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Evaluation of swab materials in forensic DNA testing: a systematic review

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Abstract

Background Forensic biology technology advances quickly: DNA typing technologies are increasing in sensitivity, resolving DNA mixtures is improving, and isolating and targeting male-specific DNA continues to become more streamlined. However, one part of the forensic biology workflow that has remained relatively unchanged is the type of swab used to collect samples. Swab composition technology has advanced, yet cotton swabs continue to be the primary choice for biological evidence collection. This report describes the results of a systematic literature review and analysis to determine which swab types work best for collecting biological evidence.

Results An article was included in the review if it is an original research article, discusses more than one swab brand or material, has a forensic focus, and reports data from real-time PCR (qPCR) or forensic DNA typing. Articles were excluded if they were not primary research (literature/systematic review) or not written in the English language or if the product was a thesis or dissertation. The literature was collected through Web of Science, PubMed, and EBSCO searches in September 2023. Removal of duplicates and selection of articles were performed in Rayyan. Additional articles were identified in the bibliographies of initially selected articles. The analysis was organized by substrate (porous, $n=9$; nonporous, $n=8$) and source of DNA ($n=5$). Forty-one substrate-DNA source combinations have been researched, and 13 substrate-DNA source combinations have an identified best-performing swab type. There are several limitations in the study, including heterogeneity of data and selection bias during the literature search and study identification.

Conclusions Primary conclusions are (1) DNA extraction chemistry needs to be considered with swab type, (2) swabs made of the same material do not perform the same when compared to each other, and (3) inter-operator swabbing is not different. This work highlights research gaps that should be addressed for substrate-DNA source combinations and can guide practitioners in making evidence-based decisions on implementing different swab types into their workflow.

Keywords Forensic, Swab, Systematic review, Meta-analysis, Biological evidence

Background

The cotton swab is arguably the most ubiquitous forensic evidence collection tool. However, the introduction of different swab materials and configurations has changed the landscape of possible forensic evidence collection tools. Since 2009, forensic biologists have had non-cotton swab choices to collect biological evidence (Benschop et al. 2010). Today, swabs may be made of nylon, rayon, polyester, foam (polyurethane), or more than one material. The fibers of the swab may be configured in a flocked arrangement, dissolve in the presence of solvents, or have been miniaturized — all with the goal of imparting specific properties to the swab to improve performance. These manufacturing advances have led to a multitude of research products studying intra- and intercomparisons

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of swabs, leaving the forensic biologist to select the "best" swab for their evidence.

Prompted outside the forensic science community, swab evolution addresses the need for different transport systems for clinically significant microorganisms. Researchers needed materials other than cotton and swabs with a variety of physical properties to meet the challenges presented by a range of microorganisms and substrates. Later attributes allowed for the preservation of the collected material in different storage conditions and media. The adoption of swabs as a tool for applications outside of microbiology has significantly affected swab size, shape, and packaging. Timothy Templet, executive vice president of sales at Puritan Medical Products, notes the following: "Every swab has a purpose, has a performance characteristic that is unique to the use" (It's the golden age of the swab a 99-year-old invention that has never been more crucial 2023). A forensic biologist's ideal swab will collect all the biological material from an item of evidence and then release all that material into the next phase of the workflow (i.e., extraction, direct amplification).

With such a variety of swabs available to forensic practitioners and a growing body of research comparing swab types, evaluating and implementing a new swab type can be burdensome. The purpose of this systematic review and analysis is to determine which swab material provides the best forensic DNA results. Research studies provide evidence-based findings for the best swab types based on the DNA's source and the substrate the biological material is on. This current research helps guide forensic practitioners and decrease their burden when selecting a swab for evidence collection. The review also identifies shortcomings, gaps, and recommendations for future research.

Methods

Article selection

We used the following inclusion criteria to select articles or journals for the review: (1) it is an original research article, (2) it discusses more than one swab brand or material, (3) it has a forensic focus, and (4) it reports data from real-time PCR (qPCR) and/or forensic DNA typing. The exclusion criteria for articles were as follows: (1) it is not primary research (e.g. literature/systematic review), (2) it was not in the English language, and (3) it is a thesis or dissertation.

Search strategy

Three reviewers used the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) protocol for review. Journals were taken from PubMed, Web of Science, and EBSCO on September 27, 2023. The keywords used were "swab," "comparison," and "forensic." Files from the three databases were uploaded into Rayyan. Duplicates were identified and removed using Rayyan's "remove duplicates" function as well as manually by two reviewers. Two reviewers also manually reviewed each article by title and abstract for inclusion or exclusion in the systematic review. Bibliographies of articles that met the inclusion criteria were searched for additional articles that were not captured in the database searches. A Zotero file of the included articles was uploaded into ResearchRabbit, and "earlier work" and "later works" were searched to identify articles that were not captured in the database and bibliography searches.

For all studies that met the inclusion criteria, the following data were extracted: author(s), year, swab types studied, swabbing method, substrate(s) type, sample size, quantification kit, quantification results, STR amplification kit, and STR results. Two reviewers per article helped to minimize the risk of selection bias.

Analysis

First, to analyze the data within the journal articles, substrate-DNA source combinations were identified for each journal or experiment within each article; the data was extracted and entered in Microsoft® Excel®. The data included whether statistics were performed on qPCR or STR data and the outcome as well as whether DNA testing was performed and its outcome. The number of replicates was noted. The criteria for the analysis of the best-performing swab type for each substrate-DNA source combination required the inclusion of at least three replicates, statistics were performed and showed significance ($p < 0.05$) for a swab type, and statistics were calculated on one substrate-DNA source combination or only the swab type as a variable. Because the data was heterogeneous between studies, parametric and non-parametric statistics were included and used a p-value of 0.05. If replicates or statistical methods were not present, the study did not meet the criteria for analysis. Due to minimal experimental detail, no analysis conclusions were made on new technologies papers.

Results

Systematic review and analysis

The three search terms (swab," "comparison," and "forensic) identified 263 articles (Fig 1). Rayyan removed 116 duplicates, leaving 147 articles to be screened for the inclusion criteria. Following the abstract review, 18 articles were retrieved with 89% concordance between reviewers on article selection. Six articles were removed because the entire paper's content did not meet the inclusion criteria. Further articles were identified through bibliographic searches of the remaining 12 articles ($n = 41$)

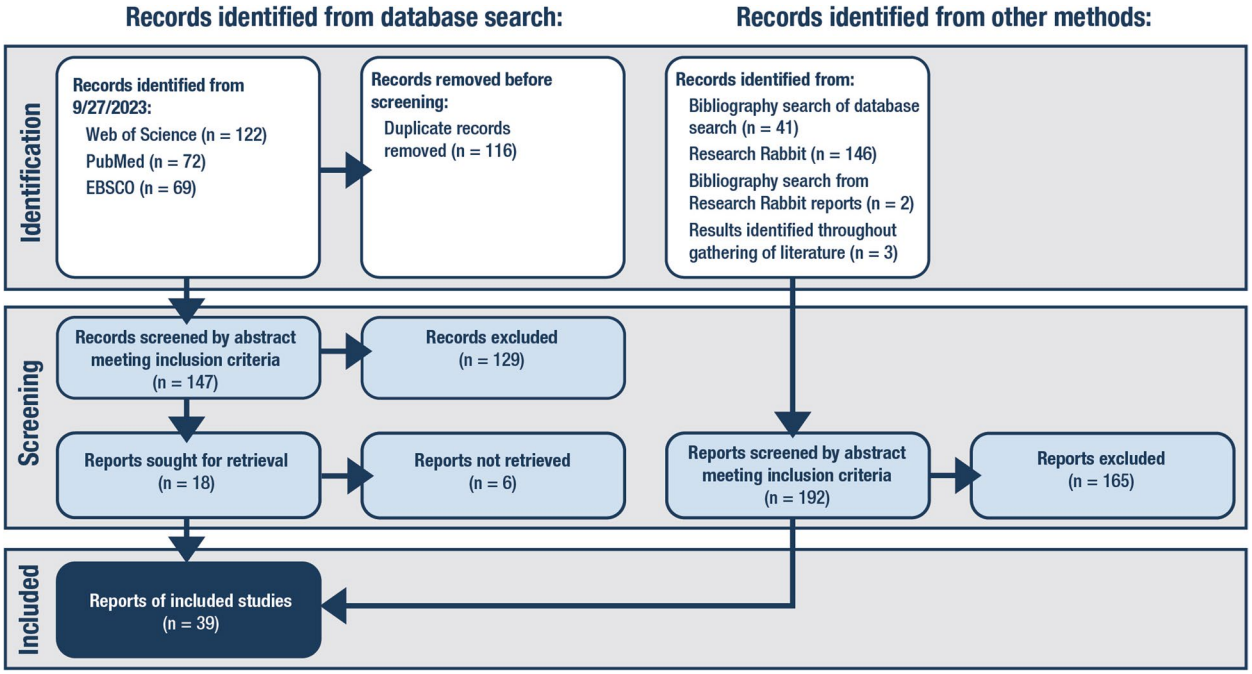

Fig. 1 Identification of studies through systematic review. Flow chart describing the articles identified for inclusion and exclusion during the systematic review

of which 27 were excluded (S1 Table). ResearchRabbit yielded eight additional articles, and their bibliographies yielded two additional articles. Three additional articles were identified from recommended similar articles on websites. The number of excluded recommendations was not captured. In sum, a total of 39 articles were included in our analysis (S1 Table).

