

Research paper

The DNA-Buster: The evaluation of an alternative DNA recovery approach

Jonathan Währer^{a,1}, Sabrina Kehm^{a,1}, Marie Allen^b, Linnéa Brauer^b, Oliver Eidam^c,
Ilona Seiberle^a, Sarah Kron^a, Eva Scheurer^a, Iris Schulz^{a,*}

^a Institute of Forensic Medicine, Department of Biomedical Engineering, University of Basel, Switzerland

^b Department of Immunology, Genetics and Pathology, Uppsala University, Sweden

^c OTR-Performance GmbH, Cologne, Germany



ARTICLE INFO

Keywords:

Dry-vacuuming
Swabbing
Taping
Wet-vacuuming
Performance comparison
Full DNA profile

ABSTRACT

Touch DNA recovery techniques can have limitations, as their effectiveness depends on the substrate on which the DNA of a person of interest can be found. In this study, an in-house dry-vacuuming device, the DNA-Buster, was compared to traditional methods for its DNA recovery performance from items typically examined in forensic casework. The aim was to evaluate whether this dry-vacuuming approach can recover DNA efficiently, potentially complementing the well-established recovery strategies. For this, the performances of swabbing, taping, wet- (M-Vac®) and dry-vacuuming (DNA-Buster) were investigated quantitatively and qualitatively for touch DNA deposited on carpet, cotton sweater, stone, tile and wood. For the sweater, both vacuuming methods outperformed the other collection tools quantitatively. While the highest DNA amounts for the carpet were yielded by swabbing and taping, dry-vacuuming was equally good in reaching full DNA profiles, whereas less complete profiles were observed for the M-Vac®. For stone and tile, swabbing was optimal, whereas dry-vacuuming clearly underperformed for these substrates. Taping was the best recovery method for wood. Despite applying single donor DNA after thoroughly cleaning the items, undesired DNA mixtures were detected for all recovery techniques and all substrates. The overall research findings show first that the novel dry-vacuuming method is suited for DNA recovery from textiles. Secondly, they indicate that more attention should be paid to the substrate-collection dependency to ensure best practices in recovering genetic material in a precise, confident and targeted manner from the variety of forensic casework material.

1. Introduction

While mainly body fluids like blood and saliva were investigated in the past, nowadays, touch DNA is a significant evidence type that has grown in use in forensic laboratories, and the forensic investigations they support [1,2]. At a crime scene, these invisible traces are collected from items assumed to have been in contact with, besides other biological material, all kinds of skin surfaces [2,3], leaving behind a group of different material, such as cell-free and residual DNA, nucleated cells, and anucleated corneocytes [2]. For these DNA samples, the recovery process is usually "blind" and often associated with sparse amounts of genetic material, limiting its short tandem repeat (STR) profiling success for a potential match to a person of interest (i.e., suspect). Unlike body fluids, which are mostly visible and contain much larger DNA amounts, the sampling strategy requirements for this type of sample are more demanding in order to increase the recovery efficiency as much as

possible. In this context, we use the term "touch DNA", referring to the study's mock samples, prepared by skin contact with defined objects, i.e. with known source and action [4].

This initial step, the efficient recovery of human biological material from a substrate, is determinative for the subsequent STR profiling success. For the collection approach, different techniques such as single or double swabbing with various swab types, tape-lifting, scraping, cutting or using the complete evidence material, and vacuuming have been compared to each other's power to recover appropriate genetic material [1,5–17]. Mostly swabs with cotton, rayon or nylon heads are used for non-porous traces [9], rubbed in a circular motion over a spot where the forensic scientist or police operator suspects human DNA. Porous evidence material is preferred to be handled with tape-lifting or stamping with a DNA stub [13,18], both using adhesive foils for trace recovery. Nevertheless, sampling practices are often based on personal experience, familiar usage and/or historical reasons than on scientific

* Correspondence to: Institute of Forensic Medicine, University of Basel, Health Department, Basel-Stadt, Pestalozzistrasse 22, CH-4056 Basel, Switzerland.

E-mail address: iris.schulz@unibas.ch (I. Schulz).

¹ These authors contributed equally to this work.

recommendations. This is not surprising since various factors, besides collection tools, influence the subsequent profiling success, e.g., DNA extracting platforms or reagents. Hence, comparing different methods' efficiencies is complicated, making it delicate to define a golden sampling approach. Optimally, a full DNA profile of currently 16 STRs should be sought to evaluate a potential match with a subject of interest.

Further obstructive aspects to successful DNA acquisition are more practical but equally important. For example, the collection of biological material can be physically challenging to reach (e.g. ceilings), suspected in poorly accessible areas of an object (e.g. the backing fabric of a carpet with long fibers) or on substrates that cannot be effortlessly transported to a laboratory (e.g. car seats) to allow an appropriate and contamination-free sampling procedure. Thus, trace accessibility itself can limit its collection efficiency, besides a given tool's recovery capacity [17]. Moreover, for transportable objects, every additional handling increases the risk of contamination by the operator, whose DNA might be accidentally transferred. Finally, relocating the evidence can also change the distribution of a trace on an item or reduce the original trace amount [19], potentially losing it inside a particular transport bag, as recently published [20] and also demonstrated in internal casework. Thus, the less a trace is moved, the more likely it is to find the original DNA present on the item and in its initial position.

From experience, standard techniques can be time-consuming, especially when examining large objects, for example, searching for foreign DNA on a victim's suit using the tape-lifting technique can take an entire day. Next, the sampling circumstances outside a laboratory are usually not optimal for trace recovery (e.g., poor light and/or weather conditions). Further, the sampling is often combined with high time pressure in view of potential environmental DNA modifications or loss and - depending on the severity of the crime - due to urging public pressure to solve the case. The aforementioned factors can also increase the potential for contamination or loss of DNA. Accordingly, an efficient and mobile method at the crime scene could improve DNA recovery and accelerate thereby the overall investigative progress.

To overcome some limitations of traditional approaches in securing forensic evidence, the Microbial Vacuum Wet-Vacuum System (M-Vac® Systems, Inc., Sandy, UT) was deployed [14]. Compared to conventional methods, equivalent or better performance was observed for specific surfaces such as porous material [12] or fabrics with long fibers [12,15,21,22]. However, the instruments' method of spraying a buffer solution onto the surface of interest causes aerosols to disperse in the sampling area [14,15]. Due to this so-called "mini-hurricane" [23], DNA has spread from the original area up to 10 cm away [15]. Furthermore, when traces from different contributors are close to each other, originally unmixed DNA can be mixed during sampling, distorting the integrity of the trace [14]. When compared to swabs and tapes, an increase of undesirable DNA mixtures of fictitious aggressor and wearer was observed [17,22] and recently confirmed when retrieving DNA from a T-Shirt [24]. And unfortunately, a large buffer volume is required for trace uptake, leading to an extra concentration step, possible cell loss and osmosis of cells due to different salt concentrations, as well as uncontrolled lysis [12].

Recently, another vacuum system was described, invented explicitly for non-destructive DNA recovery from handwritten documents [25]. This method utilizes a moistened cotton swab inserted into a glass tube connected to a benchtop vacuum system. In principle, this approach makes it possible to collect DNA from copy paper. Unfortunately, only 50% of the manila envelopes examined gave sufficient DNA quantities, and the yield for magazine pages and bank deposit slips was insufficient for DNA typing. Another disadvantage of this method is that swabs and glass tubes must be trimmed to fit the swab into the glass tube without gaps to ensure proper sampling. On the other side, the vacuuming did not negatively affect the fingerprints, which have a different probative value in crime-scene investigations [25]. In routine police investigations, though, such dactylograms are primarily secured before DNA collection [26], potentially solving the associated crime directly.

An adapted alternative technique could counterbalance the drawbacks of both existing vacuuming systems while making more traces better accessible. For this purpose, the DNA-Buster was developed and intensively tested in comparison to conventional methods. Besides its performance, the alternative method aimed to be mobile and simple. Mistakes arise more easily whenever an application's method or technique is complex or deviates too much from standard processes [27]. Also, operators are more likely to handle new technologies with confidence if they are understandable and implementable. For this reason, the collection tool was designed as a regular vacuum cleaner without special training requirements, with size and weight similar to a cordless screwdriver.

The study's goal was to compare the existing touch DNA recovery techniques to the new dry-vacuuming method on a variety of forensically relevant substrates, potentially complementing the established collection strategies. Especially for non-transportable and poorly accessible evidence, the DNA-Buster could help police and laboratory investigators to collect also challenging samples in a precise, confident and targeted manner.

