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Temporal Assessment of DNA Shedding from Human Hands After Handwashing: Implications for Touch DNA Recovery

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ABSTRACT

This study examines the temporal dynamics of DNA shedding from human hands following handwashing, with a focus on its implications for forensic touch DNA recovery. The research investigates fluctuations in DNA levels over time post-handwashing and highlights significant individual differences in shedding patterns. Statistical analysis revealed that DNA recovery levels at 30 minutes post-handwashing were significantly higher than those at 5 minutes ($p < 0.01$), demonstrating rapid DNA reaccumulation. Male participants shed significantly more DNA than female participants on average ($p < 0.01$), underscoring a potential influence of gender on DNA shedding tendencies. Results further emphasized the challenges of accurately categorizing individuals as "high," "medium," or "low" shedders, although the methodology provided a robust framework for assessing shedding abilities. This variability in DNA shedding rates has critical forensic implications, particularly regarding the timing of evidence collection and the interpretation of touch DNA evidence. By accounting for these individual differences, forensic professionals can improve the accuracy and reliability of DNA analysis, ultimately strengthening forensic investigations. Future research should explore additional factors influencing DNA shedding, including skin type, ethnicity, and long-term activity levels. Investigating the effects of different handwashing agents, environmental conditions, and extended timelines on DNA recovery could further refine forensic methodologies, enhancing the utility of touch DNA evidence in forensic science.

Keywords: Touch DNA; Forensic Science; Forensic Genetics; DNA Shedding Status; Trace DNA Analysis; QIAamp® DNA Investigator Kit; Quantifiler® Trio DNA Quantification Kit

Introduction

The Touch DNA, also referred to as trace DNA, has become an essential tool in forensic science, allowing for the recovery of DNA from surfaces that individuals have come into contact with [1]. Unlike DNA obtained from biological fluids, touch DNA is typically found in smaller quantities, making its recovery and analysis particularly challenging [2-5]. Nevertheless, its capacity to identify individuals through the skin cells left on surfaces has significantly advanced forensic investigations, especially in cases where traditional biological evidence is unavailable [6].The recovery and analysis of touch DNA are affected by various factors, including the time elapsed between DNA deposition and recovery [7-9], which can increase the risk of DNA contamination [10-13]; the type of surface involved [14-15]; the methods and techniques used for DNA collection [16-25]; environmental conditions [7,17,26,27]; and the individual's DNA shedding tendencies [28]. Research has shown that different DNA collection methods yield varying levels of success. For instance, minitape and nylon swabs have demonstrated superior performance compared to traditional cotton swabs on certain surfaces [14,18,19].

Additionally, the time since DNA deposition is critical, as environmental exposure leads to DNA degradation, reducing the amount recoverable over time [7,26]. Furthermore, the choice of quantification methods can significantly impact the efficacy of touch DNA analysis, underscoring the importance of selecting appropriate techniques to ensure accurate outcomes in forensic casework [29]. Advancements in forensic science and the development of innovative methodologies have further improved DNA recovery and analysis processes, demonstrating the field's continual evolution [30]. One of the most unpredictable factors in touch DNA analysis is individual variability in DNA shedding. Certain individuals, classified as "high shedders," release substantial amounts of DNA, whereas others, termed "low shedders," deposit minimal DNA onto surfaces [28]. This variability complicates predictions about the likelihood of successful touch DNA recovery in forensic contexts. The effect of handwashing on DNA shedding is particularly relevant, as handwashing can substantially reduce the amount of DNA present on the skin [31]. However, this reduction is often short-lived, as skin cells are continuously shed, leading to the reaccumulation of DNA on the surface over time [32].

