples^{1,60}. Accordingly, direct amplification strategies that bypass extraction and quantification have gained attention as a means of preserving limited biological material while reducing analytical turnaround times^{21,27,61}.

Despite extensive investigation into swab type, collection motion, and substrate category, one practical parameter remains poorly defined in forensic sampling protocols: the volume of liquid used to moisten swabs prior to sampling. Current guidance commonly recommends that swabs be “moistened” without specifying a standardized volume, resulting in substantial procedural variation between practitioners and laboratories²⁰. This lack of standardisation is particularly consequential for fabric substrates, which exhibit marked differences in absorbency and capillary behaviour depending on fibre composition, yarn structure, and fabric geometry^{62–66}. Hydrophilic fibres such as cotton and blended textiles retain water through fibre swelling and internal moisture reservoirs, whereas hydrophobic synthetic fibres rely primarily on capillary transport within pore spaces, producing distinct liquid uptake and retention dynamics^{62–65}. Liquid movement within textiles is governed by capillary forces and pore geometry, which control both vertical and lateral transport and determine how readily moisture is drawn away from an external source^{66–69}. Variations in fabric construction, including knit versus woven structures and surface finishing, further modulate moisture retention and wicking behaviour^{70,71}.

When considered in the context of forensic sampling, these substrate-dependent liquid transport mechanisms have direct implications for trace DNA recovery. During pressure-based rotational swabbing, porous fabrics may draw liquid away from the swab head through capillary action, thereby reducing the effective moisture available at the swab–fabric interface. Reduced available liquid may limit the mobilisation and transfer of epidermal cells and extracellular DNA at lower wetting volumes, potentially constraining recovery efficiency on highly absorbent substrates. Similar substrate-driven effects on DNA recovery have been observed in studies of trace DNA transfer and persistence on textiles and worn items^{10,18,46–49}. Consequently, differences in fabric absorbency and moisture transport properties represent a plausible mechanistic source of variability in DNA yield and STR profile quality that has not been systematically addressed through controlled manipulation of swab wetting volume.

This study aimed to determine whether controlled increases in swab wetting volume enhance trace DNA recovery and STR profile quality from fabric substrates with differing absorbency, and to mechanistically link biological outcomes with measurable physical parameters of fabric absorbency and swab wetness loss. Specifically, the effects of three wetting volumes (100, 150, and 200 µL) were evaluated across multiple fabric types commonly encountered in forensic casework.

2 Materials and methods

2.1 EXPERIMENTAL DESIGN

A factorial experimental design was used to investigate the effects of swab wetting volume and fabric type on trace DNA recovery and STR profile quality. Two independent variables were examined: swab wetting volume (100 µL, 150 µL, and 200 µL of sterile distilled water) and fabric type (six substrates representing a wide range of absorbency properties).

A within-item split-zone design was employed so that each sampling region on a garment served as its own internal control across the three wetting volumes. This approach minimised variability attributable to differences between donors and between garments. All wetting, weighing, and swabbing procedures were conducted under controlled laboratory conditions, with ambient temperature maintained between 20 and 23 °C and relative humidity between 40 and 55%.

2.2 FABRIC TYPES AND GARMENT CATEGORIES

Six fabric substrates were selected to represent the diversity of fibre structures and absorbency characteristics commonly encountered in forensic casework. These included 100% cotton knit (T-shirt fabric), a cotton/polyester blend (65/35), polyester sports fabric, nylon windbreaker fabric, denim (cotton twill), and fleece/terry fabric with high capillarity. For each fabric type, fabric thickness (mm) and basis weight (g/m²) were recorded using standard textile measurement methods to support mechanistic interpretation of absorbency and wicking behaviour.

These fabrics were incorporated into four garment categories in order to reflect realistic forensic clothing evidence and to capture variation in contact patterns, sweat exposure, and frictional loading. The garment categories and corresponding fabric allocations are

summarised in **Table 1** and consisted of T-shirts (cotton, poly-cotton, and polyester), hoodies or jackets (inner cuffs composed of cotton and fleece), trousers (denim and poly-cotton), and caps (cotton or polyester sweatbands with fleece or woven lining).

Caps were included because the inner sweatband represents a highly absorbent, sebum-rich, and friction-intensive substrate and was therefore expected to be particularly sensitive to variation in swab wetting volume during trace DNA collection.

2.3 GARMENT PREPARATION

All garments were new and prepared prior to participant use. Each item was washed at 50 °C using standard detergent without bleach additives, thoroughly rinsed, air-dried, and sterilised by ultraviolet irradiation for 25 minutes. Where applicable, both sides of each fabric were exposed to UV radiation at a consistent distance and intensity. Following sterilisation, garments were individually sealed in sterile packaging and stored until issued to participants.

2.4 DEPOSITION MODEL

Fifteen volunteers (60% male and 40% female) wore the garments for an 8-hour period during routine daily activities. Participants were instructed not to intentionally rub, wipe, or clean the garments during wear. Activities were limited to normal daily movements, including walking, sitting, and light arm movement, and excluded strenuous physical exercise.

After wear, high-contact regions were selected for sampling. For T-shirts, the inner collar and underarm regions were sampled; for hoodies or jackets, the inner cuff region was sampled; for trousers, the waistband and pocket opening were sampled; and for caps, the inner sweatband was sampled. No cleaning, shaking, or wiping of garments was performed prior to sampling.

Table 1. Fabric types, garment categories, and sampling zones included in the study.

Fabric ID	Fabric type	Fibre composition	Garment category	Sampling zone(s)	Absorbency measurements to be recorded
F1	Cotton knit	100% cotton	T-shirt	Inner collar, underarm	Thickness, basis weight, absorption capacity
F2	Poly-cotton blend	65% cotton / 35% polyester	T-shirt, trousers	Inner collar, waistband, pocket opening	Thickness, basis weight, absorption capacity
F3	Polyester sports fabric	100% polyester	T-shirt, cap sweatband	Inner collar, sweatband	Thickness, basis weight, absorption capacity
F4	Nylon windbreaker fabric	100% nylon	Jacket cuffs	Inner cuff	Thickness, basis weight, absorption capacity
F5	Denim (cotton twill)	100% cotton	Trousers	Waistband, pocket opening	Thickness, basis weight, absorption capacity
F6	Fleece / terry	Cotton or polyester blend	Hoodie cuffs, cap lining	Inner cuff, sweatband	Thickness, basis weight, absorption capacity

2.5 WITHIN-ITEM SAMPLING LAYOUT

Each sampling region was subdivided into three adjacent, equally sized zones using sterile single-use templates. The zones were assigned to the three wetting volumes (100 µL, 150 µL, and 200 µL). Zone positions were randomised

to minimise spatial bias, and small buffer gaps were maintained between zones to prevent cross-transfer.

2.6 SWAB WETTING PROCEDURE

Copan cotton swabs (150C) were moistened using calibrated micropipettes. Sterile distilled water was

applied to the cotton head in multiple small aliquots (two to four deposits) to ensure uniform distribution. The swab was gently rotated between gloved fingers to promote even absorption and was applied to the fabric within 5 seconds of wetting in order to minimise evaporative loss. A new sterile pipette tip was used for each swab.