Five DNA sources were identified in this literature: blood, saliva, semen, touched surfaces, and acellular DNA. "Touched surfaces" were denoted as such since the studies collected biological material from substrates following donors touching or handling a surface or item in a research setting. Previously purified DNA samples were characterized as "acellular DNA". The studies' substrates can be characterized into 17 different groups, displayed in Fig. 2. The selected articles studied 41 substrate-DNA source combinations. Based on the analysis criteria, an optimal swab type was identified for 13 substrate-DNA source combinations (Fig. 2). No significant difference was calculated in 11 substrate-DNA source combinations, indicating that these swab types had equivalent performances. Four substrate-DNA source combinations had outcomes that depended on the volume or amount of DNA. The journal articles associated with new technologies (rapid DNA, direct DNA, and next-generation sequencing (NGS)) were not classified according to substrate-DNA source due to minimal reporting and data heterogeneity. Common analyses in systematic reviews and meta-analysis, such as identifying bias or types I and II errors, were not conducted due to the investigatory nature and heterogeneous data within the literature. The heterogeneous, and often incomplete, data did not allow for direct or indirect comparisons of swab types. A more detailed analysis of this literature, characterized by substrate-DNA source, is provided below.

Blood

Porous substrates

The recovery of blood from a porous substrate such as drywall, paper, or clothing is anticipated within a forensic context. With respect to the DNA sources evaluated in this review, visible blood should be a highly successful DNA source that results in a successful DNA profile. Multiple studies ($n=4$) evaluated the recovery of DNA from blood placed directly onto a swab; each study used different swab materials. Although having blood deposited directly onto swabs is not as likely as swabbing blood off a surface, it helps ensure consistent volumes of blood on a swab are used in research. In three studies (using cotton, nylon, and rayon swabs), cotton swabs were the most consistent in performance (Verdon et al. 2014; Seiberle et al. 2022; Comment et al. 2023). Comment et al. studied rayon, nylon, and three cotton swabs and found that the cotton Bode SecurSwab2 recovered significantly more DNA than the others at all dilutions (Comment et al. 2023). A cotton swab was the best

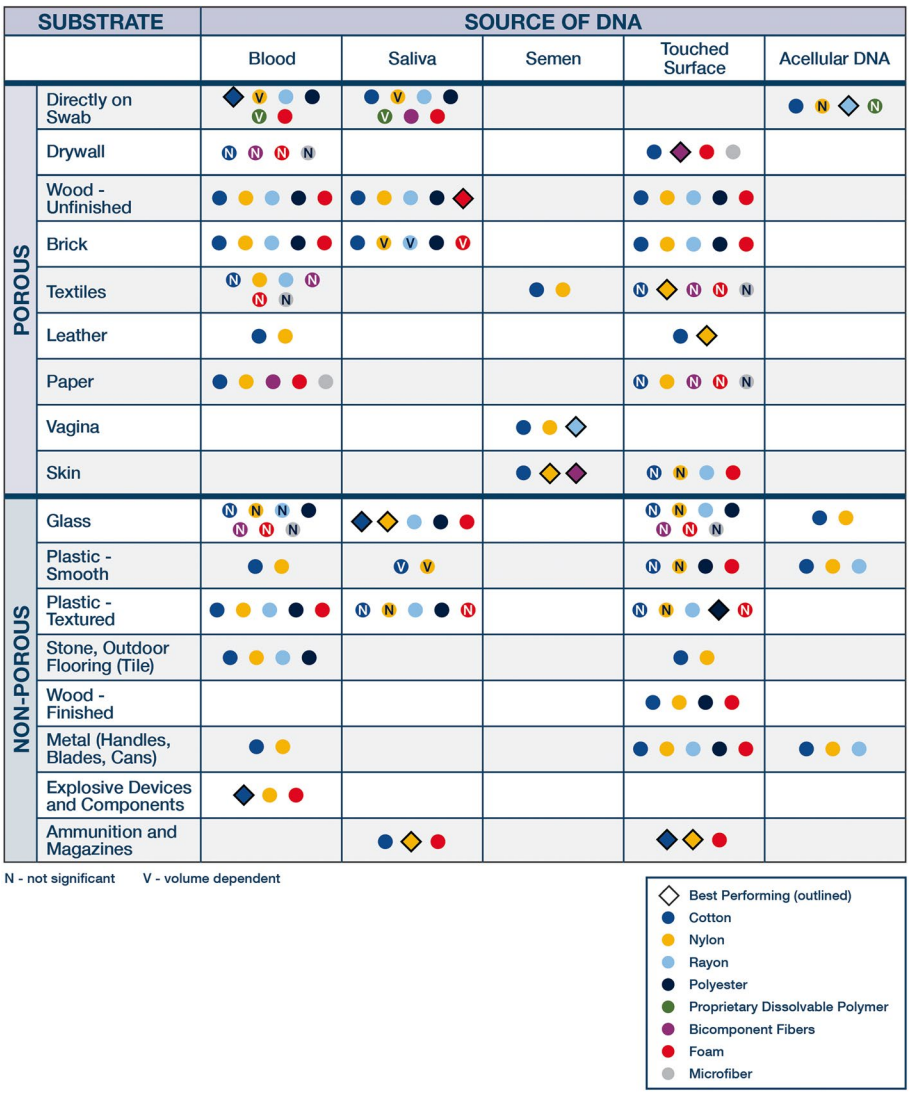

Fig. 2 Classification of substrate source of DNA combinations. Circle and diamonds represent a substrate source of DNA combination where at least one article's experiment used a swab composed of the material (noted by the color). "V" denotes that the results of the experiment are volume dependent; "N" denotes results that were not statistically significant

ranked for neat blood directly added to a swab in the Verdon study (Verdon et al. 2014). Although a rayon swab (MWE Rayon) performed best with a diluted blood sample added directly to it in the Verdon et al. study (2014), rayon performed poorly in the Sieberle et al. study (2022) with neat blood (Sarstedt Forensic Swab). This provides our first evidence that swabs made of the same material but by different manufacturers may not perform similarly, and that the amount of a source's DNA may impact swab performance (Verdon et al. 2014; Seiberle et al. 2022). A fourth study compared a proprietary dissolvable polymer swab (Diomics X-Swab™) with a nylon flocked swab and found that when neat blood was added directly to the swab, no significant difference in yield was obtained (Marshall et al. 2014). When diluted blood was added, the dissolvable swab yielded significantly more DNA than the nylon swab at 1:10 and 1:50 dilutions (Marshall et al. 2014). Of note, these four studies based their conclusions on quantitative data from qPCR, and samples were not taken through to DNA testing. Together with the analysis criteria, these results indicate cotton is the best swab type for this use. Nylon and dissolvable polymer swab performance depends on the amount of blood placed on the swab, the swab brand, or both.

Additional research studies deposited blood on various porous surfaces that may be encountered at crime scenes.

