Science & Justice 66 (2026) 101485

Contents lists available at ScienceDirect

Science & Justice

journal homepage: www.elsevier.com/locate/scijus

Police field trial and exploratory laboratory optimization of the DNA-Buster

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ARTICLE INFO

Keywords:
DNA collection
Dry-vacuuming
Swabbing
Taping
Touch DNA
Police field trial

ABSTRACT

DNA analysis is a powerful tool in crime scene investigations and has become increasingly sensitive in recent years. This is particularly relevant when dealing with touch DNA, a challenging type of evidence due to its invisible nature, and often limited quantity and quality. Conventional collection methods such as swabbing or taping can have certain limitations, including low recovery efficiency, handling with increased risk of contamination, and difficulties in sampling large surfaces or accessing small niches and corners. To address these challenges, the DNA-Buster was developed, a dry-vacuum DNA collection device that has demonstrated superior performance on textile substrates in previous research. However, the device has not yet been tested by its intended end users.

Thus, this study aimed to evaluate the feasibility of implementing the DNA-Buster within police forces using touch DNA samples from worn T-shirts collected by the crime scene investigators themselves. The study sought to gain user feedback regarding the device's secure and intuitive handling and to identify areas for optimization, such as a 3D-printed pipette tip prototype for sampling. A mock aggressor-victim scenario was additionally investigated to examine how the DNA-Buster performs on textiles compared to standard collection methods. Lastly, for the first time, the individual shedder tendencies of the crime scene investigators acting both as donors and collectors were assessed, aiming to increase the awareness of the contamination potential when handling evidence.

The study outcomes confirm that the DNA-Buster outperforms swabbing on textiles and shows comparable results to taping regarding DNA concentrations and profiling success of touch DNA. These findings highlight the importance of selecting most suitable methods for each substrate to obtain optimal results. This is particularly relevant as some police forces, for various reasons, still rely on swabbing for textiles, despite its lower profiling success. Next, the DNA-Buster was operated efficiently by all collectors after minimal training and generated consistent DNA concentrations independent of collection time. Compared to taping, its advantages lie in more practical application with respect to evidence handling by police investigators and easier laboratory processing. With respect to the shedding test, observed intra- and interindividual variability among donors underscore the challenges associated with touch DNA experiments.

Following this successful trial in collaboration with the intended end users, the DNA-Buster is now suitable, as a stationary tool, for the collection of trace DNA from textile evidence. However, its mobile use at crime scenes requires further evaluation based on future feedback from crime scene investigators.

1. Introduction

The collection and analysis of biological material to support judicial authorities in criminal investigations follows Locard's principle that ‘every contact leaves a trace’ [1]. Crime scene DNA can originate from various biological sources, the most obvious being body fluids, such as blood, saliva, or semen. In recent years, however, touch DNA has gained increasing importance and constitutes a substantial proportion of forensic casework samples [2–4]. In this study, touch DNA refers to DNA transferred from the skin when an object or a person is handled or touched [5]. Unlike body fluids, this trace is invisible to the naked eye [6,7] and typically contains far less genetic material. Its exact

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https://doi.org/10.1016/j.scijus.2026.101485
Received 4 February 2026; Received in revised form 6 July 2026; Accepted 7 July 2026
Available online 11 July 2026

composition has not yet been agreed upon and is thought to extend beyond shed skin cells, potentially including a mix of cellular and cell-free DNA as well as various endogenous and exogenous materials [8].

For collecting biological traces at a crime scene, forensic crime scene investigators typically choose a traditional approach, such as swabbing, tape lifting, or cutting, based on factors like experience, routine, or trace type [5,9]. With respect to the latter, the performance of the method depends not only on the physical trace characteristics (e.g., porous, smooth, or absorbent substrates and their respective counterparts), but also on practical considerations such as ease of use, analyst habit, the extent to which destructive collection is permitted (for example, whether the item or piece of evidence may be cut), and surface size. Among existing techniques, swabbing is the most widely used and performs well on smooth, non-porous surfaces such as glass or tile [6,10]. However, its effectiveness may decrease on rough and porous materials. Swab heads may fray on bricks [11,12], and perform poorly on textiles [7,13,14]. Tape lifting, although less commonly applied in routine work, can be more effective on porous and absorbent substrates [7,13–15], with some studies showing that tapes outperform swabs on textiles [13,14]. Yet, taping has disadvantages: first, its adhesive strength diminishes with the number of tape lifts, making the sampling of large surfaces difficult [14]. Here, laboratories often apply various numbers of tape lifts (if declared) [14,16] or pursue tape lifts until the adhesiveness of the tape is exhausted (as determined by the operator) [17], while internal testing has shown a reduction in adhesiveness occurred after 20 to 30 lifts. Second, the tape's inherent stiffness and stickiness can increase contamination risks at a crime scene and complicate its transfer into reaction vessels in the laboratory. With larger tapes, cutting adds an extra handling step and further increases the contamination risk again. In addition, pressing the tape onto the substrate brings the handler's glove close, allowing any DNA on the glove surface to be unintentionally transferred onto the evidence.

Transfer of touch DNA can occur directly, when DNA is deposited through immediate contact with a surface (primary transfer), or indirectly, when DNA is first deposited on one substrate and subsequently passed on to another (secondary or multiple transfer). Transfer steps are influenced by a complex interplay of factors, including individual characteristics, such as age, lifestyle habits, and shedder status [18–21], the properties of the touched object (e.g., material, size, shape, and cleanliness) [22–24], the type and duration of contact [21,23,25], and the time elapsed between primary and secondary contact [18,25,26]. With respect to shedder status, the term “shedder range” of an individual is preferred to better reflect intraindividual variability, while still acknowledging that some individuals may lie at either extreme, shedding very large or very small amounts of DNA, whereas most are likely to fall in between [27,28]. Nevertheless, it could be argued that high shedders pose a greater risk of contamination when collecting biological material, making awareness of their potentially high shedding tendency particularly important when touch DNA is the target. Therefore, a shedding test was implemented for the forensic crime scene investigators to estimate individual shedding tendencies and examine potential correlations with study outcomes.

In addition to the traditional methods, some research groups have developed new or tested and refined existing wet or dry-suctioning alternatives for collecting touch DNA. One example is the M-Vac® wet-vacuuming device (CSI Equipment Ltd., UK), which is intended for mobile use but limited by its considerable weight and by the risk of DNA spread or loss due to large buffer volumes and filtration. Additionally, the 3.5 cm head restrict sampling in small or confined areas, and the high cost appears to have hindered so far, its broader adoption in forensic work [10,29,30]. The in-house Pulse Lavage System (PLS), originally an orthopedic surgical instrument, has also been explored as a potential tool for biological material collection, showing promising results when compared to the M-Vac®. Since the PLS has only been tested on (diluted) blood samples [31], it would be valuable to assess its performance against traditional DNA collection tools. Further, a non-destructive vacuum method was designed for securing biological material from handwritten documents, which is currently limited to paper-like surfaces and to stationary use, though future developments can be expected [32]. More recently, a micro-trace vacuum kit (Coloprint GmbH) with a vacuum cleaner and a specialized filter unit was introduced for floor sampling to collect total human DNA at crime scenes, while also usable for vegetation trace collection [33]. The new approach showed promising DNA results but requires post-cutting of the filters into approximately 50 subsamples to keep mixture levels low, which is both time- and cost-intensive [33]. In line with the authors' recommendation, its use as a supplementary collection procedure in major scene cases appears valuable, while initiating the analysis only if all other collected samples fail to yield DNA profiles.

Another dry vacuuming device, the DNA-Buster, is intended to serve as an alternative or complementary tool to swabbing and taping for both stationary and mobile use [10]. The device uses a battery-powered diaphragm pump connected to a reusable hose, with a filtered pipette tip inserted at the sampling end. During collection, the vacuum pump generates suction, drawing biological material onto the protected filter in the tip, with the filter then transferred into a standard testing tube for DNA extraction [10]. Direct comparisons showed that swabbing and taping performed better on hard surfaces (wood and tile), whereas the DNA-Buster outperformed the swabbing approach, yielding complete DNA profiles from carpet and a cotton sweater, with best results achieved on the sweater [10]. The results obtained so far indicate that the DNA-Buster has the potential to become a more efficient tool than swabbing when textile evidence serves as a substrate. However, to date, the device has only been tested under laboratory conditions by experienced laboratory operators, circumstances that do not reflect real-world situations or demonstrate intuitive use by police personnel.

Thus, this study aimed to evaluate the feasibility of using the DNA-Buster for collecting touch DNA samples from T-shirts, worn and sampled by police themselves, with minimal training or handling guidance. Additionally, a mock aggressor-victim scenario was designed to compare the performance of the DNA-Buster with standard collection methods, sampling, and evaluating DNA mixtures from a T-shirt. As the experiment involved touch DNA with potentially increased contamination risks, both generally and specifically due to the introduction of a new collection tool, the contamination was assessed. Further, the study participants' DNA shedding range was evaluated, inspired by the work of Lowe et al. [21]. Finally, based on the feedback, the study sought to optimize the pipette tip design of the device to enable smoother movement during collection from textiles.

