

Touch DNA viability on various substrates from different shedder levels

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ABSTRACT

BACKGROUND Touch DNA samples are frequently discovered at crime scenes, including those found at the scene, on the victim, with the suspect, or on objects related to the incident. This study aimed to investigate 3 key factors affecting touch DNA samples: the characteristics person that shed the DNA, surfaces variants where the DNA was deposited, and different sampling methods effectiveness that influence DNA quantity, quality, and detection.

METHODS 9 participants grouped into high, intermediate, and low shedder levels simultaneously tied 2 types of ropes, non-porous and porous. The first person will hold a rope for 5 min then pass it to the second person to hold on the same spot for another 5 min. DNA was collected from each rope using the double swab and tape-lift method, extracted, and quantified using real-time polymerase chain reaction (PCR). Touch DNA profile at 20 short tandem repeat loci was amplified in PCR system and detected on capillary electrophoresis.

RESULTS Type of substrate ($p = 0.97$) or sampling method ($p = 0.053$) used for touch DNA collection did not significantly impact the DNA yield or profiling outcomes. A notable difference ($p < 0.001$) was found in DNA quantity between high, intermediate, and low shedders, regardless of the substrate or method used.

CONCLUSIONS Individual shedder level has a greater influence on the results of touch DNA analysis regarding the DNA quantity and profiling quality than substrate type and sample procedure.

KEYWORDS DNA profiling, forensic science, sampling methods, touch

Advances in molecular biology have significantly influenced forensic science, particularly the development and application of DNA analysis for human identification. DNA analysis is essential for identifying individuals involved in criminal cases and mass disaster scenarios.¹ In victim identification, DNA analysis can be used to help confirm identities, especially in cases where fingerprint analysis fails. DNA analysis is pivotal in criminal investigations to link individuals to specific incidents and provide vital evidence for legal

proceedings. This enhances investigation accuracy and strengthens cases against criminals.² Short tandem repeats (STRs) are a key component of this process, offering highly distinctive genetic profiles that are invaluable for victim and perpetrator identification. STRs are effective because they represent a form of DNA polymorphism characterized by variations in the lengths of repeated nucleotide sequences. The Federal Bureau of Investigation has identified 20 core STR loci critical for forensic identification due to their high

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variability among individuals. The strategic use of these core STR markers ensures a standardized approach to DNA profiling, enhancing the reliability and consistency of forensic analyses and improving the accuracy of criminal investigations and legal proceedings.³

Touch DNA, derived from skin cells that are inadvertently transferred onto objects through physical contact at crime scenes, has become the cornerstone of forensic analysis owing to its effectiveness and widespread application. It provides substantial DNA traces that enhance the ability to link suspects to crime scenes or specific objects. Identifying individuals who have handled or come into contact with the evidence is crucial.^{4,5} However, analyzing touch DNA presents significant challenges, including sample degradation and contamination. Forensic experts must consider various factors that impact DNA quality, including the amount of DNA shed, type of substrate, pressure and duration of contact, and sampling and extraction methods. In addition, environmental conditions play a critical role. Addressing these complexities is essential for optimizing touch DNA analysis and ensuring its reliability and effectiveness for identifying individuals involved in criminal investigations.^{6,7} Numerous studies have explored the impact of various factors on touch DNA analysis. Alketbi examined the influence of sampling methods on two types of substrates (porous and non-porous), the duration of contact and environmental conditions, and extraction methods.⁸⁻¹⁴ Several studies have also investigated the influence of shedders on touch DNA analysis outcomes,¹⁵⁻¹⁸ however, no study has specifically elucidated the intricate relationship between individual shedder levels, substrate types, and effective sampling methods for acquiring touch DNA samples across diverse substrate categories.

The current study thoroughly assessed touch DNA profiles derived from individuals categorized into three shedder levels: high, intermediate, and low,¹⁹ examined their correlation with two distinct substrate types, porous (fabric) and non-porous (plastic), and employed two distinct sampling methods: double swab and tape-lift. This study investigated the nuanced influence of individual shedder levels and substrate characteristics and scrutinized the efficacy of different sampling methods in touch DNA analysis. The correlation between shedder levels and DNA profiles was examined, shedding light on the potential interplay between the inherent shedding

tendencies of individuals and the successful recovery of DNA across various substrates. Furthermore, we explored how touch DNA profiles may overlap in mixture scenarios involving multiple contributors. This study highlighted the critical need to determine the sequence of individuals who have touched an object to accurately link them to specific events or crime scenes, distinguishing the most recent contributor from others. This complex process underscores the importance of understanding the shedding dynamics, substrate characteristics, and interactions between genetic materials and objects. As forensic science advances, these insights are crucial for refining methodologies and enhancing the accuracy and reliability of touch DNA analyses in legal contexts.