2. Materials and methods

2.1. The DNA-Buster

The in-house DNA-Buster prototype utilizes a Boxer® 3KQ diaphragm pump powered by a Bosch GBA 12 V 3.0 Ah battery. A pipette filter tip is inserted into the hose for DNA collection and serves as a recovery vessel. During sampling, the material is then sucked onto the filter of the tip by turning on the vacuum pump and can be retrieved by transferring the filter into a testing tube for DNA extraction (Fig. 1).

Previous research showed that the choice of the inserted filter tip construction is a crucial step and heavily affects the efficiency of the device (Table S1). The ideal filter tip for the DNA-Buster has a minimal filter-to-opening distance and the capability to maintain a high airflow rate. A specific low distance ensures that less material sticks to the inner rim of the pipette tip, which otherwise would be inaccessible for the lysis process. A larger filter pore ensures a high airflow rate, which must still be small enough to retain cell-free DNA. In our study, Brand Filter Tips [29] were the most efficient as they provided a low filter opening distance while maintaining the highest air flow among the compared tips (data not shown).

The collected biological material adheres to the filter inside the pipette tip, thereby being protected from outside contaminations. Thus, the DNA recovery process of the DNA-Buster may be less prone to contamination in comparison to standard DNA recovery methods such as an "unprotected" swab head for which unintended touches can be possible.

2.2. Optimizing the DNA-recovery

A natural conclusion would be that increasing the airflow will subsequently result in a higher DNA yield. However, changing to a more powerful pump would increase the device's size and weight, therefore sacrificing mobility. In order to assess the extent to which an increase in air flow would benefit the vacuuming power, the DNA yield after recovery with five different airflow levels was compared. For maximizing the airflow, the Boxer® 3KQ diaphragm pump was coupled in parallel to a Thomas® G07-15-Watt rotary vane pump. The pneumatic circuit diagram of the experimental apparatus is displayed in Fig. 2.

Touch DNA was collected from cloth (86% cotton, 14% polyester) to assess the recovery efficiency. As three pieces of cloth were prepared, a total of nine replicates could be analyzed for the DNA-Buster at maximum airflow and six replicates for each airflow-gradation. Then, touch DNA was applied as described in the section *Application of Touch DNA*. An exemplary allocation of the recovery areas for one piece of cloth and the used recovery methods are displayed in Fig. 3.

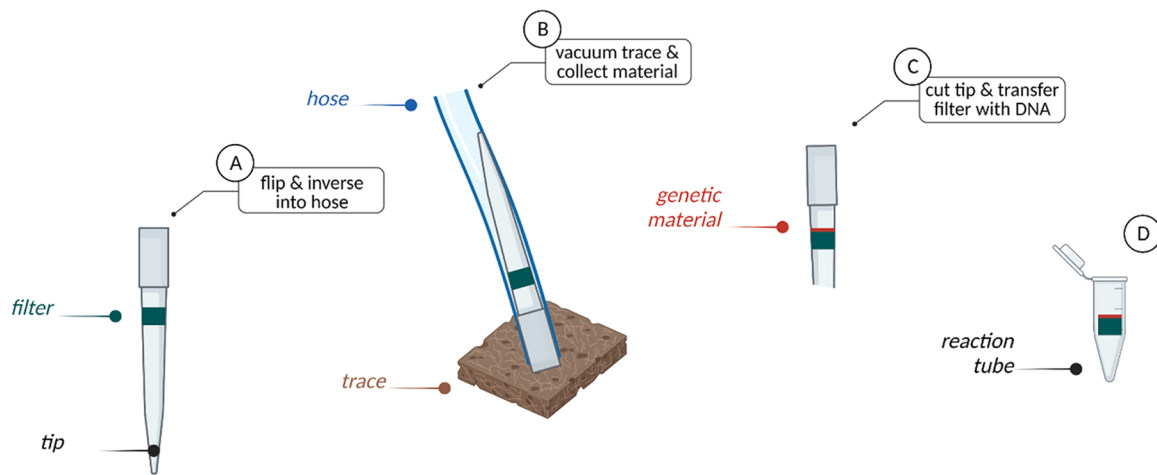


Fig. 1. The basic workflow of the dry-vacuuming DNA recovery process starts with a filtered pipetting tip (A), which is flipped and inverted into the device's hose (B). After recovery of the genetic material, the tip is taken out, and its conical end is cut off (C). The upper part is then turned upside down, and the filter can be transferred into a testing tube (D) by plunging it with another clean pipette tip. DNA lysis and extraction can then be performed with any commercially available kit for forensic DNA extraction. Created with BioRender.com [28].

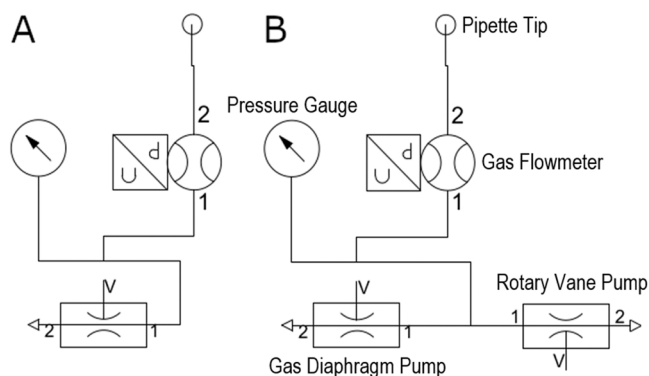


Fig. 2. Pneumatic circuit diagram of the experimental setup for evaluating different pipette tips. A) Airflow measurement with a single diaphragm pump. B) Airflow measurement with two coupled pumps in parallel (diaphragm pump and rotary vane pump). Graph created with the Bosch Rexroth Scheme Editor, version 6.3 [30].

2.3. Application of touch DNA

Touch DNA from one donor with written consent was applied by rubbing the previously cleaned and fixed items for 8×5 min with 30 min breaks in between, as described for Method 3 by Seiberle et al., 2022 [13], but with 16 cm² squares. The hands were rubbed from one item's edge to the next with constant pressure, rotating the item after each break to ensure an even application of touch DNA. Washing hands during the breaks were not permitted because studies demonstrated that the DNA from epidermal cells or cell-free DNA from sweat and sebum could be washed off [31,32]. Per day, only one item was prepared to allow biological material regeneration of the DNA donor. Hence, the biological material was provided on the same item the same day and under the same donor's condition to be subsequently recovered by the different methods per item to ensure quantity- and quality-wise consistent DNA starting amount. Due to the known variability of touch DNA, a high amount of replicates (n = 12) were prepared per item and method. The prepared material was stored in DNA-free plastic bags until the sample collection was performed at the DNA laboratories of the Institute of Forensic Medicine, Department of Biomedical Engineering, University of Basel, Switzerland (further referred to as Basel laboratory) and the Department of Immunology, Genetics and Pathology, Uppsala University, Sweden (further referred to as Uppsala laboratory).

2.4. Substrates

Mock touch DNA samples were deposited by the donor, touching the five different surfaces as described in the section *Application of Touch DNA*. To reflect various substrate characteristics, such as porous, non-porous, absorbing, non-absorbing, even and uneven, an uncoated tile (ceramic), wool sweater (100% cotton), deep-pile carpet (fibres 1–1.5 cm long), untreated wooden plate (beech) and stone (limestone) were chosen. The scheme of sampling allocation on the items for the different DNA recovery methods is given in Fig. 4.

Cleaning was done by washing the items with sodium hypochlorite in a washing machine at 60 °C for 1 h (carpet, sweater) or by swiping with ethanol (70%) and water, air-dried, and UV irradiated using the UV Airclean Workstation (UVC/T-M-AR, 25 W) for 20 min per side (tile, wood, stone) to minimize the occurrence of unintentional DNA-mixtures. Exemplarily, a reference sample was taken from each item to verify the absence of amplifiable genetic material.

DNA recovery was performed with the forensic cotton swab (For-ensiX Collection Swab SafeDry, Prionics AG, Schlieren-Zürich, Switzerland), adhesive tapes (SceneSafe Fast™ Minitapes, SceneSafe, UK), the M-Vac® and the DNA-Buster. Per substrate, 36×16 cm² fields were prepared, with 12 replicates each for taping, swabbing and dry-vacuuming at the Basel laboratory. The experimental setup was duplicated and prepared under the same conditions but using wet-vacuuming (n = 60) at the Uppsala laboratory. An extra 12 fields were provided for the five substrates and collected with adhesive tapes (n = 60). These tape-lifted samples were used as controls to assess potential bias due to slightly longer storage or transport effects that might affect the comparison of both vacuuming methods of both DNA laboratories. Sampling fields were randomized for each item. In total, 300 samples with 180 samples (Basel laboratory) and 120 samples (Uppsala laboratory) were investigated. This procedure was validated under consideration of comprehensive statistical analysis (Fig. S1). All substrates at both sites included negative controls with no touch DNA applied.