2.7 SWABBING PROCEDURE

All sampling was performed by a single trained operator, and gloves were changed between garments and between zones. Swabbing was standardised with respect to angle, duration, motion, and pressure. The swab was held at an angle of approximately 60°, and each zone was sampled for 2 minutes using 30 horizontal strokes, 30 vertical strokes, and 30 circular motions with continuous rotation of the swab. Moderate and constant pressure was applied throughout sampling. Following collection, swabs were placed into sterile tubes for downstream processing.

2.8 FABRIC ABSORPTION CAPACITY

Fabric absorbency was quantified as a continuous explanatory variable using a gravimetric mass-gain approach. For each fabric type, swatches measuring 5 × 5 cm (25 cm²) were cut from unused prepared material and allowed to equilibrate for at least 30 min under laboratory ambient conditions. Each swatch was weighed dry (W_0 , mg).

A volume of 200 µL of sterile distilled water was then applied evenly to the surface of each swatch in multiple small deposits to ensure uniform wetting. After 60 s of contact time, the swatch was placed between two sheets of standardised lint-free blotting paper and compressed with a 500 g weight for 10 s to remove unbound surface liquid. The swatch was immediately reweighed (W_1 , mg).

Absorption capacity was calculated as ($W_1 - W_0$) divided by the swatch area (25 cm²) and expressed in mg/cm². A minimum of five replicate swatches per fabric type were analysed, and results were reported as mean ± standard deviation.

2.9 SWAB WETNESS LOSS

To quantify liquid loss from swabs during fabric sampling, swab wetness loss was measured using the same swabbing protocol applied for DNA collection. Each dry swab was weighed (S_0 , mg), moistened with 100, 150, or 200 µL of sterile distilled water, and immediately reweighed (S_1 , mg). The swab was then used to sample fabric under the standardised protocol and reweighed immediately after sampling (S_2 , mg).

Liquid loss was calculated as $S_1 - S_2$, and the fraction of liquid lost was calculated as:

$$\text{Fraction lost (\%)} = (S_1 - S_2) / (S_1 - S_0) \times 100.$$

To account for evaporative loss, an air-exposure control was included in which moistened swabs were weighed, held for the same duration as fabric sampling without contacting any surface, and then reweighed. This allowed evaporative loss to be distinguished from liquid transfer to the fabric.

This measurement was used to quantify the proportion of applied liquid sequestered into the fabric during sampling and therefore unavailable for biological mobilisation at the swab–fabric interface.

2.10 DNA EXTRACTION, QUANTIFICATION, AND STR PROFILING

All samples were processed using identical downstream workflows. DNA extraction was performed using the PrepFiler Express™ Forensic DNA Extraction Kit on the AutoMate Express™ Forensic DNA Extraction System. The entire swab head was subjected to extraction, and DNA was eluted in 50 µL of elution buffer for all samples.

DNA quantification was carried out using the Qiagen Investigator Quantiplex Pro Quantification Kit on a QuantStudio™ 5 Real-Time PCR System, with data analysed using HID Real-Time PCR Analysis Software v1.3.

STR amplification was performed using the GlobalFiler™ PCR Amplification Kit on an ABI GeneAmp® 9700 Thermal Cycler with 29 amplification cycles. Capillary electrophoresis was conducted on an ABI 3500 Genetic Analyzer using a 36-cm capillary array and POP-4™ polymer. Injection mixtures contained 1 µL of PCR product, 9.6 µL of Hi-Di™ formamide, and 0.4 µL of GeneScan™ 600 LIZ®. Allelic ladders were included on every 96-well plate.

Samples were denatured at 95 °C for 5 minutes and snap-cooled prior to capillary electrophoresis. Injection was performed at 1.2 kV for 24 seconds. STR profiles were analysed using GeneMapper® ID-X version 1.5 with an analytical threshold of 75 RFU. Extraction blanks, reagent blanks, and amplification blanks were included with each batch and were required to be negative.

2.11 OUTCOME MEASURES

Primary outcome measures were DNA concentration (ng/µL and total ng), STR profile completeness (classified as full single-source or partial single-source), mean STR allele count, and mean RFU per locus for full profiles. Allele count was used as a quantitative proxy for profile completeness because it captures cumulative locus success and dropout across the profile.

2.12 STATISTICAL ANALYSIS

Statistical analyses were performed using RStudio (version 4.2.3). Linear mixed-effects models were fitted with wetting volume, fabric absorption capacity, and swab wetness loss as fixed effects, and donor and garment as random effects. Interaction terms between wetting volume and absorption capacity, and between wetting volume and wetness loss, were tested, as were quadratic terms for wetting volume. Statistical significance was set at $p < 0.05$.

3 Results

3.1 PHYSICAL AND ABSORBENCY PROPERTIES OF FABRICS

The six fabrics exhibited significant differences in their physical and absorbency characteristics (**Table 2**). Fabric thickness varied significantly across substrates (one-way

analysis of variance, $p < 0.001$), ranging from 0.16 ± 0.02 mm for nylon windbreaker material to 1.52 ± 0.11 mm for fleece/terry fabric ($n = 5$ measurements per fabric). Basis weight also differed significantly between fabrics ($p < 0.001$), spanning from 72 ± 6 g/m² to 385 ± 15 g/m².

Absorption capacity differed markedly among fabric types (Figure 1), with a significant effect of substrate on mass gain per unit area ($p < 0.001$, $n = 5$ swatches per fabric). Mean absorption capacity values ranged from 1.9 ± 0.3 mg/cm² for nylon to 12.6 ± 1.0 mg/cm² for fleece/terry fabric. Polyester sports fabric exhibited low absorbency (3.2 ± 0.4 mg/cm²), whereas the poly-cotton blend demonstrated intermediate behavior (5.6 ± 0.5 mg/cm²). Cotton knit and denim fabrics showed higher absorption capacities of 7.4 ± 0.6 mg/cm² and 8.3 ± 0.7 mg/cm², respectively.

Ranking the substrates by increasing mean absorption capacity yielded the following order: nylon < polyester < poly-cotton < cotton < denim < fleece (Figure 1). Post hoc comparisons indicated that low-absorbency synthetic fabrics (nylon and polyester) differed significantly from high-absorbency pile and cotton-based fabrics (cotton, denim, and fleece) (adjusted $p < 0.01$ for all pairwise comparisons between these groups), whereas intermediate fabrics (poly-cotton) showed partial overlap with both groups.

Together, these results confirm that the selected fabric panel spans a statistically distinct and continuous range of absorbency properties, providing a quantitative physical basis for subsequent evaluation of substrate-dependent moisture loss and wetting-volume effects during swab-based DNA collection.

Table 2. Physical characteristics and absorption capacity of the fabrics used in the study.