METHODS

Touch DNA collection

This experimental cohort study was conducted at the Human DNA Laboratory of the Centre Forensic Laboratory of the Indonesian National Police. This study selected two types of rope substrates: a porous fabric variant and its non-porous plastic counterpart. Each rope, 20 cm in length and 1 cm in diameter, was thoroughly sterilized. They were first sprayed with DNAzap™ PCR DNA Degradation Solution (Invitrogen™, USA) before being exposed to ultraviolet radiation in a laminar airflow chamber for 30 min to eliminate any residual DNA effectively.

Nine participants (five males and four females) were chosen based on the sample size formula²⁰ with unknown shedder levels for the experimental cohort study. Standardized protocols were implemented to ensure consistency across experimental procedures. The participants were instructed to wash their hands with soap, dry them using sterile tissue, and engage in various activities for 15 min. They were prohibited from making physical contact with others, consuming food with their hands, or using gloves during this interval. The researchers closely monitored the movements and actions of each participant throughout the observation period.

Each participant held the rope with both hands for 5 min while simultaneously tying the knots with both substrates on separate days. Participants executed the task twice for the tape-lift sampling and double-swab sampling. After repeating the process 3 times, 108 samples were obtained. This systematic methodology

ensures the acquisition of a comprehensive dataset, facilitating the robust analysis of touch DNA samples on diverse substrates.

Shedder level

The shedder levels were classified into three categories based on the obtained DNA concentration and resultant DNA profile. These categories are delineated as follows:^{21,22} (1) high shedder level: concentration of >0.25 ng/μl, for a complete profile result; (2) moderate shedder level: concentration of 0.0625–0.25 ng/μl, resulting in a partial to complete profile; and (3) low shedder level: concentration of <0.0625 ng/μl, resulting in either no profile or a partial profile.

Mixed-touch DNA analysis

The evaluation of mixed-touch DNA in this study was conducted using a specific experimental design. The procedure involved the first participant holding the rope for 5 min and then passing it on to the second participant who held it in the same position for another 5 min. This sequential process of multiple individuals interacting with the same rope simulated mixed-touch DNA scenarios. The study design (Figure 1) employed a structured approach to assess and analyze the dynamics of mixed-touch DNA samples. This controlled experimental setup allowed the exploration of interactions between different individuals that contributed to touch DNA profiles on a shared substrate.

Touch DNA sampling

Two distinct methodologies were applied to retrieve touch DNA from objects: the tape-lift and double-swab methods. The tape-lift procedure used adhesive tape (3M Scotch®, Indonesia), which was affixed to the area the participant touched, emphasizing the knot area and the spaces between knots. Meanwhile, the double swab method saturated the 4N6FIOQSwabs® Crime Scene nylon swab (COPAN, USA) with nuclease-free water. The swab was pressed against the touched part of the rope, followed by swabbing the same area using a dry nylon swab. Combining these methods provides a comprehensive collection of touch DNA samples, ensuring diverse approaches to maximize the retrieval of genetic material from the examined objects.

DNA analysis

DNA extraction from all samples was performed based on the established protocol of PrepFiler™ BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific Inc., USA). The concentration of all extracted DNA samples was determined through real-time quantitative polymerase chain reaction (PCR), employing specific human primers from the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific Inc.) as per the provided instructions.²³ The PCR Amplification Kit (Thermo Fisher Scientific Inc.) was utilized for the subsequent STR locus analysis, with with 25 μl reaction volume and 29 amplification cycles. The fragmented amplification products were separated and identified