A study by Plaza et al. (2016) evaluated and compared different swab types and adhesive lifters in their ability to recover DNA from blood on porous and nonporous substrates (Plaza et al. 2016). The adhesive lifters are outside of the scope of this review. The Plaza study included a swab composed of bicomponent fibers, the HydraFlock® flocked swab (Puritan®) (Plaza et al. 2016). A bicomponent fiber is made of two materials, for example, polyester and nylon or polyester and polyester, which are conjugated creating the fiber yarn (Young et al. 2012). The HydraFlock swab is described as having "multi-length split-end fibers" and a "proprietary floret structure." The arrangement of fibers increases the swab's surface area and rapidly elutes biological specimens (Puritan flocked swabs: HydraFlock® vs. PurFlock Ultra® - what's the difference [cited 4 xxxx). This swab type is studied multiple times in the literature and reviewed herein.

Blood was applied to painted drywall and 100% cotton material in the Plaza study. Samples were swabbed with cotton, bicomponent fibers (HydraFlock), microfiber (MiraSWAB®, Foamtec International), and foam swabs (Critical Swab®, VWR) (Plaza et al. 2016). Results were reported as the percent of DNA profile generated and the forensic DNA profile index (FI) where an index score of 10 indicates the highest quality profile (Hedman et al. 2010). The bicomponent fiber and foam swabs recovered the highest percent profile from blood samples on painted drywall and 100% cotton, respectively (Plaza et al. 2016). However, using the FI value, no statistically significant differences were present among these four swab types and two nonporous substrates (Plaza et al. 2016).

Throughout the literature, blood was added to other porous substrates that may be encountered as evidence. Blood deposited on paper in the Plaza study generated a limited partial profile (9% profile) with a foam swab, while the cotton, bicomponent fibers, and microfiber swabs did not produce a profile (Plaza et al. 2016). Without profiles, an FI value could not be calculated. Quantification was done in this study; however, no results were shared. Therefore, a correlation between the quantity of DNA obtained and profile completeness could not be determined. Although brief, a study was conducted with blood deposited on paper and collected with three different cotton swabs following dactyloscopic powder dusting. However, the results do not detail the comparisons between these swab types, and this research group has not published further work (Tozzo et al. 2015). A second brief report studied two different extractions in conjunction with blood (in the form of lymphoctyes) on leather and wood, collected with nylon and cotton swabs(Dadhania et al. 2013). Nylon swabs with Prepfiler® and DNA IQ™ increased DNA recovery compared to cotton swabs (Dadhania et al. 2013). Blood was deposited on carpet in the Seiberle et al. study and collected with cotton, nylon, and rayon swabs (Seiberle et al. 2022). These results were combined with other sample types to determine if differences existed among operators and police units. The results show that cotton and rayon swabs yielded higher concentrations of DNA than the nylon swabs (Seiberle et al. 2022). Because there are limited replicates and a lack of statistics, we can draw no conclusions about paper-blood, leather-blood, and textiles-blood substrate-DNA source combinations.

The Verdon et al. (2014) study evaluated ten different swabs — three cotton, two foam, two nylon, one polyester, one rayon, and one layered cotton — and ranked them by their efficient recovery of DNA from blood and saliva (Verdon et al. 2014). A layered cotton swab was removed early from the research study due to its poor performance, especially on wood (Verdon et al. 2014). Ranks ranged from a score of 1.00, indicating a more effective swab, to a high of 9.0, indicating a less effective swab. The scores were based on quantification data of blood and saliva together. The swabs described above were tested with recovering blood from two porous substrates, brick and unfinished wood. Among the cotton swabs tested, the Puritan FABSwab consistently outperformed the other two (Copan 150C and Bode SecurSwab). It roughly doubled the amount of DNA recovered from the wood substrate (Verdon et al. 2014). The Puritan 1PF foam swab consistently recovered more DNA from wood throughout the study, earning a perfect rank score of a 1.00 (Verdon et al. 2014). The second foam swab studied, the Puritan Minipopule, produced curious results, ranking the lowest for effectiveness of DNA recovery from wood with a score of 9.00 (Verdon et al. 2014). These two swabs differ in multiple ways. Most noteworthy is the isopropyl alcohol that floods the tip of the minipopule swab when a capsule is popped; it was not dried after collection and prior to being placed into the extraction tube (Verdon et al. 2014). Overall, the foam swab scored the lowest (therefore better) for recovery of biological material (blood and saliva) from unfinished wood. The MWE Rayon swab was the lowest scoring swab (therefore better) for the collection from brick; however, the use of quantitative saliva and blood data to conduct the ranking limits our analysis and conclusions on optimal substrate-DNA source combinations (Verdon et al. 2014).

Nonporous substrates

Glass was the most researched smooth, nonporous substrate used to study blood collection with various swab types. Microscope slides were commonly used for the glass substrate as they are cheap and easy to obtain in research settings. Blood and saliva sample data were

ranked based on their extraction efficiency from quantification and other variables. A cotton swab outperformed polyester, nylon, foam, and rayon swab types, with rayon swabs as the runner-up (Verdon et al. 2014). STR profile completeness from blood samples on glass and collected with cotton, microfiber, foam, and bicomponent fiber swabs showed no significant differences (Plaza et al. 2016). Yet, the bicomponent fiber swab only produced 65% profile on average (percent profile = number of donor alleles detected/total number of donor alleles), whereas the other three swab materials recovered greater than 95% profile (Plaza et al. 2016). One study looked at ranges of blood deposited on glass microscope slides (Frippiat and Noel 2016). No significant differences were observed in DNA recovery between a rayon swab and three nylon swabs. The comparison between nylon and cotton swabs from glass slides in Dadhania et al. showed an increase in DNA recovery with nylon swabs; however, no statistics were calculated (Dadhania et al. 2013). An ideal swab for the glass-blood substrate-DNA source combination cannot be determined as no significance was calculated among the swabs studied.

A brief report of the Tozzo et al. (2015) study evaluated blood deposited on plexiglass, ceramic, glass, and metal; it was collected with cotton and nylon swabs following fingerprint powder dusting (Tozzo et al. 2015). DNA profiles were generated for 92% of the samples, yet the results were not relayed in substrate-DNA source combinations (Tozzo et al. 2015). A similar short report by Dadhania et al. (2013) used lymphocytes from fresh blood on plastic, a metal knife handle, and plastic gun grips coupled with varied DNA extraction methods (Dadhania et al. 2013). Average total DNA recovered was reported for each substrate-swab-extract chemistry combination studied. However, sample size information is not provided, nor were statistics calculated (Dadhania et al. 2013). The Verdon et al. (2014) study deposited blood and saliva on pitted plastic (Verdon et al. 2014). For this nonporous, textured surface, the polyester swab ranked the best, followed by a cotton swab and then a foam swab (keeping in mind the ranking used blood and saliva data) (Verdon et al. 2014). In the plastic (smooth and textured)-blood and metal-blood combinations, studies do not meet the analysis criteria. Thus, no conclusions are rendered.

One study used an interesting experimental design that included depositing blood on outdoor tile flooring, swabbing the blood, and observing the color differences and changes over time — with the assumption that bacteria are present and impacting DNA integrity (Ip et al. 2021). The rayon swab and one cotton swab were red over the 14-day study, indicating they were better preserved than the bicomponent polyester and another cotton swab (Ip et al. 2021). This observation was confirmed with DNA testing. The rayon, cotton, and nylon swabs generated complete profiles, while the MW104F cotton and Puritan bicomponent polyester swabs produced no profile after 2 days (Ip et al. 2021). The absence of statistics and qualitative observations meant the study's results did not meet the analysis criteria for a best-performing swab type.