2.5. Sample collection

One operator per laboratory collected the donor's touch DNA from the items. After the respective collection procedure, except for the M-Vac® and control tape samples of the Uppsala laboratory, the specimens were directly transferred into a spin basket from the Investigator Lyse & Spin Basket Kit (QIAGEN GmbH, Hilden, Germany [33]). The Uppsala laboratory samples were sent back in the original packing and then



Fig. 3. Exemplary sampling allocation on a piece of cloth for different recovery methods with 4 cm × 4 cm sized sampling areas. Sampling areas were separately randomized across the cloths to minimize any bias related to the handling of the item. Created with BioRender.com [28].

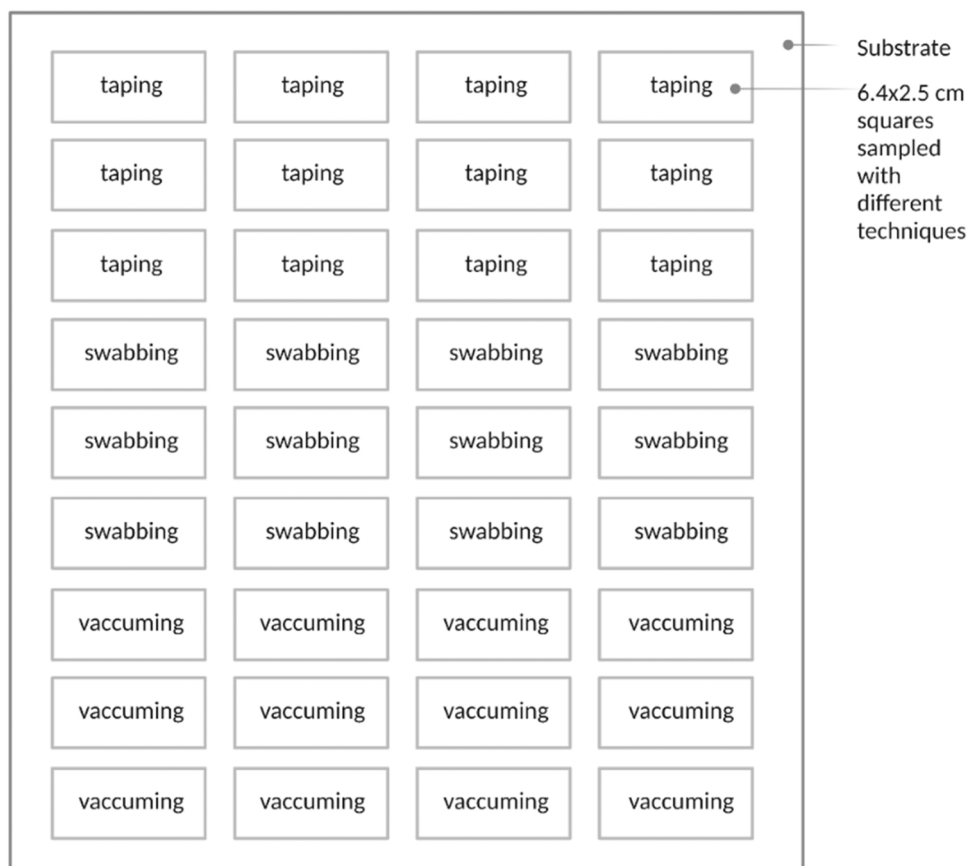


Fig. 4. Exemplary sampling allocation on an item for different recovery methods with 2.5 cm x 6.4 cm sized sampling areas. Fields were separately randomized across the substrate to minimize any bias related to the handling of the item. Created with BioRender.com [28].

transferred to the spin baskets at the Basel laboratory. The potential bias due to longer storage and transportation of the samples from Uppsala to Basel was assessed by comparing the respective control tapes, as shown

in Fig. S1. In all substrates, no potential bias was observed concerning DNA quantity. However, for the successful typing of all 16 STRs as a complete DNA profile between the Uppsala and Basel laboratories

control tapes, significant differences were observed on the substrates 'cotton sweater' and 'stone'. Thus, statements comparing the number of complete DNA profiles between samples from Uppsala and Basel laboratories should be handled with care for these items.

2.5.1. Tape-lifting

The DNA recovery technique with adhesive tape consisted of ten tape-lifts per sampling field. After five contacts with the tape, each item was rotated by 180°, sampling the entire area again but with a different angle, applying moderate pressure. This protocol was applied at both sampling sites.

2.5.2. Swabbing

A single wet swabbing technique was applied. For absorbing (carpet, sweater, wood) or porous materials (stone), the swabs were pre-moistened with 50 µl nuclease-free water (amplification grade ddH₂O, Promega Corporation, Madison, WI, USA) directly pipetted on the swab head. The recovery of touch DNA from the non-absorbing tile was performed by pipetting 10 µl of nuclease-free water onto its surface. Then, the swab was rotated gently with moderate pressure for 15 s over the respective area with applied genetic material.

2.5.3. Dry-vacuuming

All experiments were performed with the airflow setting of 3.75 liters per minute and a single diaphragm pump and using 50–1000 µl Brand Filter Tips for the experiments. For vacuuming, a filtered tip was inversely put into the hose of the DNA-Buster, vacuuming each area of every item once with moderate speed and without losing contact with the surface [25]. After recovery, the filter was transferred into a testing tube by plunging it with a clean pipette tip (Fig. 1). Downstream genetic analysis was performed as described in the respective section.

2.5.4. Wet-vacuuming

At the Uppsala laboratory, touch DNA was collected with the M-Vac® system by applying pressure to the sterile collection solution (Butterfield's Buffer, M-Vac® Systems) and the vacuum force according to the manufacturer's protocol [34]. The hand-held nozzle (MS Kit 100, M-Vac® Systems) was held with moderate pressure on the substrate's surface, moving backwards within the marked area. The pressurized buffer was delivered to the collection head and directly expelled onto the substrate. At the same time, the liquid residuals were immediately recovered by the collection head with vacuum applied, i.e., the solution spray was off [35]. Each collection activity ended with leaving the vacuum on while turning off the spray to collect the few remaining liquid droplets on the collection head. The liquid was then stored in a sterile collection bottle (M-Vac® Systems). This movement was applied four times for each sample, collecting approximately 25 ml of trace-buffer solution.

The collected DNA was concentrated using Nalgene™ Rapid Flow™ Sterile Single Use Vacuum Filter Units with polyethersulfone (PES) membrane (Thermo Fisher Scientific, Massachusetts, USA) with a pore size of 0.45 µm. The trace-buffer solution was swirled in the collection bottle to dislodge genetic material from the plastic walls. During vacuum application, the trace-buffer solution was poured onto the membrane, filtrating the solution and leaving the DNA on the filter. After drying overnight, each filter was cut out with a sterile scalpel and dissected into smaller pieces. For instant shipment to the Basel laboratory, the filter pieces were transferred into LowBind Micro tubes (Sarstedt).

2.6. Downstream genetic analysis

All 300 samples were extracted using the DNA IQ™ Casework, and Extraction Kit (Promega Corporation) loaded on a Maxwell® RSC Instrument (Promega) with 50 µl elution buffer [36,37]. DNA was quantified once with the Plexor® HY Kit (Promega) on an Applied

Biosystems™ 7500 Real Time PCR System (Thermo Fisher Scientific) according to the manufacturers' protocols [38,39]. Single STR amplification was done with the PowerPlex® ESX 17 Fast System Kit (Promega) on a 96-well Applied Biosystems Veriti™ Thermal Cycler (Thermo Fisher Scientific) according to the manufacturers' instructions [40,41]. The kit amplifies the following loci, including the extended European Standard Set D18S51, D21S11, TH01, D3S1358, D16S539, D2S1338, D1S1656, D10S1248, FGA, D8S1179, vWA, D22S1045, SE33, D19S433, D12S391, D2S441 and Amelogenin [42–44]. PCR products were analyzed on the capillary fragment analyzer Applied Biosystems™ 3500xL Genetic Analyzer for Human Identification (Thermo Fisher Scientific, [45]).

