



Research Paper

Touching Without Contact: Glove-Mediated Secondary DNA Transfer in Forensic Casework

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Abstract

The inadvertent transfer of DNA via gloves poses a significant risk to the integrity of forensic evidence, particularly in trace and touch DNA investigations. This study systematically evaluated the extent of DNA contamination on glove surfaces, the effectiveness of common cleaning agents, and the potential for secondary DNA transfer to mock evidence. Twelve participants, pre-classified by DNA shedding status, donned nitrile, latex, and vinyl gloves under controlled laboratory conditions. Glove surfaces were sampled after donning and subjected to four cleaning conditions: no cleaning, 0.3% sodium hypochlorite, RNase AWAY™, and 70% ethanol. DNA was extracted and quantified, and STR profiling was performed to assess the presence and completeness of genetic profiles.

Results demonstrated significant variability in DNA retention based on glove type and cleaning agent. Vinyl gloves retained the highest DNA levels, while nitrile gloves showed the least contamination. Sodium hypochlorite was the most effective cleaning agent, reducing recoverable DNA by up to 94%, followed by RNase AWAY™, with ethanol being the least effective. Notably, even post-cleaning gloves frequently retained sufficient DNA to yield partial STR profiles, underscoring the persistent risk of secondary transfer. Controlled glove-to-cloth contact experiments further confirmed that uncleaned gloves transferred full STR profiles in 80% of cases, while sodium hypochlorite-treated gloves minimized this risk. Ultraviolet fluorescence visualization revealed contamination hotspots concentrated at the fingertips and palm, highlighting key zones of contact and transfer potential.

These findings emphasize the need for standardized glove decontamination protocols, careful selection of glove materials, and contamination-aware handling procedures in forensic workflows. Incorporating these practices will enhance the reliability of trace DNA interpretation and reduce the risk of misattribution in forensic casework.

Keywords: Forensic Genetics, Forensic science, DNA Profiling, STR profiling, Touch DNA, Trace DNA, DNA Transfer, Secondary Transfer, Glove Contamination, Forensic Gloves, DNA Decontamination, Sodium Hypochlorite, RNase AWAY, Forensic DNA Recovery, Contamination Control, Forensic Casework, Shedding Status, Forensic Best Practices

1. Introduction

Forensic DNA analysis has undergone a transformative evolution with the development of highly sensitive short tandem repeat (STR) profiling, enabling the generation of complete genetic profiles from trace quantities of biological material—sometimes as little as 100 picograms [1,2]. This advancement has elevated the probative value of touch DNA, also known as trace DNA, which is frequently recovered from everyday objects such as doorknobs, tools, and clothing in criminal investigations [3–5]. Unlike bodily fluids, touch DNA is typically transferred via brief, incidental contact, often without the donor's awareness [6–9].

However, the very sensitivity that makes STR profiling a powerful tool also introduces new challenges. Trace DNA recovery is notoriously variable due to substrate composition [10,11], environmental exposure [12–14], and collection technique inconsistencies [10,11,15–18]. For instance, the type of swab or adhesive material, the wetting agent used, and even the number of lifts applied can significantly impact DNA yield [19–25]. Extraction and quantification methods further compound this variability [2,4,12,26–30], while inter-individual differences in DNA shedding and the ever-present risk of contamination contribute to analytical complexity [31–38].

Collection success hinges on aligning recovery tools with surface characteristics: cotton swabs are preferable for smooth materials, while tape lifting is favored for porous substrates like fabric [10,20,39–44]. In response to these limitations, hybrid techniques have emerged, including microFLOQ® swabs for direct amplification, microbial wet-vacuum systems, and improved decontamination agents [23,44–46]. These innovations emphasize the need for adaptive, site-specific collection protocols as DNA yields vary across substrates and environmental conditions [47–50].

Complementing technological advances, forensic laboratories are under increasing pressure to maintain sample integrity. Improper evidence handling can result in false inclusions or erroneous interpretations,

especially in low-template DNA scenarios [51,52]. Silica-based extraction workflows, while common, often lead to DNA loss, prompting the adoption of direct amplification protocols that bypass extraction altogether [1,17,22,53,54].

Contamination control has thus become a cornerstone of trace DNA analysis. Among the most critical concerns is the inadvertent transfer of DNA via personal protective equipment (PPE), particularly gloves. These are used universally in forensic practice to protect both personnel and evidence. Yet, studies show that even unopened glove boxes can contain detectable human DNA—possibly introduced during manufacturing or packaging [55]. Once worn, gloves can readily acquire DNA from touched surfaces and subsequently deposit that DNA onto unrelated objects, thereby facilitating secondary transfer [2,4,56].

The phenomenon of glove-mediated secondary transfer—where DNA is transmitted to an object the wearer has never touched directly—is well documented [2,5,57]. Shedding status further modulates this risk: "good shedders" transfer significantly more DNA onto gloves and tools than "poor shedders," increasing the chance of contaminating subsequent exhibits [5,38]. In burglary simulations, handlers' DNA was recovered from items handled indirectly via gloves, raising serious interpretive challenges in casework [2,5].

Moreover, glove material (e.g., latex vs. nitrile), the timing of DNA deposition, and sequential contact patterns all influence transfer potential [33,34,37]. While rigorous PPE protocols, including frequent glove changes—can reduce contamination, such standards are inconsistently applied across laboratories. Some reports show contamination rates exceeding 30% in labs lacking standardized glove practices, compared to less than 5% in labs with strict controls [36].