Fabric ID	Fabric type	Fibre composition	Thickness (mm), mean \pm SD (n = 5)	Basis weight (g/m ²), mean \pm SD (n = 5)	Absorption capacity (mg/cm ²), mean \pm SD (n = 5)
F1	Cotton knit	100% cotton	0.62 ± 0.05	165 ± 8	7.4 ± 0.6
F2	Poly-cotton blend	65% cotton / 35% polyester	0.54 ± 0.04	185 ± 10	5.6 ± 0.5
F3	Polyester sports fabric	100% polyester	0.28 ± 0.03	140 ± 7	3.2 ± 0.4
F4	Nylon windbreaker fabric	100% nylon	0.16 ± 0.02	72 ± 6	1.9 ± 0.3
F5	Denim (cotton twill)	100% cotton	0.92 ± 0.07	385 ± 15	8.3 ± 0.7
F6	Fleece / terry	Cotton/polyester blend	1.52 ± 0.11	265 ± 12	12.6 ± 1.0

Table Footnote: Thickness, basis weight, and absorption capacity values are reported as mean \pm standard deviation based on five replicate measurements per fabric. Absorption capacity was calculated as $(W_1 - W_0) / 25$ using 5×5 cm swatches (25 cm²).

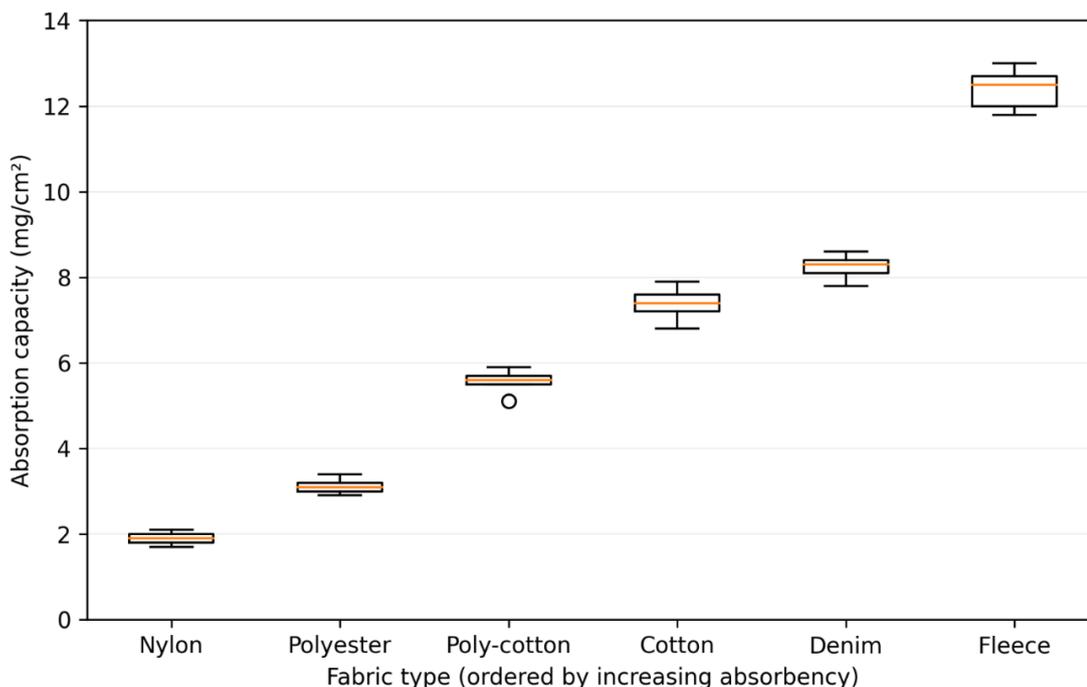


Figure 1. Absorption capacity (mg/cm²) of six fabrics measured by a standardized mass-gain method ($n = 5$ per fabric). A total of 200 μ L sterile distilled water was applied to 5×5 cm swatches (25 cm²), allowed to dwell for 60 s, and excess liquid removed by blotting under a 500 g load for 10 s. Boxes show median and interquartile range; whiskers indicate full range. Fabrics are ordered by increasing mean absorption capacity.

3.2 SWAB WETNESS LOSS DURING FABRIC SAMPLING

Swab wetness loss varied significantly as a function of both fabric type and wetting volume (**Figure 2**). Across all wetting volumes, the fraction of liquid lost during sampling increased progressively with fabric absorbency, with the lowest losses observed for nylon and polyester and the highest losses observed for denim and fleece.

Inferential analysis confirmed strong main effects of fabric type and wetting volume on the fraction of liquid lost during swabbing (fabric effect: $p < 0.001$; volume effect: $p < 0.001$). In addition, a statistically significant fabric \times volume interaction was observed ($p < 0.001$), indicating that the magnitude of the wetting-volume effect depended on substrate properties.

Descriptively, at 100 μL the mean fraction of liquid lost spanned from approximately $\sim 24\%$ for nylon to $\sim 63\%$

for fleece, demonstrating substantial moisture uptake by high-absorbency fabrics during swabbing. Intermediate losses were observed for polyester ($\sim 30\%$) and poly-cotton ($\sim 42\%$), whereas cotton and denim showed higher losses of approximately $\sim 50\%$ and $\sim 55\%$, respectively (**Figure 2**).

Increasing the wetting volume reduced the fraction of liquid lost for all fabrics. At 150 μL , mean liquid loss decreased across substrates (approximately $\sim 17\%$ for nylon and $\sim 50\%$ for fleece), and at 200 μL losses were further reduced (approximately $\sim 12\%$ for nylon and $\sim 39\%$ for fleece). The interaction pattern was evident in the widening separation between volume curves as absorbency increased: differences between 100 μL and 200 μL were modest for low-absorbency fabrics (nylon, polyester) but substantially larger for high-absorbency fabrics (cotton, denim, fleece) (**Figure 2**).