Research has confirmed that DNA levels on the hands decrease immediately after handwashing due to the removal of surface DNA, dead skin cells, and contaminants [33]. However, this decrease is temporary, as natural skin cell shedding and the production of oils and sweat cause DNA levels to increase again. Environmental contamination and incidental contact with surfaces further contribute to this reaccumulation [6,11]. Despite these observations, the rate of DNA reaccumulation and the variability in shedding patterns among individuals remain poorly understood. Factors such as skin type, activity level, and environmental exposure likely play significant roles in these processes [28,34]. Given these complexities, the aim of this study was not to establish the overall shedding status of participants but rather to evaluate their shedding abilities over time. By gaining a more detailed understanding of these patterns, forensic scientists can better optimize touch DNA recovery strategies and enhance the accuracy of forensic DNA analysis. Specifically, this research sought to investigate how DNA shedding fluctuates over a seven-day period, focusing on the temporal dynamics of DNA reaccumulation and individual variability. Additionally, the study aimed to evaluate the immediate and progressive effects of handwashing on DNA recovery by analyzing samples collected at 5-minute, 15-minute, and 30-minute intervals post-handwashing.

Materials and Methods

Participant Selection

Five participants (two females and three males), aged between 25 and 35 years, were selected for this study. Prior to sample collection, participants washed their hands for 45 seconds using antibacterial soap (LabGUARD). To assess the immediate and progressive effects of handwashing on DNA levels, samples were collected from participants at three time intervals following handwashing: 5 minutes, 15 minutes, and 30 minutes. In addition, DNA shedding patterns of the five participants (three males and two females) were monitored over a seven-day period, with samples collected at 30-minute intervals following handwashing each day. During the collection periods, participants refrained from any activity to minimize potential contamination. They were advised to avoid contact with other individuals (e.g., shaking hands or hugging) and to limit touching surfaces not designated for personal use. However, behaviors such as touching their face or body were not actively monitored. Participants remained indoors at a controlled room temperature of 20-25 °C during the time between handwashing and sample collection. To standardize the sampling area and promote even DNA distribution on the skin surface, participants were asked to rub their hands together for 15 seconds immediately before each sample collection. This step ensured that any shed skin cells or DNA-containing material on the palms and fingers was evenly distributed across both hands.

DNA Sample Collection Protocol

DNA samples were collected using Copan cotton swabs (15 ^{0}C) pre-moistened with 100 µL of sterile distilled water, applied via a plastic spray bottle technique [1]. Each swab was rubbed carefully over the palms and fingers of both hands with medium pressure, following a systematic approach. The swab head was rotated as it was moved from top to bottom and left to right to ensure even coverage of the skin surface. To optimize DNA recovery, a single swab was used for both hands of each participant. The use of moistened swabs is a well-established technique in forensic DNA collection. Moisture enhances cell adhesion to the swab fibers, increasing the yield of DNA recovered from skin surfaces [1]. Sterile distilled water serves as a non-invasive agent, aiding in cell transfer without introducing contaminants. Rotating the swab head during collection ensures contact with different skin areas, further optimizing sample recovery. This method is particularly effective for recovering low quantities of DNA, which is often the case with touch DNA samples.

DNA Extraction and Quantification

DNA was extracted immediately after sample collection using the QIAamp® DNA Investigator Kit (Qiagen) in accordance with the manufacturer's protocol. Full swab heads were utilized for each sample to maximize recovery, with a final elution volume of 50 µL. The extracted DNA was quantified using the Quantifiler® Trio DNA Quantification Kit and QuantStudio 5 Real-Time PCR (qPCR) system. HID Real-Time PCR Analysis Software v1.3 (Thermo Fisher Scientific) ensured precise measurement of DNA concentration.

DNA Amplification and Analysis

Selected DNA samples, including controls, underwent amplification using the GlobalFiler™ PCR Amplification Kit on the ABI GeneA-

mp® 9700 PCR System (Thermo Fisher Scientific). A total of 29 amplification cycles were conducted per the kit's protocol. Amplified DNA fragments were analyzed on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific). Each sample was prepared with 1 µL of PCR product mixed with 9.6 µL of Hi-Di™ formamide and 0.4 µL of GeneScan™ 600 LIZ® Size Standard v2.0. Additionally, at least 1 µL of allelic ladder was added to each injection on a 96-well plate. Denaturation at 95 °C for 5 minutes was followed by cooling on ice for 5 minutes, and electrophoresis was performed on a 36-cm capillary array containing POP-4™ polymer (Life Technologies) using standard injection parameters (1.2 kV, 24 seconds).The resulting Short Tandem Repeat (STR) data were analyzed using GeneMapper® ID-X Software Version 1.4 (Life Technologies), with an analytical threshold set at 75 RFU. DNA profiles obtained were complete and single-source, corresponding to the participants, with no evidence of contamination or mixtures. Negative controls used during collection and extraction confirmed the absence of DNA contamination, ensuring the validity of the procedures.