Environmental exposure also plays a critical role in touch DNA persistence and recovery. Dusty or sandy surfaces can impair DNA extraction, and high humidity or temperature may accelerate degradation

[12–14,58–60]. In vehicle interiors—common in hit-and-run investigations—DNA recovery success varies widely by site, highlighting the need for situational adaptability [61]. Furthermore, emerging research into the recovery of human DNA from ambient air and contactless surfaces points to increasingly complex contamination routes [47–49,59].

These findings are especially pertinent in drug-related casework. DNA has been successfully recovered from the outer surfaces of drug packaging, offering investigative leads on those involved in trafficking [56–58,62]. However, samples often originate from end-level handlers. Airborne deposition during production processes presents another avenue for upstream contamination—highlighting the potential for indirect DNA deposition even in the absence of direct contact [59,62].

While gloves are essential in forensic practice to safeguard both evidence integrity and examiner safety, they can paradoxically become sources of DNA contamination. Research has shown that even unused gloves—regardless of whether they are drawn from sealed or open packaging—can carry trace amounts of human DNA, likely introduced during manufacturing or handling processes [63]. Once worn, gloves may accumulate biological material from exhibits or surfaces and unintentionally transfer it to unrelated items or tools, thereby complicating the interpretation of DNA profiles [64].

Of particular concern is secondary DNA transfer, whereby DNA is deposited onto objects indirectly via intermediaries such as gloves, tools, or surfaces. Numerous controlled studies have demonstrated that gloves can act as conduits for such indirect transfer. For example, DNA from a primary handler has been recovered from secondary objects like ropes or screwdrivers, even when the person wearing the gloves never directly contacted those items [65,66]. These findings highlight the nuanced and often unpredictable pathways through which DNA can be spread in forensic environments, underscoring the need for robust contamination control measures.

This study addresses critical gaps in forensic contamination control by systematically evaluating glove-mediated DNA transfer under controlled laboratory conditions. By examining the influence of glove material, user shedding status, and pre-use decontamination protocols, the research aims to establish evidence-based strategies to mitigate secondary DNA transfer. The findings are intended to inform standardized contamination prevention guidelines, enhance the interpretive reliability of trace DNA evidence, and support best practices in forensic laboratory and field operations.

2. Materials and methods

2.1 Study Design and Objectives

This experimental study was conducted to investigate glove-mediated DNA transfer under controlled laboratory conditions. Specifically, it aimed to evaluate the extent of DNA transfer from bare hands to the external surfaces of gloves during donning, assess the efficacy of different glove-cleaning agents in reducing DNA contamination, and determine the impact of these cleaning treatments on downstream STR profiling. The study adhered to ISO 17025 quality assurance frameworks and contamination control procedures aligned with ISO 18385:2016. A total of 144 glove surface samples were analyzed, collected from three glove types and subjected to four distinct cleaning protocols. All experiments were conducted in quadruplicate per condition, and appropriate statistical models were applied to assess differences in DNA yield and transfer risk.

2.2 Participants and Ethical Compliance

Twelve volunteer participants (six males and six females), all trained forensic laboratory personnel, were recruited for the study. Each participant had been pre-categorized as a good, intermediate, or poor DNA shedder based on standardized prior assessments using a validated shedding index [2]. Informed written consent was obtained from all participants, and the research protocol received ethical clearance from the relevant institutional review board, classifying the project as low-risk human subject research.

Participants were blinded to the assigned glove-cleaning treatment during secondary transfer trials to mitigate potential experimental bias.

2.3 Glove Types and Handling Conditions

Three types of disposable gloves commonly encountered in forensic practice were selected for evaluation: nitrile gloves (powder-free and ISO 18385-certified), latex gloves (powdered and non-certified), and vinyl gloves (non-powdered and non-certified). All gloves were obtained from factory-sealed boxes and handled within a Class II biosafety cabinet to prevent exogenous contamination prior to experimental use. Participants donned the gloves using a standardized two-handed technique designed to replicate standard operating procedures in forensic laboratories. For simulations involving secondary DNA transfer, a double-gloving method was employed, wherein a fresh glove was layered over a previously worn, DNA-contaminated glove to model realistic contamination scenarios.

2.4 Fluorescent Tracer Visualization

To assess the spatial distribution of DNA transfer during glove donning, a fluorescent tracer composed of GlowTec™ UV germ powder suspended in mineral oil was applied uniformly to the participants' bare hands. After glove application, the external surfaces of the gloves were visualized using a 365 nm ultraviolet light source. Fluorescent signals were photographed and categorized according to regional zones of the glove, including the palm, fingertips, thumb, and wrist areas. The intensity of fluorescence was rated using a 0–3 ordinal scale to generate contamination heatmaps, enabling visual identification of high-contact areas most susceptible to secondary transfer.

2.5 Sampling and Transfer Simulation

DNA sampling was performed using Copan 150C cotton swabs pre-moistened with 100 microliters of sterile distilled water applied via a calibrated spray dispenser to ensure uniform wetting [2,19,24]. Each glove was sampled at three anatomically relevant contact points—thumb, index finger, and wrist—using

standardized circular motions for 10 seconds per site. Swabs were individually sealed in sterile, barcoded microcentrifuge tubes.

To simulate secondary DNA transfer to crime scene materials, participants wearing gloves (either uncleaned or post-treatment) made contact with sterile cotton cloth squares (3 × 4 cm) that had been pre-irradiated with UV light at 900 mJ/cm² for 60 minutes to eliminate background DNA. Contact points were marked in advance, and each area was subsequently swabbed using the same collection technique. Negative controls, consisting of untouched cloth areas, were included to monitor for procedural contamination. All collected swabs were air-dried in a sterile laminar flow cabinet and stored at 4°C until DNA extraction.