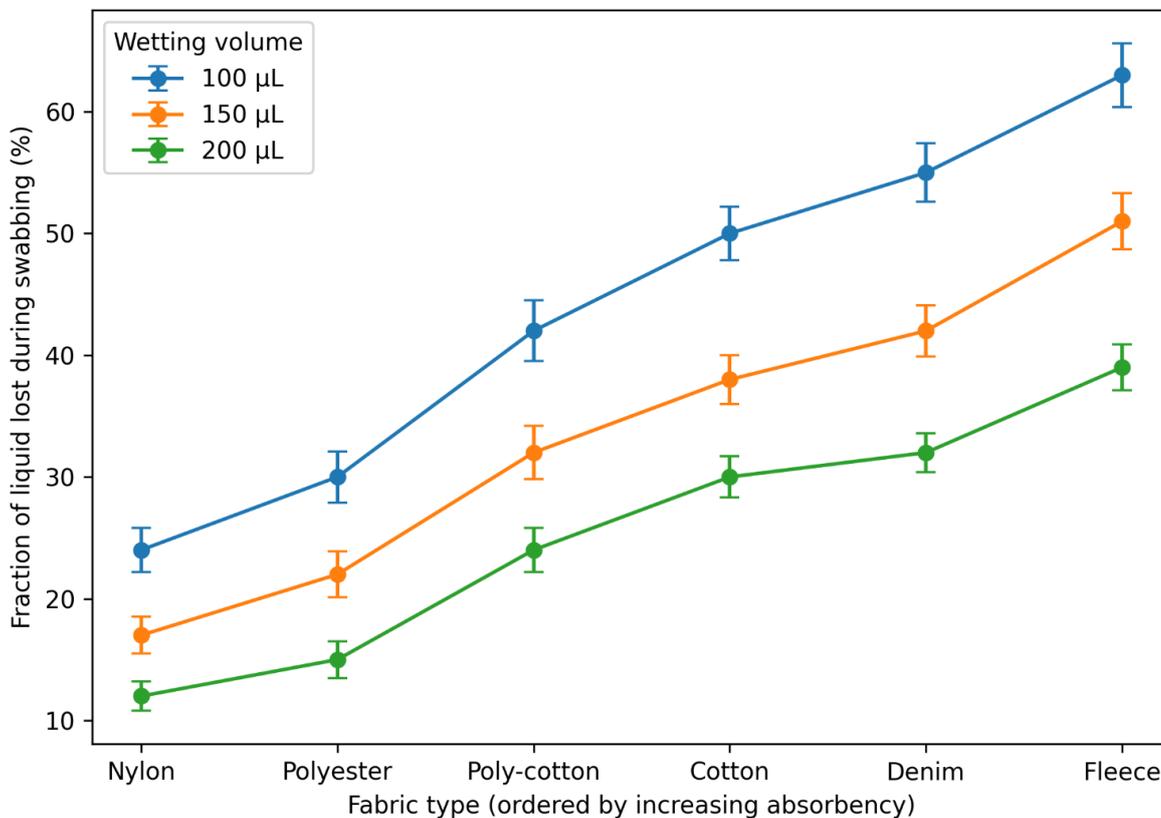


Figure 2. Fraction of liquid lost from cotton swabs during standardized fabric sampling ($n = 5$ per fabric per volume). Swabs were moistened with 100, 150, or 200 μL sterile distilled water, and liquid loss was calculated as $(S_1 - S_2) / (S_1 - S_0) \times 100$. Bars represent mean fraction lost; fabrics are ordered by increasing absorption capacity. Linear mixed-effects modelling showed significant effects of fabric type, wetting volume, and their interaction (all $p < 0.001$).

Collectively, these findings demonstrate that both fabric properties and swab wetting volume significantly influence the effective moisture retained on the swab during sampling, with the strongest volume sensitivity occurring on highly absorbent fabrics, thereby providing a quantitative mechanistic basis for subsequent DNA recovery analyses.

3.3 EFFECT OF WETTING VOLUME ON DNA YIELD
DNA yield recovered from worn fabric substrates increased with swab wetting volume across all fabric types (**Figure 3; Table 3**). Linear mixed-effects modelling

demonstrated a significant main effect of wetting volume on DNA yield ($p < 0.001$) and a significant main effect of fabric type ($p < 0.001$). A significant wetting volume \times fabric interaction was also observed ($p < 0.001$), indicating that the magnitude of the volume effect varied according to substrate properties.

For low-absorbency fabrics (nylon and polyester), increases in DNA yield with higher wetting volumes were modest. Median yields for nylon increased from approximately 0.10–0.12 ng at 100 μL to approximately 0.15–0.17 ng at 200 μL , while polyester

increased from approximately 0.15–0.18 ng to approximately 0.23–0.26 ng over the same volume range. In contrast, fabrics with greater absorbency exhibited more pronounced volume-dependent increases. Poly-cotton and cotton showed substantial gains between 100 and 150 µL, with further increases at 200 µL. The largest absolute increases were observed for cotton and fleece, where median yields at 200 µL were approximately 40–50% higher than those obtained at 100 µL.

Post hoc comparisons confirmed that, for high-absorbency fabrics (cotton, denim, and fleece), DNA yields obtained at 150 µL and 200 µL were significantly greater than those obtained at 100 µL (Tukey-adjusted $p < 0.01$). For

low-absorbency fabrics (nylon and polyester), differences between 100 and 150 µL were smaller and not consistently significant after correction, whereas the increase from 150 to 200 µL reached significance only for polyester ($p < 0.05$). Variability in DNA yield increased with fabric absorbency, with wider interquartile ranges observed for cotton, denim, and fleece compared with nylon and polyester (**Figure 3**). This pattern reflects greater heterogeneity in DNA recovery from highly porous substrates. Collectively, these findings demonstrate that increasing swab wetting volume enhances DNA recovery from fabric surfaces, with the greatest benefit observed for substrates with higher absorption capacity.

Table 3. Summary of DNA and STR outcomes by fabric type and swab wetting volume (n = 15 donors per fabric per volume).

Fabric	Volume (µL)	Mean DNA yield (ng)	Mean allele count	% FS
Nylon	100	0.12	20	33
Nylon	150	0.14	24	40
Nylon	200	0.17	28	53
Polyester	100	0.18	24	40
Polyester	150	0.22	30	60
Polyester	200	0.26	34	73
Poly-cotton	100	0.29	30	60
Poly-cotton	150	0.37	36	80
Poly-cotton	200	0.41	38	87
Cotton	100	0.45	34	73
Cotton	150	0.56	38	87
Cotton	200	0.69	41	93
Denim	100	0.39	32	67
Denim	150	0.52	37	80
Denim	200	0.61	40	93
Fleece	100	0.50	35	73
Fleece	150	0.63	40	93
Fleece	200	0.71	41	100

Table Footnote: Mean allele count was calculated across autosomal STR loci (heterozygous = 2 alleles; homozygous = 1; dropout = 0). Amelogenin and Y-markers (e.g., DYS391, Y-indel if present) were excluded to avoid sex-linked bias and ensure donor comparability. “%FS” denotes the percentage of samples yielding a full single-source profile (n = 15 per fabric/volume; increments of 6.7%).

3.4 EFFECT OF WETTING VOLUME ON STR PROFILE COMPLETENESS

The proportion of full single-source STR profiles (FS) increased with swab wetting volume across all fabric types (**Figure 4**). Mixed-effects modelling demonstrated a significant main effect of wetting volume on profile completeness ($p < 0.001$) and a significant main effect of fabric type ($p < 0.001$). A significant wetting volume × fabric interaction was also observed ($p < 0.001$), indicating that the effect of wetting volume differed between substrates.

For low-absorbency fabrics (nylon and polyester), FS rates remained relatively low at all volumes and

increased only modestly with higher wetting volumes. Nylon exhibited FS rates of approximately 33%, 40%, and 53% at 100, 150, and 200 µL, respectively, while polyester increased from approximately 40% at 100 µL to approximately 73% at 200 µL. In contrast, fabrics with higher absorbency showed marked improvements in profile completeness with increasing wetting volume. Poly-cotton increased from approximately 60% FS at 100 µL to approximately 87% FS at 200 µL, while cotton increased from approximately 73% FS to approximately 93% FS over the same volume range. Denim and fleece followed similar trends, with fleece achieving FS rates approaching 100% at 200 µL.

