Review article

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# Forensic potential of toothbrush derived DNA: A narrative review on human identification

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Human identification is a cornerstone of forensic science, encompassing both the confirmation of unknown individuals and the linkage of biological material to known persons. Although DNA profiling remains the gold standard for individual identification due to its exceptional discriminatory power, its application is frequently constrained by the unavailability of direct reference samples. This limitation has driven growing interest in alternative DNA sources derived from personal items encountered in daily life. Among these, the toothbrush has received particular attention, as its bristles routinely retain buccal epithelial cells and salivary residues that may yield sufficient human DNA for forensic profiling. This narrative review synthesizes the available literature on the use of toothbrushes as a DNA source within the context of forensic personal identification, with specific attention to extraction methodologies, factors influencing DNA recovery including toothpaste-mediated PCR inhibition, rinsing intensity, storage duration, and oral health status. The review further addresses the potential interference of the oral microbiome, the forensic challenges posed by mixed DNA profiles and secondary transfer, the applicability of contemporary techniques including massively parallel sequencing and probabilistic genotyping, and the integration of toothbrush-derived DNA into disaster victim identification protocols. Although complete and interpretable short tandem repeat profiles have been obtained across multiple studies, the primary evidence base remains limited to a small number of studies with heterogeneous methodologies and modest sample sizes. Standardized collection and extraction protocols have not yet been established. Future research should prioritize inter-laboratory validation, the systematic evaluation of oral health variables, and the integration of toothbrush sampling into evidence-based forensic frameworks.

Keywords: DNA, forensic science, human identification, toothbrush

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## Introduction

Personal identification constitutes a fundamental process in forensic science, integrating knowledge from medicine and related scientific disciplines to establish or confirm the identity of an individual, whether deceased or living [1]. This process extends beyond simple recognition to encompass the identification of dismembered body parts, skeletal remains, and biological traces such as blood and other bodily fluid stains recovered from crime scenes [2]. The importance of reliable identification is particularly evident in mass disaster scenarios, such as aviation accidents, tsunami events, or acts of terrorism, where human remains may be severely compromised by decomposition, fragmentation, or thermal damage, rendering visual recognition unreliable or impossible [3]. Under such circumstances, the application of scientifically rigorous methods becomes indispensable.

Conventional identification methods, including facial recognition, examination of

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distinguishing marks, tattoos, personal belongings, and visual identification by relatives, remain inherently subjective and susceptible to error [4,5]. These limitations increase the risk of misidentification, with potentially grave legal, ethical, and humanitarian consequences. Modern forensic practice accordingly relies on scientific methods characterized by high specificity and reproducibility: dental identification, fingerprint analysis, and above all, DNA profiling, which is currently regarded as the gold standard owing to its exceptional discriminatory power [6,7].

Deoxyribonucleic acid (DNA) is the fundamental genetic material present in virtually every nucleated cell of the human body. Inherited in equal measure from both biological parents, DNA profiles exhibit a high degree of individuality that renders them exceptionally valuable for forensic identification [8]. The only known exception to individual uniqueness is monozygotic (identical) twins, who share virtually identical genomic sequences and therefore cannot be distinguished by standard short tandem repeat (STR) profiling alone, though emerging approaches including epigenetic analysis and somatic mutation profiling offer potential solutions in such cases.

Despite its robustness, DNA-based identification faces a critical practical limitation: while DNA can often be extracted from unidentified remains or biological traces, the comparative reference sample is frequently unavailable. Most individuals do not have pre-existing DNA profiles stored in accessible forensic databases, and family-based kinship analysis, though useful, reduces the certainty of identification compared to direct reference matching [9]. In cases where no relatives can be located, comparative analysis may be entirely precluded. These challenges have prompted sustained research into alternative sources of reference DNA recoverable from an individual's personal belongings [3,10]. Among these, the toothbrush has emerged as a particularly promising candidate, given its universal daily use, its individual-specific nature, and its propensity to retain buccal epithelial cells and salivary material on its bristles [11]. This narrative review critically evaluates the evidence base supporting toothbrush-derived DNA as a forensic identification tool, addressing extraction methodology, influencing variables, analytical challenges, and integration into contemporary forensic practice.