2.7. Data analysis

The Plexor Analysis Software (Promega, version 1.5.6.7) was used for quantification. Samples with a DNA concentration below the limit of quantification (LoQ) were assigned a DNA concentration of 0 ng/µl. STR profiles were analyzed using GeneMapper® ID-X Version 1.5 (Thermo Fisher Scientific) with default stutter filters and a validated analytical threshold of 50 fluorescence units (RFU). The profile quality was analyzed concerning peak morphology, peak balance, artefacts and stutters. A complete STR profile consisted of 16 STR loci and Amelogenin from the donor, either as a single or as a main component (i.e. with the highest signal intensities) in a mixed profile, occurring despite the thorough item cleaning and single DNA donorship. Non-donor alleles refer to any alleles meeting the criteria that could not be assigned to any reference persons (i.e., staff). With more than three STR systems also showing non-donor alleles, the corresponding DNA profile was classified as a mixture, with more than two non-donor alleles per STR system as a complex mixture. The used classification allows discriminating true mixtures from drop-in phenomena [46]. Partial profiles contained less than a complete profile but at least four alleles. Below this, the result was considered as no profile.

2.8. Evaluation criteria and statistical analysis

For the different collection techniques, DNA concentration, generation of full DNA profiles (counting donor alleles) and occurrence of the unintended DNA mixtures (counting extra non-donor alleles) were statistically analyzed. An allele was counted if above the analytical threshold. For each item, the resulting DNA concentrations (ng/µl) were compared using Kruskal-Wallis tests followed by pairwise Wilcoxon-Mann Whitney tests for post-hoc comparison of the different airflow levels and recovery methods, respectively. Correction for multiple testing was applied according to the Bonferroni-method. The Wilcoxon-Mann Whitney implementation from the package coin [47] was used to handle better the ties introduced by replacing DNA concentrations below LoQ with 0 ng/µl.

Binomial tests were used to separately assess the tendencies to generate complete DNA profiles and to yield mixed profiles for each method on each item according to the following procedure: (i) A hypothesized probability of success was calculated for each item as an item-wise baseline for all methods. For the completeness (in percentage) of DNA profiles, the amount of all samples with ≤ 3 allelic drop-outs on an item were divided by the total amount of samples (n = 60). This ratio yields a baseline probability of generating complete profiles with ≤ 3 allelic drop-outs, which then can be used to compare the individual methods to this baseline. For mixed profiles, the total amount of generated mixed profiles was divided by the total amount of samples for each item. (ii) For each method (and per item), the number of profiles with ≤ 3 allelic drop-outs reflected the number of successes. Similarly, the number of mixed profiles was used to assess the tendency to show mixed profiles. (iii) The number of samples yielded by each method on an item represents the number of trials (n = 24 for tapes and n = 12 for Swab, DNA-Buster and M-Vac®). Again, the item-wise performance of a

method regarding the tendency to generate mixtures could be evaluated for a common, item-related baseline. Two-tailed tests were performed to compare the completeness of profiles to the baseline. One-tailed tests were performed to assess whether a method had a higher tendency to generate DNA mixtures concerning the common baseline. Again, all instances of multiple testing were adjusted using the Bonferroni method. Statistical testing and data visualization were performed using R [48]. Plots were generated with ggplot2 [49] and ggpubr [50].

3. Results and discussion

3.1. DNA-yield with increasing airflow

Technically, changing the airflow from 3.44 L/min to 3.75 L/min significantly improved the DNA recovery using the DNA-Buster (Wilcoxon Mann-Whitney $p < 0.05$). However, a further airflow increase did not significantly enhance the DNA uptake according to the post hoc test (Fig. 5). The mean DNA concentration was 0.03 ng/μl for the minimum and 0.11 ng/μl for the maximum airflow rate. The highest mean DNA concentration was quantified at 6.54 L/min with 0.13 ng/μl. Furthermore, a noticeable but insignificant drop in DNA concentration was observed at 4.68 L/min with a mean of 0.05 ng/μl, potentially due to outliers and inaccuracies during the collection procedure.

The DNA yield after increasing the airflow did not significantly change beyond an airflow of 3.75 L/min. For the tool, this outcome implies that there is no need to increase the device in size and weight in favor of a more powerful pump. However, further experiments on different objects and surface conditions should be conducted to confirm the redundancy of a stronger or second pump since these results only refer to a single type of item (i.e., a piece of cloth). Moreover, the insignificant change of the DNA yield beyond the airflow of 3.75 L/min could also be due to the DNA-Buster being able to recover the deposited touch DNA already to a full extent. Repeating the experimental setting with traces of higher amounts of DNA could verify these findings. Also, further tests can be performed using different tips to overcome the below-mentioned problematic access to the genetic material.

3.2. DNA results per sampling method and substrate

The total DNA yields retrieved from the different substrates are visualized in Fig. 6. The results show that DNA yields depend on the DNA collection method and the substrates.

The qualitative STR results are presented in Fig. 7, displaying the

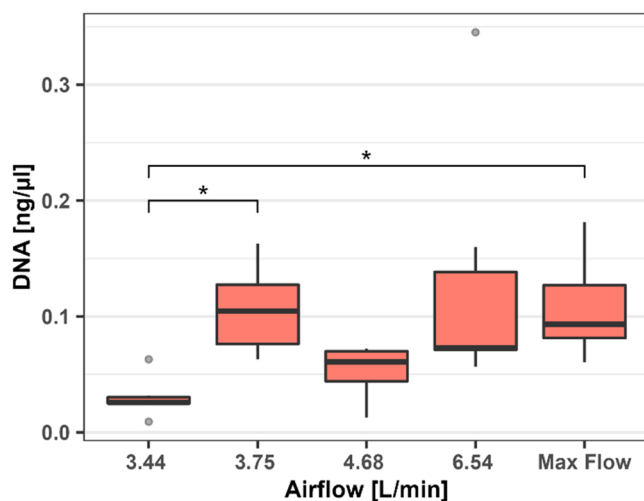


Fig. 5. Quantified DNA concentrations after recovery of touch DNA from a piece of cloth with increasing airflow of the DNA-Buster. Asterisks represent statistically significant differences ($p < 0.05$).

completeness of donor alleles in the DNA profiles. The distribution of single, mixed, complex mixed and no profiles are given in Fig. 8. DNA (complex) mixtures were detected despite the thorough cleaning of items and single donorship.

3.2.1. Carpet

Swabbing and taping yielded similar quantification and DNA profile results for recovering touch DNA from the carpet, while the DNA-Buster reached lower concentrations. With respect to DNA profiles, the carpet was the only substrate where the three methods swabbing, taping and dry-vacuuming yielded almost exclusively complete profiles. The quantitative yield was similar among both vacuuming methods, but the M-Vac® performed less well in the qualitative analysis. This result does not support the observation of McLamb et al., who reported that the M-Vac® achieved a higher DNA yield than wet-swabbing on a carpet. However, blood was used in that study instead of touch DNA, with likely a different recovery success [22]. At the same time, they pointed out that by recovering more DNA the M-Vac® approach can unnecessarily increase the complexity of DNA mixtures.

Both vacuuming approaches were expected to be more efficient due to the complete enclosure of the single fibres. Nevertheless, swabbing and tape-lifting were able to gain more and congruent amounts of DNA, a finding that contrasts with two other studies in which tape-lifting reached higher DNA concentrations on different textiles [10,17]. In addition, more mixed profiles were observed with taping in contrast to all other methods. With respect to the single donor, the detected (complex) mixtures are potentially due to DNA from non-donor persons that persisted the cleaning activities of the items. Contamination can be excluded since the detected alleles did not correspond to any reference person. For the observed increased mixture in tape-lifting, one speculation could be that the mixed DNA components were only revealed by peeling them off with the comparably strong adhesive tape. Also, the weave thickness, fiber types and potential electrical charges might impact the absorbance and recovery of the deposited or persistent touch DNA [51]. In real casework, the ideal solution would be to collect all present DNA and thus, taping would be recommended for substrates like carpets.

3.2.2. Cotton sweater

The high efficiency of adhesive tapes on fabrics has been demonstrated in several studies [7,13,52–54]. Particularly for cotton tissue, tape-lifting has shown better results than the swabbing method [17,53], which is consistent with the presented findings. One hypothesis for the poor performance of swabs is their tightly wound tips. With these, it could be challenging to physically reach the biological material which may have entered the tissue cavities. Moreover, the wet swab could potentially uptake more dirt and inhibitors, such as the clothing dye [10], reducing overall downstream analysis efficiency. Dry swabbing, on the other hand, could be associated with fraying, as shown for the DNA recovery from the stone. Garments are often relevant in aggressor-victim scenarios, while offenders frequently grab the victims' clothing tight, leaving potential traces in addition to the victim's DNA [55–59]. Thus, the highest occurrence of DNA mixtures is observed for this substrate, with their ratio depending, besides other factors, on the individual's DNA shedding status [35]. Here, much of this unwanted wearer DNA was reported to be co-sampled with wet swabs [53].