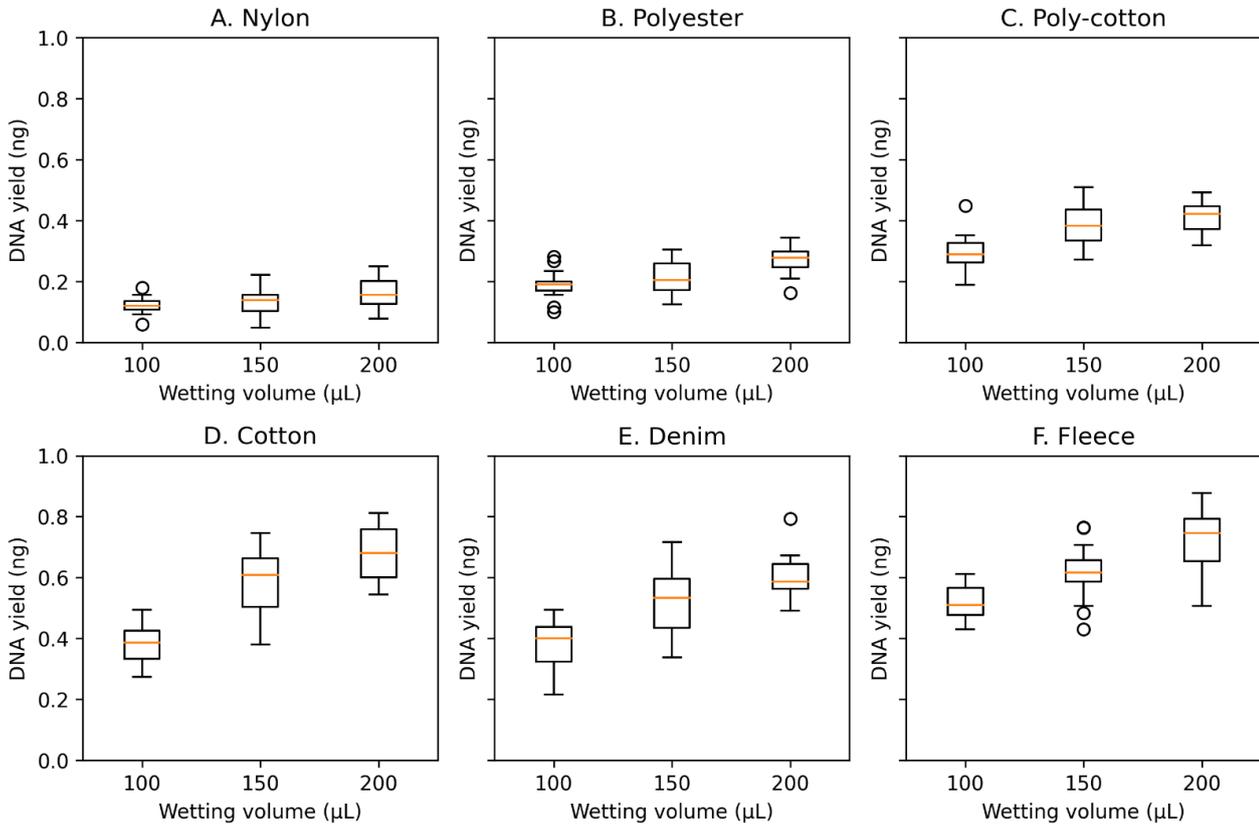


Figure 3. DNA yield recovered from worn fabric substrates by swab wetting volume ($n = 15$ donors per fabric per volume). Boxplots (panels A–F) show median, interquartile range, and full range. Swabs were moistened with 100, 150, or 200 μL sterile distilled water under standardized sampling conditions. Linear mixed-effects modelling revealed significant effects of wetting volume, fabric type, and their interaction (all $p < 0.001$).

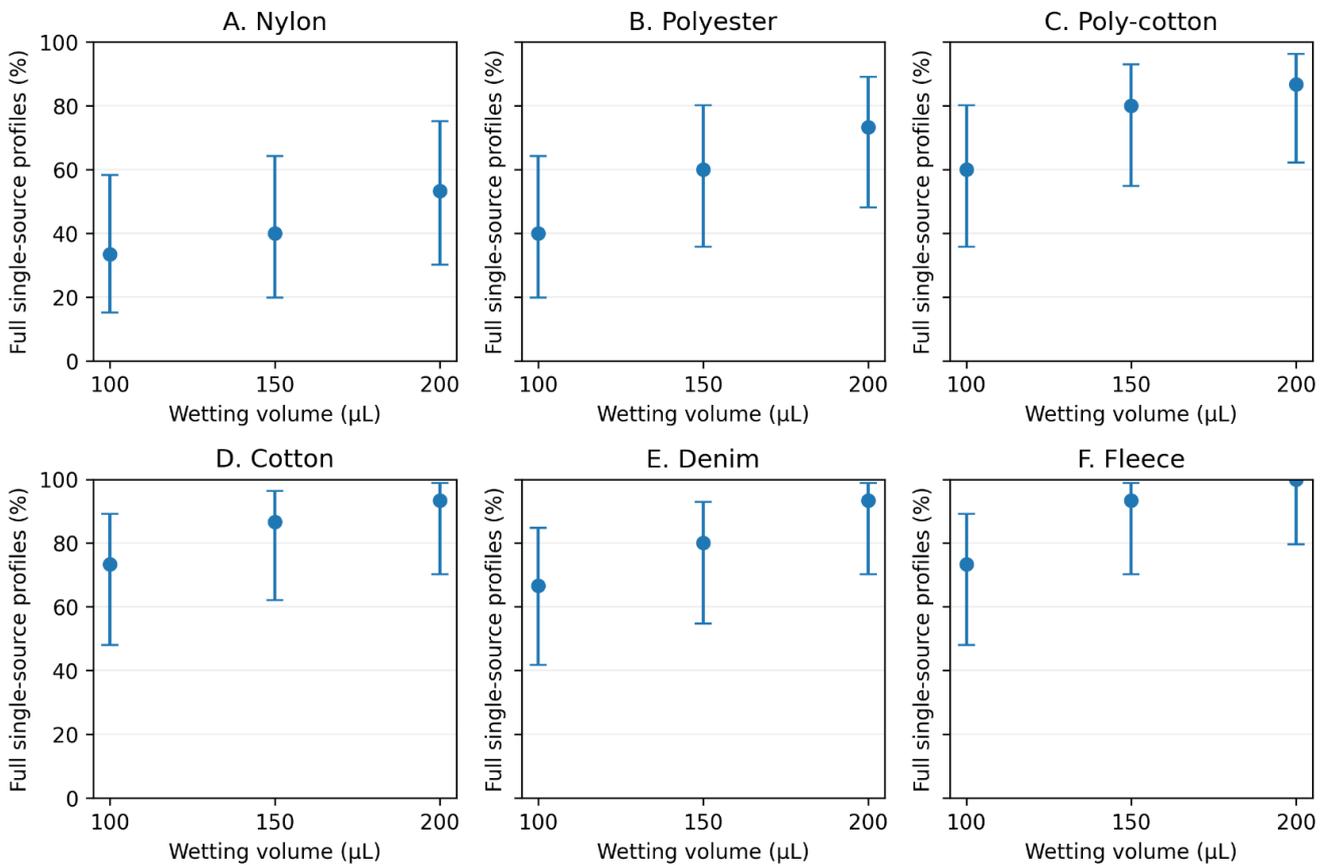


Figure 4. Percentage of full single-source STR profiles recovered from worn fabrics by swab wetting volume ($n = 15$ donors per fabric per volume). Panels A–F represent nylon, polyester, poly-cotton, cotton, denim, and fleece; points show observed proportions at 100, 150, and 200 μL with 95% binomial confidence intervals (Wilson). Swabs were moistened with sterile distilled water and applied under standardized conditions. Mixed-effects modelling showed significant effects of wetting volume, fabric type, and their interaction (all $p < 0.001$).