2.6 Glove Cleaning Treatments

Four glove-cleaning conditions were examined in the study. The control condition involved no cleaning, representing the baseline contamination risk. The three cleaning protocols tested were: a 0.3% sodium hypochlorite solution (Actisan™), RNase AWAY™ (Thermo Fisher Scientific), and 70% ethanol (certified DNA-free). For each cleaning condition, the glove surface was wiped thoroughly using DNA-free paper towels saturated with the assigned cleaning agent. Gloves were allowed to air-dry for 30 seconds prior to being used in secondary transfer simulations. The efficacy of each cleaning treatment was evaluated based on subsequent DNA yield, STR profile completeness, and contamination risk.

2.7 DNA Extraction, Quantification, and Amplification

Genomic DNA was extracted from all collected swabs using the QIAamp® DNA Investigator Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's protocol. Each swab head was incubated in 370 microliters of ATL buffer and 20 microliters of Proteinase K at 56°C for one hour. DNA was eluted in 50 microliters of ATE buffer.

Quantification was performed using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific) on a QuantStudio 5 Real-Time PCR System. Each quantification plate included internal positive controls, no-template controls, and quantification standards. Total DNA concentration (ng/μL), degradation index, and male-to-total ratios were recorded for all samples.

PCR amplification of STR loci was carried out using the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) with 29 amplification cycles. Amplified products were separated by capillary electrophoresis on an ABI 3500xL Genetic Analyzer using a standard injection mix composed of 1.0 μL PCR product, 9.6 μL Hi-Di™ formamide, and 0.4 μL GeneScan™ 600 LIZ® Size Standard v2.0. Each amplification batch included an allelic ladder, a known positive control (2800M), and a negative control.

2.8 STR Analysis and Interpretation

Resulting STR profiles were analyzed using GeneMapper™ ID-X Software v1.6. A minimum analytical threshold of 50 relative fluorescence units (RFU) was applied. Allelic profiles were assessed for peak height, heterozygote balance, and presence of drop-in or drop-out events. Mixtures were interpreted using a major/minor contributor framework. All electropherograms were independently reviewed by two forensic analysts who were blinded to the glove type and cleaning treatment to ensure objective assessment and to resolve any discrepancies in allele calling or profile completeness.

2.9 Quality Control and Contamination Prevention

All procedures were conducted within an ISO 17025-accredited framework, and quality assurance protocols conformed to ISO 18385:2016 requirements for minimizing DNA contamination in forensic products. Certified DNA-free consumables were used throughout the study. Laboratory spaces were physically segregated into pre- and post-PCR zones, and a strict unidirectional workflow was enforced to prevent cross-contamination. All instruments, pipettes, and bench surfaces were thoroughly decontaminated

before and after each experiment using a two-step process: 0.5% sodium hypochlorite followed by 70% ethanol. Each experimental run included reagent blanks, extraction controls, and PCR negative and positive controls. Routine surface monitoring and documentation were performed in accordance with internal SOPs and external audit criteria. All analysts involved in the study were trained in contamination prevention and record-keeping protocols, ensuring data traceability and procedural integrity.

3. Results

3.1 DNA Recovery from Glove Surfaces under Varying Cleaning Conditions

A total of 144 glove surface samples were collected from 12 participants using three different glove types—nitrile, latex, and vinyl—across four glove-cleaning conditions: no cleaning, sodium hypochlorite, RNase AWAY™, and 70% ethanol. Each experimental condition was repeated in quadruplicate per participant to ensure statistical robustness. DNA was successfully extracted and quantified from all collected samples.

Quantitative results indicated that uncleaned gloves consistently exhibited the highest DNA concentrations. Among glove types, vinyl gloves retained the greatest levels of DNA (mean ± SD: 0.063 ± 0.030 ng/μL), followed by latex (0.051 ± 0.028 ng/μL), and nitrile (0.034 ± 0.021 ng/μL). Regarding cleaning efficacy, sodium hypochlorite was the most effective treatment, achieving an average reduction of up to 94% in recoverable DNA across all glove types. RNase AWAY™ performed comparably but slightly less effectively. Ethanol proved the least efficient, with residual DNA levels remaining at approximately 80% of those observed in uncleaned controls. These results are illustrated in Figure 1.

3.2 STR Profiling of Glove-Derived DNA

Chromatographic All DNA extracts obtained from glove surfaces underwent short tandem repeat (STR) amplification and fragment analysis. The completeness of STR profiles was found to be strongly influenced by

the glove-cleaning treatment, with uncleaned gloves yielding the most robust genetic signals.

Specifically, 88% of uncleaned glove samples produced full or near-complete STR profiles, defined as $\geq 90\%$ allele recovery. In contrast, gloves cleaned with 70% ethanol retained partial profiles in approximately 32% of cases, indicating suboptimal decontamination. RNase AWAY™ further reduced allele recovery, with partial profiles observed in about 21% of treated samples. Sodium hypochlorite demonstrated the highest decontamination efficiency, with only 16% of samples yielding partial profiles and

the majority producing little to no interpretable STR signal.

These findings confirm that detectable and potentially interpretable STR profiles may persist even after cleaning, particularly when suboptimal decontamination protocols are used. The presence of full genetic profiles on uncleaned gloves underscores their potential as vectors for secondary DNA transfer and highlights the critical importance of implementing effective glove-cleaning protocols. These results are illustrated in Figure 2.