The alternative vacuuming methods are assumed to suck in any biological material lingering between the textile's pores, bearing the risk of resulting in a major contribution of victim DNA and masking an interpretable profile from the perpetrator. In our study, both vacuuming methods showed high quantitative and qualitative results but resulted mainly in (complex) mixed profiles, which had already been described for the wet-vacuuming method [21,24]. Thus, the findings suggest, on the one hand, that vacuuming may not be ideal for touch DNA sampling of clothes, but on the other hand, the DNA-Buster and M-Vac® achieved higher DNA yields during quantification for the cotton sweater.

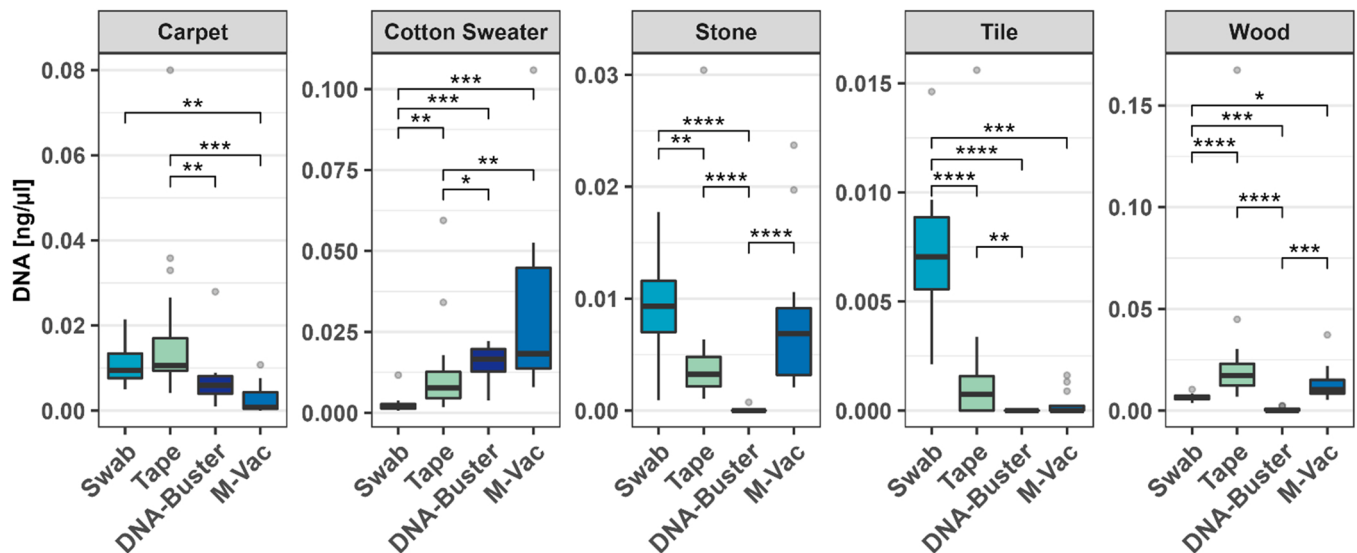


Fig. 6. DNA concentrations recovered with the different sampling methods: swab (n = 12), tape (n = 24), DNA-Buster (n = 12), M-Vac® (n = 12) are shown in boxplots for each material. The boxplots show interquartile ranges, medians, outliers and minimum and maximum scores (excluding the outliers). The sampling method with significantly different DNA yields compared to the other method/s is indicated with asterisks (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

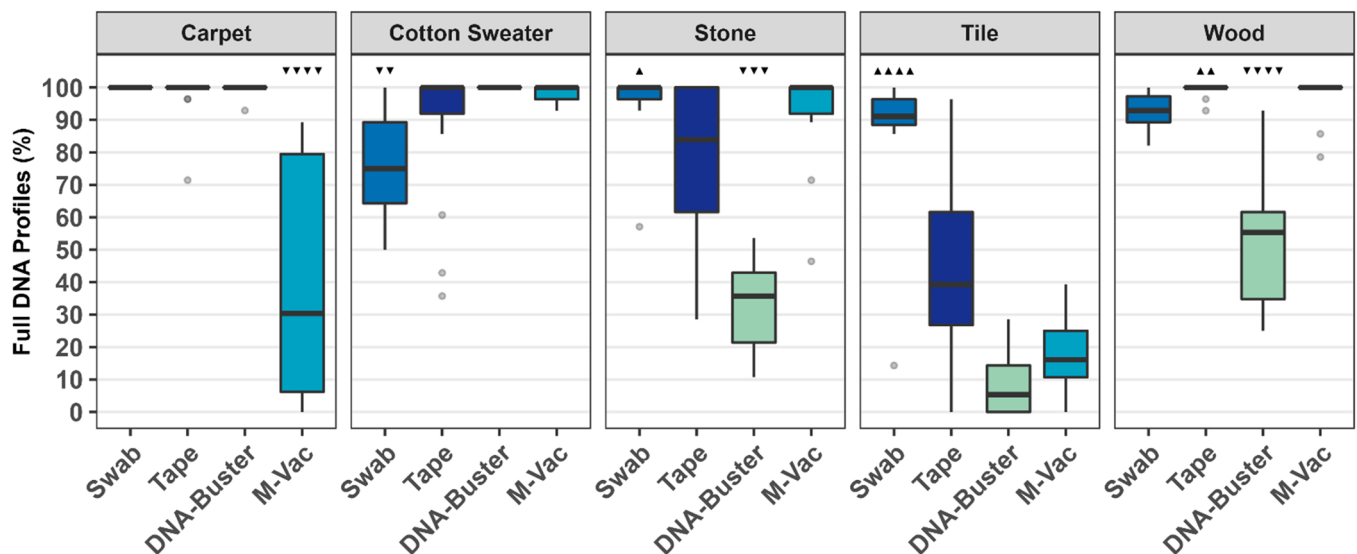


Fig. 7. Profile results of the STR Systems amplified with ESX17 kit. Box plots show the completeness of DNA profiles per sampling method (swab, DNA-Buster, M-Vac®: n = 12 each; tape: n = 24) for the five surfaces examined. Boxplots represent interquartile ranges, medians, outliers and minimum and maximum scores (excluding the outliers). The top arrows represent the outcome of the item-wise testing (two-tailed) of a method comparing the completeness to the baseline. Arrows pointing up indicate significantly higher results, and arrows pointing down significantly lower results.

Concerning the qualitative analysis, the DNA-Buster showed several mixed and complex mixed profiles with major components and numerous interpretable minor components. In contrast, the M-Vac® samples were all (complex) mixed profiles, with only incomplete or no major components. These results indicate that vacuuming methods have the potential to capture the relatively small amount of biological material that originated from the offender. Further analysis of DNA ratios of victim-perpetrator mock scenarios is required to determine if the detected mixed profiles are suitable for profiling purposes. With respect to the comparability of the two vacuuming methods and the completeness of the DNA profiles, an equal performance was observed. However, considering the potential bias due to storage and transport, these results could be better for the M-Vac® (Fig. S1, and section *Sample Collection*).

3.2.3. Stone

Stone and rock are problematic substrates since the small surface cavities and coarse nature of stones present inherent challenges for DNA collection techniques as they trap the biological material efficiently and lead to swab fray and loss of adhesiveness from tapes [12]. Dry-vacuuming was expected to achieve better results than the other sampling methods due to the suction's ability to access every corner of an uneven surface. However, the potential capability of vacuuming DNA which may be deposited in the porous limestone surface's multiple perforations was not confirmed. This was probably caused by the plastic tip that cracked during sampling due to the hard and rough surface. Consequently, as the rigid characteristics of the stone severely impaired the vacuum function, no vacuum could be generated. Thus, the DNA-Buster had the lowest quantity and fewest complete profiles, mainly partial single profiles. Also, the more static recovery of