Post hoc comparisons indicated that, for high-absorbency fabrics (cotton, denim, and fleece), the proportion of FS profiles obtained at 150 μL and 200 μL was significantly greater than that obtained at 100 μL (Tukey-adjusted $p < 0.01$). For low-absorbency fabrics, differences between 100 and 150 μL were smaller and not consistently significant after correction, whereas the increase from 150 to 200 μL reached significance for polyester ($p < 0.05$) but not for nylon.

Taken together with the observed increases in DNA yield (Section 3.3) and the reduction in swab wetness loss at higher volumes (Section 3.2), these results indicate that the improvement in STR profile completeness at higher wetting volumes is mediated by enhanced moisture retention on absorbent substrates. This improved wetness availability likely facilitates more efficient mobilization and transfer of cells and extracellular DNA during pressure-based rotational sampling.

4 Discussion

4.1 PRINCIPAL FINDINGS

The present study demonstrates that increasing the volume of sterile distilled water used to moisten cotton swabs enhances trace DNA recovery from worn fabric substrates and improves downstream STR profile completeness. This finding aligns with the broader body of trace DNA research showing that recovery success is highly sensitive to controllable pre-analytical variables, particularly when template quantities are low and stochastic effects dominate amplification outcomes^{1,3,4,22,31}. Importantly, the magnitude of the wetting-volume effect was substrate-dependent, with the largest improvements observed for highly absorbent fabrics such as cotton, denim, and fleece, and more modest gains for low-absorbency fabrics such as nylon and polyester. Substrate-driven variability is well documented in trace DNA recovery and is repeatedly highlighted as a major contributor to inconsistent casework outcomes^{4,10–12,22,31}. By experimentally manipulating swab wetting volume and integrating quantitative measures of fabric absorbency and swab liquid loss, this study establishes a direct mechanistic link between textile liquid-transport behaviour and forensic trace DNA recovery outcomes.

A central contribution of this work is the integration of physical measurements of fabric absorbency and direct quantification of swab wetness loss during sampling, allowing biological outcomes (DNA yield, allele counts, and full-profile rates) to be interpreted mechanistically. Evidence recovery from porous substrates is frequently less predictable than from non-porous substrates, and prior fabric-focused studies have shown that recovery efficiency depends strongly on fabric type and collection method^{10–12,46–50}. By explicitly measuring absorption capacity and moisture loss, the present study supports the conclusion that wetting volume is a controllable parameter whose impact is mediated by substrate-driven moisture sequestration at the swab–fabric interface. This provides a defensible explanation for why similar wet-swabbing approaches can produce very different outcomes across fabric types in operational settings^{4,10–12,22,46,49}.

When positioned in the broader fabric-trace DNA landscape, these findings also help reconcile apparently conflicting conclusions in the literature regarding optimal collection tools for textiles. Some controlled clothing studies have reported strong performance for adhesive-based approaches (e.g., mini-tape lifting) and scraping compared with wet swabbing across multiple clothing types⁷². Those findings underscore that “best method” is often conditional rather than universal, and that performance depends on how the biological material is retained on or within the textile, the sampled area size, and the extent to which the method maintains effective contact at the collection interface. The present study complements that line of work by demonstrating that, when cotton swabs are used on porous fabrics, the dose of moistening liquid becomes a critical determinant of recovery—particularly on absorbent fabrics where liquid is rapidly drawn away from the interface.

4.2 MECHANISTIC INTERPRETATION: WHY WETTING VOLUME MATTERS ON FABRICS

Trace DNA swabbing is fundamentally a transfer process that depends on mobilisation of cellular material and extracellular DNA from the substrate, followed by uptake into the swab matrix. The importance of the surface–collection interaction is well recognised in trace DNA literature, where identical collection tools can behave differently depending on substrate properties and environmental context^{4,11,12,22}. For porous and fibrous substrates such as textiles, capillary uptake and internal liquid transport can draw moisture away from the swab–surface interface, thereby reducing the effective wet contact required for efficient cell and DNA mobilisation.

The present findings show a tight coupling between fabric absorbency and swab wetness loss during standardised sampling, supporting a physical model in which the swab functions as a transient liquid reservoir and the textile substrate acts as a competing sink. At low wetting volumes, a substantial proportion of available liquid can be rapidly sequestered into the fabric, leaving an increasingly dry interface where recovery becomes dominated by friction rather than fluid-mediated transfer. This is consistent with broader discussions that swab-based sampling can be recovery-limited and strongly parameter-dependent (swab type, wetting agent, substrate, and technique), and that the absence of robust standardisation is a continuing weakness in trace DNA field practice⁷³.

Increasing the wetting volume increases the absolute amount of liquid available, thereby maintaining a hydrated interface despite capillary uptake. This likely promotes (i) detachment of corneocytes and epithelial fragments from fibres, (ii) solubilisation and redistribution of extracellular DNA, and (iii) improved transport of biological material into swab fibres during pressure-based rotation and controlled stroke patterns. Evidence that “heavier wetting” can be beneficial on absorbent substrates is supported by controlled swabbing work showing that, for an absorbing surface, increased wetting can improve recovery when combined with optimised technique (notably $\sim 60^\circ$ swab angle and continuous rotation)⁷⁴. Additional experimental work also indicates

that swabbing technique and duration can materially influence yields on porous substrates, with certain double-swabbing approaches and longer swabbing durations improving recovery on cotton swatches under controlled conditions⁷⁵.

Finally, the mechanistic rationale for moisture-driven effects on trace DNA recovery is also consistent with a broader “porous media” perspective: nucleic acids can interact with porous matrices through adsorption and transport processes that modulate recovery depending on substrate surface properties and microstructure⁷⁶. While those studies are not textiles-focused, they reinforce a key point relevant to trace DNA on fabrics—namely that substrate properties can influence where DNA resides (surface-accessible vs sequestered) and how efficiently it can be mobilised into a collection medium.

4.3 SUBSTRATE-SPECIFIC EFFECTS AND THE ROLE OF FABRIC STRUCTURE

The substrate-dependent response to wetting volume reflects fundamental differences in fibre chemistry and textile microstructure. Nylon and polyester fabrics are generally characterised by lower intrinsic hydrophilicity and limited internal moisture reservoirs, with liquid transport dominated by capillary flow through pore spaces rather than absorption into fibres^{62,64,66,69}. These properties are consistent with the low absorption capacity and limited swab wetness loss observed for these fabrics and help explain why increasing wetting volume produced only modest gains in DNA recovery and profile completeness. It is also important to recognise that apparent “polyester” performance can vary widely depending on finishing and surface engineering; hydrophilic treatments can substantially alter liquid transmission and absorptivity behaviour in polyester textiles, potentially shifting where moisture accumulates and how quickly it is transported through or along the fabric⁷⁷.