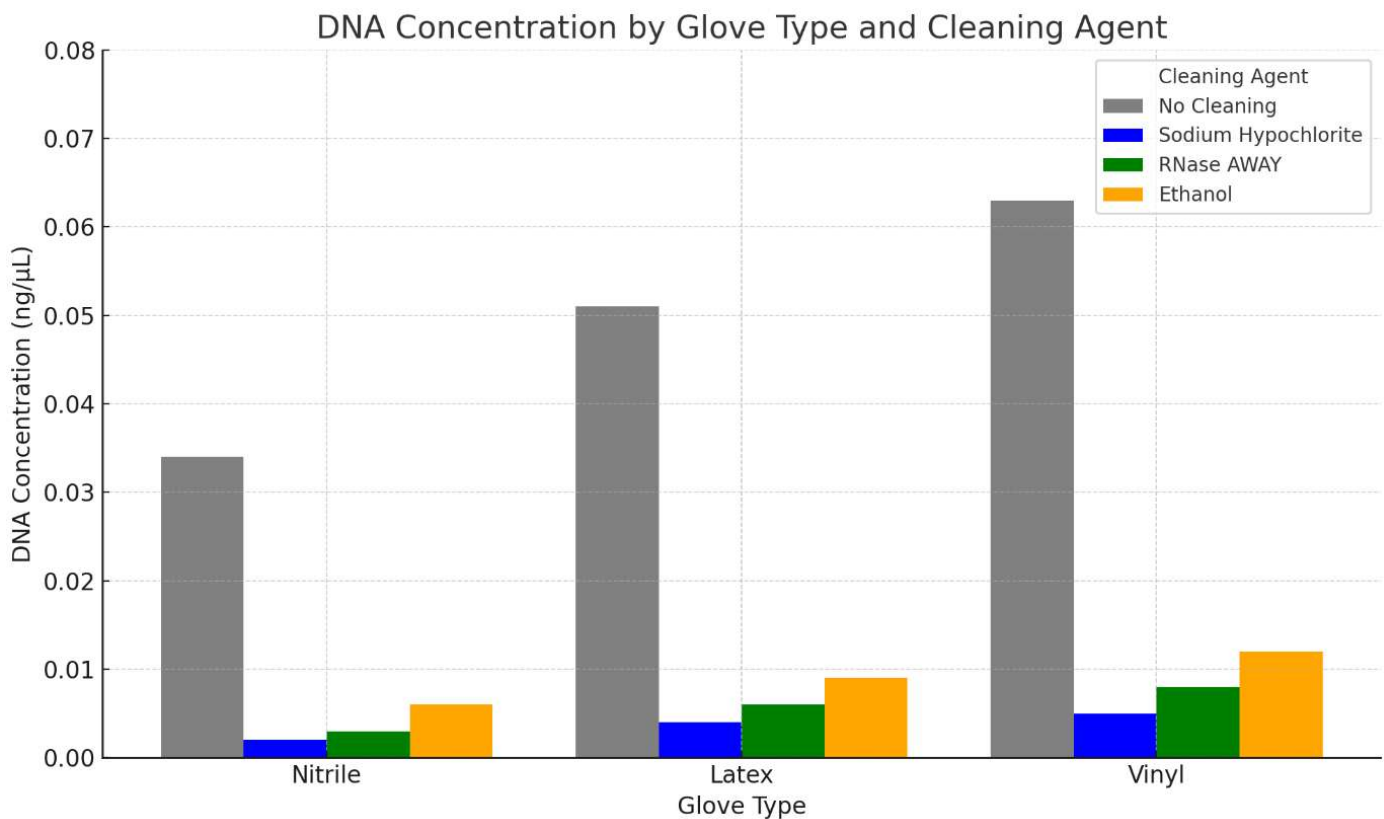


Figure 1. This figure illustrates the average DNA concentration (ng/μL) recovered from the outer surfaces of three glove types—nitrile, latex, and vinyl—subjected to four cleaning conditions: no cleaning, 0.3% sodium hypochlorite, RNase AWAY™, and 70% ethanol. Each glove and cleaning combination was tested across twelve participants in quadruplicate (n = 12 per condition; total n = 144). Gloves with no decontamination retained the highest DNA levels, with vinyl gloves showing the greatest contamination (mean ± SD: 0.063 ± 0.030 ng/μL), followed by latex (0.051 ± 0.028 ng/μL) and nitrile (0.034 ± 0.021 ng/μL). Sodium hypochlorite achieved the most substantial reduction in DNA concentration, with average decreases of up to 94% relative to uncleaned gloves. RNase AWAY™ was moderately effective, while ethanol was the least effective, retaining ~80% of baseline DNA levels. These results highlight the critical influence of both glove material and cleaning agent on contamination potential and emphasize the importance of evidence-based decontamination practices in forensic DNA handling.