The study by Phetpeng et al. (2015) is the only paper captured in this review that used buffy coat as a source of DNA. Because buffy coat is part of blood, it is classified as blood for DNA source (Phetpeng et al. 2015). This study deposited the buffy coat onto the kind of nonporous surfaces used to make improvised explosive devices (IEDs), including electrical tape, batteries, PVC piping, and copper wire (Phetpeng et al. 2015). Notably, the authors conducted parametric statistical methods, determining that swab type, swab moistening agent, and the substrate all affect the amount of DNA recovered. The swab type is responsible for 47% of the variance observed (Phetpeng et al. 2015). Four cotton swabs, one nylon swab, and one foam swab were evaluated; regardless of moistening agent or substrate, the HI-VAN Lab swab (cotton) and the Puritan nylon flocked swab performed the poorest (Phetpeng et al. 2015). The Puritan DNA-Free Cotton Tipped Applicator performed consistently well on all four substrates. But varied results were observed among the four cotton swabs, indicating again that not all swabs of the same material perform similarly (Phetpeng et al. 2015). Using data, the authors gathered for best swab type and swab moistening agent, and 56 cases were worked with 195 samples. This allowed the authors to compare their previous protocol to their data-informed protocol by replacing the HI-VAN Lab swabs with the EO cotton swabs (Phetpeng et al. 2015). Although the Puritan cotton swab performed better throughout their research, EO swabs were selected due to pricing. When the researchers calculated the number of full, high partial, low partial, and no profile generating samples, they found the new method (using the EO cotton swab) increased profile completeness and decreased the number of no profiles (Phetpeng et al. 2015). In conclusion, a cotton swab met our analysis criteria for best-performing swab for the explosives-blood substrate-DNA source combination.

Saliva

Porous substrates

Applying saliva directly to a swab when conducting research can mimic reference-type buccal swabs or the collection of saliva from an item of evidence while maintaining a consistent amount of biological material. Both studies that have put saliva directly on the swab are anomalous to the majority of studies evaluating swab differences. The Garvin study (2013) compares two swabs:

one with a provided desiccant and the other ventilated for passive drying. The Marshall study (2014) compares a nylon swab to a proprietary dissolvable polymer swab that no longer is available (Marshall et al. 2014; Garvin et al. 2013). Nonetheless, the dissolvable polymer swab (Diomics X-Swab) performed similarly to the nylon swab when very low amounts of saliva were pipetted onto them. The polymer swab performed significantly better than nylon when more saliva was present (Marshall et al. 2014). When comparing cotton (ForensiX evidence collection tube with SafeDry) and rayon (Sarstedt Forensic Swab) swabs with and without assisted drying (desiccant and ventilation blockage), data show that the drying assistance has a positive impact on DNA recovery (Garvin et al. 2013). For example, when the cotton swab was stored with its desiccant, it recovered 95% DNA relative to controls. Without the desiccant, only 4.8% DNA recovery was observed (Garvin et al. 2013). The study recorded no difference in DNA recovery between the rayon and cotton swabs. However, it is unclear if this is a statistically supported result (Garvin et al. 2013). Our analysis criteria did not identify a top-performing swab material.

In addition to the Verdon et al. study described in the blood/porous substrates section above, a second study deposited saliva on wood and collected it with various swabs. The unfinished wood-saliva combination was studied by Hedman et al. (2010) in a stepwise testing approach that assessed 13 swabs; the best performing, based on DNA quantification, was selected for further comparison (Hedman et al. 2021). They studied three cotton swabs, four nylon-flocked swabs, and six foam swabs (divided into small and large foam swabs). It is the only study captured in this review to further characterize foam swabs and evaluate multiple types (Hedman et al. 2021). In the study's first step, four swabs retrieved significantly less DNA than others: Bode Crime Scene Collectors (cotton), 4N6FLOQSwabs regular tip size Crime Scene (nylon flocked), Sigma Virocult (small foam), and Puritan Foam Tipped Applicator (small foam) (Hedman et al. 2021). The Macrofoam Critical Swab (large foam) recovered significantly more DNA from wood than the four DNA swabs mentioned above (Hedman et al. 2021). In the second step, the Selefa swab (cotton), 4N6FLOQSwabs regular-sized tip Crime Scene swab (nylon flocked), Critical Swab medium swab (foam), and the Macrofoam Critical Swab were further evaluated on a larger surface area of wood (Hedman et al. 2021). Again, the large foam swab performed better than the nylon and cotton swabs; however, the difference was not significant (Hedman et al. 2021). Based on the study's sample size and statistics, foam swabs meet our criteria for best-performing swab for the unfinished wood-saliva combination. Together, the Verdon et al. and Hedman et al. studies support the use of foam swabs to collect saliva from unfinished wood surfaces. The Verdon study first hypothesized in 2014 that foam swabs are more efficient for collecting biological fluids, such as saliva, because the flexible foam matrix can penetrate the pores of the wood. The Hedman study data in 2021 further supports this hypothesis (Verdon et al. 2014; Hedman et al. 2021).

The Cahill and Chapman study (2020) evaluated nylon, foam, and rayon swabs for their ability to recover saliva from bricks (Cahill and Chapman 2020). The results depended on the number of cells deposited. No significant differences were calculated between swabs when a low number of cells (1000 cells) were deposited. The nylon swab performed significantly better with a moderate number of cells (10,000 cells) and the foam swab performed significantly better with a high number of cells (20,000 cells) (Cahill and Chapman 2020). The Cahill and Chapman and Verdon et al. studies vary as to the better-performing swab type for the brick-saliva combination. But we exclude the Verdon data because the statistics are not separated by DNA source and conclude that the use of nylon, rayon, and foam swabs is volume dependent.

Nonporous substrates

Studies that applied saliva onto nonporous substrates can be characterized by smooth and textured surfaces. Of four studies that evaluated the recovery of DNA from saliva on glass, three found a cotton swab to be the top performer; one study showed this as a significant difference compared to nylon and foam swabs (Verdon et al. 2014; Seiberle et al. 2022; Hedman et al. 2021). One study found that nylon recovered significantly more DNA than two cotton swabs, and after 40 days of room temperature storage, the nylon swab contained more quantifiable DNA (Mawlood et al. 2015). The ForensiX nylon and cotton swabs used in this study were stored in a tube with an active desiccant system. This is a significant difference from the other three studies in which cotton swabs performed better (Verdon et al. 2014; Seiberle et al. 2022; Hedman et al. 2021; Mawlood et al. 2015). Based on analysis criteria, cotton and nylon are identified as the best-performing swabs for the glass-saliva combination of substrate-DNA source.

For textured, nonporous surfaces, ridged or pitted plastic was a substrate studied by Verdon and Hedman (Verdon et al. 2014; Hedman et al. 2021). The results from the former are presented above (Verdon et al. 2014). Although the results were not significant, Verdon found the large foam swabs outperformed the cotton and nylon swabs in the amount of DNA recovered from textured plastic (Hedman et al. 2021). It is possible the foam

swabs can reach pits and grooves of these textured surfaces much as researchers hypothesized about the foam swab collecting DNA from wood. However, the current data indicates the swabs perform similarly (Verdon et al. 2014). One study compared nylon and cotton swabs to collect diluted saliva from petri dishes at high and low quantities of DNA with three different DNA extraction methods: one manual and two robotic (Brownlow et al. 2012). Statistically different combinations of swab type-extraction methods yielded different results. Swab type data was also pulled out of the dataset and showed that with high quantities of DNA, the nylon swab produced significantly higher yields than the cotton swab with a manual extraction. But the cotton swab performed better on automated platforms. During manual extractions of low DNA quantities, nylon yielded significantly more, and no difference between swab type was determined with robotic extraction (Brownlow et al. 2012). When putting aside extraction method (the third variable), the use of cotton and nylon swabs to collect saliva from smooth plastic surfaces is volume dependent.

One article evaluated cotton, foam, and nylon swabs for collecting DNA from saliva deposited on fired cartridge cases (FCC) (Jansson et al. 2020). Although the nylon and foam swabs initially performed better than cotton when all were wetted with a detergent prior to swabbing, the foam swab was not considered further (Jansson et al. 2020). The nylon and cotton swabs were then directly compared. The nylon swab recovered significantly higher amounts of DNA from fired brass cartridges as well as more DNA from unfired cartridges (Jansson et al. 2020). Based on these results, nylon is the best swab for the ammunition-saliva substrate-DNA source combination.