Statistical Analysis and Visualization

Descriptive statistics, including the mean and standard deviation, were calculated to summarize DNA shedding rates over the seven-day study period. The following visual tools and statistical methods were employed to analyze and present the data:

1) A line chart was created using Matplotlib (Python) to depict trends in DNA shedding over time for each participant. Error bars indicating the standard deviation were included to illustrate the variability in DNA recovery across different days.

2) A box plot, also generated using Matplotlib, represented the distribution of DNA recovery values for each participant. This visualization included the median, interquartile range (IQR), and any potential outliers, providing a comparative overview of individual shedding patterns.

To assess statistical significance between groups (e.g., male vs. female participants) and across different days, a repeated-measures analysis of variance (ANOVA) was conducted using the SciPy library in Python. This analysis evaluated whether the differences in DNA shedding rates over time and between participants were statistically significant. Post hoc pairwise comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to identify specific days or groups with significant differences. For all statistical tests, a p-value of less than 0.05 was considered statistically significant. Results from these analyses were annotated on the visualizations to highlight significant trends or differences where applicable. These methods provided a robust framework for assessing variability in DNA shedding and identifying patterns relevant to forensic applications.

Results

DNA recovery levels immediately following handwashing showed significant variations across time intervals (see Figure 1). Samples collected at 5, 15, and 30 minutes post-handwashing indicated a progressive increase in DNA levels over time. For example, Participant 1 (Male) exhibited DNA recovery levels of 0.4 ng/µL at 5 minutes, 0.6 ng/µL at 15 minutes, and 0.9 ng/µL at 30 minutes. This trend was consistent across participants, with all individuals demonstrating a notable increase in DNA recovery levels over the three intervals. Statistical analysis confirmed that DNA recovery levels at 30 minutes were significantly higher than those at 5 minutes ($p < 0.01$), indicating rapid reaccumulation of DNA on the skin surface after handwashing. Over the seven-day period following handwashing, DNA shedding patterns varied significantly across participants (see Figure 2). Despite this variability, some general trends were observed. Most participants demonstrated a reduction in DNA shedding after Day 1. For example, Participant 1 (Male) had the highest DNA recovery on Day 1 (1.232 ng/µL), followed by a decline on Day 2 (0.89 ng/µL). Similarly, other participants showed a general decrease in DNA shedding after the first day. However, fluctuations were common throughout the study.

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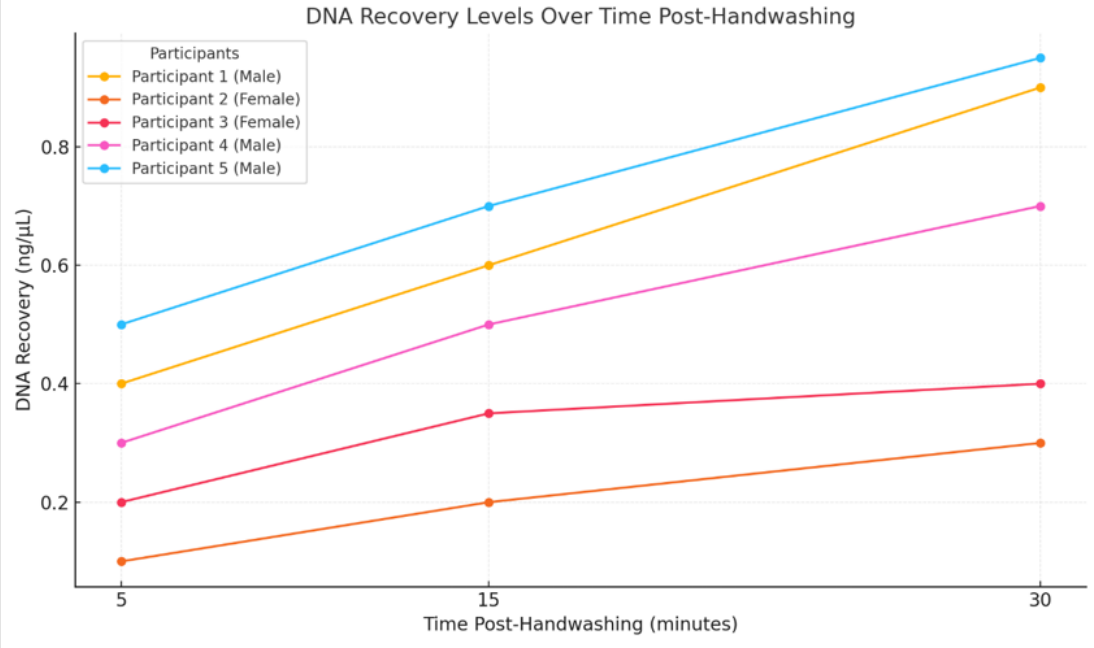