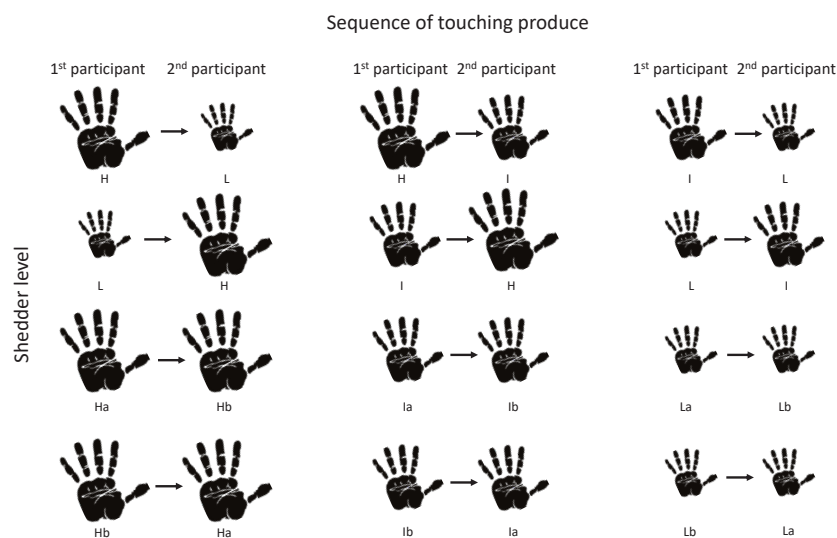


Figure 1. Study design on mixture touch DNA analysis. H=high shedder, Ha=high (a) shedder, Hb=high (b) shedder, I=intermediate shedder, Ia=intermediate (a) shedder, Ib=intermediate (b) shedder, L=low shedder, La=low (a) shedder, Lb=low (b) shedder

using an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific Inc.). The separation medium consisted of 8.7 μ l HI-DiTM Formamide and GeneScan™ 600 LIZ™ dye Size Standard v2.0 (Thermo Fisher Scientific Inc.).²²

The evaluation of the results involved determining whether all alleles at each locus were successfully amplified, indicating a complete DNA profile. A partial profile was defined as the successful amplification of alleles at specific loci, whereas no profile indicated the absence of successful amplification across all alleles at each location. To ensure quality control, a buccal swab from each participant served as the positive control DNA profile (reference sample), whereas the negative controls included swabs and tape-lifts from disinfected ropes untouched by the participant. These procedures confirmed the reliability and accuracy of the DNA profiling process used in this study.

Statistics

The data acquired from the experiment included the touch DNA concentration, degradation index (DI) of the touch DNA, and the total number of loci amenable to amplifying DNA profile generation. Statistical analyses were conducted using SPSS software version 16 (SPSS Inc., USA) to discern the patterns and associations within the dataset. The Kolmogorov–Smirnov test was used to assess the normality of the data distribution, followed by the Mann–Whitney non-parametric test to ascertain the impact of different substrates and sampling methods on DNA concentration and profiling outcomes.

The Kruskal–Wallis test explored the association between the shedder level, touch DNA concentration, and DI. In the context of DNA profile data, the Wilcoxon test was conducted to determine the most suitable

sampling method for each type of substrate. These detailed statistical analyses provide comprehensive insights into the factors influencing the touch DNA characteristics and profiling outcomes across various substrates and sampling methodologies.

Ethical clearance

This research has received approval from the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga (Number: 191/HRECC.FODM/II/2023).

RESULTS

Touch DNA quality and quantity obtained from the different substrate types

The effect of the substrate type, whether porous or non-porous, on the concentration of touch DNA extracted from the rope was not statistically significant ($p = 0.97$). The porous rope exhibited an average DNA concentration of 0.2273 ng/l, whereas the non-porous rope displayed a concentration of 0.2384 ng/l (Figure 2). These findings suggest that the porous or non-porous nature of the substrate does not significantly influence the amount of touch DNA recovered.

Touch DNA quality and quantity with different sampling methods

The sampling methods did not significantly differ ($p = 0.053$) in the extraction of touch DNA from the substrate, with a DI value of 0.186. While the double-swab method exhibited a slightly higher average concentration of touch DNA (0.2961 ng/ μ l) than the tape-lift method (0.1696 ng/ μ l), this difference lacked statistical significance (Figure 2). Conversely, the