2. Material and methods

2.1. Type and preparation of substrates

Twenty 100% cotton T-shirts were used: 18 for the police field trial, one for the *mock aggressor-victim experiment* and one for the *3D-printed tips* test. Prior to DNA application, the T-shirts were machine-washed at 60 °C using bleach pods, air-dried indoors, and stored in paper envelopes. The inner front and back of five randomly selected T-shirts were swabbed using 60 µL of nuclease-free water (Promega Corporation, Madison, WI, USA) and a cotton swab (ForensiX Evidence Collection Kit, Prionics AG, Schlieren, Switzerland) as negative controls. As accredited approach for screening substrates for background DNA, swabbing was used. No foreign DNA was detected in these negative controls by real-time PCR (see *Genetic Analysis*). The DNA collection areas were defined using hard plastic stencils (12.5 × 27.5 cm; see Fig. 1). The stencils were cleaned with 70% ethanol (Thommen-Furler AG, Rüti bei Büren, Switzerland, ETOH) and UV-irradiated (LTF Labortechnik GmbH & Co. KG, Wasserburg (Bodensee), Germany) for 20 min. The light source was a UVC lamp (Philips TUV 25 W T8, Amsterdam, Netherlands), emitting short-wavelength UV radiation in the 100–280 nm range with a peak emission at 253.7 nm, while the lamp's glass

Fig. 1. Stencil fixation and vacuum direction. Left: Stencil that is secured with six clips in the middle of the T-shirt and touching the sewing line of the collar at the top. Right: Sampling area (green) that was vacuumed vertically from top to bottom (created with BioRender.com). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

envelope filters out the ozone-producing 185 nm radiation. Between each sampling, the stencils were wiped with 70% ethanol again. The metal clips used to fix the stencils were soaked in 70% ethanol for 30 min and air-dried. The 3D-printed tips were soaked in 70% ethanol (Thommen-Furler AG) for 20 min, air-dried, and then UV-irradiated for a further 20 min. Negative controls of the tips were performed using a ForensiX swab (Prionics AG) moistened with 60 µL of nuclease-free water. Again, no foreign DNA was detected.

2.2. Application of touch DNA

The T-shirts were worn for eight hours during normal daily activities. For the police field trial, each of the six crime scene investigators wore three T-shirts on separate days. For the mock *aggressor–victim* and *3D-printed tips* experiments, one T-shirt was worn by a female laboratory member per day. After wearing the T-shirts, they were stored in paper envelopes until sampling the next day. For the police field trial, the crime scene investigators also completed a questionnaire (**Fig. S1**), documenting wear duration, physical activity, contact with other people, and any additional clothes worn over the T-shirt.

In the mock *aggressor–victim experiment*, a male donor once applied his DNA by rubbing the front of the T-shirt for five minutes the day after it had been worn by a female laboratory member. DNA was collected immediately afterwards. The experiment was repeated with eight five-minute rubbing sessions, with 30-min breaks in-between [9]. Handwashing was minimized as this has been shown to reduce the amount of cell-free DNA and epidermal cells in sweat and sebum [34]. Written informed consent was obtained from all participants included in the study. The local ethics committee approved the conduct of the outlined experiments (ID: 2025–00643).

2.3. Sample collection: police field trial

The six crime scene investigators who wore the T-shirts also performed the sample collection one day later, with each T-shirt being sampled by a participant who had not worn that particular T-shirt (randomized; Table 1).

Prior to sampling, the crime scene investigators received training in stencil placement, operation of the DNA-Buster, and the contamination-free transfer of pipette tips into the hose of the DNA-Buster. During the collection process, the crime scene investigators wore disposable laboratory coats, disposable head cover, masks, and gloves. The DNA-Buster's hose was wiped with 70% ethanol (Thommen-Furler AG) between sampling activities but not re-tested for DNA residues, as neither the area behind the filter within the tip nor the hose had shown any biological residues in the contamination assessment tests (see below) or previous tests, which is consistent with the intended function of such filters to protect pipettes from contamination. Provided that the hose does not contact the substrate, which should be avoided for any collection tool and was ensured in this study, carryover was not expected. However, as improper handling (e.g., accidental contact with the substrate) cannot be entirely excluded, wiping the hose was included as an additional precautionary measure when vacuuming. DNA was collected from the inside of the T-shirts using a stencil fixed with six clips and aligned with the collar seam (front and back; Fig. 1).

Table 1
Summary of the crime scene investigators who collected the samples (C1–C6) from each T-shirt (T1–T3). The T-shirts were worn by the same persons (P1–P6); however, the individuals who wore the T-shirts were not the same individuals who collected the DNA samples. C4 collected twice the T-shirts of P6 (T1 and T3).

	P1 ♂	P2 ♀	P3 ♀	P4 ♀	P5 ♂	P6 ♂
T1	C3	C5	C1	C6	C2	C4
T2	C4	C3	C2	C1	C6	C5
T3	C2	C1	C4	C5	C3	C4

Using clean gloves, an inverted pipette tip with filter (Brand, Wertheim, Germany; 50–1000 µL, ⌀ 7.9 mm inner diameter, filter pore size of 29 µm) was inserted into the hose of the DNA-Buster. Switching on the device, the sampling area was then vacuumed vertically from top to bottom (Fig. 1, right). The tip was then removed from the hose and stored in a 15 mL Falcon Tube (Greiner Bio-One GmbH, Kremsmünster, Austria) for transport to the forensic laboratory. The person collecting the DNA completed a questionnaire (**Fig. S2**), documenting the date and duration of the collection (in minutes), observations during the DNA collection, and suggestions for improvement of the DNA-Buster.

2.3.1. Sample collection: mock aggressor-victim experiment

DNA was collected from the outside of the T-shirt, both from the back (with no additional male DNA) and from the front where the male had touched the T-shirt (containing additional male DNA). Sampling was performed by the same person for all methods, who was neither a donor nor had direct physical contact with the donors.

Each side of the T-shirt was divided into 36 sections of 16 cm^2 (Fig. 2), and replicates from each sampling method (swabbing, taping and vacuuming, each $n = 12$) were randomly assigned to a section. Taping consisted of twelve lifts per 16 cm^2 section using one tape, moving from left to right and top to bottom within each section (Coloprint GmbH, Hilden, Germany, size of adhesive surface ≈ 2 cm^2). Swabbing involved pipetting 60 µL of nuclease-free water directly on a ForensiX cotton swab (Prionics AG) and rubbing the swab across the T-shirt surface for 30 s with moderate pressure while rotating. For dry-vacuuming, the DNA-Buster was used as described above, with standard filtered Brand tips for 40 s, in a meander pattern across per section once (Fig. 1, right). All samples were then transferred immediately into Investigator Lyse & Spin Baskets (QIAGEN, Hilden, Germany) for same-day DNA extraction. This experiment was repeated once using only taping and the DNA-Buster under the same conditions but with repetitive male DNA application.

2.3.2. Sample collection: 3D-printed tips

DNA was collected from the inside of a T-shirt worn by a female laboratory member. Both sampling methods were randomly assigned to twelve sections of 16 cm^2. First, the DNA-Buster was used with standard filtered Brand tips; then with 3D-printed, translucent polyethylene terephthalate glycol-modified (PETG) tips (Fig. 3, manufactured by OTR-Performance GmbH, Cologne, Germany). The 3D-printed tips were used instead of the brand tips, by inserting them into the hose of the DNA-Buster. PETG is characterized by its high resistance to UV radiation and chemical agents. Each 3D-printed tip contained a circular filter

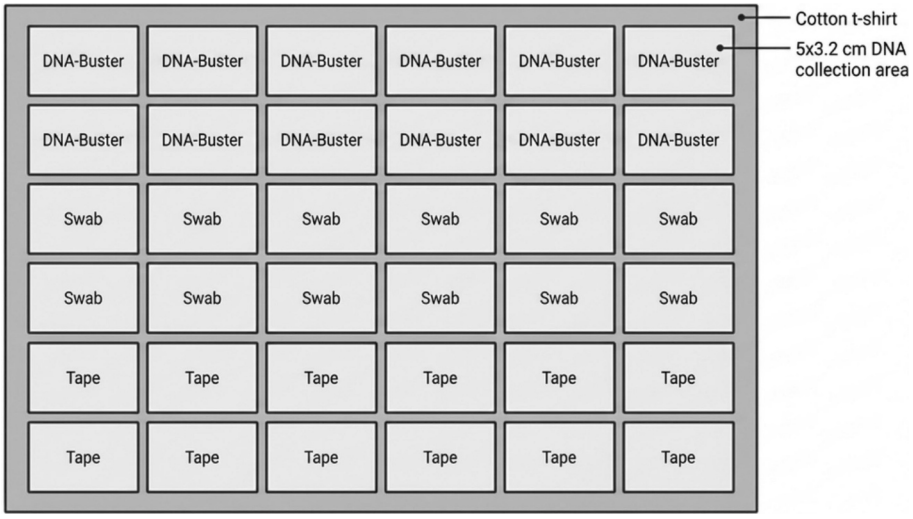

Fig. 2. Exemplar sample allocation to cotton T-shirts for the different recovery methods, with sampling areas sized 5 × 3.2 cm (DNA-Buster, swabbing, taping, each n = 12). The sections were randomized separately across the substrate to minimize bias. Created with BioRender.com.