Semen

Porous substrates

Semen is the most common DNA source collected from sexually motivated offenses. The evidence is collected with sexual assault kits which provide swabs routinely made of cotton. They are used by forensic medical professionals to collect samples. Studies conducted by Egger et al. (2022) and Benschop et al. (2010) evaluated cotton (Prionics ForensiX Evidence Collection Kit; Prionics ForensiX Evidence Collection Tube Safe-Dry; Deltalab), nylon (FLOQSwab), and rayon (Sarstedt Forensic Swab XL) swabs that were self-collected vaginally, post-coitus (Benschop et al. 2010; Egger et al. 2022). In the recovery of DNA from the sperm fraction, samples from rayon swabs yielded significantly more DNA than the ForensiX Evidence Collection Kit cotton swab and significantly more DNA in the non-sperm fraction than both cotton swabs (Egger et al. 2022). Considering no significant variation in dropout for all swabs tested in the Egger study, further analysis of the DNA profiles was not conducted (Egger et al. 2022). The Benschop study found higher DNA yields for nylon swabs in the sperm fraction and a lower yield in the non-sperm fraction compared to the cotton swab (Benschop et al. 2010). Through dual-target quantification of total DNA and male DNA, more male DNA was present in the sperm fraction following collection with nylon swabs (Benschop et al. 2010). Upon DNA analysis, both swabs produced allelic information up until 60-h post-coitus, and more male alleles were present in the non-sperm fraction samples collected with nylon swabs. This did not detract from the female being the major contributor (Benschop et al. 2010). As the data stand, rayon swabs show a better extraction efficiency than cotton swabs, and nylon recovers more DNA than cotton while performing similarly during DNA analysis (Benschop et al. 2010; Egger et al. 2022). From these data and the analysis criteria, the vagina-semen combination is best collected with a rayon swab.

In one study, semen was dried on human skin to compare cotton, nylon, and polyester bicomponent fiber swabs (Ferreira-Silva et al. 2019). Significantly greater amounts of DNA were recovered from the skin-semen substrate-DNA source combination when collected with the nylon or polyester bicomponent fiber swabs than with cotton. No significant differences were calculated between the two non-cotton swabs (Ferreira-Silva et al. 2019). During DNA analysis, all samples from the three swab types generated full male profiles (Ferreira-Silva et al. 2019). In analyzing the DNA profile results, the authors used a semicontinuous likelihood ratio calculation of the male and female participants in each sample; yet, the results are not presented (Ferreira-Silva et al. 2019). The DNA typing results presented were the percentage of samples that produced mixed profiles: 85.7% of samples collected on cotton swabs produced a mixture, whereas 57.1% of the nylon swabs and 47.6% of the polyester bicomponent swabs produced mixtures (Ferreira-Silva et al. 2019). Notably, non-mixed profiles were of the male donor. Thus, in a real-case scenario, nylon and polyester bicomponent swabs would have yielded less complex mixtures for interpretation.

As clothing items are common pieces of evidence collected during sexual assault investigations and may be included in a sexual assault kit, it is important to research the swabbing of clothing for semen evidence. One article briefly studied collecting of semen on cotton T-shirts with nylon and cotton swabs, yet the results did not detail this substrate-DNA source type (Seiberle et al. 2022).

Touched surface

Porous substrates

Building materials have been used as substrates to evaluate the collection of touch DNA. In a study comparing nylon and cotton swabs on unfinished wood, a second dimension, the extraction performed, was combined with the data (Alketbi and Goodwin 2019). Although not statistically significant, collecting a touch DNA sample with a nylon swab and then using the QIAGEN QIAamp® DNA Investigator for extraction recovered more DNA compared to cotton swabs. Cotton swabs yielded more DNA than nylon when paired with the PrepFiler Express BTA™ kit for extraction (Alketbi and Goodwin 2019). Seiberle et al. (2022) also studied unfinished wood surfaces as touched substrates with cotton, nylon, and rayon swabs and found that the nylon and cotton performed better in DNA recovery than rayon. However, no statistics were calculated (Seiberle et al. 2022). Verdon et al. (2014) looked at five different swab compositions and found a foam swab to have the best collection and extraction efficiency, with cotton and polyester swabs performing well (Verdon et al. 2014). The Verdon study also used a ranking system to capture all the touched surface quantification data and make a conclusion on swab performance. The touched DNA sourced ranks were calculated separately from the blood and saliva ranks presented previously in this paper. Fingerprints were deposited on drywall in the Plaza et al. study where the forensic DNA profile index (FI) was also calculated (Plaza et al. 2016). In this study, the HydraFlock® swabs yielded a 100% profile and had a 9.11 average FI (10 being the highest quality profiles), which was significantly higher than the microfiber, cotton, and foam swabs also studied (Plaza et al. 2016). The cotton swab sample yielded the lowest quality profiles, with an average FI value of 1.72 (Plaza et al. 2016). The Verdon study evaluated touch DNA deposited on a clay paver and found that two cotton swabs (Puritan FABSwab and Copan 150C) and one nylon swab (Puritan 1PN Flock) had ranking ratios below 2 (a value of one being the most ideal) (Verdon et al. 2014). DNA profiles were generated; however, profiling data was not separated by substrate type. Although the wood-touched and brick-touched surface combinations have been researched, the analysis criteria were not met to render a conclusion. Based on the results of the Plaza study (2016), the drywall-touched surface combination is best collected with a bicomponent fiber swab.

Although newer collection techniques for the recovery of touch DNA from textiles exist, like vacuuming, swabbing remains the prevalent approach due to the higher cost of vacuum technology. Plaza et al. looked at four different swab types and their ability to collect touched 100% cotton fabric and found no significant difference in swab quality of DNA profiles (Plaza et al. 2016). The HydraFlock swab yielded the most alleles and had the highest quality profile, while the other three swabs generated poor quality profiles (Plaza et al. 2016). Hansson and colleagues (2009) found the same outcome — no differences were observed among the cotton, nylon, and foam swabs for the quantity of DNA recovered on cotton shirts; however, this was not based on a statistical calculation (Hansson et al. 2009). Qualitatively, the foam and cotton swabs generated full profiles, while the nylon swab only produced partial profiles (Hansson et al. 2009). The Comte study (2019) questioned whether the cotton swab collection kit used in evidence collection was the best for their police forensic unit. Comte assessed three different cotton swabs from different manufacturers and two nylon swabs from the same manufacturer using shirt and t-shirt collar samples (Comte et al. 2019). The nylon swabs (4N6FLOQSwabs Crime Scene and Genetics) gathered and extracted significantly more DNA from the collars of t-shirts than the police forensic unit's current cotton swab from the Prionics cardboard evidence collection kit (Comte et al. 2019). The three cotton swabs that performed equally well were the Prionics, the Puritan FAB-MINI-AP, and the Sarstedt Forensic. Nylon was a better performing swab for the textiles-touched surface combination in one study. Conversely, cotton, bicomponent fibers, foam, and microfiber swabs may perform just as well.

Leather steering wheels were a substrate in one study which found that nylon swabs significantly outperformed cotton (from the Prionics evidence collection kit) in the recovery of touch DNA (Comte et al. 2019). A significant difference was also observed between two cotton swabs; the Puritan FAB-MINI-AP swab, a mini-tip swab, yielded more DNA than its competitor (Comte et al. 2019). This study meets our analysis criteria and shows the best-performing swab for the leather-touched surface combination is nylon.

When documents are in question, it is possible that probative information can come from swabbing a surface for touch DNA. One study evaluated DNA recovery following fingerprint dusting with dactyloscopic powders on paper. It found 76.3% and 55% DNA typing success with nylon and cotton swabs, respectively (Tozzo et al. 2015). In a comparison of cotton, bicomponent fiber, microfiber, and foam swabs, the study calculated no significant difference in alleles recovered, nor profile quality (Plaza et al. 2016). In 2019, Alketbi and Goodwin found that swab performance and DNA extraction influence each other when collecting DNA from touched paper (Alketbi and Goodwin 2019). Cotton swabs, followed by the use of the PrepFiler Express BTA kit, yielded more DNA. Nylon swabs followed by the use of the QIAamp

DNA Investigator Kit yielded more DNA from touched paper samples (Alketbi and Goodwin 2019). Examining three different types of paper — magazine paper, paper used for bank checks, and copier paper — one study concluded that nylon swabs performed better than bicomponent fiber swabs. However, the data are unclear in supporting this conclusion (Yonar et al. 2022). This study met the criteria for inclusion in our systematic analysis with DNA typing. However, agarose gel electrophoresis was conducted to observe DNA in samples where no DNA was visualized in the HydraFlock swabbed samples. "DNA was more or less observed" from those samples collected with the 4N6FLOQSwabs (Yonar et al. 2022). From the Plaza study, we can conclude that there is no difference in swab performance among cotton, bicomponent fiber, foam, and microfiber swabs. The other studies mentioned do not meet the analysis criteria.