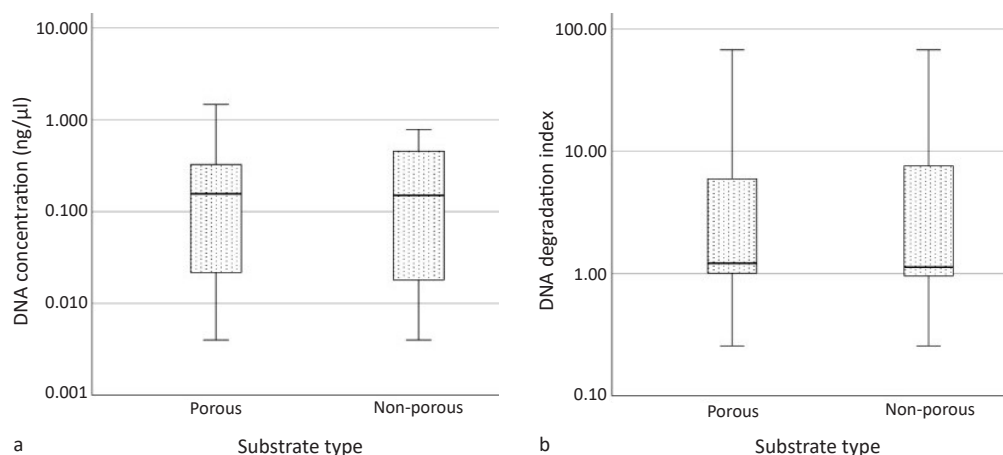


Figure 2. Touch DNA viability based on DNA concentration (a) and DNA degradation index (DI) (b) collected from different substrate. There is no significant difference in the average DNA concentration between porous and non-porous substrates, nor in the average DI

Figure 3. Touch DNA viability based on DNA concentration (a) and DNA degradation index (DI) (b) collected from different sampling method

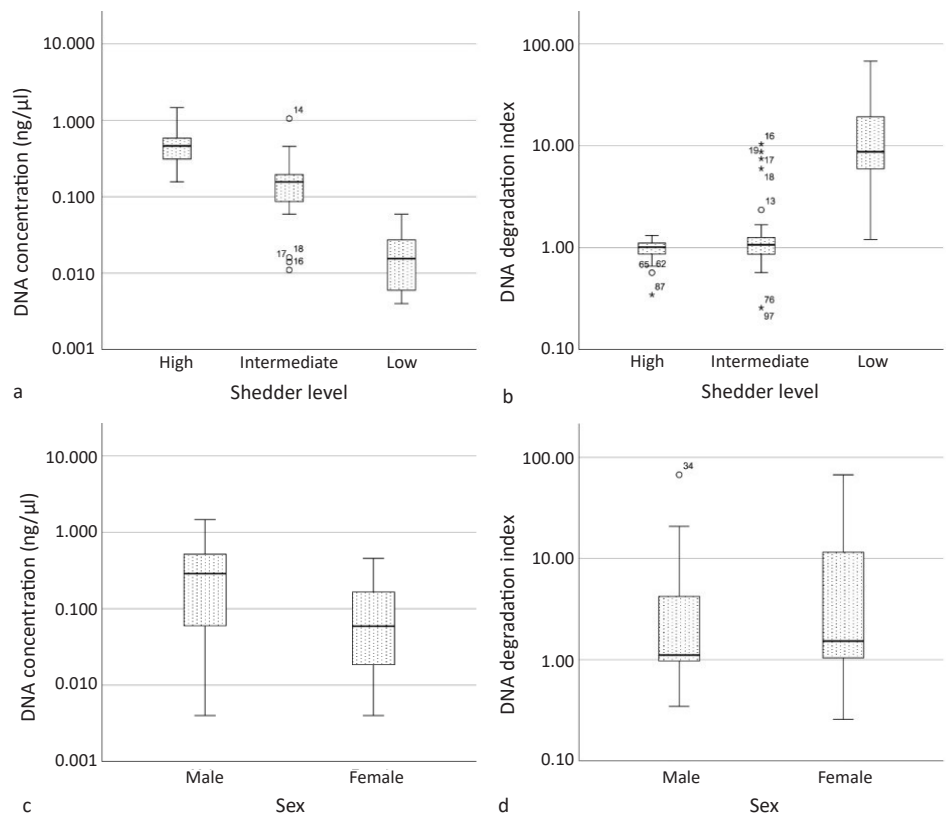
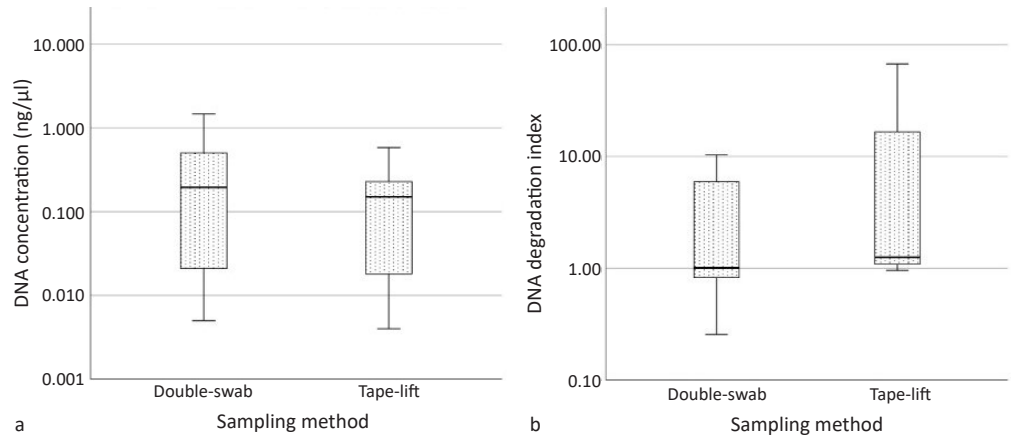