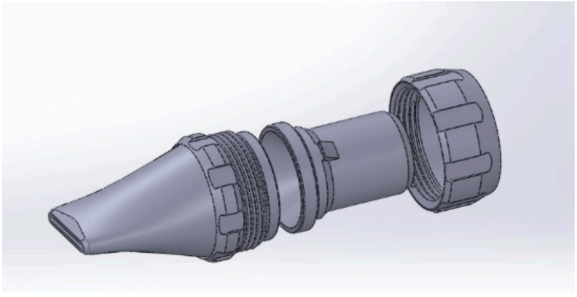

Fig. 3. Computer-Aided Design (CAD) model of the 3D-printed tips. This tip consists of a suction nozzle and an unscrewable attachment to insert and remove the filter. This tip is made of translucent polyethylene terephthalate glycol-modified (PETG). Created on Solidworks by OTR-Performance GmbH, Cologne, Germany.

paper (Whatman Maidstone, UK; Cat. No. WHA1004047, 20–25 µm pore size, 205 µm thickness) cut to a diameter of 18.5 mm and UV-irradiated for at least 20 min to reduce potential DNA contamination. After sampling, the filter was removed with tweezers, cut into three equal pieces, and placed in Investigator Lyse & Spin Baskets (QIAGEN).

2.3.3. Contamination assessment

With respect to contamination, substrate contact occurs only via the filtered pipette tip inserted into the hose of the DNA-Buster, while the device itself cannot be assumed to be DNA-free, similar to routinely handled crime scene equipment. Consequently, contamination assessment focused on the hose and the post-filter area of the pipette tip to verify that no DNA had passed through the filter. For this assessment, the inner side of a worn T-shirt was sampled twelve times using the DNA-Buster as described before. After each sample was taken, the inside of the hose and the post-filter part of the commercially available pipette tip (DNA-free, sterile and forensic grade) were swabbed (ForensiX, Prionics AG). To facilitate swab insertion, the narrow end of the tip was cut with clean scissors for this contamination test. Swabbing was performed by applying 60 µL of nuclease-free water to the swab head and rubbing the area of interest for 30 s. Negative controls included swabbing an unused pipette tip and the hose before any sampling. All swabs were transferred to spin baskets (Investigator Lyse & Spin Basket Kit, QIAGEN) for DNA extraction.

2.3.4. Reference DNA profiles and shedder test

Reference DNA profiles were generated using two buccal mucosa swabs collected from each participant. Sampling of the six crime scene investigators was performed as part of this study. Reference profiles for the forensic laboratory staff members were already available prior to the commencement of the study, thus, no additional analyses were required. When sampled, the investigators were designated as person P1-P6, when they collected biological material, they were designated as collectors C1-C6, with the numbers referring to the same individuals in both roles

Additionally, a shedding test was performed on the six crime scene investigators, following a modified protocol [21]: Each investigator held a commercially available, DNA-free and sterile packed 50 mL Falcon tube (CELLSTAR® Greiner Bio-One, Huberlab, Aesch, Switzerland), using both hands, completely encircling the tube with their fingers, and maintained the grip without movement for one minute. Immediately afterwards, the entire tube was swabbed, using a swab moistened with 60 µL of nuclease-free water. This procedure was repeated six times on the same day with a 45-min break in-between sampling. Normal daily work activities were continued throughout the test period. An additional 20-min waiting period was included following handwashing. P1-P5 were sampled on the same day, while P6 was sampled the following day.

2.3.5. Genetic analysis

The test samples were extracted using the Maxwell® FSC DNA IQ™ Casework Kit (Promega), according to the manufacturer's protocol on solid substrates [35]. For the complete immersion of the respective substrate (tape, filter, or swab head) during lysis, a total lysis volume of 600 µL was used (386 µL Casework Extraction Buffer D103A, 10 µL Proteinase K MC111A, 4 µL 1-Thioglycerol A208B for cell elution and lysis, and an additional 200 µL Lysis Buffer A826G for purification) but with an adapted elution buffer volume of 60 µL. DNA was quantified once using the PowerQuant® System (Promega) on the Applied Biosystems 7500 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol [36] but with internally validated halved reaction volume. Single short tandem repeat (STR) amplification was performed using the AmpFℓSTR® NGM Detect™ kit (Thermo Fisher Scientific) on a Life Technologies Veriti® 96-well thermal cycler, according to the manufacturer's protocol [37,38]. This kit amplifies the following STR marker: D2S1338, SE33, D16S539, D18S51, TH01, D12S391, D3S1358, FGA, VWA, D21S11, D1S1656, D2S441, D8S1179, D19S13, D22S1045 and D10S1248, and Amelogenin.

The reference buccal mucosa swabs were treated with the Swab

Solution Kit (Promega) at 70 °C for 30 min according to the manufacturer's protocol [39], but with an internally validated solution volume of 500 µL. Reference samples underwent dual amplification using the AmpFLSTR™ NGM SElect™ Express Kit (Thermo Fisher Scientific) and the PowerPlex® ESI 17 Fast Kit (Promega), with the latter targeting the same STR markers at different genomic positions.

All PCR products were analyzed using the Applied Biosystems™ 3500xL Genetic Analyzer for Human Identification (Thermo Fisher Scientific). The run parameters were set as follows: run voltage of 15 kV, injection voltage of 1.2 kV and injection time of 24 s.

2.3.6. Data analysis

The quantification results were analyzed using the PowerQuant Analysis Software (Promega, version 4.8.0.0), according to the manufacturer's protocol. Samples declared as undetermined were assigned a DNA concentration of 0 ng/µL. STR profiles were analyzed using the GeneMapper ID-X v.1.6 Software (Applied Bioystems) with default settings and a validated analytical threshold of 50 relative fluorescence units (RFU).

Profile completeness was determined by counting the number of alleles present from the donors (donor alleles) in comparison to their expected profiles. A complete STR profile contained all genotypes of the 16 STR loci, and Amelogenin, assignable to the respective donor, either as a single sourced profile or as the main contributor of a DNA mixture. Partial STR profiles were defined as those with at least one allelic dropout, but with a minimum of four available STR loci. Below this, the results were classified as non-interpretable. Profiles without any alleles did not occur. Profiles with three or more STR loci showing additional non-donor alleles were classified as DNA mixtures, while mixtures with more than four non-donor alleles were defined as complex mixtures [40].

Individuals with unequal DNA contributions in mixed STR profiles were classified as main and minor contributors, respectively, while STR profiles with equal contributions were considered as balanced [41]. For two-person mixtures, the average peak height per contributor was calculated by summing their respective peak heights and dividing by the number of expected alleles per contributor. If the contributors shared a heterozygous allele, only the unshared heterozygous allele was included in the calculation. For unshared homozygous alleles, the peak was included only once. The contributor ratio was then determined by dividing the higher average peak height by the lower average peak height according to [42,43].

The donor alleles were statistically assessed, either as single sources or as the main contributors in mixed profiles. Beyond this, it was checked whether the minor contributor(s) of the mixtures matched another crime scene investigator (e.g., the DNA collector) or could be assigned to any other laboratory staff to identify potential contaminations during operating or subsequent handling.

2.4. Statistical analysis

Statistical analysis and data visualization were performed using R (technical specifications are listed in **Table S1**) [44]. Data on DNA concentrations (ng/µL), the number of single-sourced and (complex) mixed profiles, and profile completeness were visualized as box or bar plots using the *ggplot2* [45] and *ggpubr* [46] packages, and assessed descriptively by calculating the mean, standard deviation (SD), median and interquartile range (IQR) for DNA concentrations and profile completeness, and the absolute frequency of characterized profile types. The mean profile completeness (MPC, %) across the samples was calculated by dividing the average number of reportable and concordant alleles by the total number of alleles present in a known reference profile. For the *mock aggressor-victim* experiment, male profile completeness in the DNA mixture was assessed using only the unshared male donor alleles ($n = 19$) rather than the total number of male alleles ($n = 31$). Alleles shared with the female donor ($n = 12$) were excluded because they could not be assigned unambiguously to either the male or the female donor in the mixed profiles. Accordingly, male profile completeness (male MPC) was calculated based on the 19 unshared male alleles, with detection of all 19 alleles corresponding to 100% completeness. The IQR method was used to identify outliers [47].