## Methods

This article was prepared as a narrative review. Electronic databases including PubMed (MEDLINE), Scopus, and Google Scholar were searched in April 2025 using the following keyword combinations: ("toothbrush" AND "DNA"), ("toothbrush" AND "forensic identification"), ("toothbrush" AND "PCR inhibition"), ("buccal epithelial cells" AND "bristles"), ("oral microbiome" AND "DNA extraction" AND "forensic"), and ("personal items" AND "DNA" AND "forensic identification"). No date restriction was applied at the search stage; however, a deliberate effort was made to identify publications from 2020 to 2025 to supplement older foundational studies. Reference lists of included articles were manually screened for additional relevant publications. Studies were included if they reported original data or systematic synthesis on DNA extraction from toothbrushes, factors affecting DNA yield or profile quality from toothbrushes, or related forensic DNA topics directly applicable to the toothbrush context.

### The Toothbrush as a Source of Biological Material for DNA Analysis

The toothbrush is a universal oral hygiene implement, used by the majority of the global

population on a daily basis. During brushing, mechanical friction between bristles and the oral mucosa produces an accumulation of buccal epithelial cells, keratinocytes, and salivary residues on the bristle surface. Because toothbrushes are inherently personal items, not typically shared between individuals, a DNA profile successfully derived from a toothbrush can theoretically be attributed to a single known user with a high degree of confidence [11].

Tanaka *et al.* systematically investigated this potential and demonstrated that nucleated buccal mucosal cells were consistently detectable on the bristle surfaces of used toothbrushes, with complete STR-based DNA profiles generated from all toothbrush samples examined [11]. Similarly, Jobim *et al.* reported successful human identification using DNA purified from cellular residues on used toothbrushes, highlighting the toothbrush as a viable alternative reference source when family DNA is unavailable [10]. The systematic review by Sujatha *et al.* subsequently confirmed these findings across five eligible primary studies, concluding that a toothbrush constituted a reliable source of DNA for both gender determination and personal identification regardless of the duration of use [12].

More recently, Torres-Pérez *et al.* evaluated DNA profiles from both toothbrushes and underwear using two different extraction methods, finding that toothbrushes, despite yielding lower absolute DNA quantities than underwear, consistently produced single-contributor profiles without the mixed DNA profiles observed in underwear samples [13]. This is a forensically important distinction, as single-contributor profiles are considerably easier to interpret and attribute with confidence than mixed profiles.

Despite this promise, the quantity of DNA extractable from toothbrushes is typically low. This necessitates amplification by polymerase chain reaction (PCR) prior to STR profiling, introducing susceptibility to stochastic effects such as allelic dropout and drop-in at low template concentrations. This low-template character of toothbrush-derived DNA is a central forensic challenge and is discussed further in subsequent sections.

## DNA Extraction Methods from Toothbrushes: A Structured Comparison

Several extraction strategies have been described for toothbrush-derived DNA, each with

Table 1 Structured comparison of DNA extraction methods from toothbrushes.

| Parameter | Whole Head Immersion | Bristle Bundle Removal | Partial Head Sectioning (1/4–1/3) | Key References |
|---|---|---|---|---|
| DNA yield potential | High (max. surface area) | Variable; paradoxically lower with more bundles | Moderate–high; balanced approach | Tanaka *et al.* (2000); Bandhaya & Panvisavas (2008) |
| Lysis solution volume | Large (full submersion required) | Small | Reduced vs. whole head | Tanaka *et al.* (2000); Riemer *et al.* (2012) |

Table 1 Structured comparison of DNA extraction methods from toothbrushes. (continued)

| Parameter | Whole Head Immersion | Bristle Bundle Removal | Partial Head Sectioning (1/4–1/3) | Key References |
|---|---|---|---|---|
| Repeat extraction possible? | No (material fully consumed) | Yes (remaining bundles usable) | Yes (remaining portion preserved) | Riemer *et al.* (2012) |
| Toothpaste contamination risk | Moderate | High (increases with bundle count) | Moderate | Bandhaya & Panvisavas (2008); Sujatha *et al.* (2021) |
| Handling difficulty | Low | High (small, loose bristles) | Low (bristles remain attached to head) | Riemer *et al.* (2012) |
| Contamination risk during handling | Low | High | Low–moderate | Sujatha *et al.* (2021) |
| Cost | Higher (reagent volume) | Lower | Lower than whole head | Riemer *et al.* (2012) |
| Recommended for casework? | Possible; not preferred | With caution; limited bundles | Yes — currently preferred method | Riemer *et al.* (2012) |

distinct trade-offs with respect to yield, cost, reproducibility, and handling risk. These are compared in Table 1.