One study compared cotton, rayon, nylon, and foam swabs by swabbing the palm of a donor's hand followed by using enhancement methods to visualize the biological material (Panjaruang et al. 2022). Statistics were not calculated, and the data presented in a table were misaligned with the text. Thus, no conclusions can be drawn (Panjaruang et al. 2022). A second study looked at the skin (female wrists) touched by a donor (male); DNA was collected with both wet (moistened with water) and dry cotton and nylon swabs (Kallupurackal et al. 2021). The dry cotton swabs rubbed on the wrist, and the moistened nylon swabs produced the best DNA results as measured by profile quality (Kallupurackal et al. 2021). These results meet our criteria for analysis; cotton and nylon swabs perform similarly. We advise keeping in mind the additional variable of a wetting agent on the swab and method of swabbing.

Nonporous substrates

Touched surfaces on smooth, nonporous substrates of glass or plastic were the most studied combination analyzed in this review. Two of the studies were short and did not present the data with the glass or plastic substrate-touched surface combination pulled out (Tozzo et al. 2015; Haase et al. 2019). Four studies compared combinations of nylon, cotton, rayon, microfiber, bicomponent fiber, and foam swabs. They found no significant differences among the swabs within each study (Seiberle et al. 2022; Plaza et al. 2016; Comte et al. 2019; Alketbi and Goodwin 2021). One of these studies observed a significant increase in DNA collected when comparing wet and dry swabbing with cotton and nylon swabs, yet the swab types made no difference (Alketbi and Goodwin 2021). A second study in this set of four provides a figure in which nylon and cotton swabs appear to yield similar amounts of DNA (amounts higher than rayon swabs) (Seiberle et al. 2022). In all or portions of a second set of four studies, either no tests for significance were conducted or pairwise comparisons or statistics were not described for sufficient interpretation of the substrate-DNA source combination (Verdon et al. 2014; Alketbi and Goodwin 2019; Hansson et al. 2009; Hartless et al. 2019). Of these, the Hartless study (2019) found the order of most to least donor alleles recovered from plastic surfaces to be polyester, foam, and cotton; the order was reversed for glass surfaces (Hartless et al. 2019). In contrast, Verdon et al. (2014) found the polyester swab excelled at extraction and collection from glass (Verdon et al. 2014). Another study within the no statistics or insufficient interpretation set states that a foam swab outperformed a cotton and nylon swab, and the nylon swab performed the worst (Hansson et al. 2009). The Panjaruang et al. (2022) study visualized the "touch DNA" first with SYBR® Green and selected the foam swab to continue on to DNA typing. However, this choice does not align best with the study's quantification data (Panjaruang et al. 2022). A 2019 study by Alketbi and Goodwin coupled the swab type with the extraction; nylon swabs with a column-based extraction recovered the most DNA, followed by cotton swabs with a bead-based extraction (Alketbi and Goodwin 2019). From these numerous publications, we can only conclude that no significant differences have been observed among cotton, nylon, bicomponent fibers, foam, and microfiber swabs. The remaining results do not meet the analysis criteria.

Textured plastic surfaces (e.g. lids to containers, knobs, and components in vehicles) are an additional nonporous substrate that may hold evidence. A study by Williams and team (2013) compared cotton and nylon swabs to collect touch DNA from screw top lids and found no significant difference in the amounts of DNA recovered (Williams et al. 2013). Comparatively, Alketbi and Goodwin retrieved more DNA with nylon swabs than with cotton swabs when wet at the manufacturer's recommended volume (Alketbi and Goodwin 2021). In the Verdon study of 10 different swabs, all three cotton swabs ranked highest for combined collection and extraction efficiency (Verdon et al. 2014). These three cotton swabs are COPAN 150C, Puritan FABSwab, and Bode SecurSwab. The Bode swab provided comparable DNA typing results to the other swabs studied when results were not separated by substrate (Verdon et al. 2014). Giovanelli and colleagues (2022) added polyester into the nylon versus cotton comparison and found that it significantly outperformed the other swabs when collecting samples from textured plastic surfaces within vehicles (Giovanelli et al. 2022). Nylon swabs yielded significantly lower DNA quantities and alleles typed (Giovanelli et al. 2022). Based on the analysis criteria, polyester is ideal for the textured

plastic-touched surface combination. When polyester was not included, a separate study saw no difference among nylon, foam, and cotton swabs.

Two additional textured, nonporous substrates studied are finished wood and stones. Finished wood described as varnished was classified as nonporous, although we recognize it may be slightly porous. A foam swab (Medical Wire) recovered more donor alleles from a wooden handle on a screwdriver than a polyester and cotton swab: however, statistics were calculated for different swab types across four different substrate materials (Hartless et al. 2019). Stones may be opportunistic nonporous weapons where touch DNA is left behind. Therefore, they were evaluated as a substrate in a study by Seiberle and colleagues(Seiberle et al. 2022). After identifying the best method for deposition of touch DNA onto sample stones, the study found that cotton swabs (ForensiX Collection Swab SafeDry, Prionics) yielded more DNA than a nylon swab (4N6FLOQSwabs Genetics). Evaluating swabbing in a stepwise manner, the group observed that when equal amounts of water (10 µL) were used as a wetting agent prior to swabbing, the cotton swab recovered significantly more DNA than the nylon swab (Seiberle et al. 2022). Based on the texture of stone and meta-analysis results, foam swabs may be advantageous to collect DNA from this substrate. With no replicates provided in this study, a conclusion cannot be made for the stone-touched surface combination.

Firearm-associated evidence is likely made of metal: aluminum or brass. Three studies used cartridge cases, both fired and unfired, and magazines as substrates to collect touch DNA with various swab materials (Jansson et al. 2020; Haase et al. 2019; Tasker et al. 2020). One study compared nylon and cotton swabs followed by using the One-Touch™ Kit with extraction from samples deposited on shell casings. The cotton swab recovered a median of 10 times more DNA than that of the nylon swab (Haase et al. 2019). In contrast, Jansson and colleagues (2020) did a stepwise study to find that the nylon swab outperformed their current cotton swab (Jansson et al. 2020). The research's first phase considered a foam swab but quickly eliminated it for poor performance (Jansson et al. 2020). Upon implementing this new nylon swab, DNA recovery from cartridge cases significantly improved. The percentage of samples above the amplification cutoff increased from 11.1 to 28.6%, and the number of usable DNA profiles grew from 5.0 to 8.0% (Jansson et al. 2020). A multilayered cotton swab (SimpleSwab2™, Gentueri, Inc.) was compared to a traditional wound cotton swab and a nylon flocked swab for their ability to recover touch DNA from magazines and rifle ammunition (Tasker et al. 2020). The SimpleSwab2 and the nylon swab (4N6FLOQSwab Genetics) yielded significantly better STR profiles compared to the wound cotton swab; no difference was calculated between these two swabs (Tasker et al. 2020). Based on the analysis criteria, cotton and nylon swabs perform best for the ammunition-touched surface substrate-DNA source combination.

For metal surfaces not associated with firearms, limited research has been conducted to determine the best swab for touch DNA. In two studies, a cotton swab recovered more DNA than nylon, rayon, foam, and polyester swabs; however, no statistics were calculated that focused on the substrate-DNA source combination (Seiberle et al. 2022; Hartless et al. 2019). In a short third study, DNA analysis was done for various porous and nonporous substrates, including metal. However, data was not separated out by substrate type (Tozzo et al. 2015). There is insufficient data about this substrate-DNA source combination to meet our analysis inclusion criteria. However, research based on observations with metal substrates should be explored (Bonsu et al. 2023, 2021).