The distribution of the data was assessed using the Shapiro-Wilk normality test and Q-Q and density plots. DNA concentrations (ng/µL) from the police field trial were compared using multiple linear regression with the following fixed effects: the person wearing the T-shirt; the person collecting the DNA; whether the sample was taken from the front or back; and the duration of the collection. The assumption that the model's error distribution is normally distributed was met. The DNA concentrations from the *mock aggressor-victim* experiment and *3D-printed tips* experiment were compared using non-parametric Kruskal-Wallis tests, followed by pairwise Dunn tests for post hoc comparisons of the different DNA recovery or collection methods. Normality was assessed for both the raw and log-transformed data using Shapiro-Wilk tests. As the normality assumption was not met for all variables, and given the small sample sizes, non-parametric tests were deemed more appropriate and consistent [48]. Correction for multiple testing was applied according to the Bonferroni method. Profile completeness from the *mock aggressor-victim* experiment was also compared using Kruskal-Wallis and Dunn tests. A significance threshold of p-value $= 0.05$ was used for all statistical tests. For statistically significant results, Hedges' g was calculated as a measure of effect size. As Hedges' g represents a bias-corrected version of Cohen's d, effect sizes were interpreted using conventional benchmarks for standardized mean differences (small ≈ 0.2, medium ≈ 0.5, large ≥ 0.8) as proposed by Cohen [49].

3. Results

3.1. Police field trial

3.1.1. Questionnaire results of DNA application

The mean T-shirt wear duration was 8:37 h (SD = 41 min). None of the crime scene investigators reported engaging in physical activity while wearing the T-shirt. All indicated routine daily contact with colleagues or partners, without any unusual events. A jacket or jumper was worn over the T-shirt in all cases, except P2's third T-shirt, where it was worn alone.

3.1.2. Questionnaire results of DNA collection

Suggestions on handling the DNA-Buster were minimal and concerned only improving the motion by using a slight zigzag pattern to better flatten the T-shirt and using slanted rather than flat tips to improve gliding. Overall, the device was described as simple and intuitive to use, even when used alone.

3.1.3. DNA concentration

Fig. 4 shows the DNA concentrations (ng/µL) per crime scene investigator ($n = 6$) on the front and back of the three independently worn T-shirts. The DNA concentrations ranged from 0.0001 ng/µL to 0.3769 ng/µL. The medians for P1 to P6 were 0.0239 ng/µL, 0.1420 ng/µL, 0.0048 ng/µL, 0.0031 ng/µL, 0.0252 ng/µL and 0.0813 ng/µL, respectively. P2 showed the highest (0.1793 ± 0.1292 ng/µL), while P3 and P4 showed the lowest mean DNA concentrations (0.0047 ± 0.0040 ng/µL and 0.0062 ± 0.0069 ng/µL, respectively). The mean DNA concentrations for P1, P5 and P6 were 0.0535 ± 0.0620, 0.0360 ± 0.0399 and 0.0760 ± 0.0198 ng/µL respectively. Multiple linear regression analysis revealed that the person wearing the T-shirt, and its side (back or front) had a significant effect on DNA concentration ($p = 0.00014$ and 0.01064, respectively).

The difference in DNA concentrations between the front and back of the T-shirt varied by individual. P1, P2, and P4 exhibited consistently higher mean DNA concentrations from the back of their T-shirts (0.0677 ± 0.0748 ng/µL, 0.2795 ± 0.1632 ng/µL, and 0.0087 ± 0.0074 ng/µL)

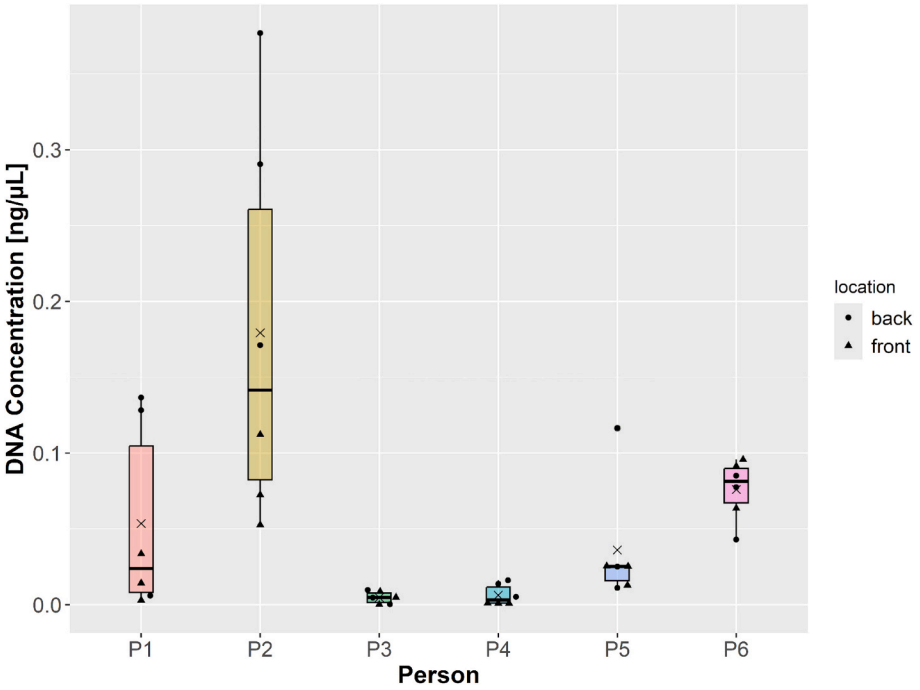

Fig. 4. DNA concentration by person wearing the T-shirt. The boxplots show the DNA concentration (ng/µL) of the six crime scene investigator (P1–P6) wearing the T-shirts, including interquartile range, median, minimum and maximum scores (excluding outliers). The mean is represented by a black cross. The sample size is n = 6 per person (n = 3 from the front and n = 3 from the back of the T-shirts).

in comparison to the front (0.0123 ± 0.0152 ng/µL, 0.0593 ± 0.0466 ng/µL, and 0.0007 ± 0.0004 ng/µL). Conversely, P6 had higher mean DNA concentrations in the front (0.0835 ± 0.0441 ng/µL) compared to the back (0.0513 ± 0.0388 ng/µL), while results from P3 and P5 showed no side-dependent trends. Both genders were equally found in high and low DNA concentrations groups.

In contrast to the T-shirt wearer, the person who collected the DNA did not significantly impact the DNA concentrations (linear regression model: $p > 0.05$). Fig. 5 presents a boxplot indicating that the six crime scene investigators performed with statistically comparable effectiveness when collecting DNA using the DNA-Buster, even though descriptive inspection might suggest differences, such as operator C6 appearing less effective that the others. The median and mean DNA concentrations were as follows: C1, 0.0116 ng/µL (0.0870 ± 0.1199); C2, 0.0294

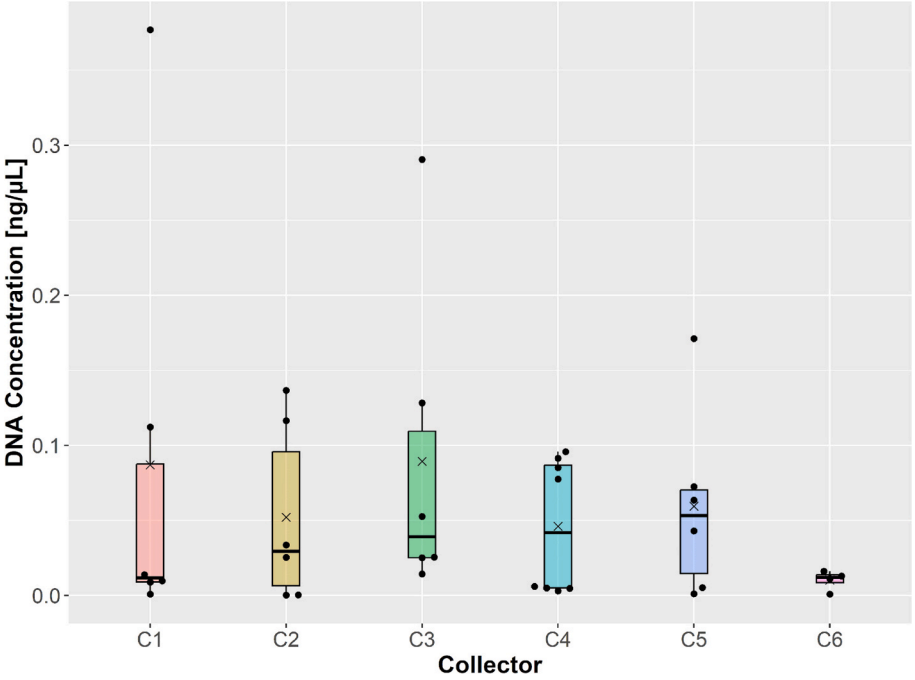

Fig. 5. DNA concentration by person collecting the DNA from a T-shirt worn by a different person. Boxplots showing the six people (C1–C6 who collected the DNA using the DNA-Buster, including interquartile range, median, minimum and maximum scores (excluding outliers). The mean is represented by a black cross. The sample size is n = 6 per person, except for C4 and C6 ($n = 8$ and $n = 4$, respectively).