Figure 1: DNA recovery levels (ng/µL) measured at 5, 15, and 30 minutes post-handwashing (n = 45; three replicates per participant). The data demonstrate a progressive increase in DNA recovery over time across all participants, indicating the reaccumulation of DNA after an initial decrease due to handwashing. Statistical analysis revealed that DNA recovery levels at 30 minutes were significantly higher than those at 5 minutes ($p < 0.01$), underscoring the rapid rate of DNA reaccumulation. Variability between participants highlights differences in individual DNA shedding patterns.

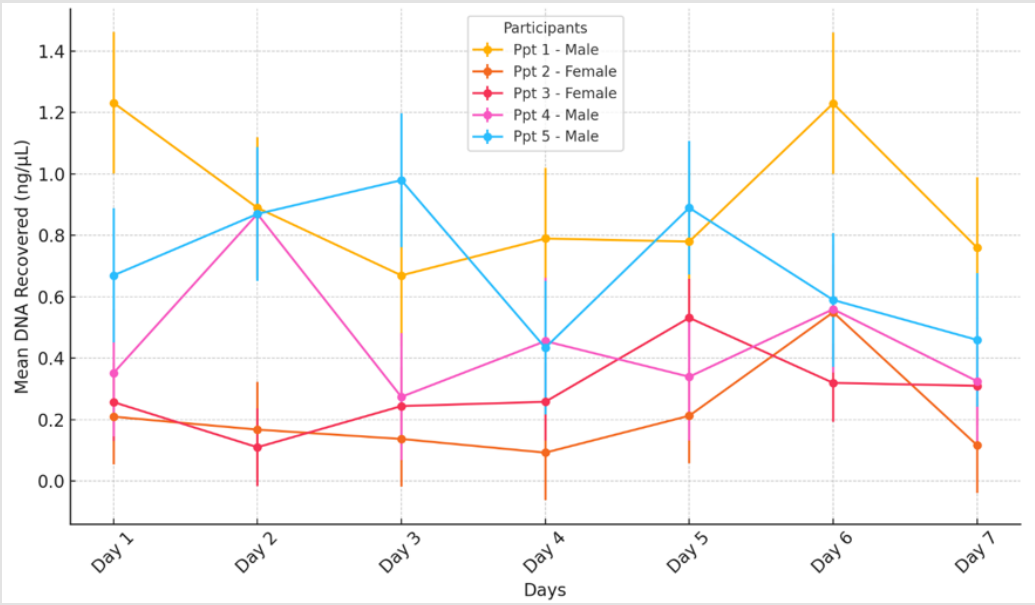

Figure 2: DNA shedding patterns of the five participants (three males and two females) over the seven-day period following handwashing (n= 105; three replicates per participant). The x-axis represents the days (Day 1 to Day 7), and the y-axis shows the mean DNA recovered (ng/µL). Each participant is represented by a distinct line, with markers indicating DNA shedding levels on each day. Error bars highlight the standard deviation, illustrating the variability in DNA shedding across the seven days. Statistical analysis revealed significant reductions in DNA shedding from Day 1 to Day 7 for most participants ($p < 0.05$), with notable day-to-day fluctuations observed in individuals such as Participant 5 (Male) on Day 3 and Participant 4 (Male) on Day 2 ($p < 0.05$). These results emphasize the temporal dynamics of DNA reaccumulation and individual differences in shedding patterns.