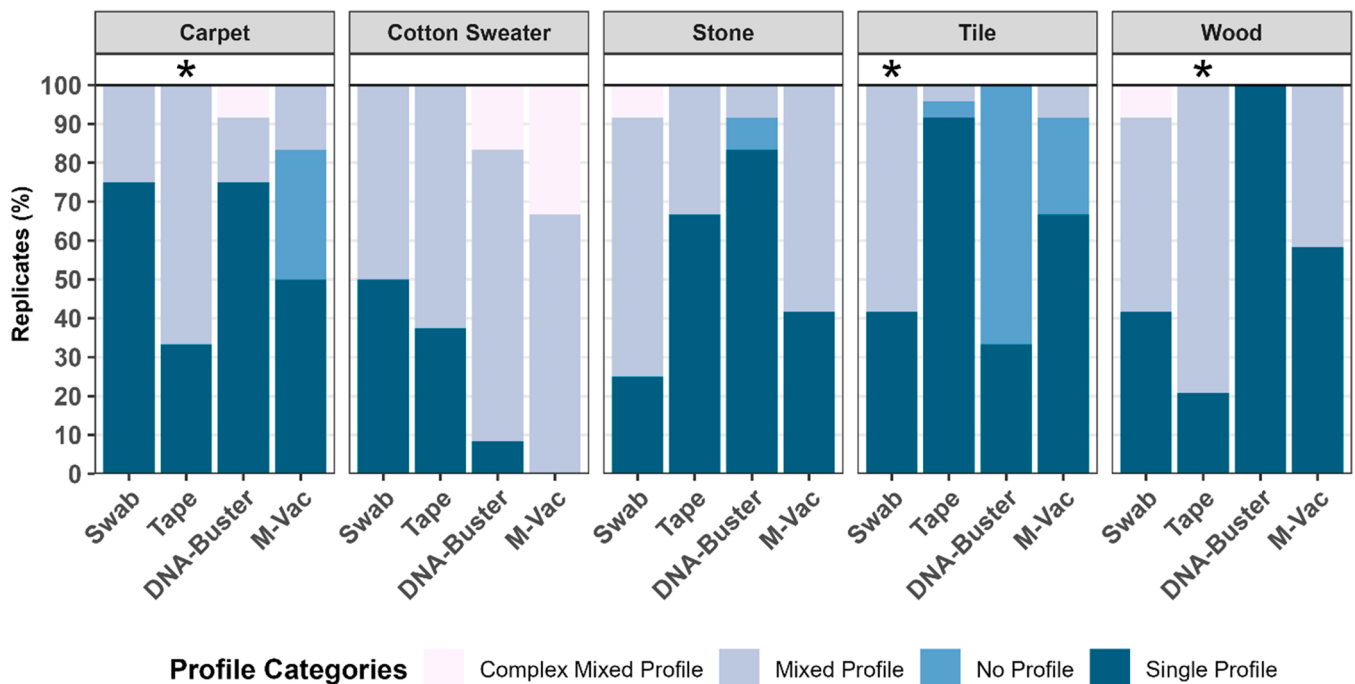


Fig. 8. Profile distribution for each sampling method (swab, DNA-Buster, M-Vac®: n = 12 each; tape: n = 24) per substrate in percent. The asterisks indicate a significantly higher occurrence of mixed profiles with respect to the common baseline (one tailed $p < 0.05$). Incomplete or complete profiles are not shown separately in the interest of clarity.

vacuuming lacks a mechanical or scrubbing interaction with the substrate, which could explain the poorer performance when compared to the more active swabbing and taping sampling procedures. However, a certain superiority in DNA quantity (but not at the DNA profiling level) was described for the M-Vac® when compared to the swabbing technique by Vickar et al. [12], which was confirmed by our findings. As noted in the section *Sample Collection*, here the results could be affected by a potential bias due to storage and transport (Fig. S1) negatively impacting the wet-vacuuming. Since, in this case, the M-Vac® showed significantly better performance, the extent of this bias was deemed to be neglectable.

The highest amount of recovered touch DNA on stone was achieved with the swabbing technique, although the cotton tip was frayed to pieces during the sampling process. Verdon et al. also found that fibers from cotton swabs may be discarded on rough surfaces, limiting their collection efficiency [16]. Further challenges include retained loose fibers and other impurities in the reaction mixture, which can inhibit PCR and potentially distort quantitative PCR results [11] or other downstream processes. For instance, the DEPArray™ technology, a single-cell separation and identification device, was disrupted by fibers released during sample preparation [60]. In our study, wet swabbing was associated with an increased non-donor DNA level compared to tape-lifting and dry-vacuuming. In one sample, it was impossible to deduce a major component of the donor and another replicate showed a minor profile of unknown origin. The occurrence of more unwanted background DNA was also observed by Pfeifer and Wiegand when swabbing was compared to tape-lifting [58].

3.2.4. Tile

In three out of four sampling methods, the tile was the only substrate for which at least one replicate resulted in a high number of loci drop-outs, thus, no DNA profiling was feasible (except for swabbing). The surface characteristics of tiles are smoothness and non-porosity. Several studies have demonstrated that rough, porous surfaces tend to collect and retain skin cell DNA better than smooth and non-porous surfaces such as ceramics and glass [61–63]. This could be attributed to the

abrasive nature of a rough surface, which causes friction by touching it and increasing the chances of DNA retention. At the same time, the amount of DNA recovered from such uneven surfaces is supposed to be lower, possibly due to needing to reach the relevant deep or hidden surface areas [63].

In contrast, due to the direct 'availability' of biological material, there are greater chances to recover DNA from smooth and non-porous surfaces such as glasses and tiles than from rough, porous surfaces. Oorschot et al. suggested that DNA is not trapped within the smooth surface matrix, which makes the genetic material easier to collect [56]. Additionally, smooth and non-porous surfaces increase the rate of transpiration during interactions, stimulating the detachment of skin cells and the release of cell-free DNA, mostly transported with sweat [63–65]. Following this theory, more DNA would be deposited on such surfaces. Some studies investigated DNA recovery from non-porous surfaces like glass with cotton swabs and concluded that smooth surfaces facilitate DNA recovery [66–68].

However, compared with other substrates, the recovery of trace DNA from the tile resulted in the lowest means of complete STR profiles for all sampling methods in our study. One explanation could be that the DNA donor re-collected the DNA by trying to deposit it during mock sample preparation. Since the DNA is more 'available' on smooth surfaces, each deposit could bear the risk that DNA is directly re-transferred to the donor's hands. Another possible reason for the poor profile quality might be the influence of the oil, grease and sweat secreted from the donor's skin when frequently touching the surface. The sticky and oily coating could make the DNA unavailable for uptake [69,70]. In our study, the swabbing technique yielded the highest DNA concentrations and most full DNA profiles, which is consistent with other studies [66–68]. This might be due to the application of water, which increases the general solubility. Internal studies investigated several swabbing solutions besides the use of nuclease-free water. Here, detergent-based solutions like Triton X-100 and sodium dodecyl sulfate (SDS) led to significantly greater DNA yields. Fats, lipids, and proteins become suspended in these solutions. These findings support the hypothesis that the oiled coating decreases the overall DNA recovery efficiency [69,70].

Despite the application of a solution spray, the M-Vac® showed poor performance, as well, which is consistent with research on its performance compared to the double-swabbing technique, also from a tile [12]. Garret et al. reported that on such even and smooth surfaces, the solution spraying pressure from the M-Vac® propels DNA up to 10 cm from the collection area, which could contribute to the loss of DNA [15]. In addition, Vickar et al. observed that the nubs on the device's head, designed to impede the head from suctioning on surfaces, prevented the expelled solution from being vacuumed back into the collection bottle. As a result, the M-Vac® potentially leaves valuable DNA on the surface that other strategies, e. g., swabbing, would collect [12].

While swabbing and taping are more active sampling procedures, dry- and wet-vacuuming are more static, lacking a mechanical or scrubbing interaction with the substrate, which again could explain the poorer performance when vacuuming. Even though the DNA-Buster generated a high vacuum power forced by the tiles' smooth surface during sampling, virtually no DNA yield was detected. This is in contrast to one study, which showed that dry-vacuuming is most effective on glass compared with stubbing, double-swabbing and cutting, proving the potential of vacuuming for forensic purposes [71]. In our study, four dry-vacuuming replicates provided an incomplete DNA profile, despite all samples being below the detection threshold during quantification. This could be attributed to the limited sensitivity level of the Plexor® HY Kit, as reported by Poetsch et al. [72], or quantification methods in general, as they tend to underestimate the DNA amount in a given sample [73].

3.2.5. Wood

The highest quantity and quality results were achieved using adhesive tapes on wood, which had also been observed by Daly et al. [74]. The adhesive tapes picked up vast numbers of sprouts from wood, probably also carrying DNA. Verdon et al. investigated the performance of different swabs from various substrates and found that the recovery of touch DNA from wood with the swabbing technique tends to be difficult [16]. Their findings are consistent with this study as mainly partial profiles were obtained. A potential negative interaction between the wooden matrix and the cotton swab tip could be a reason. In addition, the inflexible woven tip allows no penetration into the substrate pores, where cellular material might be trapped [16]. Another study suggested adopting the tip head to foam material to be more effective when recovering touch DNA from a wooden surface [75], which also could be a promising approach when vacuuming.