(0.0520 ± 0.0956); C3, 0.0390 (0.0765 ± 0.0974); C4, 0.0417 (0.0409 ± 0.0839); C5, 0.0532 (0.0593 ± 0.0899); and C6, 0.0120 (0.0102 ± 0.0363) ng/μL. The three datapoints outside the boxplots in Fig. 5 correspond to samples from P2's T-shirts who also showed the highest DNA concentrations in Fig. 4.

The crime scene investigators were instructed to document the duration of each sample collection, which varied from five to eleven minutes, with a mean of 8:20 min ± 1:14 min. The duration of collection did not statistically affect the DNA concentration (linear regression model: p-value >0.05). The average DNA concentrations for the three time slots were 0.0608 ± 0.0606 ng/μL (5–7 min), 0.0640 ± 0.1090 ng/μL (7–9 min), and 0.0525 ± 0.0511 ng/μL (9–11 min) (**Fig. S3**). The lowest variability was observed for C6, which, however, should be treated with caution as the DNA amounts were low and only a limited number of data points were available.

3.1.4. STR profile quality

All DNA profiles from P1, P2, P5 and P6 were complete (**Fig. S4**, n = 6 per donor), while for P3 and P4 three complete and three partial profiles could be obtained, respectively. As can be seen in Fig. 4, these two crime scene investigators had the lowest amounts of DNA on their T-shirts (0.0047 ± 0.0040 ng/μL and 0.0062 ± 0.0069 ng/μL, respectively). P3's partial profiles were 21%, 34% and 97% complete (**Fig. S4**, n = 6 per donor), with corresponding DNA concentrations of 0.0001 ng/μL, 0.0002 ng/μL and 0.0046 ng/μL, respectively (Fig. 4). P4's partial profiles were 59%, 78%, and 89% complete (**Fig. S4**, n = 6 per donor), with corresponding DNA concentrations of 0.0010, 0.0008, and 0.0008 ng/μL, respectively (Fig. 4).

There were ten single-sourced profiles, 20 two-person mixtures, two complex mixtures with a minimum of three contributors, and four 'not interpretable' partial profiles samples which were obtained from P3 and P4 (Fig. 6). One of the mixed profiles was a sample taken from P5's T-shirt that was collected and contaminated by C6, containing the latter's complete DNA profile. The ratio of contributors between P5 and C6 was 1.07, reflecting hence a balanced mixture. The other mixtures showed a clear main contributor, always corresponding to the person who wore the T-shirt, while the minor contributors could not be attributed to any person.

Lastly, the DNA concentrations (ng/μL) obtained from the shedder test are shown in Fig. 7, for the right and left hand of the participants. Due to personal time constraints, P6 performed the shedder test one day later than the other crime scene investigators. The means for both hands combined for P1 to P6 were 0.0075 ± 0.0115, 0.0116 ± 0.0054, 0.0057 ± 0.0041, 0.0024 ± 0.0015, 0.0280 ± 0.0399, and 0.0515 ± 0.0202 ng/μL.

3.1.5. Mock aggressor-victim experiment

Fig. 8 represents the DNA concentrations (ng/μL) obtained with the three collection methods (DNA-Buster, swab, tape). Samples from areas collected from the back of the T-shirt and without additional male touch DNA (light blue) represent the baseline DNA after eight hours of wear. No significant differences were observed among methods (Kruskal-Wallis, $p > 0.05$), although swabbing yielded the lowest concentrations (median 0.0005 ng/μL; mean ± SD 0.0006 ± 0.0005 ng/μL) compared with the DNA-Buster (0.0011 ng/μL; 0.0016 ± 0.0016 ng/μL) and tape (0.0023 ng/μL; 0.0023 ± 0.0014 ng/μL). After explicitly introducing additional male touch DNA (dark blue), swabbing again yielded significantly lower concentrations (0.0018 ng/μL; 0.0017 ± 0.0010 ng/μL) than both the DNA-Buster (Dunn test, $p = 0.0017$, $g = 1.23$) and tape ($p = 0.000021$, $g = 1.61$), indicating large effect sizes. Tape showed the highest values (0.0070 ng/μL; 0.0102 ± 0.0071 ng/μL), followed by the DNA-Buster (0.0041 ng/μL; 0.0089 ± 0.0078 ng/μL), with their difference being not significant (Dunn test, $p > 0.05$, $g = 0.17$), corresponding to a small effect. Across all methods, mean and median concentrations increased following male DNA deposition, as expected.

With respect to DNA profiling, the male MPC was significantly higher for taping than both swabbing (Dunn test, $p = 0.0004$, $g = 2.00$) and the DNA-Buster ($p = 0.0282$, $g = 1.21$), while no significant difference was observed between the DNA-Buster and swabbing ($p > 0.05$) although the effect size indicated a medium effect ($g = 0.60$). The male MPC was 66.67 ± 24.62% when using the DNA-Buster, 51.75 ± 23.09% for swabbing and 92.11 ± 14.97% for taping (Fig. 9). The not-target female DNA was found in all samples, with an MPC ranging from 45.6% to 88.1%, depending on the respective method.

Fig. 10 presents DNA concentrations (ng/μL) from the repeated experiment with repetitive male touch DNA applications using the DNA-Buster and tape. For areas without additional male touch DNA (light blue), concentrations were significantly higher after vacuuming than taping (Dunn test, $p = 0.0179$). In contrast, no significant differences were observed for areas with additional male touch DNA (Kruskal-Wallis, $p > 0.05$). With male DNA present, the mean and median concentrations were 0.0470 ± 0.0283 ng/μL and 0.0406 ng/μL for the DNA-Buster, and 0.0450 ± 0.0412 ng/μL and 0.0359 ng/μL for tape. All samples from both methods yielded complete male DNA profiles.

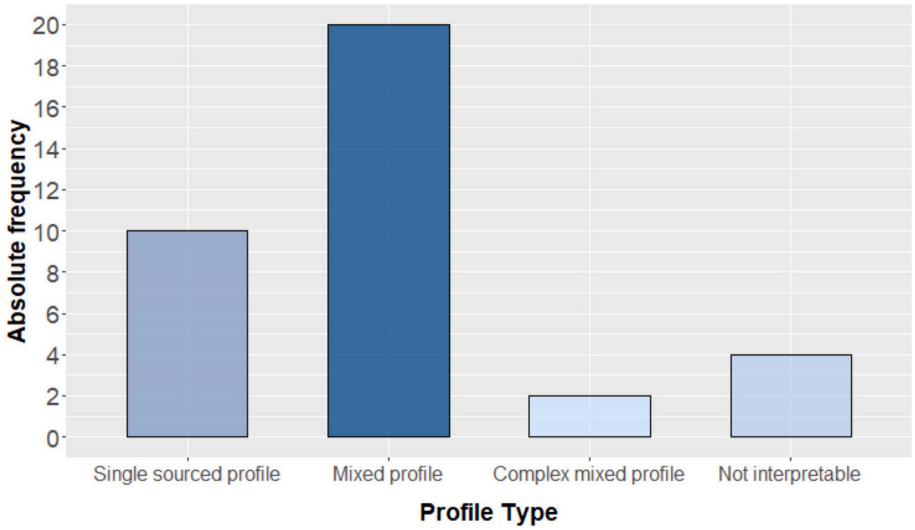

Fig. 6. DNA profile type distribution for the police field trial. Bar chart depicting the absolute frequency of the four profile types. The samples (n = 6 per donor) are distributed into single sourced profiles ($n = 10$), mixed profiles ($n = 20$), complex mixtures (n = 2), and not interpretable profiles (n = 4).

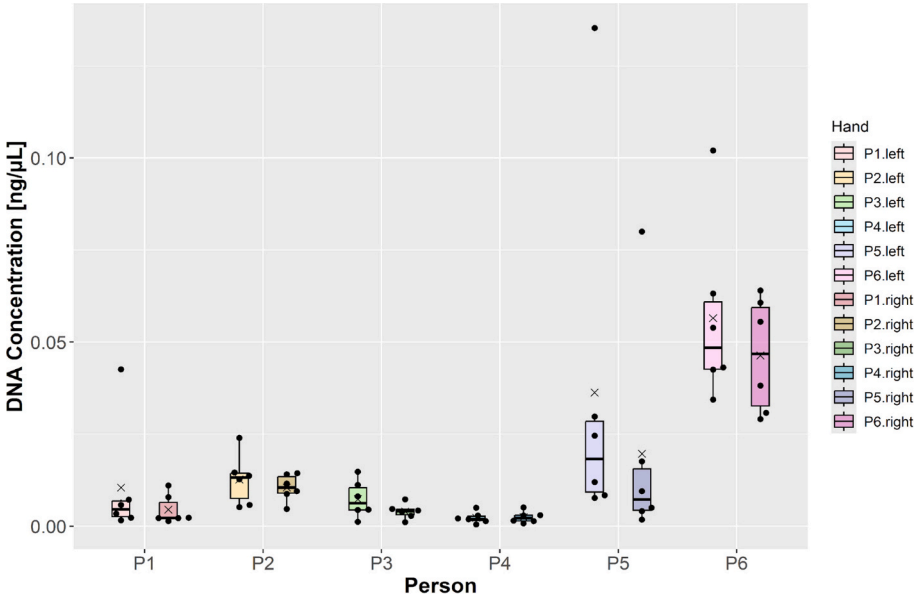

Fig. 7. DNA concentration by person, for the shedder test. Boxplots with the two hands per person (P1-P6) on the x-axis, and the DNA concentration (ng/µL) on the y-axis. The boxplots show interquartile ranges, medians, and minimum and maximum scores (excluding outliers). The mean is represented using a black cross.