## Whole Head Immersion

The earliest and most straightforward approach involved excision of the entire toothbrush head using a sharp instrument, followed by immersion of the head in lysis solution to maximize cellular contact and DNA yield [11]. Tanaka *et al.* employed this approach and demonstrated complete DNA profiles from all samples, establishing proof of concept for toothbrush-derived profiling [11]. However, this method requires a substantially larger volume of lysis solution to submerge the toothbrush head, increasing reagent costs. Moreover, because the entire head is consumed in a single extraction, no material remains available for repeat analysis should procedural errors, such as PCR failure or sample contamination, occur during downstream processing.

## Selective Bristle Bundle Removal

An alternative approach involves the removal of individual bristle bundles rather than the entire head. Bandhaya and Panvisavas reported a counterintuitive finding in this context: extraction from five bristle bundles yielded greater amplifiable DNA than extraction from ten bundles [14]. This paradoxical result was attributed to the inhibitory effect of residual toothpaste entrapped among the bristles. As Sujatha *et al.* noted, common toothpaste constituents, including fluoride compounds, surfactants such as sodium lauryl sulfate (SDS), preservatives, and abrasive particles including titanium dioxide, are

well-recognized PCR inhibitors that interfere with Taq polymerase activity [12]. Increasing the number of bristle bundles proportionally increases the co-extracted toothpaste load, partially suppressing PCR amplification and resulting in lower-than-anticipated DNA yields.

While this method reduces reagent consumption and permits repeat extraction from remaining bristle bundles, handling small quantities of loose bristles substantially elevates the risk of sample contamination and analyst exposure. This is a meaningful concern given that toothbrush-derived DNA is already low-template and therefore highly vulnerable to contaminating exogenous DNA.

## Partial Head Sectioning (Recommended Approach)

To reconcile the competing limitations of the preceding methods, Riemer *et al.* evaluated a pragmatic compromise: using aviation snips to remove approximately one-quarter to one-third of the toothbrush head for extraction [15]. This approach reduced the volume of lysis solution required relative to whole-head immersion, preserved the remainder of the toothbrush head for potential repeat extraction, and maintained the structural integrity of the bristle bundles, thereby mitigating the contamination risks associated with loose bristle handling. In a study involving 55 adult subjects divided across three groups by duration of use, Riemer *et al.* found no statistically significant differences in DNA quantity or quality across groups, and concluded that partial sectioning represented a practical, standardizable technique suitable for forensic laboratory adoption [15]. This method has since been cited as the recommended approach in multiple subsequent reviews.

## PCR Inhibition: A Broader Forensic Perspective

PCR inhibition in toothbrush-derived extracts must be understood within the broader forensic context of inhibitory interference. As reviewed by Vajpayee *et al.*, PCR inhibitors encountered in forensic samples include hematin from blood, collagen from bone and soft tissue, humic and fulvic acids from soil, bile salts from feces, and melanin from skin, each interfering with polymerase function through distinct mechanisms [16]. Toothpaste-derived inhibitors (SDS, fluoride, polishing agents) represent a substrate-specific inhibitor class that is unique to oral hygiene item evidence.

Established mitigation strategies for PCR inhibition include: dilution of the DNA extract (which reduces inhibitor concentration at the cost of template quantity); the addition of bovine serum albumin (BSA), which sequesters inhibitory compounds; use of inhibitor-resistant polymerases or enhanced PCR master mixes containing facilitators; centrifugal filter-based purification using products such as Microcon® or Centricon®; and magnetic bead-based purification, which has demonstrated superior profile completeness in toothbrush samples compared to silica-based methods [13,16]. Torres-Pérez *et al.* specifically demonstrated that magnetic particle extraction (PrepFiler®) recovered higher DNA quantities and generated more complete profiles from toothbrushes than silica suspension methods [13]. Internal amplification controls should be routinely incorporated into toothbrush-derived DNA analyses to detect inhibition and prevent false-negative interpretation. Sidstedt *et al.* additionally demonstrated that inhibitor tolerance can be enhanced through the use of polymerase synergy strategies, where combinations of polymerases outperform single-enzyme systems in the presence of inhibitory compounds [17].

## Factors Affecting DNA Recovery from Toothbrushes

### Duration of Use Prior to Collection

One of the most practically relevant questions in toothbrush-based forensic analysis is how long a toothbrush must have been in use before sufficient DNA can be collected. Bandhaya and Panvisavas addressed this directly by comparing DNA yields from toothbrushes used for 1, 7, 14, and 30 days [14]. They found no statistically significant differences in extractable DNA quantity across these intervals when an appropriate extraction protocol was employed, indicating that even a single day of use can yield sufficient cellular material for profiling. Riemer *et al.* corroborated this finding, observing comparable profile quality from toothbrushes used for one month, three months, and random periods of undisclosed duration [15].