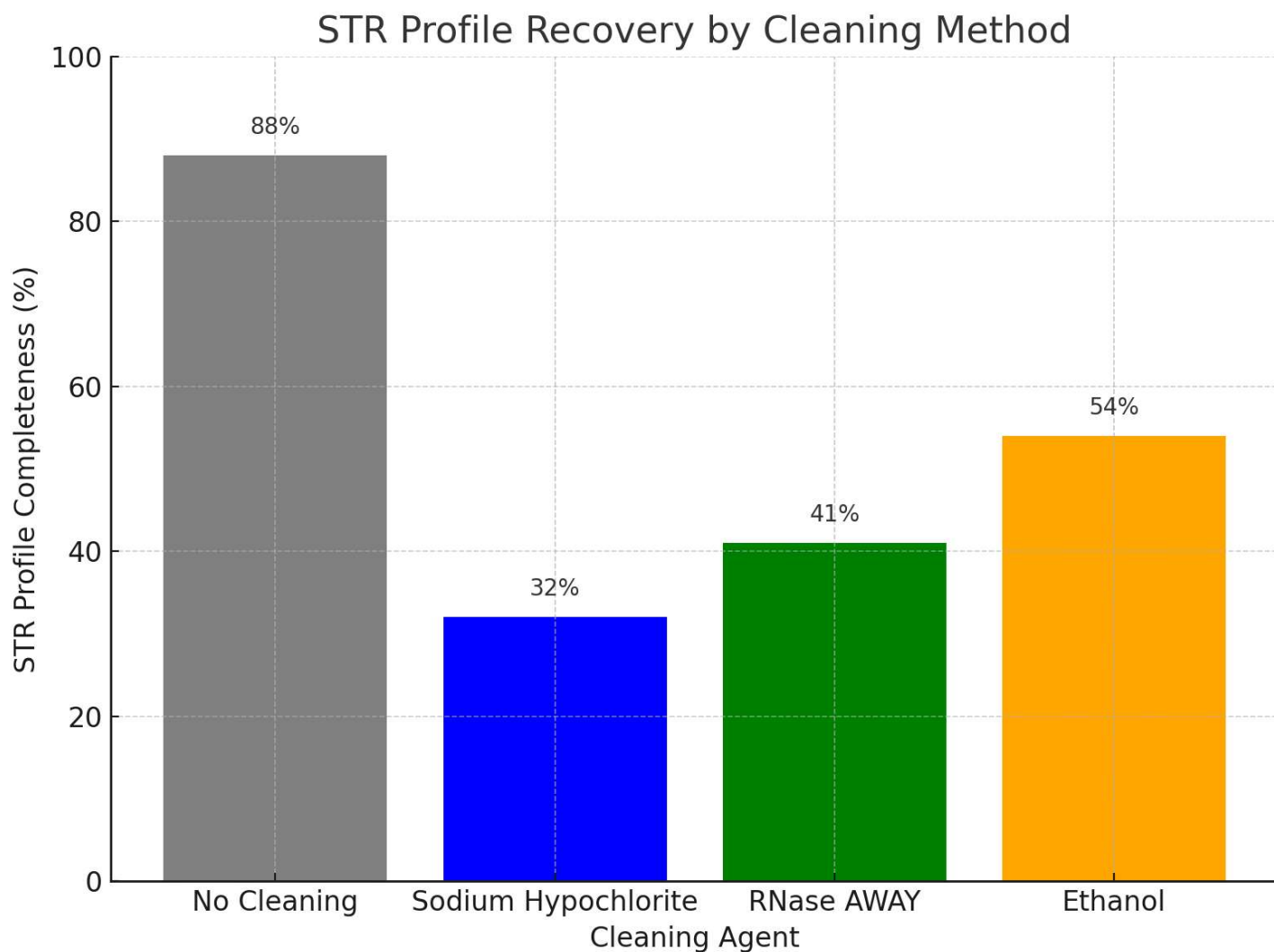


Figure 2. This figure depicts the average STR profile completeness (%) obtained from DNA transferred during glove-to-substrate contact under four distinct glove-cleaning conditions: no cleaning, 0.3% sodium hypochlorite, RNase AWAY™, and 70% ethanol. Each cleaning condition was evaluated using twelve replicate glove-contact events (n = 12 per group, total n = 48). Uncleaned gloves generated STR profiles with a mean completeness of 88%, reflecting the high potential for full genotype transfer when gloves are used without decontamination. Sodium hypochlorite demonstrated the greatest effectiveness in limiting profile recovery, reducing allele detection to an average of 32%. RNase AWAY™ yielded intermediate results, with 41% mean profile completeness, while ethanol-treated gloves retained sufficient DNA to produce partial profiles in over half the cases (mean 54%). Although all cleaning agents reduced STR detectability to varying degrees, partial profiles remained observable across conditions, emphasizing that incomplete decontamination can still result in secondary transfer of interpretable DNA. These findings highlight the necessity of using highly effective cleaning protocols when glove reuse or contact with trace evidence is anticipated in forensic workflows.

3.3 Secondary Transfer to Mock Evidence

To evaluate the risk of secondary DNA transfer from contaminated gloves to forensic substrates, a total of 40 mock transfer events were conducted. In each event, gloved hands were used to press onto sterile cotton cloth squares, simulating contact with potential evidence items during crime scene handling. The gloves had been subjected to one of four cleaning conditions: no cleaning, ethanol, RNase AWAY™, or sodium hypochlorite.

The resulting STR profiles recovered from the cloth surfaces demonstrated a clear relationship between cleaning protocol and contamination risk. Uncleaned gloves transferred full STR profiles in 80% of the events (8 out of 10) and partial profiles in the remaining 20%. Gloves treated with ethanol still produced one full profile and five partial profiles, representing a 60% rate of detectable DNA transfer. In contrast, gloves cleaned with either RNase AWAY™ or sodium hypochlorite yielded no full STR profiles, and only limited partial allelic recovery, indicating a significantly reduced potential for downstream contamination.

These results highlight the real-world forensic implications of inadequate glove decontamination, particularly when handling trace evidence or low-template DNA. The data underscore the effectiveness of chemical decontaminants, especially hypochlorite-based solutions, in minimizing transfer risk. These outcomes are illustrated in Figure 3.

3.4 Statistical Analysis of DNA Quantification Data

A one-way analysis of variance (ANOVA) was performed to assess the effect of cleaning treatment on DNA concentration. The results demonstrated a statistically significant main effect of cleaning method ($F(3, 140) = 45.23, p < 0.001$). Subsequent Tukey's Honest Significant Difference (HSD) post-hoc tests indicated that DNA concentrations recovered from uncleaned gloves were significantly higher than those from any of the cleaned glove conditions ($p < 0.01$).