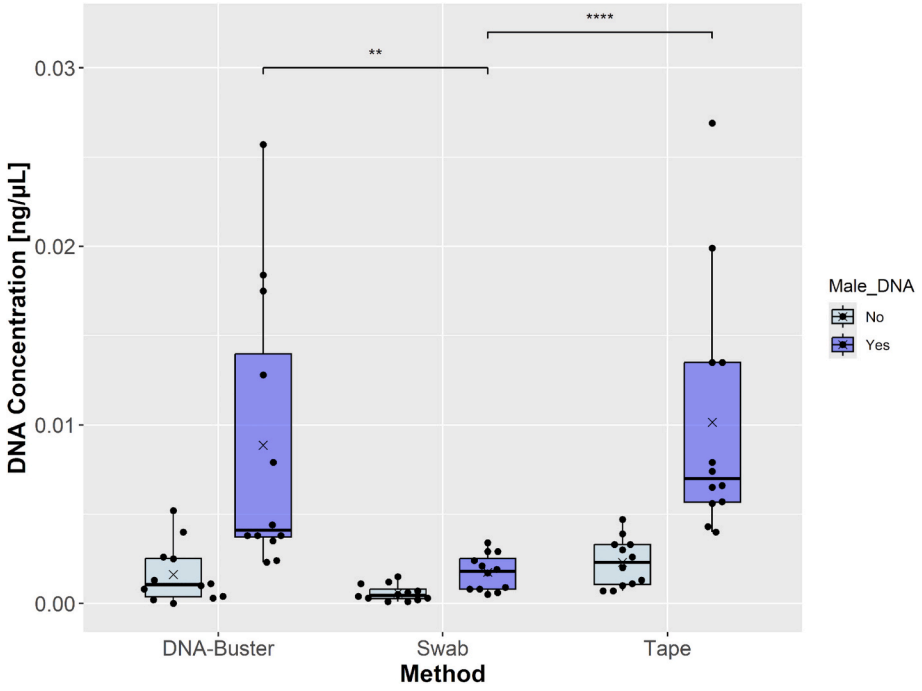

Fig. 8. DNA concentration by method, with and without additional male touch DNA applied once for five minutes. Boxplots with the method of DNA collection on the x-axis, and the DNA concentration (ng/µL) on the y-axis. The boxplots show interquartile ranges, median and minimum and maximum scores (excluding outliers). The mean is represented using a black cross. The sample size is $n = 24$ per method ($n = 12$ without male DNA, light blue; n = 12 with male DNA, dark blue). The sampling method with significantly different DNA concentrations compared to the other method/s is indicated with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.1.6. 3D-printed tips

The DNA concentration (ng/µL) when using the 3D-printed tips and the standard Brand tips are shown in Fig. 11. Three datapoints, identified as outliers by the IQR method, were removed for the following analysis to improve readability of the central data distribution, as the extreme values compressed the y-axis. The plot containing the corresponding outliers can be found in **Fig. S5**. The mean and the median were 0.0041 ± 0.0016 ng/µL and 0.0043 ng/µL for the 3D-printed tips, and 0.0079 ± 0.0026 ng/µL and 0.0080 ng/µL for the normal tips. Using the standard tips resulted in a significantly higher DNA concentration (Kruskal-Wallis: p = 0.0017, g = 0.67), representing a medium to large effect.

3.1.7. Contamination assessment

Of the 26 negative control samples, 21 showed no detectable autosomal DNA during quantification. The remaining five samples contained minute amounts of DNA (0.0001 to 0.0002 ng/µL). To check for contamination, these samples were further genotyped, and no DNA

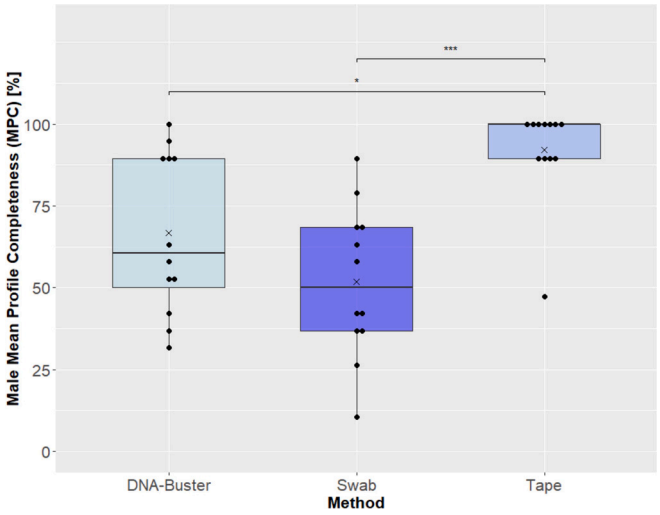

Fig. 9. Male mean profile completeness (MPC) per method. Boxplots with the DNA collection method on the x-axis, and the male MPC on the y-axis. The boxplots show interquartile ranges, median and minimum and maximum scores. The mean is represented using a black cross. The sample size is n = 12 per method. The sampling method with significantly different male MPC compared to the other method/s is indicated with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

profiles were obtained.

4. Discussion

4.1. Police field trial

The DNA-Buster, which can be constructed according to [10], has previously demonstrated promising performance in collecting DNA from textiles, outperforming the swabbing method. However, these experiments were conducted by experienced staff already familiar with the device, rather than by crime scene investigators as the intended end users.

After minimal training, the crime scene investigators found the DNA-Buster intuitive to use, achieving statistically comparable DNA concentrations and collection times across participants. Collection times were not deliberately specified, as the aim was to evaluate whether collection duration correlates with DNA recovery, and to determine the time span the crime scene investigators would intuitively consider appropriate for balancing feasibility and time constraints with maximal material collected. As neither collectors nor collection time significantly influenced DNA concentrations, despite some descriptive trends, it can be assumed that either all available material had already been collected, or the filter's capacity had been reached. The latter warrants further investigation to determine how frequently the filtered tip should be replaced, particularly when sampling larger surfaces. However, expanding the sampled area, regardless of the sampling method, may result in complex DNA mixtures, that are more challenging or even impossible to interpret. The same applies to combining several tapes, swabs or filters; here up to four filters could technically be pooled in one extraction tube. Before pooling or sampling larger areas, subsampling a test area may help to estimate the number of contributors; however, this may consume or compromise potentially relevant evidence. In addition, the complexity observed in such a test may not necessarily reflect that of subsequent sampling, even in directly adjacent areas. Alternatively, targeting several smaller areas may be preferable when a high number of contributors is expected, although this comes at the risk to yield (too) low DNA amounts, while increasing time and resources. In all cases, the true number of contributors is inherently unknown and can only be inferred.

Based on the evaluated median collection time of eight minutes and a surface area of 344 cm^2, approximately 43 cm^2 can be sampled per minute with the DNA-Buster. However, it remains unclear whether shorter (< 5 min) or longer (> 11 min) sampling times affect the overall

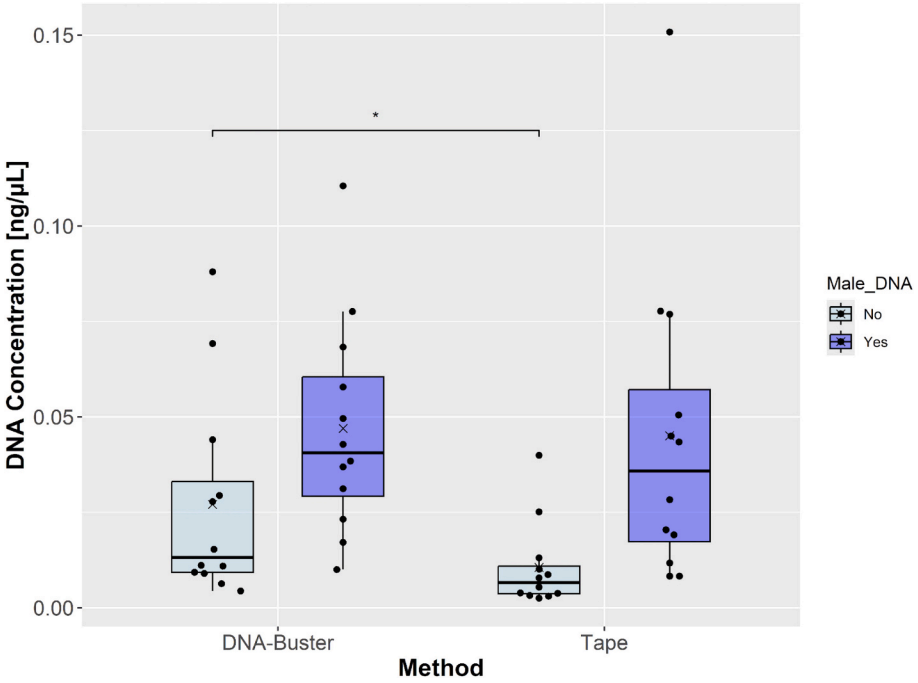

Fig. 10. DNA concentration by method, with and without additional male touch DNA, repetitively applied. Boxplots with the method of DNA collection on the x-axis, and the DNA concentration (ng/µL) on the y-axis. The boxplots show interquartile ranges, median and minimum and maximum scores (excluding outliers). The mean is represented using a black cross. The sample size is n = 24 per method (n = 12 without male DNA, light blue; n = 12 with male DNA, dark blue). The sampling method with significantly different DNA concentrations compared to the other method/s is indicated with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