For instance, Participant 5 (Male) exhibited an increase in DNA shedding on Day 3 (0.98 ng/µL) after an initial decline on Day 2, and Participant 4 (Male) saw a rise in DNA recovery on Day 2 (0.87 ng/µL) compared to Day 1. By Days 6 and 7, DNA shedding levels had generally stabilized for most participants at lower levels than at the start of the study, suggesting a potential equilibrium in DNA shedding after an initial period of variability. Statistical analysis revealed that the reduction in DNA shedding from Day 1 to Day 7 was significant for most participants ($p < 0.05$). Variations observed on specific days, such as the increases on Day 2 for Participant 4 and Day 3 for Participant 5, were also statistically significant ($p < 0.05$), indicating notable fluctuations in shedding patterns across the study period. Significant inter-individual variability in DNA shedding was observed, with patterns emerging that appeared to be related to gender (see Figure 3).

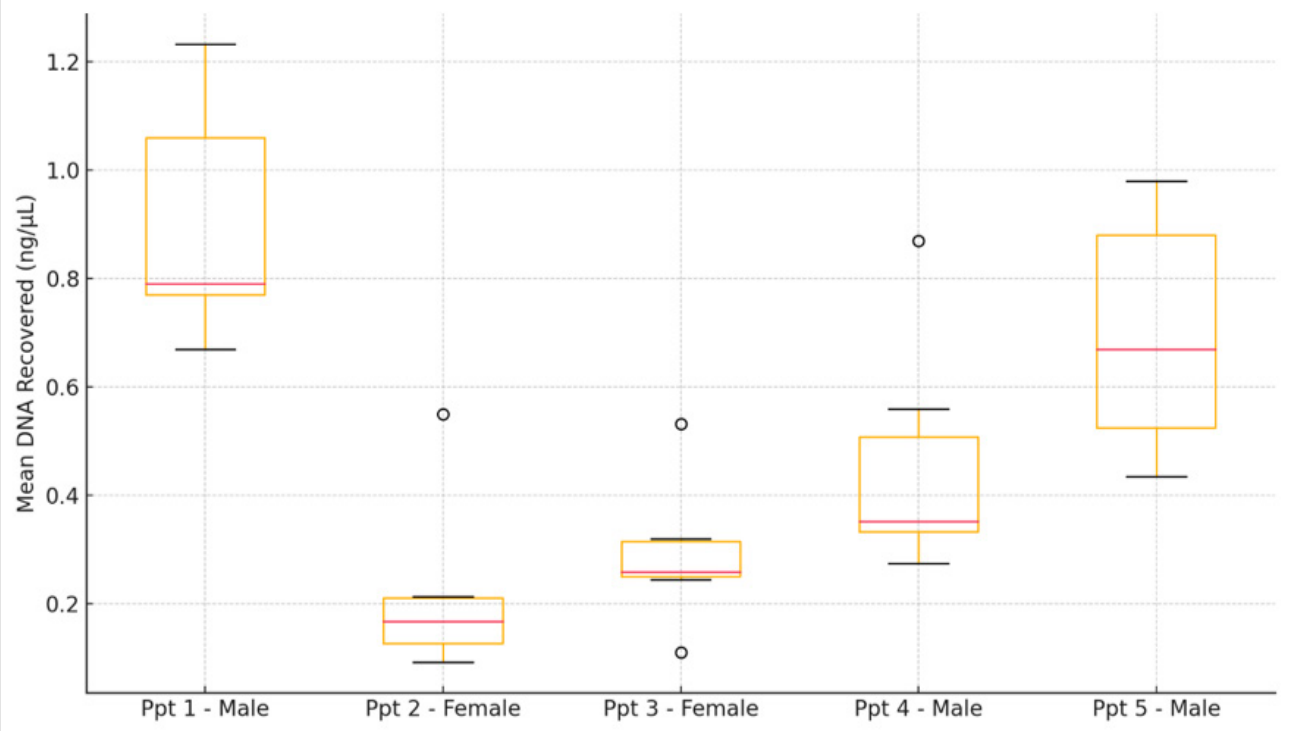

Figure 3: Summary of the distribution of DNA shedding for each participant over the seven days (n= 105; three replicates per participant). The mean DNA recovery for Participant 1 (Male) was 0.85 ± 0.12 ng/µL, for Participant 2 (Female) was 0.162 ± 0.04 ng/µL, for Participant 3 (Female) was 0.267 ± 0.08 ng/µL, for Participant 4 (Male) was 0.428 ± 0.10 ng/µL, and for Participant 5 (Male) was 0.803 ± 0.21 ng/µL. The box plot illustrates the central tendency and spread of DNA shedding data for each participant. The central line within each box represents the median DNA recovery value, while the edges of the box show the interquartile range (IQR), capturing the middle 50% of the data. Whiskers extend to indicate the range of values, excluding any outliers, which are plotted as individual points. Statistical comparisons using repeated-measures ANOVA indicated significant differences in mean DNA recovery between male and female participants ($p < 0.01$), with males consistently shedding more DNA than females over the study period. These results highlight both inter-individual variability and gender-based differences in DNA shedding patterns.

On average, male participants shed more DNA than female participants over the course of the seven days. The mean DNA recovery for male participants (e.g., Participant 1, 0.85 ± 0.12 ng/µL; Participant 5, 0.80 ± 0.21 ng/µL) was consistently higher than that of female participants (e.g., Participant 2, 0.16 ± 0.04 ng/µL; Participant 3, 0.27 ± 0.08 ng/µL). Statistical comparisons using repeated-measures ANOVA revealed a significant difference in DNA shedding rates between male and female participants ($p < 0.01$), supporting a potential gender-based influence on DNA shedding rates. Among the participants, certain individuals stood out as higher shedders. Participant 1 (Male) and Participant 5 (Male) consistently exhibited higher DNA recovery values compared to the others. Participant 1 maintained the highest DNA recovery values, even as overall shedding decreased over time. In contrast, Participants 2 and 3 (Females) exhibited lower overall DNA recovery, with Participant 3 showing more pronounced