Sodium hypochlorite was found to be significantly more effective than ethanol ($p = 0.04$) and RNase AWAY™ ($p = 0.03$) in reducing DNA levels. An additional ANOVA examining glove type revealed that nitrile gloves retained significantly lower DNA concentrations compared to both latex and vinyl gloves ($p < 0.05$), regardless of cleaning method. These findings support the hypothesis that both glove material and cleaning protocol contribute independently and interactively to the risk of DNA contamination.

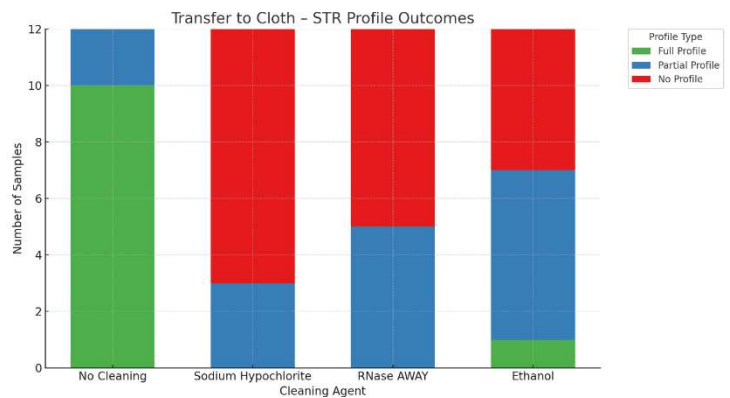


Figure 3. This figure shows the frequency distribution of STR profile outcomes—categorized as full, partial, or no profiles—obtained from cotton cloth swatches contacted by gloved hands under four glove-cleaning conditions: no cleaning, 0.3% sodium hypochlorite, RNase AWAY™, and 70% ethanol. Each condition was tested in twelve replicate contact events ($n = 12$ per condition; total $n = 48$). Gloves that had not undergone any cleaning produced full STR profiles in 10 of 12 samples (83%), confirming the high risk of secondary DNA transfer sufficient to generate complete genetic profiles. In comparison, gloves treated with sodium hypochlorite produced no detectable STR profiles in 75% of cases (9/12), with only three samples yielding partial profiles. RNase AWAY™ resulted in five partial and seven no-profile outcomes, while ethanol-treated gloves produced one full and six partial profiles, indicating intermediate effectiveness. Overall, all cleaning agents led to a $\geq 70\%$ reduction in full-profile recovery relative to uncleaned gloves. These results emphasize the potential for forensic contamination through glove-

mediated transfer and underscore the necessity of selecting effective decontamination strategies to mitigate the risk of generating interpretable STR profiles from secondary contact.

3.5 Visualization and Mapping of Glove Contamination via UV Fluorescence

To spatially characterize the distribution of DNA contamination on glove surfaces, a visualization experiment was performed using UV-sensitive fluorescent tracer applied to participants' bare hands prior to glove donning. Following brief contact and handling, gloves were exposed to ultraviolet light, and contamination intensity was assessed using a standardized 0–3 scoring system, where 0 indicated no signal and 3 indicated very strong fluorescence.

The resulting fluorescence patterns, presented in Figure 4, consistently revealed concentrated signals in high-contact regions such as the palm, fingertips, and thumb. The average glove surface exhibited 3.6 distinct contaminated hotspots, independent of glove material. This spatial distribution highlights the immediacy of contamination following glove donning and underscores the importance of targeted cleaning protocols that prioritize anatomically high-risk areas for DNA transfer.

3.6 Summary of Key Findings

Across all experimental phases, the results consistently demonstrated that glove type and cleaning treatment significantly influence DNA contamination risk. Vinyl gloves exhibited the highest capacity for DNA retention, followed by latex and nitrile. Sodium hypochlorite emerged as the most effective decontamination agent, substantially reducing both DNA quantification values and STR profile completeness.

Importantly, uncleaned gloves were capable of transferring sufficient DNA to yield complete genetic profiles on secondary substrates, highlighting the potential forensic implications of inadequate glove decontamination. Fluorescent tracer mapping further confirmed that contamination is spatially concentrated

in predictable anatomical regions, offering practical insights for targeted cleaning interventions.

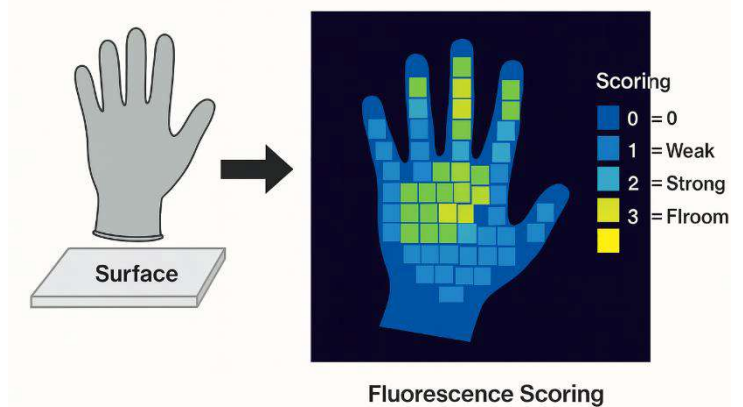


Figure 4. This figure presents a schematic and heatmap visualization of glove contact contamination patterns following glove-to-surface interaction under controlled conditions. High-shedding individuals wore gloves treated with a fluorescent tracer and contacted sterile fabric surfaces, after which the gloves were immediately examined under ultraviolet (UV) light at 365 nm. Fluorescence intensity was scored using a standardized 0–3 scale, where 0 indicates no visible fluorescence, 1 represents weak speckled traces, 2 indicates moderate smearing, and 3 corresponds to dense contact imprints. The resulting fluorescence heatmap reveals consistent contamination across high-contact glove regions, particularly the fingertips, thumb, and palmar surface—zones associated with the highest friction and contact pressure during routine handling. These visual patterns confirm that DNA-bearing residues are not randomly distributed but instead follow predictable spatial dynamics, supporting a risk-based approach to glove cleaning and monitoring in forensic workflows.