In contrast, cotton, denim, and fleece exhibit higher capillarity and moisture retention driven by fibre morphology, yarn structure, and increased surface area. Cotton fibres possess a twisted ribbon-like structure that promotes capillary action and fibre swelling when wet, while denim incorporates dense woven architectures with substantial inter-fibre space. Fleece and terry fabrics further enhance liquid retention through looped or raised fibres that increase contact area and moisture trapping^{62,63,65,66}. Modern textile science further emphasises that directional liquid transport and transplanar wicking (transport across thickness) are designable properties governed by wetting, wicking pathways, and structural gradients—features that can vary substantially even within “similar” clothing categories^{78,79}.

These textile principles strengthen the interpretation that “absorbency” is not a single property but an emergent consequence of fibre wettability, swelling, yarn geometry, and inter-yarn spacing. For example, wicking behaviour is influenced not only by yarn-level wicking but also by yarn-to-yarn liquid migration and inter-yarn spaces, which can strongly shape how quickly liquid is

redistributed away from the swab–fabric interface during sampling⁸⁰. In woven cotton twills, structural parameters such as weft yarn properties measurably influence wetting time, spreading speed, and wetted radius, demonstrating that even within one fibre class (cotton), liquid transport can differ markedly due to fabric construction⁸¹.

Taken together, these findings demonstrate that a wetting volume sufficient for one substrate (e.g., nylon) may be suboptimal for another (e.g., cotton or fleece). This substrate-specific behaviour reinforces the need to consider material properties when optimising collection protocols for fabric-based trace DNA evidence^{11,12,22,46,49}. It also suggests that protocol generalisation based solely on “porous vs non-porous” may be inadequate; instead, a more useful operational framework may be “low-wicking synthetics vs high-wicking/retentive textiles,” which better reflects how quickly moisture is withdrawn from the interface.

4.4 RELATIONSHIP BETWEEN DNA QUANTITY AND STR PROFILE QUALITY

The observed increases in DNA yield at higher wetting volumes translated directly into improvements in STR profile completeness. This reflects well-established stochastic effects in low-template STR profiling, where insufficient template quantity increases the probability of allelic dropout, locus imbalance, and partial profiles^{4,31}. In trace DNA samples—where biological material may include variable proportions of intact cells, cell fragments, and extracellular DNA—small differences in recovered quantity can have large downstream effects^{4,22,31}.

The relationship between DNA yield and STR outcome was not strictly linear. For several fabrics, relatively small increases in DNA quantity produced disproportionately large increases in the proportion of full profiles, consistent with a threshold effect in which samples move from a stochastic regime into a more stable amplification range. This behaviour is operationally important: modest changes in upstream collection (e.g., moving from a low wetting volume that becomes effectively “dry” on absorbent textiles to a volume that maintains a hydrated interface) can convert partial profiles into full, interpretable profiles—changing both evidential utility and interpretability.

It is also notable that recovery is not solely constrained by mobilisation at the interface; the swab itself can act as a binding matrix that retains DNA and cellular material, limiting elution efficiency. Work on improving recovery from cotton swabs highlights that swab-bound DNA can be difficult to release efficiently without appropriate chemistry, and that extraction buffer composition and downstream clean-up can substantially influence the amount of amplifiable DNA ultimately available for PCR⁸². This reinforces the principle that collection and extraction should be treated as an integrated system rather than isolated steps—a point also emphasised in broader evaluations of sampling materials and their compatibility with downstream chemistry⁸³.

4.5 COMPARISON WITH EXISTING LITERATURE AND COLLECTION METHOD CONTEXT

Previous studies have demonstrated that moistening swabs improves DNA recovery relative to dry swabbing and that fabric substrates generally yield lower DNA quantities than non-porous surfaces^{10–12,22,46,48–50}. Numerous investigations have evaluated swab types, sampling motions, and collection strategies, consistently showing that recovery depends on the interaction between substrate and method^{11,12,19,22}. However, much of the operational literature has treated “wet swabbing” as a binary condition and has not systematically varied or quantified the volume of liquid applied, particularly for fabric substrates.

The present study extends this body of work by explicitly quantifying wetting volume, fabric absorbency, and swab wetness loss, and by linking these variables to DNA yield and STR success through a factorial experimental design. By introducing continuous mechanistic variables, this work moves beyond descriptive comparisons and provides a causal framework for understanding how textile properties interact with sampling conditions. Importantly, broader research has now begun to show that solution volume itself can influence sampling efficiency and that the effect can depend on the swab type and the item being sampled, reinforcing the need to validate “first-step” parameters in context rather than assuming portability across workflows⁸⁴.

The findings must also be interpreted within the broader context of trace DNA collection method selection for fabrics. Controlled comparisons on clothing have shown that mini-tape lifting can be highly competitive for recovering trace DNA from textiles and may outperform wet cotton swabbing under certain experimental conditions⁷². At the same time, method performance can shift depending on sampling area size and textile type; it has been reported that adhesive mini-tapes may be particularly effective on small, localised sampling areas, whereas cotton swabs can be equally effective when larger fabric areas are sampled⁴⁹.

Environmental context further modulates method performance. For wearable fabrics exposed to particulate-rich outdoor environments, swab-based collection combined with an appropriate extraction workflow has been shown to outperform mini-tape-based approaches across DNA yield, STR profile completeness, and allele recovery¹⁸. In such contexts, particulate contamination may reduce adhesive contact with fibres and limit the ability of tape to access biological material within the textile structure, whereas swab-based approaches may facilitate recovery by mobilising biological material while displacing surface debris¹⁸. Collectively, these observations reinforce that no single collection method is universally optimal for trace DNA recovery from fabrics. Instead, performance depends on fabric structure, sampling area size, and environmental exposure⁷².

The present study complements this literature by demonstrating that, when cotton swabs are selected as the collection tool, wetting volume becomes a critical

determinant of recovery efficiency on absorbent substrates. These findings do not challenge the demonstrated utility of adhesive methods on fabrics, but rather identify a critical optimisation parameter when cotton swabs are selected as the collection tool.

Finally, the broader trace DNA collection literature increasingly recognises that optimisation of collection protocols has lagged behind analytical sensitivity improvements, and that standardisation of practitioner technique remains an unresolved gap^{73,85}. Evidence syntheses focusing on swab materials also highlight substantial heterogeneity across studies and reinforce that swabs made of the same material do not necessarily perform equivalently, and that extraction chemistry must be considered alongside swab choice⁸³. These points strengthen the rationale for the present approach: by quantifying and controlling a key field-variable (wetting volume) and tying it to substrate physics, the study generates actionable knowledge that is less susceptible to practitioner-to-practitioner variability than unstandardised “wetness by eye” practice.

4.6 OPERATIONAL AND FORENSIC IMPLICATIONS

From an operational standpoint, these findings have direct relevance for crime scene and laboratory practice. In many workflows, swabs are moistened without precise volumetric control, often using sprays or visually estimated droplets. Reviews of swab-based sampling emphasise that recovery is often low, strongly parameter-dependent, and not governed by any consistent international standard—conditions that increase between-operator variability and reduce reproducibility⁷³. The present results indicate that visually estimated low volumes are likely to be particularly suboptimal on absorbent fabrics, where moisture is rapidly withdrawn from the interface.