Paradoxically, extended periods of use may result in reduced DNA yield rather than accumulation. This counterintuitive observation is attributed to the cumulative dislodgement of retained cellular material during repeated post-brushing rinses. Toothbrushes used over longer periods experience a greater total number of rinsing events, each of which may wash away deposited epithelial cells. The net effect may be lower cellular retention on older brushes despite their longer contact history with the oral mucosa [14,15].

### Rinsing Intensity and Water Pressure

The mechanical removal of cellular material by rinsing represents one of the most significant practical determinants of DNA yield. Alfadaly *et al.* examined this variable in a study of both toothbrushes and siwak (miswak) sticks used by participants in Saudi Arabia across durations ranging from the same day to four months [18]. Their findings demonstrated that vigorous rinsing with high-pressure water markedly reduced recoverable DNA from both item types, independent of use duration. This finding has direct implications for forensic recovery: toothbrushes that have been rinsed under high-pressure tap water immediately before or after collection may yield substantially less DNA than those that have been rinsed gently or left in an unwashed state.

These observations suggest that forensic guidance should specify collection of toothbrushes before the next use, avoiding additional rinsing post-collection, and documenting the brushing habits of the putative owner wherever possible. Standardized collection instructions analogous to those established for buccal swab reference samples are needed.

### Post-Collection Storage Duration and Conditions

Storage duration after collection significantly affects the reliability of toothbrush-derived DNA, particularly for gender determination. Alqarni *et al.* conducted a longitudinal study in which 40 volunteers used new toothbrushes for four days, with DNA analyses performed at collection and at one-, two-, and six-months post-collection [19]. The accuracy of sex determination using real-time PCR targeting the SRY gene declined to 80% at two months and to 35% at six months, differences that achieved statistical significance. This trajectory of degradation is considerably steeper than that typically observed for reference buccal swab samples stored under equivalent conditions, likely reflecting the lower initial template quantity and the greater vulnerability of toothbrush-retained cells to environmental degradation.

These findings have important operational implications: toothbrushes collected at a scene should be sealed and transported to the laboratory

as rapidly as practicable, and analyses should be performed without unnecessary delay. Where immediate analysis is not feasible, low-temperature storage in sealed packaging may slow DNA degradation, though the optimal storage conditions for toothbrush-derived cellular material have not been systematically studied.

### Temperature, Humidity, and Bathroom Environment

The bathroom represents an inherently challenging storage environment for biological specimens. Elevated ambient temperatures and chronic humidity promote microbial proliferation and accelerate the enzymatic degradation of DNA within retained cells. These environmental insults act on toothbrushes in situ during the period of active use, before forensic collection even occurs, meaning that the DNA recovered from a bathroom-stored toothbrush may already be substantially degraded prior to extraction.

An often-overlooked additional hazard is fecal plume aerosolization, generated during toilet flushing in bathrooms where toothbrushes are stored without protective covers. Sujatha *et al.* drew specific attention to this phenomenon, noting that aerosolized particles from flushing events may deposit exogenous microbial and viral DNA onto bristle surfaces, introduce substances that act as PCR inhibitors, and thereby both contaminate the sample and reduce the reliability of forensic results [12]. This is not merely a theoretical concern: studies of bathroom microbiology have documented that flushing a toilet without a closed lid can project fecal-origin particles up to 1.5 meters from the ground [20]. Toothbrushes stored within this radius without caps or covers may accumulate such material over time. Forensic collection protocols should therefore document whether the toothbrush was kept in a shared bathroom and whether it was stored in a closed container or exposed to the open bathroom environment.

## Modern Forensic DNA Techniques Applicable to Toothbrush-Derived DNA

### Standard STR Profiling

Short tandem repeat (STR) profiling using capillary electrophoresis (CE) remains the standard approach in forensic genetics worldwide. Commercial multiplexes such as GlobalFiler™, PowerPlex® Fusion, and Investigator® 24plex simultaneously amplify 20–24 CODIS-compatible loci, generating profiles suitable for database searching [21]. These kits have been validated for use with low-template and degraded samples and have been applied to toothbrush-derived DNA in multiple studies, demonstrating their suitability for this sample type.