Figure 4. DNA viability based on DNA concentration and DNA degradation index (DI) collected from different shedder level (a and b) from male and female (c and d)

tape-lift sampling method showed a higher average DI value (8.7911) than the double-swab sampling method (2.8989) (Figure 3). These findings underscore the importance of scrutinizing various sampling methodologies in forensic touch DNA analysis to comprehensively understand their implications for DNA recovery and quality.

Touch DNA quality and quantity from different shedder levels

The shedder level significantly influenced the attachment, retrieval, and extraction of touch DNA ($p < 0.001$). This substantial impact is evident in the

mean concentration of DNA obtained from different shedder states, including high (0.4949 ng/μl), intermediate (0.1850 ng/μl), and low (0.0187 ng/μl) (Figure 4). Touch DNA viability is reflected in quantity and quality, as observed through the DI and total loci that can be amplified.

The shedder level, which denotes an individual’s ability to release epithelial cells and other biological material through the skin surface,¹⁵ was scrutinized in the context of touch DNA viability in both male and female participants. The examination, illustrated in Figure 4, suggests a seemingly elevated concentration of touch DNA in male participants compared to

female participants; however, it is crucial to note that this disparity was not statistically significant ($p = 0.494$). Research on shedder dynamics contributes valuable insights into the understanding of touch DNA characteristics across sex lines, highlighting the nuanced intricacies of DNA shedding and deposition.

Interrelationships between sampling methods, substrate types, and shedder levels in touch DNA concentration and quality

The relationship between the sampling method, substrate type, and shedder level on the concentration of touch DNA and the resulting DNA profile is summarized in Table 1 below.

Touch DNA mixture analysis

The presence of touch DNA on an object often originates from multiple individuals, given how easily the DNA samples adhere to the surface. Analysis of DNA profiles from such samples usually reveals allele mixtures, which can be interpreted as mixed DNA profiles. This study examined how the sequence of touch DNA deposition and shedder levels affected the viability of touch DNA. The dominance of DNA profiles was visible through the comprehensive success of the amplified alleles and the prominence of allele peaks within the DNA profile. A detailed presentation of the outcomes defining the dominance of DNA profiles under various shedder level conditions is presented in Table 2. This tabular representation provides nuanced insights into the differential impacts of shedder levels on the efficacy and prominence of DNA profiles, offering a comprehensive view of the interplay between shedder dynamics and DNA profiling outcomes.

The complete profile indicated that all alleles in the 20 STR loci were successfully detected. A partial profile indicated that not all the alleles at the 20 STR loci were

successfully detected. A full mixture profile indicated that the complete DNA profiles of both participants were successfully detected.