fluctuations. Within-participant variability was notable for some, such as Participant 5, who exhibited substantial fluctuations with a standard deviation of 0.218 ng/µL. Participant 4 (Male) also demonstrated variability, with notable increases in DNA shedding on Days 2 and 4, which were statistically significant compared to other days ($p < 0.05$). Conversely, female participants displayed more consistent, albeit lower, DNA shedding levels, with fluctuations that were not statistically significant ($p > 0.05$).

Discussion

The findings from this study provide significant insights into the dynamics of DNA shedding following handwashing, contributing to the broader understanding of touch DNA. The results confirm existing assumptions while introducing new considerations critical for forensic applications.

Immediate Effects of Handwashing on DNA Levels

As anticipated, DNA levels on the hands decreased immediately after handwashing, consistent with previous research indicating that handwashing effectively removes loose skin cells, surface DNA, and other contaminants [1,35]. This immediate reduction aligns with the temporal dynamics presented in Figure 1, where DNA recovery progressively increased at 5, 15, and 30 minutes post-handwashing. The physical removal of loose skin cells and surface oils during handwashing likely contributed to the initial decrease in DNA levels. Following the initial reduction, DNA levels began to reaccumulate as skin cells were naturally replenished and oils and sweat were produced. These processes facilitate the transfer of DNA back to the skin surface, restoring DNA availability for recovery [36]. Environmental factors such as room temperature and humidity during the 30-minute post-handwashing period may also influence the rate of DNA reaccumulation. The consistent recovery pattern observed in this study highlights the predictable nature of DNA reaccumulation under controlled conditions and suggests that the timing of sample collection post-handwashing could critically impact DNA recovery rates in forensic applications.

Variability in DNA Shedding Rates and Shedder Status

One of the most striking findings of this study was the variability in DNA shedding rates among participants, challenging the assumption that DNA shedding follows a uniform pattern. As shown in Figure 2, participants were categorized into "high shedders," "medium shedders," and "low shedders" based on their DNA recovery levels. Participants 1 and 5 consistently exhibited higher DNA recovery, while Participants 2 and 3 displayed significantly lower levels, consistent with classifications established in forensic science [37,38]. High shedders are likely influenced by physiological factors such as elevated sebum production, which facilitates the transfer of DNA-containing cells to surfaces [39]. Faster skin cell turnover may also increase the availability of shed DNA. In contrast, low shedders may have drier skin, which reduces cell detachment, or slower turnover rates, resulting in less DNA transfer. Genetic factors and behavioral habits, such as frequent handwashing or the use of drying skincare products, could further contribute to lower DNA recovery [40].