### Massively Parallel Sequencing (Next-Generation Sequencing)

Massively parallel sequencing (MPS), also referred to as next-generation sequencing (NGS), has emerged as a transformative technology in forensic genetics, enabling the simultaneous analysis of thousands of genetic markers across diverse marker categories [22]. For low-template samples such as those derived from toothbrushes, MPS offers several potential advantages over CE-based STR profiling: superior sensitivity for detecting low-abundance alleles, the capacity to simultaneously interrogate STR loci, single nucleotide polymorphisms (SNPs), mitochondrial DNA, Y-chromosome markers, and phenotypically informative SNPs within a single analytical workflow, and the ability to resolve isoalleles at STR loci that are indistinguishable by fragment length analysis alone.

Carratto *et al.* reviewed the main forensic applications of MPS, highlighting its capability to analyze simultaneously thousands of genetic markers and its potential utility for samples at

low quantity or quality — precisely the conditions characteristic of toothbrush-derived DNA [22]. Browne and Freeman further noted that MPS technologies enable higher sensitivity detection of mitochondrial DNA heteroplasmy and more comprehensive mitochondrial genome sequencing, which could be valuable for toothbrush samples that yield insufficient nuclear DNA for full STR profiling [23]. As of 2024, the uptake of MPS in forensic laboratories remains limited by cost, infrastructure requirements, and the complexity of data analysis, with surveys indicating that over half of forensic scientists working with NGS platforms do not feel sufficiently confident to train other staff in its use [23]. Nevertheless, MPS represents a natural evolution for the analysis of challenging samples including those derived from toothbrushes.

### Rapid DNA Technology

Rapid DNA systems, integrated platforms capable of producing full STR profiles from buccal reference swabs in approximately 90 minutes without specialized laboratory infrastructure, have been validated for reference sample processing and are approved for booking station use in the United States [24]. Their potential application to toothbrush-derived DNA has not been formally evaluated, as current cartridge-based systems are optimized for high-quality buccal swab reference DNA rather than low-template or inhibitor-containing samples. However, the conceptual appeal of rapid in-field profiling from a decedent's personal items, including toothbrushes, in mass disaster scenarios is considerable. The INTERPOL Disaster Victim Identification Guide (2023) explicitly includes personal items from the victim's place of residence as antecedent DNA sources for reference comparison [25], and rapid toothbrush-based DNA analysis could theoretically contribute to the ante-mortem data collection phase of DVI operations if appropriate cartridge chemistry were developed and validated.

### Y-Chromosome STR and Mitochondrial DNA Analysis

In cases where nuclear DNA yield is insufficient for full autosomal STR profiling, supplementary marker systems may be employed. Y-chromosome STR (Y-STR) analysis provides information about the paternal lineage of male contributors, allowing familial identification via the father's line even when autosomal template is degraded or insufficient. Mitochondrial DNA (mtDNA) analysis targets a high-copy-number genome, each cell contains hundreds to thousands of mitochondria, and is therefore particularly valuable for highly degraded low-template samples. Both marker systems have been applied in forensic cases involving degraded biological material and may represent useful adjuncts when toothbrush-derived nuclear DNA is insufficient for full autosomal profiling [21,22].

## Contamination, Mixed DNA Profiles, and Secondary Transfer

### The Oral Microbiome and Non-Human DNA Contamination

The human oral cavity harbors one of the most diverse microbial ecosystems in the body, comprising over 700 bacterial species as well as fungi, viruses, and archaea [26]. This complex community, collectively forming the oral microbiome, is transferred directly onto toothbrush bristles during brushing. Consequently, DNA extracts from toothbrushes contain not only human genomic DNA but also substantial quantities of microbial DNA.

The variation in an individual's oral microbiome during disease states significantly influences the biological composition of a sample. Its impact depends on whether the analytical goal is microbial sequencing or human DNA profiling [27-29]. Standard forensic DNA fingerprinting relies on STRs, which are highly specific to human genomic DNA and do not amplify or cross-react with bacterial or fungal material [29]. However, microbial DNA can compete for PCR resources at low template concentrations, and high microbial loads may dilute the effective human DNA fraction, potentially contributing to stochastic amplification failures at individual loci.

Inter-individual variability in the oral microbiome is substantial, influenced by diet, age, antibiotic exposure, systemic disease, and oral hygiene. A person with a dysbiotic oral microbiome may deposit considerably more non-human DNA onto their toothbrush than an orally healthy individual under comparable conditions. This variability is not currently accounted for in forensic toothbrush protocols and warrants further investigation.