DISCUSSION

The internal factors influencing the quality of touch DNA are associated with the intricate process of cellular and DNA release from an individual's body, coupled with the subsequent attachment of touch DNA to an object. This multifaceted factor is contingent on various variables, including the activities engaged in by the person, environmental conditions prevailing at the moment of attachment, and the nature of the substrate constituting the object.²⁴

This study's environmental conditions were strictly controlled, maintaining a consistent setting across all treatments (22°C and 70% humidity). The individual shedder levels, either high, intermediate, or low categories, were established based on the DNA concentration and quality of the DNA profile. This meticulous approach allowed researchers to systematically examine the release of touch DNA, considering both the shedder level and the substrate type, categorized as either porous or non-porous. As shown in Figure 2, the analysis revealed that the type of substrate did not significantly affect the attachment of the touch DNA to an object, as evidenced by the concentration of DNA and quality of the DNA profile; however, it is noteworthy that touch DNA on a porous substrate exhibited a higher DNA concentration compared to the non-porous substrate. This observation underscores the importance of the substrate characteristics in influencing the quantitative aspects of touch DNA, thereby providing valuable insights into forensic considerations. Furthermore, these findings are consistent with those of several

Table 1. Touch DNA concentration and DNA profile quality between sampling methods, substrate types, and shedder level

Sampling methods	Substrate types	High shedder		Intermediate shedder		Low shedder	
		DNA concentration (ng/μl)	Full DNA profile (%)	DNA concentration (ng/μl)	Full DNA profile (%)	DNA concentration (ng/μl)	Full DNA profile (%)
Double swab	Porous	0.6281	100	0.1892	67	0.0194	0
	Non-porous	0.6337	100	0.282	67	0.0242	0
Tape-lift	Porous	0.3755	100	0.1312	33	0.0202	0
	Non-porous	0.3422	100	0.1375	33	0.0109	0

Full profile DNA was achieved when alleles on CODIS STR loci were successfully amplified. CODIS=20 combined DNA index system; STR=short tandem repeat

Table 2. Mixture touch DNA analysis

Samples	Sequence of touching procedure with shedder level	DNA concentration (ng/ μ l)	DNA profile
1	1 st high and 2 nd low	0.216	Partial profile (both participant)
2	1 st low and 2 nd high	3.132	Full profile of 2 nd participant, partial profile of 1 st participant, and 2 nd participant dominant
3	1 st high1 and 2 nd high	0.9	Full mixture profile
4	1 st high and 2 nd high	0.96	Full mixture profile
5	1 st high and 2 nd intermediate	0.612	Full mixture profile
6	1 st intermediate and 2 nd high	0.684	Full mixture profile
7	1 st intermediate and 2 nd intermediate	0.504	Full mixture profile
8	1 st intermediate and 2 nd intermediate	0.324	Full mixture profile
9	1 st intermediate and 2 nd low	0.504	Full mixture profile
10	1 st low and 2 nd intermediate	0.252	Partial profile
11	1 st low and 2 nd low	0.54	Partial profile both participant
12	1 st low and 2 nd low	0.54	Partial profile both participant

previous studies^{8,25} reporting that touch DNA obtained from porous substrates demonstrated a higher DNA concentration than that from non-porous substrates.

Moreover, porous and non-porous substrate types have implications on the DNA quality. In this study, the touched area was pre-demarcated, so it was easier to locate the area that needed to be swabbed. This phenomenon can be attributed to the enhanced binding capacity of porous substrates for epithelial cells and other biological materials compared to non-porous substrates. The rough and porous structure of these substrates facilitates the detachment of cells from the skin, contributing to the increased DNA concentration observed in touch DNA samples recovered from porous surfaces.⁷

In this study, we comprehensively explored the influence of diverse sampling methods on the recovery of touch DNA across various substrates. Mann–Whitney tests found no statistically significant differences in touch DNA concentrations or DI when employing different sampling methodologies. Within the framework of touch DNA analysis, the double-swab method provided a marginally higher average concentration than the tape-lift method, although this disparity was not statistically significant. Conversely, the tape-lift sampling method yielded a higher average DI than the double-swab sampling method. This implies the potential proclivity of the tape-lift approach towards DNA degradation, signifying a compromise in DNA quality and a potential diminution in the efficacy of successfully obtaining a comprehensive DNA profile.

Numerous studies^{25–27} have consistently demonstrated the enhanced efficacy of the double-swab method in providing more reliable DNA profiling outcomes. The double-swab approach involves a two-step process in which the biological material on the substrate undergoes initial hydration with the first wet swab, followed by the retrieval of touch DNA using the second dry swab. This methodology ensures a dual-sampling effort, where genetic material from both swabs is combined during the extraction process, thereby amplifying the overall touch of the DNA samples. This valuable insight serves as a guidance tool for forensic scientists, helping in the judicious selection of sampling methods for touch DNA evidence collection and ultimately bolstering the reliability and accuracy of DNA analysis in criminal investigations.