4. Discussion

This study presents a comprehensive evaluation of glove-mediated DNA transfer and the effectiveness of various decontamination strategies under controlled forensic conditions. By quantifying DNA retention, STR profile recovery, and secondary transfer to mock evidence, the findings provide critical insights into

contamination dynamics and support practical improvements in forensic workflows.

4.1 Glove Material Influences DNA Retention

DNA retention on glove surfaces was strongly influenced by glove material. Vinyl gloves yielded the highest DNA concentrations, followed by latex and nitrile. This ranking is likely due to differences in surface porosity, electrostatic properties, and frictional interaction with skin. Nitrile gloves, which are smoother and less porous, exhibited the lowest retention. This aligns with previous work indicating that nitrile gloves reduce the risk of secondary transfer by limiting DNA adherence and shedding contact points [65,67]. Consequently, nitrile gloves are recommended for tasks involving trace-level DNA evidence, particularly where contamination risk must be minimized.

4.2 Cleaning Agents Exhibit Differential Efficacy

Decontamination efficacy varied significantly between cleaning agents. Sodium hypochlorite (0.3%) was the most effective, reducing recoverable DNA by up to 94%, followed by RNase AWAY™, while 70% ethanol was the least effective, with only modest reductions in DNA concentration. These results are supported by earlier research that identified chlorine-based reagents as significantly more efficient than alcohol- or acid-based solutions in eliminating nucleic acid residues [68,69]. Ethanol, while commonly used, lacks nuclease activity and cannot reliably degrade surface DNA, a concern previously noted in both diagnostic and forensic settings [68,70]. Our findings confirm that ethanol is insufficient as a standalone decontamination agent for gloves intended for DNA-sensitive work.

4.3 STR Profiling Confirms Presence of Amplifiable DNA Post-Cleaning

Despite chemical treatment, gloves still retained amplifiable levels of DNA capable of generating interpretable STR profiles. Partial profiles were recovered from gloves cleaned with sodium hypochlorite in 16% of cases, and with ethanol in 32% of cases. These results are consistent with studies

showing that trace amounts of residual DNA, when combined with highly sensitive STR amplification kits, can yield usable genotypes [65,66,67]. Importantly, such profiles may introduce ambiguity in casework if gloves are reused or if glove contamination is not accounted for during interpretation. Even partial STR signals may influence mixed DNA profile analysis and affect evidentiary value if source attribution is misapplied [67].

4.4 Gloves as Vectors for Secondary Transfer

The mock evidence simulations clearly demonstrated that gloves can act as intermediaries for secondary DNA transfer. Uncleaned gloves transferred full STR profiles to 80% of contacted cloth samples, while ethanol-cleaned gloves still led to partial or full profiles in over half the trials. In contrast, RNase AWAY and sodium hypochlorite significantly reduced transfer risk, yielding either partial or no profiles in the majority of trials. These findings reinforce previous simulations and casework observations where DNA profiles appeared on items not directly handled by the individual in question, attributed to indirect contact via gloves or other tools [65,66,67]. This has substantial implications in scenarios involving contested DNA evidence, as indirect transfer mechanisms must be considered in evaluating activity-level propositions.

4.5 Fluorescence Mapping Reveals High-Risk Contact Zones

Ultraviolet visualization of fluorescent tracer distribution revealed consistent contamination zones across glove surfaces. The fingertips, palm, and wrist areas were repeatedly identified as high-risk regions, consistent with primary contact points during handling. These observations mirror prior studies that highlighted frequent and varied contact zones during evidence processing, including glove-to-object and glove-to-surface interactions [67]. Such visual tools can be integrated into training programs to improve practitioner awareness, reinforce good handling techniques, and support risk-based glove cleaning or replacement strategies.

4.6 Statistical Validation Supports Experimental Findings

Quantitative results were supported by rigorous statistical testing. A one-way ANOVA confirmed that cleaning agent had a significant impact on DNA recovery ($p < 0.001$), and post-hoc Tukey's tests demonstrated that sodium hypochlorite was significantly more effective than ethanol ($p = 0.04$) and RNase AWAY ($p = 0.03$). Additionally, glove material influenced DNA retention independently of cleaning protocol, with nitrile gloves showing significantly lower DNA concentrations than vinyl or latex ($p < 0.05$). These findings strengthen the evidence base for recommending nitrile gloves and chlorine-based cleaning agents in trace DNA protocols.

4.7 Practical Implications and Recommendations

This study provides several evidence-based recommendations with direct relevance to forensic laboratories and crime scene units. First, nitrile gloves should be prioritized for all DNA-sensitive operations, as they demonstrated the lowest DNA retention and the least propensity for secondary transfer across all cleaning conditions tested [65,67]. Their smoother surface texture and reduced porosity likely contribute to this reduced contamination risk.