In patients with periodontal disease, severe local inflammation causes a massive influx of host immune cells (e.g., neutrophils) into the oral cavity, often increasing the yield of human genomic DNA and enhancing material available for STR typing. At the same time, the dysbiotic shift toward Gram-negative biofilm bacteria complicates microbial analysis unless mechanical bead-beating lysis is employed [28,29]. Gingival bleeding, a hallmark of periodontitis, blood-derived compounds such as hematin, a well-established PCR inhibitor [16], may simultaneously suppress amplification. The net effect of periodontal disease on toothbrush DNA yield and STR profile completeness has not been directly studied, representing an important gap in forensic literature.

Similarly, in oral candidiasis, extracting human DNA from a toothbrush remains feasible. *Candida albicans* readily forms a resilient biofilm on synthetic bristles that traps shed human epithelial cells alongside fungal cells [30,31]. Recovering fungal DNA requires specialized enzymatic digestion (e.g., lyticase) to breach its chitin cell wall, but co-isolated human "touch DNA" remains accessible to standard forensic protocols [29,31,32]. In immunocompromised individuals, including those with HIV/AIDS, hematological malignancies, or undergoing immunosuppressive therapy, oral candidiasis may be chronic and extensive, leading to elevated fungal burdens. Whether this impacts STR profile completeness remains an open empirical question. From a forensic standpoint, the medical history of the toothbrush owner, when ascertainable, should be considered when interpreting extraction efficiency.

Xerostomia, which reduces salivary flow, may decrease cellular transport onto bristles and reduce the adhesive medium that retains cells. Conversely, oral mucositis secondary to chemotherapy or radiotherapy causes extensive mucosal breakdown and shedding, potentially increasing epithelial cell deposition, though DNA integrity may be compromised by treatment-related mutagenicity. Age-related changes in mucosal cellularity and saliva composition may similarly affect deposition. Brushing habits, including duration, pressure, and technique, further contribute to variability in cellular transfer. None of these factors have been systematically investigated in the context of forensic toothbrush DNA analysis.

## Household and Environmental Contamination

When toothbrushes are evaluated as sources of evidential or touch DNA, microbial contamination does not interfere with human STR

typing because forensic multiplex PCR primers are species-specific [11,33]. Yet, a notable gap in the literature concerns exogenous human contamination, shared storage, and secondary transfer within household environments [33,34].

Although toothbrushes are generally considered personal items, they are often stored in shared domestic spaces such as bathrooms or communal containers. Sujatha *et al.* emphasized the importance of confirming exclusive ownership of a toothbrush prior to forensic collection, noting the risk of contamination from household members [12]. This risk is not merely hypothetical. In shared bathrooms, toothbrushes frequently produce mixed DNA profiles due to storage in common cups, exposure to aerosolized droplets from toilet flushing, sink splashes, or direct bristle-to-bristle contact [34,35]. Even post-collection packaging can facilitate passive secondary transfer between bristles and container walls [35].

Interestingly, Torres-Pérez *et al.* reported that toothbrushes in their study cohort yielded complete profiles without mixtures, contrasting with underwear samples from the same participants [13]. This may reflect the relatively protected anatomical origin of oral cells compared with dermal cells. However, such findings should not be interpreted as evidence that mixed profiles are impossible. Rather, they highlight the need to explicitly consider and investigate household contamination risks in forensic casework involving toothbrush evidence.

Forensic analysts typically distinguish the true owner's profile from exogenous contributors by examining both quantitative and qualitative aspects of DNA mixtures [35]. The primary contributor, the toothbrush owner, usually generates a "major profile" characterized by high peak heights and area ratios, reflecting the abundant deposition of buccal epithelial cells during brushing. In contrast, secondary or environmental transfer produces a low-template "minor profile" prone to stochastic effects such as allele dropout [11,35]. To isolate the true owner's DNA, laboratories employ elimination databases containing the profiles of cohabitants, relatives, and investigators, enabling systematic subtraction of background contributors [34,35].

## Interpretation Challenges in Low-Template Toothbrush DNA

Toothbrush-derived DNA often presents unique interpretive challenges due to its inherently low-template nature and susceptibility to contamination. When a toothbrush yields a mixed DNA profile, containing alleles from more than one contributor, the forensic utility of the sample is diminished unless the mixture can be confidently deconvoluted. Probabilistic genotyping software such as STRmix™ and TrueAllele® has been developed to model the contributions of multiple individuals in complex mixtures. However, case studies demonstrate that these programs can produce divergent likelihood ratio estimates from the same evidential sample, underscoring the interpretive complexity of low-template mixtures and the importance of understanding the assumptions embedded in each system [36].