Acellular DNA

Porous substrates

Three articles directly added acellular DNA to swabs to evaluate different swab types, providing results as release or extraction efficiency of DNA from the swab (Marshall et al. 2014; Bonsu et al. 2021; Wood et al. 2017). The Marshall et al. study (2014) used the purified DNA sample from the Quantifiler® Human DNA Quantification Kit derived from a cell line; the Bonsu et al. study (2021) used a commercially available, purified DNA sample from multiple donors; and the Wood et al. study (2017) does not describe the source of the acellular DNA used (Marshall et al. 2014; Bonsu et al. 2021; Wood et al. 2017). In Bonsu et al., the release efficiency of the swabs was corrected for based on an extraction efficiency of 88% (about a 12% loss of DNA). Thus, the release efficiencies were 97.9% and 57.7% for the Isohelix (described as an "optimized rayon swab" Material Safety Data Sheet (MSDS) 2020) and Puritan rayon swabs, respectively. The Isohelix swab performed significantly better (Bonsu et al. 2021). The Wood et al. study also calculated an extraction efficiency, but for nylon and cotton swabs, recording an efficiency of 84.6% (nylon) to 55.8% (cotton). However, this group did not correct for the loss of DNA when evaluating the swabs' release efficiency (Wood et al. 2017). The release efficiency for the nylon swabs was found to be higher than the cotton swabs, with 84.6% and 55.8% of DNA recovered, respectively (Wood et al. 2017). Notably, the standard deviations of the percent DNA recovered by both rayon swabs were smaller than those for the nylon and cotton swabs (Bonsu et al. 2021; Wood et al. 2017). This difference may be attributed to human variation, or

it may signify a difference in the swab material capacity. The studies cannot discern the reason for the difference. Marshall et al. calculated the release efficiency of acellular DNA by dividing the DNA recovered by the DNA applied to the swabs and multiplying by 100 to obtain the percent recovered. The nylon swab recovered more DNA with 85% and 55% DNA recovered from the 1- and 2-ng depositions, respectively (Marshall et al. 2014). When 3 ng of DNA was deposited on the swabs, the X-Swab Diomat material (dissolvable swab) recovered 60% of the DNA, while the nylon swab recovered 32%. These results were not significant (Marshall et al. 2014). The rayon swab meets the criteria for a best-performing swab for the swab-acellular DNA combination; nylon and dissolvable swabs showed no difference in performance.

Nonporous substrates

The Bonsu and Wood studies also evaluated combinations of swab types when acellular DNA was deposited on nonporous substrates (plastic, glass, and metals). Cotton swabs recovered about 25% of the DNA on glass compared to about 15% with nylon (Wood et al. 2017). This study did not provide statistics (Wood et al. 2017). In the Bonsu study (2021), the Isohelix optimized rayon swab recovered more DNA than a second rayon swab (Puritan) from brass, copper, steel, and plastic substrates (Bonsu et al. 2021). Within both these studies, the percent recovery of DNA decreased with metal surfaces to about or below 50%. Again, the Isohelix swab recovered a greater percent of DNA compared to the other rayon swab; however, there was an overall decrease with metal substrates (Bonsu et al. 2021). Of note, five times more acellular DNA was deposited on cables of an unknown metal type in the Wood et al. study. Again, less than 5% of the DNA was recovered with both cotton and nylon swabs (Wood et al. 2017). This decreased recovery of DNA from metal surfaces further supports the hypothesis of the strong metal-DNA interactions. Such interactions negatively impact the ability of any swab type to remove DNA and the complex impact that metal ions have on a qPCR reaction (Bonsu et al. 2023, 2021). None of the current studies examining acellular DNA recovery from nonporous surfaces meet our analysis criteria.

Swab use with newer technologies

Direct amplification provides an alternative workflow to traditional DNA testing schemes. Compared to a traditional workflow where the swab would be removed during extraction, direct amplification would keep the swab in the sample tube during amplification and sample aliquoting for capillary electrophoresis separation. Our analysis thus far shows that different swab materials work better for different substrates — source of DNA combinations, and together with the swab remaining in the sample during direct amplification, it is imperative to review this body of literature. However, minimal research has been conducted comparing swab types for direct amplification. In a short study about recovering acellular DNA from a smooth plastic substrate, the authors found that nylon swabs yielded almost four times more relative fluorescent units (RFU) compared to cotton swabs (Templeton et al. 2013). This study also evaluated foam swabs, which performed better than cotton — with over three times higher average RFU (Templeton et al. 2013). One lab studied nylon swabs designed for direct PCR. However, comparisons were made between the direct PCR workflow and a traditional workflow (which included a manual DNA extraction step that increased the study variables) (Ambers et al. 2018; Sherier et al. 2020). Because these studies did not compare swab type in the direct amplification, our analysis criteria were not achieved.

Similar to direct amplification, rapid DNA instruments have an alternative workflow to traditional DNA analysis that differs in what happens to the swab. In rapid DNA instruments, the swab material does not remain with the sample extract through the amplification and separation steps. Three papers briefly studied the differences in swab type that are used for reference-type samples (blood and buccal swabs) (Moreno et al. 2017; Chen et al. 2021; Wiley et al. 2017). Moreno and colleagues compared cotton and Puritan™ Hydra-Flock™ swabs used to collect buccal cells; the Hydra-Flock swabs successfully produced profiles for 90% of the samples, while cotton swabs only experienced success 71% of the time (Moreno et al. 2017). Yet, due to many cartridge malfunctions in the DNAScan/ANDE™ instrument, the authors found no differences in evaluated peak heights between the two sample types. Wiley et al. used the RapidHIT ID instrument to compare buccal swabs collected on nylon, polyester, and cotton swabs (Wiley et al. 2017). Individual sample results did not present data; however, the authors tabulated the number of runs with each swab type and concluded that nylon and polyester swabs are compatible with the RapidHIT ID system (Wiley et al. 2017). Blood was used as the DNA source when Chen et al. compared a sterile medical cotton swab to common cotton and nylon swabs. Interestingly, the medical-grade cotton swab produced no profiles, and the regular cotton swab (FineGene Biotech, China) produced full profiles. The nylon swab had 52.6% of the loci identified (Chen et al. 2021). Cotton swabs were used as the control throughout these three works, and alternative swab types were briefly used as comparisons. The research suggests that bicomponent fiber, nylon, and polyester swabs are

compatible with rapid DNA platforms for reference-type samples. However, these studies did not meet our analysis criteria.

Next-generation sequencing (NGS) continues to be implemented into casework. Although the swab containing the sample is handled the same in NGS as in a traditional DNA workflow, the comparison of swab types with an NGS workflow has been researched the least. In a conference brief, Haase and colleagues sequenced mtDNA profiles from gun shell casings and glass slides; however, the data are not analyzed by swab type (Haase et al. 2019). Because the research does not exist, it is still an open question whether swab type has an impact on NGS.

Discussion and conclusions

Swabbing to collect biological material from evidence is the most common collection method used in the crime lab and minimizes changes to the evidence. Swabs aid in gathering evidence from large items that cannot be packaged and sent to the lab, such as vehicles. Swabs also collect reference samples and evidence inside the body following a sexual assault. While the forensic use of the swab today is far from its intended purpose, the basic swab design has not changed much since it was first patented in 1874 by Moritz Leiner which included a stem with an absorbent tip for a "cheap, simple, and effective" way to clean one's ears (Leiner 1874). With the advent of 3-D printing, manufacturers now produce wound fibers, flocked fibers, or pads on tips — with wood or plastic stems (Vashist et al. 2023). The tip material, originally made of elastic, is most commonly made of cotton today — with rayon, polyester, nylon, and polyurethane tip options offering specific alternative properties (Leiner 1874; Vashist et al. 2023). This review's findings show that swabs are arguably the simplest and most influential part of the forensic DNA testing workflow.

The purpose of this systematic review was to determine which swab material provides the best DNA results. The initial literature examination quickly determined that this question does not have one answer; answers vary by the DNA source and the substrate the biological material is being collected from. By categorizing the current research results by substrate-DNA source, best-performing swabs could be realized for many source-substrate combinations (Fig. 2). Furthermore, for 58% of the substrate-DNA source combinations, we have identified a best-performing swab type, swab types that perform similarly, or a swab type that performs based on the volume of the DNA source. The procedures for the systematic review and analysis have limitations. The initially identified articles were limited to the three databases that we searched and the search terms we used. We could have missed articles that contained experiments that did a swab comparison but were not the focus of the paper. Additionally, some substrate-DNA source combinations may have been excluded from analysis because the paper did not provide replicate numbers (Seiberle et al. 2022; Dadhania et al. 2013). To keep the review and analysis scoped and consistent, additional variables, such as extraction chemistry, were not considered. This analysis can be used by practitioners to make informed decisions about their sample collection and by researchers to help guide future needed research. Three overall conclusions can be made outside our categorizations: (1) the overall workflow needs to be considered according to swab type, (2) swabs made of the same material do not perform similarly when compared to each other, and (3) inter-operator swabbing may not be different.