Second, the reuse of gloves between handling different items should be strongly discouraged unless a validated decontamination protocol is employed. Even after cleaning, gloves were shown to retain amplifiable quantities of DNA, which may still be transferred to evidentiary material. This underscores the potential for indirect contamination events that could compromise the integrity of forensic interpretations [65,66].

Regarding cleaning agents, sodium hypochlorite (0.3%) emerged as the most effective decontamination reagent across all glove types, with RNase AWAY serving as a viable secondary option. In contrast, ethanol (70%) was significantly less effective, resulting in persistent DNA recovery and partial STR profiles in a considerable number of samples. These findings support previous assessments of nucleic acid decontamination agents and call into question the

continued reliance on ethanol as a standalone cleaning solution in forensic contexts [68,69].

Importantly, the results suggest that glove cleaning while donned—that is, post-donning surface decontamination—may provide additional contamination control during active evidence handling. Van den Berge et al. (2019) [69] demonstrated that wiping the exterior of donned gloves with sodium hypochlorite or RNase AWAY significantly reduced hand-to-glove DNA transfer. Although ethanol was the least effective cleaning agent in the current study and others [68], it still reduced contamination to some extent and may represent a practical interim measure where stronger reagents are unavailable. This strategy could be particularly useful in operational environments where frequent glove changes are impractical. Future research should further evaluate the real-time effectiveness of in-use glove cleaning protocols.

The use of double-gloving protocols, where an outer glove layer is routinely changed between handling different items, is also strongly advised. This layered approach has been shown to mitigate cross-contamination and provides a physical barrier that can be rapidly removed without compromising the inner glove's integrity [70].

Finally, the integration of UV fluorescence visualization into laboratory training programs can help forensic personnel understand glove contact patterns and the spatial dynamics of contamination. This method has been shown to enhance awareness of high-risk glove zones, such as fingertips and palms, and could play a key role in improving donning and doffing practices [67].

Collectively, these recommendations should be considered for incorporation into forensic laboratory quality assurance systems, with the goal of reducing glove-mediated DNA contamination and improving the reliability of trace DNA evidence interpretation.

4.8 Study Limitations and Future Directions

Although the study design addressed several key contamination vectors, limitations remain. Only three glove types were assessed; future studies should explore glove variability across manufacturers, powder formulations, and long-term wear. Environmental variables such as humidity, temperature, and handling pressure were not modeled but may affect DNA transfer rates. While this study focused on glove cleaning prior to use, contamination introduced during active handling (e.g., from the wearer's skin or via aerosols) remains a critical concern. Prior work has demonstrated that self-DNA may be transferred to gloves during donning and handling [69], and that gloves frequently contact multiple surfaces, increasing contamination potential [67].

Future investigations should simulate real crime scene evidence handling, explore glove friction with different substrates, and test the effectiveness of in-use cleaning strategies. Longitudinal contamination audits and studies integrating activity-based DNA transfer models would further support probabilistic interpretation frameworks in forensic casework [66].

5. Conclusion

This study provides robust empirical evidence demonstrating that forensic gloves, if not properly managed, can serve as significant vectors for both primary and secondary DNA transfer. The type of glove material, efficacy of cleaning agents, and user shedding status were all shown to influence DNA retention and the potential for contamination during forensic procedures. Among the glove types tested, nitrile gloves exhibited the lowest DNA recovery levels, underscoring their suitability for trace DNA handling. Sodium hypochlorite emerged as the most effective decontamination agent, substantially reducing DNA load and transfer risk, whereas 70% ethanol proved least effective when applied prior to glove use.

Critically, even after cleaning, gloves often retained sufficient amplifiable DNA to yield partial STR profiles, with downstream transfer to mock evidence

occurring in a significant proportion of cases. This highlights the risk of misinterpretation in forensic casework, particularly in scenarios where trace DNA evidence is central to investigative conclusions.

Fluorescent tracer mapping confirmed that contamination tends to localize in high-contact glove regions—such as fingertips and palms—offering practical targets for risk-based cleaning strategies. Statistical analyses further validated these findings, confirming the combined influence of glove type and cleaning protocol on contamination potential.

Importantly, while ethanol was the least effective cleaning agent in pre-use protocols, its application after donning gloves may still offer partial mitigation by removing DNA introduced during glove handling. This approach, although not a substitute for full glove replacement or more effective agents like sodium hypochlorite, may serve as a practical interim step in field or resource-limited settings and warrants further investigation.

Taken together, these findings emphasize the need for standardized, evidence-based glove handling protocols in forensic workflows. Prioritizing optimal glove materials, implementing validated decontamination procedures, adopting double-gloving strategies, and promoting contamination awareness through visualization and training will significantly reduce the risk of inadvertent DNA transfer, thereby improving the reliability and interpretive integrity of trace DNA evidence.

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Conflict of interest

The authors declare no conflicts of interest, financial or otherwise, that could have influenced the design, execution, or reporting of this study. All procedures and analyses were conducted independently to ensure the objectivity and integrity of the findings.

Ethics Statement

This study was approved by the institutional oversight committee of the General Department of Forensic Science and Criminology, Dubai Police, and conducted in accordance with ethical standards for research involving human participants. All volunteers provided informed consent, and all procedures adhered to internationally recognized guidelines for the handling of biological materials, participant confidentiality, and data integrity.

Author Contributions

S.K.A. conceptualized and designed the study, oversaw experimental coordination, performed forensic DNA analyses, conducted statistical evaluations, and led the drafting and finalization of the manuscript. A.A.M. contributed to sample processing, laboratory analyses, and provided critical scientific input and manuscript revisions. Both authors reviewed and approved the final version of the manuscript and are accountable for its content.

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