A recent study has shown that dry-vacuuming can collect latent DNA from even and absorbent paper substrates [25] while preserving present fingerprints [76]. Additionally, the research showed that the recovered DNA quantities were sufficient to provide probative DNA profiles in 80% of the samples tested. Wet-vacuuming, however, would likely destroy latent fingerprints or the whole paper document due to applying the liquid solution. Therefore, the M-Vac® is not the optimal collection method in investigations of extortion letters or related touched paper evidence [25]. Instead, it was shown to be more effective for blood traces than swabs from wood [22]. Our results confirm this finding, as high quantitative and qualitative results were achieved with the M-Vac® compared to the dry-vacuuming using the DNA-Buster. One explanation might be that the force of air alone might not be sufficient to recover the multidimensional cells, including mucopolysaccharide and complex protein cell walls that can effectively bind to the wood's surface. Here, mechanical action or usage of a solution when wet-vacuuming is more likely to recover the valuable DNA within the cells. On the other side, using solutions could also unwillingly alter the genetic evidence (e.g. position), as demonstrated for the M-Vac® [14,15].

4. Conclusions

One outcome of this study is that the DNA-Buster has shown a good performance for DNA collection from textiles with respect to the mean

completeness of DNA profiles. Benefits are seen in the lightweight, portable device, accessing small-sized evidence in niches, corners, and confined spaces, which could aid forensic operators in accessing difficult-to-reach and/or non-transportable genetic evidence in crime-scene settings. Secondly, we conclude that much more attention should be paid to the demonstrated apparent substrate-collection dependency directly impacting the STR profiling success. Our investigations reveal that some substrates are best being swabbed (tile) while others should better be taped (wood) or vacuumed (sweater) to recover preferably all valuable genetic material which a person of interest leaves behind. Several approaches seem to work equally efficiently for some objects, such as swabbing for stones and tiles or dry-vacuuming for both "textiles". Here, further research with the different sampling methods and substrates with various properties would strengthen the current data basis, and potentially help to secure the valuable biological material from the variety of forensically relevant traces using the most promising sampling approach.

Funding

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

CRediT authorship contribution statement

Jonathan Währer: Formal analysis, Validation, Investigation, Writing – original draft preparation, Visualization, **Sabrina Kehm:** Formal analysis, Investigation, Writing – original draft preparation, Visualization, **Linnéa Brauer:** Investigation, Writing – original draft preparation, **Marie Allen:** Writing– review & editing, **Oliver Eidam:** Methodology, **Sarah Kron:** Methodology, **Ilona Seiberle:** Conceptualization, Writing – review & editing, **Eva Scheurer:** Resources, Writing – review & editing, **Iris Schulz:** Conceptualization, Writing – review & editing, Visualization, Supervision, Project administration.

Acknowledgements

The authors want to kindly thank M.Sc. Janine Schulte, Moana Heller and Dr. Alina Senst for assisting with this article. The authors wish further to thank the reviewers for their valuable feedback.

Conflict of interests

The authors declare they have no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fsigen.2023.102830](https://doi.org/10.1016/j.fsigen.2023.102830).

References

- [1] J. Comte, S. Baechler, J. Gervais, E. Lock, M.P. Milon, O. Delemont, V. Castella, Touch DNA collection - performance of four different swabs, *Forensic Sci. Int. Genet.* 43 (2019), 102113, <https://doi.org/10.1016/j.fsigen.2019.06.014>.
- [2] J. Burrill, B. Daniel, N. Frascione, A review of trace "Touch DNA" deposits: variability factors and an exploration of cellular composition, *Forensic Sci. Int. Genet.* 39 (2019) 8–18, <https://doi.org/10.1016/j.fsigen.2018.11.019>.
- [3] E.K. Hanson, J. Ballantyne, Getting blood from a stone: ultrasensitive forensic DNA profiling of microscopic bio-particles recovered from "touch DNA" evidence, *Methods Mol. Biol.* 1039 (2013) 3–17, https://doi.org/10.1007/978-1-62703-535-4_1.
- [4] R.A.H. van Oorschot, B. Szkuta, G.E. Meakin, B. Kokshoorn, M. Goray, DNA transfer in forensic science: a review, *Forensic Sci. Int. Genet.* 38 (2019) 140–166, <https://doi.org/10.1016/j.fsigen.2018.10.014>.
- [5] A.M. Garvin, R. Holzinger, F. Berner, W. Krebs, B. Hostettler, E. Lardi, C. Hertli, R. Quartermaine, C. Stamm, The forensiX evidence collection tube and its impact on DNA preservation and recovery, *Biomed. Res. Int.* 2013 (2013), 105797, <https://doi.org/10.1155/2013/105797>.