Observations from the literature

The first conclusion observed throughout preparing this review is that the best swab type depends on the entire process, extraction through DNA typing. One article evaluated nylon and cotton swabs when collecting saliva from plastic petri dishes. The authors paired manual extraction with one of two robotic extraction procedures (Brownlow et al. 2012). Significantly, more DNA was recovered from nylon than from cotton swabs during manual extraction when both high and low quantities of DNA were available. Yet, cotton swabs recovered significantly more DNA than nylon swabs when extracted on an automated platform when high amounts of DNA were available (Brownlow et al. 2012). Three other studies encountered results that depended on the extraction chemistry (or changes in the protocol) that was used to process the swab (Dadhania et al. 2013; Jansson et al. 2020; Alketbi and Goodwin 2019). This repeated observation should encourage practitioners to evaluate swab type paired with extraction chemistry when implementing a new swab or swab brand.

Considerations of swab brands bring us to our second conclusion — all swabs made of the same material do not perform similarly. Multiple studies evaluated multiple swabs of the same material. But sometimes those same swab types bracketed the range of swab performance (Verdon et al. 2014; Seiberle et al. 2022; O'Brien and Figarelli 2012). For example, one study examined multiple brands of cotton swabs, together with other swab types ($n=10$ swabs studied); one cotton swab ranked first and another last (O'Brien and Figarelli 2012). The Verdon et al. study gives another great example of this spread; the Puritan Minipopule (foam) swab performed the poorest, while the Puritan 1PF foam swab performed significantly better (Verdon et al. 2014). Although one experiment found no significant differences between two cotton

swabs, it does not negate this conclusion (Comte et al. 2019). This review studied some commercially provided swabs that are no longer available (S1 Table). Therefore, practitioners considering incorporating a new swab type should also consider the continuity of the swab provider.

Swabbing takes place at the crime scene by crime scene personnel, not just in the crime laboratory. In our final overall conclusion, we look at inter-operator swabbing differences. Two stepwise studies researched whether differences exist when different people swab. One used 10 operators, and the other used 12 operators from 4 different agencies (Seiberle et al. 2022; Hedman et al. 2021). Both studies had a sufficiently large population size to do statistical calculations and found that there were no significant differences among operators in swabbing for biological evidence (Seiberle et al. 2022; Hedman et al. 2021). Both studies used a defined swabbing protocol. For this reason, we can assume that if swab operators are trained consistently using the same protocol, the DNA testing outcomes will be the same regardless of who swabbed the evidence.

Identified research gaps

Shortcomings and gaps in the research were identified during this review. The clearest gaps are those portions of Fig. 2 that show areas where either no research has been conducted or areas that do not identify an ideal swab. For example, evidence commonly associated with sexual assaults, such as saliva and semen in and on the body, should be researched more prior to conclusions being made about the best swab type. Cotton swabs are used ubiquitously in sexual assault kit evidence collection processes, yet our review indicates that other swab types may outperform cotton swabs. Another example is foam swabs, which are hypothesized to better reach textured surfaces like wood. Comparing foam against other swabs for substrates like stone may help the community further understand foam's utility. Beyond these examples, there are more substrates, sources of DNA, and swab types that can be incorporated into future research. In addition, three additional swab factors observed in the literature should be considered: swab wetting agents, storage of swabs, and active versus passive drying of swabs (related to the first conclusion). Therefore, the current study can also help the field develop evidence-informed questions for future research. For example, do we need to study certain substrate-DNA source combinations such as textiles-semen or textiles-saliva since cutting may be a better sample collection method? Or is it necessary to find the best swab type for reference-type saliva collections?

An analysis of literature comparing wetting agents, storage, and drying of swabs was outside of the scope of our systematic review, but these factors have been shown to have an impact on results ((Verdon et al. 2014; Seiberle et al. 2022; Mawlood et al. 2015) as examples, not an exhaustive listing). Various swab wetting agents were observed in many studies: water, saline, isopropyl alcohol, detergents, and Tris–EDTA buffer; some studies compared wetting agents. The volume of wetting agent was also found to have an impact on study outcomes (Seiberle et al. 2022; Alketbi and Goodwin 2021). Further study is needed on wetting agents, including detergent concentrations and volume applied. Garvin and team took an interesting approach to studying swab storage and drying by incorporating the isolation and identification of microbial activity on various swabs (Garvin et al. 2013). A timed study, like that conducted in Mawlood et al. (2015), together with studies of microbial activity, could improve understanding about actively and passively drying swabs and subsequent storage. The critical analysis of DNA profiles following such experimentation would be of great interest.

DNA profile analysis is another critical shortfall throughout this systematic review. More than 20% of the research stopped after quantification of the DNA. The research captured in this review supports the observation that quantification values do not always correlate to DNA profiles (Phetpeng et al. 2015). This critical shortfall ties into an overall theme surrounding a lack of data and thoroughness in its presentation. Some studies that did complete the DNA workflow and follow through to STR typing provided vague results, stating in one to two sentences that the samples selected for DNA typing produced the expected profiles or indicating in the methods section that DNA typing took place without sharing results. Such situations can leave the reader with more questions than answers. Does the swab type release components that may impact amplification (Marshall et al. 2014)? If the DNA results are not different, does the swab type matter? Further, approximately 40% of the studies cited incorporated one to five replicates in their experimental design, and only about a quarter of articles used greater than 10 replicates. These small sample sizes, together with the limited DNA template analyzed being in stochastic ranges, call into question the reliability of the data. Some of the research experiments did not conduct hypothesis-based statistical calculations, which could have provided additional insights to support data figures. Calculations, such as those done in the Phetpeng et al. study, provide great insight. Their figures showed that the swab type had the single greatest impact on DNA quantification, whereas the moistening agent only influenced the outcome by 1% (Phetpeng et al. 2015). Robust, thorough, and critical analyses of all data for the forensic DNA workflow in swab type research can help further

the body of knowledge while increasing confidence in conclusions.

This systematic review shows that minimal research has been conducted comparing swab types in direct PCR, rapid DNA, and NGS workflows, yet crime labs are using these technologies, and we observe in the literature that swabs do have an impact on the outcome. As new technologies enter the forensic DNA workflow, future research should be designed with the above considerations in mind. Research in these areas, and other technologies that vary from the traditional DNA workflow, would help advance our knowledge of how swab type impacts downstream results.

Research impact

In conclusion, the best-performing swab for biological evidence collection is dependent on the DNA source and substrate. The results herein provide a resource to practitioners, researchers, and commercial swab vendors. Two examples of how practitioners may use these findings are to help guide the collection of biological evidence or provide guidance in developing a validation study to bring on a new swab type. Forensic researchers may use our findings to help guide future research where gaps exist and develop a thorough experimental design. Lastly, commercial swab vendors can look to our study to identify the needs of the field. In future work, evaluating the implementation of a new swab type is suggested. For example, practitioners working within the traditional workflow or transitioning to a new technology may do a retrospective evaluation of implementing a new swab type which helps provide real-world use cases. Two papers described how new swab type implementation has had a positive impact on casework; both showed an increase in interpretable DNA profiles (Phetpeng et al. 2015; Jansson et al. 2020). Laboratories can move towards such an implementation evaluation by conducting a baseline study to identify metrics of their current swab type (Baechler 2016). Our work takes years of research evidence and synthesizes it comprehensively to provide concise insight into swab types used to collect biological evidence which can be used broadly as a resource for those in the forensic biology field.

Abbreviations

DNA	Deoxyribonucleic acid
FCC	Fired cartridge case
FI	Forensic DNA profile index
IED	Improvised explosive device
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
PVC	Polyvinyl chloride
qPCR	Quantitative PCR
RFU	Relative fluorescent unit
STR	Short tandem repeat

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41935-025-00444-2.

Supplementary Material 1. S1 Table. Data file containing initial literature selection worksheet, where additional articles were identified, extracted data, analysis of substrate-DNA source combination, and swabs available by vendor.

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Authors' contributions

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Data is provided within the manuscript or supplementary information files.

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Competing interests

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