Understanding shedder status is crucial in forensic contexts, as it directly affects the likelihood of leaving detectable DNA at a crime scene. High shedders are more likely to contribute substantial DNA evidence even after multiple handwashes, whereas low shedders may leave insufficient traces, complicating evidence collection in cases involving minimal contact [41]. These findings emphasize the importance of tailoring forensic strategies to individual variability in DNA shedding.

Gender Differences in DNA Shedding

This study corroborates findings that gender plays a significant role in DNA shedding, with male participants generally shedding more DNA than females, as depicted in Figure 3. Male participants consistently exhibited higher DNA recovery levels (e.g., Participant 1: 0.85 ± 0.12 ng/µL; Participant 5: 0.80 ± 0.21 ng/µL) compared to females (e.g., Participant 2: 0.162 ± 0.04 ng/µL). These differences can be attributed to physiological factors, including greater sebaceous gland activity in males, which increases sebum production and facilitates DNA transfer [4]. Hormonal influences, such as elevated testosterone levels in males, may also promote sebum production and accelerate skin cell turnover rates [42]. Additionally, structural differences in skin thickness and hydration levels between genders could further contribute to variations in DNA transferability. These findings suggest that gender-specific factors should be considered when interpreting DNA evidence, particularly in cases where touch DNA plays a pivotal role.

Environmental and Activity-Related Factors

Environmental conditions and activity levels were identified as significant factors influencing DNA shedding rates. Participants exposed to higher humidity or engaging in physical activity may have experienced increased DNA shedding due to enhanced sweating and skin cell turnover [43-45]. Other environmental and behavioral factors, such as the use of body lotions, creams, or sanitizers, can also impact DNA shedding. For instance, moisturizers increase skin hydration, potentially enhancing DNA transfer to surfaces, while alcohol-based sanitizers may temporarily reduce DNA recovery by removing oils and cells but facilitate reaccumulation as the skin restores its natural cycle. Temperature conditions further influence DNA recovery, with higher temperatures promoting perspiration and increasing DNA transfer, whereas colder conditions may limit shedding by reducing sweat production. Future research should systematically investigate the effects of these factors to improve the predictability of DNA recovery rates under various conditions [46,47].

Forensic Implications of DNA Shedding Variability

The significant inter-individual variability in DNA shedding observed in this study has critical implications for forensic science, particularly in the context of touch DNA analysis. Differences in DNA reaccumulation rates post-handwashing, as highlighted in Figure 1, suggest that individual variability must be considered when interpreting DNA evidence. High shedders, such as Participants 1 and 5, may leave detectable DNA profiles even after multiple handwashes, whereas low shedders, such as Participants 2 and 3, may leave minimal or no DNA despite limited handwashing [41]. These findings

highlight the importance of adjusting forensic strategies to account for shedder status. DNA persistence and degradation rates over time may also vary based on initial deposition amounts. High shedders may leave more recoverable DNA even under adverse conditions, while evidence from low shedders may degrade faster, complicating crime scene reconstructions [48]. Temporal fluctuations in DNA shedding, as demonstrated by the reaccumulation patterns in this study, further underscore the importance of timing in evidence collection [49]. By integrating these findings into forensic protocols, professionals can refine evidence collection techniques, adapt methods to individual characteristics, and improve the reliability of touch DNA analysis. Future studies addressing the effects of environmental factors, individual behaviors, and longer-term DNA recovery trends will help enhance the utility of touch DNA evidence in forensic investigations.