Standardising wetting volume in the range of 150–200 μL for fabric sampling—especially for highly absorbent substrates—could significantly increase the probability of obtaining usable STR profiles without introducing additional consumables or analytical complexity. This recommendation is also consistent with broader swabbing-optimisation evidence demonstrating that technique (angle and rotation) and heavy wetting can improve yields on absorbent substrates when executed consistently⁷⁴, and that swabbing method and duration can influence recovery on porous materials such as cotton⁷⁵. Unlike many optimisation variables that require changes to consumables or chemistry, wetting volume can be standardised immediately using existing equipment, making it a uniquely accessible target for quality improvement.

In practice, the operational advantage of wetting-volume standardisation is that it is low-cost, implementable with existing equipment (pipette or controlled dispenser), and trainable. It also offers a route to reduce variability in outcomes across practitioners—an issue repeatedly identified as a quality risk in trace DNA recovery^{73,84,85}.

The findings also support a pragmatic decision framework for fabric evidence: collection method choice

should be evidence-specific. For small, localised areas where biological material is likely superficial and well defined, adhesive mini-tapes may be advantageous^{49,72}. For larger areas, highly absorbent fabrics, or particulate-contaminated textiles, appropriately moistened cotton swabs (with controlled wetting volume and consistent technique) may offer robust performance and operational simplicity^{18,49}. In scenarios where the substrate is highly challenging and porous and where visible stains are absent or diffuse, wet-vacuum methods can sometimes recover substantially more DNA than wet swabbing across many porous substrates, although practical constraints (cost, time, and complexity) often limit routine deployment⁸⁶.

Finally, because swab choice and extraction chemistry behave as a coupled system, operational optimisation should consider downstream compatibility. Evidence syntheses indicate that extraction chemistry should be evaluated alongside swab type rather than independently⁸³, and improvements in swab extraction approaches illustrate that recovery from cotton can be markedly increased by tailored chemistry and clean-up strategies in some contexts⁸².

4.7 METHODOLOGICAL CONSIDERATIONS

The experimental design employed controlled deposition conditions, standardised swabbing procedures, and a single extraction and amplification workflow to isolate the effects of wetting volume and substrate properties. This approach ensured that observed differences in DNA recovery and STR outcomes could be attributed primarily to the interaction between swab wetness and fabric absorbency rather than downstream analytical variability^{4,11,22,31}. The within-item split-zone approach additionally reduced confounding by donor-to-donor differences in shedding and garment-to-garment variability, supporting stronger causal inference for the wetting-volume effect at the substrate interface.

Although the study focused on a defined set of fabrics and a single wear scenario, the inclusion of multiple fabric types spanning a wide absorbency range supports the generalisability of the mechanistic conclusions. The consistent trends observed across substrates, together with the quantitative linkage between absorbency, wetness loss, and DNA yield, indicate that the relationships identified are robust and not limited to a single garment type or sampling context. Rather than limiting validity, the controlled design provides a stable foundation for future work examining additional variables such as ageing, laundering, and mixed-source deposition^{15–18,22}.

Interpretation of trace DNA on clothing should also acknowledge the broader transfer and persistence context. Trace DNA can accumulate on clothing during daily activities and may include both wearer-derived DNA and extraneous DNA from indirect transfer, with mixtures often more common in certain garment areas⁸⁷. Additional studies demonstrate that contactless transfer can occur via shaking/agitation and may generate informative transferred profiles under some conditions⁸⁸. Conversely, laundering-related transfer between textiles

without biological stains appears to be limited in some scenarios, supporting the view that not all indirect transfer pathways are equally probable or forensically meaningful⁸⁹. These lines of evidence reinforce the importance of integrating case circumstances and activity-level propositions when interpreting trace DNA profiles from fabrics, particularly where mixtures are present⁸⁷.

4.8 FUTURE RESEARCH DIRECTIONS

Future studies could evaluate additional swab materials and alternative wetting solutions, including buffered or surfactant-containing formulations, as swab–substrate interactions are known to differ by swab design, wetting agent chemistry, and surface type^{12,19,25}. Evidence indicates that detergent-based swabbing solutions can outperform water for recovering trace DNA in some touch/trace scenarios, suggesting potential gains if such solutions are validated for fabric casework while balancing downstream inhibition risks and workflow constraints⁹⁰.

The influence of fabric treatments, dyes, and finishes on moisture retention and DNA recovery also warrants investigation, particularly given that engineered finishing can markedly alter absorptivity and liquid transport behaviour in synthetic textiles⁷⁷. Additional work could explicitly compare fabrics and garment components designed for directional liquid transport and transplanar wicking, where moisture movement across thickness may strongly affect interface wetness during sampling^{78,79}. Integrating finer textile-structure metrics—yarn spacing, thickness, pile geometry, and inter-yarn migration behaviour—may also improve predictive models of swab wetness loss and recovery^{80,81}.

Environmental exposure and time since deposition remain important casework variables that should be tested alongside wetting volume, including dusty or sandy exposure and the interaction between particulate loading and collection tool performance^{15–18}. Extending the design to mixed-source scenarios and incorporating controlled activity-level transfer experiments would further enhance relevance to casework interpretation^{87,88}. Finally, validation studies that combine controlled wetting volumes with operator training and competency testing could address the widely reported gap in technique standardisation and reduce between-practitioner variability in trace DNA recovery^{73,84,85}.

5 Conclusion

This study identifies swab wetting volume as a critical, substrate-dependent determinant of trace DNA recovery from fabric evidence. By quantitatively linking fabric absorbency and swab wetness loss to DNA yield and STR profile completeness, the findings establish a mechanistic explanation for why increased wetting volume improves sampling performance on porous and highly absorbent textiles.

Rather than treating wet swabbing as a binary condition, the results demonstrate that liquid volume directly governs the balance between biological mobilisation at the fabric surface and liquid sequestration within the

textile matrix. Wetting volume therefore functions as a controllable optimisation parameter, particularly for cotton-based and fleece fabrics where absorbency is high.

From an applied perspective, standardising wetting volume represents a simple, low-cost intervention capable of materially improving evidential yield without altering consumables, instrumentation, or downstream analytical workflows. By integrating textile liquid-transport behaviour into forensic sampling strategy, this work advances a predictive, substrate-aware framework for trace DNA collection from fabrics.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial or personal interests that could have influenced the work reported in this paper. The study design, data collection, analysis, and interpretation were conducted

independently to ensure the objectivity and scientific integrity of the findings.

ETHICS STATEMENT

This study received ethical approval from the institutional oversight committee of the General Department of Forensic Science and Criminology, Dubai Police (Ref. No. D.P.L 19, September 2025). All procedures were conducted in accordance with established ethical standards for research involving human participants. Written informed consent was obtained from all volunteers prior to participation. The study adhered to internationally recognised guidelines governing the handling of biological material, protection of participant confidentiality, and maintenance of data integrity.

AUTHOR CONTRIBUTIONS

Salem K. Alketbi conceptualized and designed the study, supervised experimental coordination, performed the forensic DNA analyses, conducted the statistical evaluations, and led the drafting and finalization of the manuscript. Mohammad S. Ali and Shahad A. Khan contributed to sample processing and laboratory analyses and provided critical scientific input and revisions to the manuscript. All authors reviewed and approved the final version of the manuscript and take responsibility for its content.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed in this study are not publicly available due to institutional and privacy restrictions but are available from the corresponding author upon reasonable request.

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