Low-template samples are also vulnerable to stochastic PCR effects. Allelic dropout may cause heterozygous loci to appear homozygous, misrepresenting a contributor's genotype. Conversely, allelic drop-in, spurious alleles amplified at low concentrations, can create the false impression of a mixed profile. Stutter artefacts, normally managed by analytical thresholds, become proportionally larger relative to true peaks in low-template contexts, further complicating interpretation. Analysts must therefore apply validated low-template thresholds and avoid interpreting profiles below established quantification limits without specialist oversight.

Contamination control adds another layer of complexity. Household environments can introduce exogenous contributors, and laboratory handling may inadvertently add staff DNA. To mitigate this, forensic laboratories maintain elimination databases containing the DNA profiles of personnel who may have come into contact with evidence. The United Kingdom's National DNA Database (NDNAD), for example, operates a centralized Contamination Elimination Database (CED) that includes profiles of police officers, staff, and others in the DNA processing chain [37]. Such systems are essential for laboratories processing toothbrush evidence, where contamination is particularly consequential.

Taken together, mixed profiles, stochastic effects, and contamination risks highlight the interpretive fragility of toothbrush-derived DNA. Robust analytical thresholds, probabilistic tools, and elimination databases are indispensable safeguards, but further empirical research is needed to establish best practices for ensuring reliability in this challenging evidential context.

## Comparison with Other Personal Items as DNA Sources

Toothbrushes are one of several personal items that have been investigated as alternative sources of reference DNA in the absence of direct biological samples. A comparative assessment helps to contextualize the relative utility of the toothbrush within the broader landscape of personal item DNA evidence.

Razors and electric shaver heads retain skin epithelial cells, hair follicle cells, and occasionally blood from shaving cuts. Their potential as DNA sources has been documented, and their regular individual use makes attribution to a single person reasonably certain. Cigarette butts accumulate saliva and buccal cells from repeated lip contact and have been used forensically in real casework, including a 2023 cold case murder investigation in which discarded chewing gum from a suspect provided the confirmatory DNA match [38]. Cigarette butts offer meaningful quantities of salivary DNA but are susceptible to rapid degradation from environmental exposure, particularly moisture and UV radiation.

Chewing gum retains saliva and buccal cells embedded within the gum matrix, which can physically preserve cellular material from the immediate degradative effects of heat and humidity. Its principal limitation as a forensic DNA source is the absence of certainty that the gum was used exclusively by the individual of interest, and the difficulty of collection without contamination.

Worn clothing and underwear accumulate skin epithelial cells from direct body contact and have been compared directly with toothbrushes by Torres-Pérez *et al.* [13]. In their study, underwear yielded higher absolute DNA quantities than toothbrushes, but toothbrushes generated more complete and more easily interpretable profiles, specifically because underwear samples frequently produced mixed profiles from multiple contributors, reflecting the fact that clothing may contact not only the primary user but also laundry co-inhabitants, textile manufacturer residues, or environmental depositors. Toothbrushes, by contrast, consistently generated single-contributor profiles in this study.

Across all personal item categories, the toothbrush offers a favorable combination of attributes: it is universally present in domestic settings, is reliably attributable to a single user, is available in a preserved indoor environment (reducing post-deposition degradation), and consistently produces single-contributor

profiles amenable to direct comparison. These advantages support its continued development as a forensic DNA source, notwithstanding the methodological standardization challenges that remain.

## Integration into Forensic Casework and Disaster Victim Identification Protocols

The INTERPOL Disaster Victim Identification Guide (2023 edition) recognizes personal items from a victim's place of residence as legitimate antecedent (ante-mortem) DNA reference sources, alongside family reference samples [25]. This recognition places toothbrushes, among other personal items, within the formal DVI framework as a category of reference material to be collected during the ante-mortem data gathering phase. In mass casualty scenarios where relatives are unavailable, geographically dispersed, or where kinship analysis would provide insufficiently precise identification, toothbrush-derived DNA offers a direct reference that may match a victim's post-mortem profile without the statistical attenuation inherent in kinship analysis.

Despite this formal recognition, documented case reports in which toothbrush DNA played a definitive role in forensic identification remain rare in the published literature. Jobim *et al.* published one of the earliest accounts of successful toothbrush-based human identification and argued for its utility precisely in cases where family reference DNA was inaccessible [10]. Riemer *et al.* similarly positioned their standardized collection methodology within a decedent identification context [15]. However, the paucity of published casework examples may reflect operational reporting practices, forensic case reports are frequently confidential or unpublished, rather than an absence of real-world application.