Limitations and Future Research

Limitations

This study, while providing valuable insights into the dynamics of DNA shedding, has limitations that warrant consideration. The small sample size (n = 5) may not capture the full range of variability in DNA shedding across a broader population. The focus on handwashing with antibacterial soap further limits the generalizability of findings to other handwashing agents or conditions. The study also did not examine potential differences in DNA shedding between the dominant and non-dominant hands. Exploring whether variations in hand usage affect DNA recovery could provide valuable insights. Additionally, this research did not extend over long periods, precluding the assessment of sustained handwashing effects or behaviors such as touching the face or body, which might influence DNA reaccumulation. Finally, the study did not consider factors such as skin type, ethnicity, or age, which previous research has shown can significantly affect DNA shedding rates. Consequently, these findings may not fully represent all demographic groups.

Future Research

Future studies should address these limitations to expand on the understanding of DNA shedding dynamics. Key recommendations include:

Larger and More Diverse Sample Sizes: Future studies should include a greater number of participants from diverse demographic groups to better capture variability in DNA shedding. Variables such as ethnicity, skin type, age, and activity level should be systematically analyzed to assess their impact on DNA recovery.

Long-Term Tracking of DNA Shedding: Studies should monitor DNA shedding over extended periods, incorporating behavioral observations such as face-touching or surface contact. This approach would provide a more comprehensive understanding of DNA reaccumulation dynamics.

Comparison of Dominant and Non-Dominant Hands: Investigating differences between dominant and non-dominant hands could reveal how usage patterns influence DNA shedding.

Effects of Different Handwashing Agents: Examining the impact of various soaps, sanitizers, and washing protocols on DNA recovery could refine forensic methodologies.

Environmental Influences: Future research should evaluate the effects of environmental factors such as humidity, temperature, and light exposure on DNA degradation and recovery to optimize touch DNA evidence handling in diverse conditions. By addressing these gaps, future research can further enhance the reliability and applicability of touch DNA evidence in forensic investigations.

Conclusion

This study provides valuable insights into the temporal dynamics of DNA shedding following handwashing, offering important implications for forensic science. The key findings reveal that DNA shedding is not a uniform process but varies significantly among individuals, with shedding rates fluctuating over time as skin cells are replenished and environmental factors come into play. This variability was particularly evident in the immediate reduction in DNA levels after handwashing, followed by a gradual reaccumulation at rates and extents that differed across participants. For forensic professionals, these findings underscore the necessity of adopting more nuanced approaches to touch DNA recovery and analysis. Recognizing that DNA shedding patterns vary widely between individuals-and are influenced by factors such as time elapsed since handwashing, skin type, and environmental conditions—is crucial for the accurate interpretation of DNA evidence. By accounting for these individual differences and carefully timing evidence collection, forensic teams can enhance the accuracy and reliability of touch DNA analysis, improving the overall efficacy of forensic investigations. Although determining an individual's shedding status with absolute certainty remains challenging, the methodology employed in this study offers a robust framework for assessing individual shedding tendencies. Systematic tracking of DNA shedding over time and under controlled conditions enables researchers to better understand participants' shedding abilities.

This approach is particularly valuable in forensic research, where optimizing participant selection and experimental designs can lead to more efficient and targeted studies of touch DNA. In summary, this study highlights the need for forensic methodologies to remain flexible and adaptive to the dynamic nature of DNA shedding. The insights gained from this research provide a foundation for refining experimental designs, improving participant selection, and enhancing forensic protocols. By integrating these findings into practice, forensic professionals can navigate the complexities of touch DNA evidence with greater precision, ultimately leading to more robust and reliable forensic outcomes.

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Conflict of Interest

The authors affirm that there are no financial interests or personal relationships that could have influenced the work presented in this study.

Ethics Statement

This research adhered to the ethical guidelines established by the General Department of Forensic Science and Criminology, Dubai Police General Headquarters, Dubai, UAE. The study's methodology, including data collection and analysis, was rigorously reviewed and approved by the Department to ensure compliance with both institutional and international standards for ethical research practices. The research was conducted with the highest ethical integrity to ensure the validity of the findings and to contribute meaningfully to the advancement of forensic science practices.

Author Contributions

S.K.A. was responsible for sample collection, data analysis, and drafting the main manuscript. W.G. provided critical review and revisions to enhance the intellectual content of the manuscript. Both authors have reviewed and approved the final version of the manuscript for submission.

Data Availability Statement

Not applicable.

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