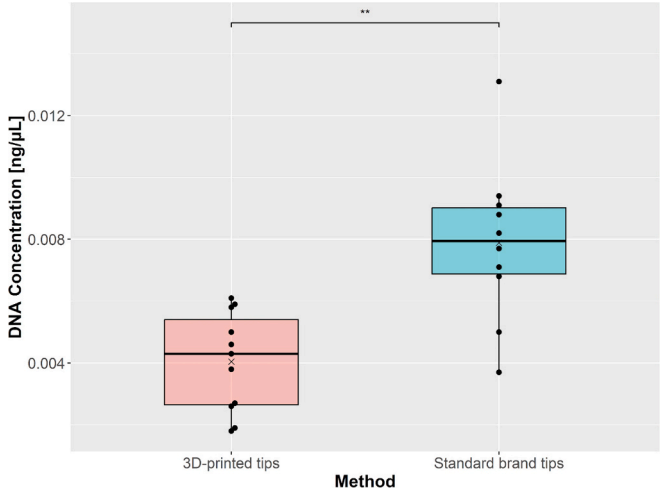

Fig. 11. DNA concentration by method. Boxplots with the method of DNA collection on the x-axis, and the DNA concentration (ng/µL) on the y-axis. The boxplots show interquartile ranges, medians, and minimum and maximum scores (excluding the outliers). The mean is represented using a black cross. The initial sample size was n = 12 per method; after outlier removal, $n = 11$ for the 3D-printed tips and n = 10 for the standard Brand tips remained. The significant difference between the DNA concentrations is indicated with asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

collected amounts of touch DNA. One could hypothesize that a very short collection duration may not recover enough touch DNA for successful profiling, whereas prolonging sampling beyond 11 min is unlikely to increase collected biological material, as the available material has likely been fully recovered.

The occurrence of partial profiles and mixed DNA profiles from touch DNA samples was generally expected based on the literature [41,50,51]. In this study, partial profiles were only observed in 50% of the samples from P3 and P4, who also showed the lowest DNA concentrations (see next section). While the main contributors of the mixtures consistently corresponded to the wearer of the T-shirt, the detected minute amounts of foreign DNA could not be assigned to a police or lab staff. Since the crime scene investigators followed their daily routines, and interacted with colleagues and partners, a secondary transfer could be assumed, being consistent with previous studies on DNA transfer [52] and background DNA in shared environments [53,54], while in this case, the DNA must have been even transferred to the inner side of the T-shirt. In general, potential background DNA was not controlled, for example by collecting 'environmental' samples from the respective working areas, an aspect that could be addressed in future studies. Furthermore, as clothing was worn over the T-shirt in all but one case (P2's third T-shirt), foreign DNA may also have originated from these overlying garments, which are likely to have come into contact with DNA from partners or co-workers.

The clear contamination observed in P5's T-shirt sample, however, was identified as a 50:50 mixture with biological material from the collector, C6. The contamination could be due to an isolated handling error by the collector (e.g., inadequate mask use or glove replacement), despite their in-depth knowledge of evidence handling and the overall low contamination risk with the collection procedure and the device itself. Another possible explanation is that P5 and C6 came into direct contact on the day of the experiment. Interestingly, given C6's notably high DNA concentrations in both the shedder test and the police field trial (see P6), it appears more plausible that contamination originated from this high shedder rather than from other crime scene investigators.

4.2. Shedder impact

Comparing DNA concentrations from the police field trial and the independent shedder test revealed discrepancies in individual DNA deposition patterns, particularly for P2 and P6. In the police field trial, P2 exhibited the highest DNA concentrations followed by P6, whereas in the shedder test, P6 produced the most DNA, followed by P5. In contrast, P2 yielded less DNA than one could have potentially expected based on the field trial outcome, while still showing higher DNA amounts than P1, P3, and P4. These differences may result from distinct experimental setups, e.g., deposition times or body parts involved in the two experiments (upper body vs. hands), as DNA shedding has been shown to vary by anatomical location [55,56]. For instance, Oleiwi et al. [56] demonstrated that fingers and palms differ in DNA shedding, possibly due to the varying thickness of the stratum corneum, while Zoppis et al. [55] showed variable activity of sebaceous glands. However, sebaceous glands are absent on palms but occur in higher densities in seborrheic regions, such as the back [57], potentially explaining P2's elevated DNA concentrations in the police field trial, if their sebaceous gland activity was comparably higher than that of the other crime scene investigators'. Further, the independent shedder test was conducted during a heatwave in mid-summer, when increased perspiration can be expected and has been shown to enhance DNA deposition [52,55,58]. As the fingers have the highest eccrine gland density [59], P6's increased sweating from the hands could explain the elevated yields observed during the shedder test. However, as P6 was sampled a day later than the colleagues, it could also be possible that the environment (e.g., temperature, humidity) was different that day.

Although direct comparison between the two experiments was not the study objective, the discrepancies underline a known and important point: shedding varies not only between individuals but also within the same person [60]. Variability is said to be influenced by numerous factors such as activity [52,55,58], environmental conditions (e.g., humidity, temperature) [61], age [62], gender [34,62], hormones [61], body part [55,56], and skin diseases [63]. Frequent contact with objects or other body parts (e.g., face) can further increase the amount of DNA transferred to and accumulated on hands [27]. Consequently, there is no universal or standardized method for assessing and categorizing shedder status. While some studies classify individuals based on DNA concentration [64,65], others use profile completeness thresholds [21,66]. As shedding remains rather an estimation than a fixed status, the participants' shedding was tested accordingly, with notably different tendencies between and ranges within individuals. Here, further improvements to the shedder test became apparent, including greater standardization (e.g., same localization), repeated sampling on multiple days to assess intra-individual variability, documentation of environmental parameters (e.g., temperature and humidity), and recording participant activity. Such standardization could also help to reduce overall heterogeneity, as also demanded for other DNA procedures (e.g., extraction, quantification [67]), and has not only been recommended but also described as challenging for touch DNA research [68,69]. On the other hand, such in-depth and highly controlled measures may reduce feasibility and still fail to account for variables such as applied pressure and contact motion (e.g., angle of rubbing or pressing).

Interestingly, no effect of hand dominance was observed, a topic controversially discussed in literature [60,64]. Notably, Phipps and Petricevic showed that dominant hands shed more DNA in one study but less in a subsequent large-scale study [60], whereas Goray et al. [64] noticed no difference, which is in line with our findings, as all participants were dominantly right-handed. Overall, despite certain limitations, the shedding trends for four of the six crime scene investigators (P1, P3, P4, P5) remained consistent across experiments, indicating that shedding tendencies were sufficiently stable for the purpose of the study and the underlying conditions.

As mentioned above and reported in [55,56], discrepancies between body regions were also observed between the back and chest in our study: P1, P2, and P4 exhibited higher DNA concentrations from the back than from the front of their T-shirts, while P6 showed the opposite. While differences in location can explain the deviating pattern for P6, it

cannot be entirely excluded that, although unlikely, P6 wore the T-shirt the wrong way round or that the front and back samples were inadvertently switched.

Considering previous reports of higher shedding in males [34,62], no clear gender-related trend was apparent in our study, as both male (P1, P6) and female (P2, P4) crime scene investigators represented the highest and lowest shedders. A larger sample size may be necessary to detect potential gender effects on DNA deposition. However, factors such as body movement during wear could also play a role, as contact pressure (e.g., sitting or leaning against a chair) may increase DNA transfer to the T-shirt when compared to a person who spent more time standing, which was not monitored in this study. In this case, it should be noted that P2 was the only person to wear one of the three T-shirts without a jacket or jumper over the top. Even though only the samples of one shirt were affected, this could, in principle, also impact DNA concentrations by accumulating more background DNA.