Human identification by DNA profiling is generally based on STR profiles. Interpretable STR profiles were obtained by successfully amplifying the target allele in every STR loci that had been analyzed depending on the quantity of DNA.²⁸ The attachment process, which determines the quantity and quality of touch DNA reserved in an object, is influenced by several factors: individual shedder level, individual activity before DNA attachment, type of substrate, and the quality of the type of touch regarding DNA attachment.⁷ In this study, it was found that the shedder level had a significant effect on the concentration and quality of the DNA profile. As can be seen in Table 1, a high shedder level resulted in a higher touch DNA

concentration and a complete profile, an intermediate shedder level showed a lower DNA concentration with several loci from some samples failing to amplify, and low shedder level individuals had the lowest touch DNA concentration and unidentified DNA profiles. Previous studies^{16-18,21} have also shown that the shedder rate depends on skin abnormalities, the amount of sweat (cell-free DNA in sweat), DNA in sebaceous fluid, skin thickness, and the habit of frequently touching other body parts before collecting DNA on the hands.¹⁷ Further analysis of touch DNA of low shedders is needed because the absence of DNA does not mean that the individual did not have contact with the evidence or the crime. However, individual shedder rates are influenced by biological mechanisms and are not yet fully understood. Even a person with high DNA concentration can have a different degree of shedder level depending on their habits, activities, and surroundings at the time of contact.¹⁶

This study showed that sex does not significantly affect the DNA concentration and touch DNA stability, as seen from the DI. As shown in Figure 2, the average concentration of male touch DNA is greater than females. This aligns with several previous studies showing that males are higher shedders of touch DNA than females.^{19,29} However, research by Manoli et al³⁰ explained that an individual's age is more influential on cell detachment and transfer to the object surface when touch occurs. Regardless of sex, the percentage of cellular deposition decreased with age. The strong inverse relationship between age and primary DNA accumulation in males could explain the correlation between aging and the reported decrease in the epidermal turnover rate or cellular senescence.³¹

The order in which participants touched objects related to the criminal act is very important because it can clarify which individuals are connected to the objects involved in the incident. In this study, the shedder level greatly influenced the dominance of mixed DNA profiles. The second person also had a higher DNA profile dominance than the first person, as demonstrated by the results of participants with the same intermediate shedder levels.

The study's findings affirmed that the different surface natures of touch DNA collection spots significantly impacted the quantity of recovered DNA and the outcomes of DNA profiling. Different sampling methods also showed no statistically significant variance for touch DNA analysis, although the double-

swab method demonstrated better DNA profiling outcomes and higher DNA concentrations than the tape-lift method. Notably, a substantial difference in the quantity of DNA was identified when comparing high shedders to intermediate and low shedders, which persisted across various substrate types and sampling methodologies. This underscores the importance of caution when analyzing mixed-touch DNA samples and emphasizes the need to consider additional evidence. The shedder level is a pivotal factor capable of exerting a substantial influence on the dominance of DNA profiles derived from such samples.

The study provides valuable insights into the factors influencing touch DNA analysis, yet it has several limitations that should be acknowledged. The small sample size of nine participants limits the generalizability of the findings, as a larger and more diverse group could yield more representative results. The focus on only two surface types (non-porous and porous) restricts the applicability of the conclusions to other materials commonly encountered at crime scenes. Finally, while the study addressed mixed-touch DNA, it did not explore the complexities of profiling such samples, which are crucial in forensic investigations. Despite these limitations, the findings clearly demonstrate that the characteristics of the individual shedding DNA have a profound impact on DNA quantity and profiling quality, emphasizing the need for further research to enhance our understanding of touch DNA evidence in forensic contexts.

In conclusion, these results highlight the reliability of touch DNA analysis across diverse surfaces and sampling methods. The significant impact of the shedder level on DNA quantity and dominance within mixed samples highlights the complexity of interpreting touch DNA evidence. Moreover, it is recommended that extraction methods (automated or manual) be identified and larger samples be analyzed in further studies. Forensic practitioners should judiciously consider shedder variability and integrate additional corroborative evidence to interpret mixed-touch DNA profiles for more comprehensive and accurate forensic analysis.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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