Standardized collection protocols for forensic toothbrush sampling are essential for its reliable integration into DVI operations. Recommended collection procedures should include: wearing double nitrile gloves and a face mask during collection; using sterile tools to handle the toothbrush without direct contact with the bristle area; sealing the toothbrush immediately in a paper envelope (to allow moisture to escape and reduce microbial overgrowth) placed inside a labeled evidence bag; documenting the storage location of the toothbrush prior to collection (including whether it was stored in an open or closed container in the bathroom); and transporting the sealed sample to the laboratory as rapidly as possible, ideally under refrigerated conditions. These steps, while not yet codified in any validated international standard specific to toothbrushes, are consistent with general forensic biological evidence collection principles.

## Limitations of Current Evidence

The most fundamental limitation of the toothbrush forensic literature is its small size. The systematic review by Sujatha *et al.* identified only five primary studies meeting strict inclusion criteria, all characterized by small sample sizes, heterogeneous extraction methods, and varying outcome measures [12]. The bibliometric analysis by the same group, examining publications from 2000 to 2021, revealed an inconsistent publication trend with a decline in output in recent years [39], suggesting that the field has not attracted the sustained research investment required to build a robust evidence base.

The lack of inter-laboratory validation is a critical gap. No multi-site validation study has been published comparing toothbrush DNA extraction outcomes across different forensic laboratories using standardized protocols. Without such data, it is impossible to establish the

reproducibility and transferability of existing methods, a prerequisite for their adoption as validated forensic procedures under international quality assurance frameworks such as ISO 17025.

Existing studies have enrolled predominantly healthy adult volunteers, often with no documentation of oral health status, brushing habits, rinsing behavior, or storage conditions. The population-level generalizability of findings from such homogeneous, controlled cohorts to real-world forensic scenarios, where the putative owner of the toothbrush may be elderly, immunocompromised, periodontally diseased, or subject to conditions that profoundly alter oral biology, is unknown.

The absence of comparative effectiveness research using standardized commercial STR kits across the full spectrum of extraction methods also limits the ability to make evidence-based recommendations. Most studies have used a single extraction method without direct comparison to alternative approaches under identical conditions. Similarly, no published study has evaluated the application of MPS or probabilistic genotyping software specifically to toothbrush-derived DNA, leaving untested the potential of these modern tools to improve profile completeness and interpretive confidence for this challenging sample type.

## Conclusion

The collective evidence indicates that toothbrushes represent a practical and forensically valuable source of reference DNA, particularly in circumstances where direct biological samples are unavailable and kinship analysis is insufficiently precise. Multiple studies have demonstrated the feasibility of generating complete STR profiles from toothbrushes across diverse usage durations, with toothbrush-derived DNA consistently outperforming other personal items in producing single-contributor profiles.

Despite its promise, the reliability of toothbrush-derived DNA remains subject to numerous modifying variables including rinsing practices, post-collection storage, bathroom environmental conditions, oral health status, and individual oral microbiome composition, none of which have been comprehensively characterized. The current primary evidence base is critically limited in both scale and methodological diversity. Accordingly, toothbrush DNA analysis cannot yet be considered a validated or standardized forensic technique. Instead, it should be regarded as a promising adjunct requiring substantial refinement before routine adoption in accredited forensic laboratories. Therefore, the present review highlights several key knowledge gaps that demand systematic investigation. Future research should prioritize:

1. **Oral health effects** — systematic evaluation of periodontal disease, oral candidiasis, and xerostomia on DNA yield and STR profile completeness.
2. **Inter-laboratory validation** — comparative studies across multiple sites using standardized extraction and profiling protocols.
3. **Advanced analytical tools** — assessment of massively parallel sequencing (MPS) and probabilistic genotyping software specifically applied to toothbrush-derived low-template DNA.
4. **Environmental influences** — characterization of bathroom conditions and storage practices on DNA degradation kinetics.
5. **Material science factors** — comparison of bristle composition and its impact on cellular adhesion and DNA retention.
6. **Applied casework** — prospective forensic case series documenting real-world use of toothbrush DNA in criminal investigations and disaster victim identification (DVI).

Achieving these goals would transition toothbrush DNA analysis from proof-of-concept toward evidence-based, standardized protocols suitable for integration into international forensic quality frameworks.

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