4.3. Mock aggressor-victim experiment and substrate dependency

With respect to total DNA, the approximately fivefold higher performance of the DNA-Buster and adhesive tape compared to swabs on cotton T-shirts is consistent with previous findings by Währer et al. [10], who observed significantly lower recovery rates with swabs than with the DNA-Buster, sampling also cotton T-shirts. The limited efficacy of swabs on textiles, compared to taping, is already documented in the literature, and may be attributed, for example, to the tightly wound swab head, restricting access to biological material trapped within fabric cavities [9,70] resulting potentially in inefficient DNA release during extraction due to strong DNA binding within the swab fibers [71], or to cell lysis caused by pre-moistening, which can lead to DNA retention inside the swab matrix [72,73]. Furthermore, an increased uptake of textile dyes and finishing chemicals when swabbing may act as PCR inhibitors [7,13,14], although the robustness of current commercial kits [74,75] likely calls this hypothesis into question. Nevertheless, according to internal communication, some police forces so far still use swabbing for textiles evidence, despite its documented risk of reduced collection efficiency and, consequently, lower profiling success; an unfortunate gap that underscores the importance of effective communication between academic research findings and law enforcement practice.

As expected, the mean DNA concentration increased with repeated touching events (eight times instead of once), with approximately fivefold and fourfold rises in DNA amount for vacuuming and taping, respectively. For both approaches, complete STR profiles could be achieved, comparable to those in [10], even though different donors were used. In contrast, touching the T-shirt once yielded considerably lower DNA concentrations for both approaches, but still showed improved DNA recovery compared to swabbing. However, in the single-touch application experiment, male profile completeness showed weak statistical significance between taping and vacuuming. One possible explanation is an incomplete surface coverage during vacuum sampling. Compared with the larger adhesive surface of the tape, the smaller diameter of the pipette tip may increase the risk of bypassing areas, particularly because touch DNA is invisible, and sampled regions are not visibly altered. Applying a second vacuuming pass perpendicular to the initial sampling direction may therefore improve surface coverage. This hypothesis is supported by Währer et al., who reported comparable efficiency of vacuuming and taping after rotating the substrate by 180° for a second sampling pass [10]. Hence, changing the sampling direction may help to reduce blind spots caused by missed areas or insufficient contact between the pipette tip and the substrate for effective aspiration.

Limited user familiarity seems unlikely to fully explain the observed difference, as users reported the device to be easy to operate with minimal instruction. However, compared with adhesive tape, which may be more tolerant of minor handling inconsistencies, vacuum sampling may still be more sensitive to operator technique. This should be re-investigated after increased user experience, and before investigating the performance under mobile, on-scene conditions. An intrinsically lower performance of vacuum sampling compared with taping appears less likely when also considering previous findings [10] and the results of the repetitive experiment. In the latter, vacuum sampling performed better than taping in female-only DNA areas and comparably in mixed DNA areas with higher overall DNA input. Therefore, the observed difference in the single-touch experiment may also be explained by stochastic effects, which are typically more pronounced at very low DNA amounts.

Currently, swabbing remains the predominant collection technique among Swiss law enforcement authorities, likely due to its simplicity, low cost, and long-standing familiarity [10]. As demonstrated in this study, using taping or dry vacuuming instead of swabbing could enhance DNA profiling success by increasing DNA recovery from textile substrates. Considering the alternatives, adhesive tapes efficiently collect DNA, are simple and cost-effective [14,76], and do not require a powered device. In contrast, the DNA-Buster requires an initial device investment, but per-sample costs are very low (i.e., one filtered pipette tip). With respect to handling, tapes may lose adhesiveness during repeated tape lifts, complicating sampling of larger areas and potentially leading to DNA redeposition [14]. Taping also places the caseworker's gloved hands close to the sample, increasing the risk of DNA transfer [23]. In addition, laboratory processing can be cumbersome, as tapes often need to be cut to fit in extraction tubes and are difficult to handle due to stickiness and limited flexibility. However, tape-processing could also be automated, thereby reducing cutting and manual handling steps. Compared with taping, the main advantage of the DNA-Buster remains the reduced amount of direct contact required between the user and the substrate, thereby lowering the contamination potential. Nevertheless, the pipette tip still needs to be handled, for example when transferred from the pipette tip box into the hose, and contamination can never be entirely excluded. However, the risk is considered low, as the filter within the tip remains protected and, during laboratory processing, is simply pushed from the tip into the reaction tube using forceps, without the need for cutting.

For the DNA-Buster, the continuous suction recovers comparable biological material regardless of the operating time up to eleven minutes, using one filtered tip. The narrow tip attached to the flexible hose permits access to confined areas, such as the crease of a car seat, that are difficult to sample with tape. Feedback from the police indicated that, for a mobile application, however, the hose should be secured during use, as it may otherwise come into unintended contact with objects or persons in the surroundings and thereby compromise sample integrity. This observation highlights the value of operational testing with end-users to identify practical and important improvements.

While consistent with previous laboratory findings for the DNA Buster [10], the present police field trial was conducted at a small-to-intermediate scale and limited to cleaned clothing items without residual background DNA. Future studies could therefore include larger sample sizes and uncleaned garments to better reflect realistic conditions and assess potential limitations of vacuum-based sampling not apparent here. On the other hand, preparing worn garments with homogeneous biological material across a larger number of samples for comparative testing of different collection approaches remains highly challenging. Overall, this study demonstrates that swabbing is less effective for textile substrates, whereas both taping and the DNA-Buster represent suitable and comparably effective alternatives.

4.4. 3D-printed tips and further optimization steps

Further feedback from the crime scene investigators suggested optimizing the pipette tip for smoother motion on the substrate. 3D-printed tips were therefore designed and tested, enabling rapid prototyping of complex geometries not readily achievable with conventional manufacturing methods (e.g., injection molding [77]). Glycol-modified

polyethylene terephthalate (PETG) was selected due to its translucency and resistance to chemical and UV light, allowing decontamination with 70% ethanol and UV exposure.

The printed 3D tip aimed at smoother movement on the substrate surface, however, it yielded considerably lower DNA concentrations than the standard tip and required cutting, potentially introducing contamination. As only one design has been tested so far, alternative geometries or materials could be explored to potentially improve recovery from surfaces besides textiles.

The distance between the tip opening and the filter was reported as another critical factor, as greater distance may increase material loss to the rim of the tip [10]. A shorter filter-opening-distance design could therefore improve material recovery. Further modifications could include adjusting suction nozzle dimensions or filter properties (e.g., thickness, pore size).

5. Conclusion

The study demonstrated that the DNA-Buster is an effective, intuitive tool for crime scene investigators yielding comparable results in DNA quantity and quality to tapes and outperforming conventional swabbing techniques. If implemented in police routine for stationary use on textiles, approximately 7% of crime evidence in our catchment area could be improved. By increasing DNA recovery, its use may contribute to an overall higher profiling success compared to currently applied swabbing and, consequently, support improved case resolution when working with textile-based evidence. Further development will extend studies for substrates other than textiles, as well as establish alternative tip designs and find a practical solution for securing the hose in preparation for intended mobile applications. The current DNA-Buster version will next be used in routine-like casework by crime scene investigators, using a bidirectional meander sampling approach to ensure surface coverage. Testing under less controlled, real-world conditions will provide further insight into its performance and enable potential optimization based on end-user feedback prior to routine implementation in forensic services.

To improve shedder assessment, potential standardization should be addressed. Monitoring environmental conditions and conducting repeated sampling of larger study cohorts over time might help to better understand inter- and intraindividual variability in DNA deposition and may increase awareness of high shedders when handling trace DNA amounts.

Finally, the study outcome once again highlights the importance of substrate-specific collection strategies. The collection step represents the first and most critical stage in recovering the full extent of biological material present on a substrate, thereby increasing overall profiling success. This consideration is particularly relevant for law enforcement authorities that, for various reasons, continue to rely on a single collection method, such as swabbing. Here, strengthening communication between forensic researchers and law enforcement and offered training programs remain essential to translate laboratory-gained insights into operational practice.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used DeepL and ChatGPT (OpenAI) to improve readability and language in specific sections. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

E. Sohrmann: Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis. **J. Schulte:** Writing – review & editing, Visualization, Validation, Software, Project administration, Investigation, Formal analysis. **S. Kron:** Writing – review & editing, Validation, Project administration, Methodology, Conceptualization. **A. Schocker:** Writing – review & editing, Resources, Methodology, Conceptualization. **O. Eidam:** Writing – review & editing, Methodology. **I. Schulz:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors want to kindly thank the DNA-Buster testers Mischa Sigrist, Till Meyer, Michela Zoia and Cora Giovanelli from the police force.

Ethical approval and informed consent

The study was approved by the ethics committee northwest/central Switzerland (BASEC-ID: 2025–00643) and conducted with the ethical standards in accordance with the Declaration of Helsinki at the Institute of Forensic Medicine of the University of Basel. Written informed consent was obtained from all participants included in the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2026.101485.

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