- [1] M.L. Hebda, A.E. Doran, D.R. Foran, Collecting and analyzing DNA evidence from fingernails: a comparative study, *J. Forensic Sci.* 59 (5) (2014) 1343–1350, <https://doi.org/10.1111/1556-4029.12465>.
- [2] D.T. Plaza, J.L. Mealy, J.N. Lane, M.N. Parsons, A.S. Bathrick, D.P. Slack, Nondestructive biological evidence collection with alternative swabs and adhesive lifters, *J. Forensic Sci.* 61 (2) (2016) 485–488, <https://doi.org/10.1111/1556-4029.12980>.
- [3] C. Fripiat, F. Noel, Comparison of performance of genetics 4N6 FLOQSwabs with or without surfactant to rayon swabs, *J. Forensic Leg. Med.* 42 (2016) 96–99, <https://doi.org/10.1016/j.jflm.2016.06.002>.
- [4] I. Wood, S. Park, J. Tooke, O. Smith, R.M. Morgan, G.E. Meakin, Efficiencies of recovery and extraction of trace DNA from non-porous surfaces, *Forensic Sci. Int.: Genet. Suppl. Ser.* 6 (2017) e153–e155, <https://doi.org/10.1016/j.fsigss.2017.09.022>.
- [5] S. Hess, C. Haas, Recovery of trace DNA on clothing: a comparison of mini-tape lifting and three other forensic evidence collection techniques, *J. Forensic Sci.* 62 (1) (2017) 187–191, <https://doi.org/10.1111/1556-4029.13246>.
- [6] B.B. Bruijns, R.M. Tiggelaar, H. Gardiner, The extraction and recovery efficiency of pure DNA for different types of swabs, *J. Forensic Sci.* 63 (5) (2018) 1492–1499, <https://doi.org/10.1111/1556-4029.13837>.
- [7] T. Vickar, K. Bache, B. Daniel, N. Frascione, The use of the M-Vac(R) wet-vacuum system as a method for DNA recovery, *Sci. Justice* 58 (4) (2018) 282–286, <https://doi.org/10.1016/j.scijus.2018.01.003>.
- [8] I. Seiberle, J. Währer, S. Kron, K. Flury, M. Girardin, A. Schocker, I. Schulz, Collaborative swab performance comparison and the impact of sampling solution volumes on DNA recovery, *Forensic Sci. Int. Genet.* 59 (2022), 102716, <https://doi.org/10.1016/j.fsiggen.2022.102716>.
- [9] Gunn L.E. Validation of the M-Vac cell collection system for forensic purposes [Thesis: Master of Science]. Boston University. 2013.
- [10] A.D. Garrett, D.J. Patlak, L.E. Gunn, A.N. Brodeur, C.M. Grgicak, Exploring the potential of a wet-vacuum collection system for DNA recovery, *J. Forensic Identif.* (2014) 64.
- [11] T.J. Verdon, R.J. Mitchell, R.A. van Oorschot, Swabs as DNA collection devices for sampling different biological materials from different substrates, *J. Forensic Sci.* 59 (4) (2014) 1080–1089, <https://doi.org/10.1111/1556-4029.12427>.
- [12] T.J. Verdon, R.J. Mitchell, R.A. van Oorschot, Evaluation of tapelifting as a collection method for touch DNA, *Forensic Sci. Int. Genet.* 8 (1) (2014) 179–186, <https://doi.org/10.1016/j.fsiggen.2013.09.005>.
- [13] J. Hedman, Y. Akel, L. Jansson, R. Hedell, N. Wallmark, C. Forsberg, R. Ansell, Enhanced forensic DNA recovery with appropriate swabs and optimized swabbing technique, *Forensic Sci. Int. Genet.* 53 (2021), 102491, <https://doi.org/10.1016/j.fsiggen.2021.102491>.
- [14] M. Goray, R.A. van Oorschot, J.R. Mitchell, DNA transfer within forensic exhibit packaging: potential for DNA loss and relocation, *Forensic Sci. Int. Genet.* 6 (2) (2012) 158–166, <https://doi.org/10.1016/j.fsiggen.2011.03.013>.
- [15] C. Mercer, J. Henry, D. Taylor, A. Linacre, What's on the bag? The DNA composition of evidence bags pre- and post-exhibit examination, *Forensic Sci. Int. Genet.* 57 (2022), 102652, <https://doi.org/10.1016/j.fsiggen.2021.102652>.
- [16] J. Hedman, J. Ågren, R. Ansell, Crime scene DNA sampling by wet-vacuum applying M-Vac, *Forensic Sci. Int.: Genet. Suppl. Ser.* 5 (2015) e89–e90, <https://doi.org/10.1016/j.fsigss.2015.09.036>.
- [17] J.M. McLamb, L.D. Adams, M.F. Kavlick, Comparison of the M-Vac® wet-vacuum-based collection method to a wet-swabbing method for DNA recovery on diluted bloodstained substrates, *J. Forensic Sci.* 65 (6) (2020) 1828–1834, <https://doi.org/10.1111/1556-4029.14508>.
- [18] M-Vac® Systems I. Why M-Vac, Forensic DNA Collection FAQ Sandy, UT [Last access: 05.12.2022]. Available from: <https://www.m-vac.com/why-mvac/forensic-dna-collection-faq>.
- [19] Irion P.R. Evaluating the Use of the M-Vac® Wet Vacuum System to Recover DNA from Cotton Fabric [Thesis: Master of Science]. West Virginia: West Virginia University. 2020.
- [20] P. McLaughlin, C. Hopkins, E. Springer, M. Prinz, Non-destructive DNA recovery from handwritten documents using a dry vacuum technique, *J. Forensic Sci.* 66 (4) (2021) 1443–1451, <https://doi.org/10.1111/1556-4029.14696>.
- [21] S. Fieldhouse, E. Oravcova, L. Walton-Williams, The effect of DNA recovery on the subsequent quality of latent fingerprints, *Forensic Sci. Int.* 267 (2016) 78–88, <https://doi.org/10.1016/j.forsciint.2016.08.003>.
- [22] L.J. Williams, C.D. White, F.J. Longstaffe, Improving stable isotopic interpretations made from human hair through reduction of growth cycle error, *Am. J. Phys. Anthropol.* 145 (1) (2011) 125–136, <https://doi.org/10.1002/ajpa.21479>.
- [23] Aoki S., Shteyn K., Marien R. BioRender. (05.12.2022).
- [24] BrandGmbH+CoKG. Brand Filterspitzen [Last access: 05.12.2022]. Available from: <https://shop.brand.de/de/>.
- [25] BoschRexrothAG. Rexroth Scheme Editor [Last access: 06.12.2022]. Available from: <https://www.boschrroth.com/de/de/produkte/konfiguratoren-und-tools/scheme-editor/>.
- [26] D. Lacerenza, S. Aneli, M. Omedei, S. Gino, S. Pasino, P. Berchiolla, C. Robino, A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers, *Forensic Sci. Int. Genet.* 22 (2016) 44–53, <https://doi.org/10.1016/j.fsiggen.2016.01.012>.
- [27] J. Helmus, J. Poetsch, M. Pfeifer, T. Bajanowski, M. Poetsch, Cleaning a crime scene 2.0-what to do with the bloody knife after the crime? *Int. J. Leg. Med.* 134 (1) (2020) 171–175, <https://doi.org/10.1007/s00414-019-02162-6>.
- [28] QiagenGmbH. Investigator® Lyse&Spin Basket Kit Handbook [Last access: 05.12.2022]. Available from: <https://www.qiagen.com/us/resources/resourceDetail?id=3850f41c-fec4-464a-8044-3211268c435b&lang=en>.
- [29] M-Vac® Systems I. SEC Series 100 and 150 User Guide [Last access: 05.12.2022]. Available from: <https://www.m-vac.com/images/pdfs/sec100usersguideervh.pdf>.
- [30] R.A.H. van Oorschot, G.E. Meakin, B. Kokshoorn, M. Goray, B. Szkuta, DNA transfer in forensic science: recent progress towards meeting challenges, *Genes* (11) (2021) 12, <https://doi.org/10.3390/genes11211766>.
- [31] PromegaCorporation. Maxwell® CSC Instrument Operating Manual 2014 [Last access: 06.12.2022]. Available from: https://ch.promega.com/-/media/files/resources/protocols/technical-manuals/101/maxwell-csc-instrument-operating-manual.pdf?rev=1affd07e67c840d198d636ff561fc9&sc_lang=en.
- [32] PromegaCorporation. Maxwell® FSC DNA IQ™ Casework Kit, Technical Manual 2021 [Last access: 06.12.2022]. Available from: https://ch.promega.com/-/media/files/resources/protocols/technical-manuals/101/maxwell-fsc-dna-iq-casework-kit-protocol.pdf?rev=e1621d575e0a41e0baf1d27b056084ad&sc_lang=en.
- [33] PromegaCorporation. Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, Technical Manual 2017 [Last access: 0

- [58] C.M. Pfeifer, P. Wiegand, Persistence of touch DNA on burglary-related tools, *Int J. Leg. Med* 131 (4) (2017) 941–953, <https://doi.org/10.1007/s00414-017-1551-4>.
- [59] A.M. Magee, M. Breathnach, S. Doak, F. Thornton, C. Noone, L.G. McKenna, Wearer and non-wearer DNA on the collars and cuffs of upper garments of worn clothing, *Forensic Sci. Int Genet* 34 (2018) 152–161, <https://doi.org/10.1016/j.fsigen.2018.02.011>.
- [60] F. Fontana, C. Rapone, G. Bregola, R. Aversa, A. de Meo, G. Signorini, M. Sergio, A. Ferrarini, R. Lanzellotto, G. Medoro, G. Giorgini, N. Manaresi, A. Berti, Isolation and genetic analysis of pure cells from forensic biological mixtures: The precision of a digital approach, *Forensic Sci. Int Genet* 29 (2017) 225–241, <https://doi.org/10.1016/j.fsigen.2017.04.023>.
- [61] Parsons R., Bates L., Walton-Williams L., Fieldhouse S., Gwinnett C. DNA from Fingerprints: Attempting dual recovery: Department of Forensic and Crime Sciences, Staffordshire University; 2016 [Last access: 04.12.2022]. Available from: <http://eprints.staffs.ac.uk/2764/3/eprints2764.pdf>.
- [62] M. Goray, E. Eken, R.J. Mitchell, R.A. van Oorschot, Secondary DNA transfer of biological substances under varying test conditions, *Forensic Sci. Int Genet* 4 (2) (2010) 62–67, <https://doi.org/10.1016/j.fsigen.2009.05.001>.
- [63] S.K. Alketbi, The affecting factors of touch DNA, *J. Forensic Res.* 09 (03) (2018), <https://doi.org/10.4172/2157-7145.1000424>.
- [64] R.A. Wickenheiser, Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact, *J. Forensic Sci.* 47 (3) (2002) 442–450.
- [65] Pesaresi M., Buscemi L., Alessandrini F., Cecati M., Tagliabracci A. Qualitative and quantitative analysis of DNA recovered from fingerprints 2003 [Last access: 08.12.2022]. International Congress Series 947–951. Available from: https://www.isfg.org/files/61bebd1f8e4d5e47364077a5adee68b15529d225.02005721_131595303851.pdf.
- [66] P. Tozzo, A. Giuliadori, E. Ponzano, L. Caenazzo, Effect of two different swabs on genetic profiling of enhanced fingerprints, *Forensic Sci. Int.: Genet. Suppl. Ser.* 5 (2015) e7–e9, <https://doi.org/10.1016/j.fsigs.2015.09.004>.
- [67] Oleiwi A.A. Experimental Approaches to Improving Trace DNA Recovery from Developed Fingerprints [Thesis: Doctor of Philosophy]: University of Wolverhampton. 2015.
- [68] J. Hedman, L. Jansson, Y. Akel, N. Wallmark, R. Gutierrez Liljestrand, C. Forsberg, R. Ansell, The double-swab technique versus single swabs for human DNA recovery from various surfaces, *Forensic Sci. Int Genet* 46 (2020), 102253, <https://doi.org/10.1016/j.fsigen.2020.102253>.
- [69] D. Aloraer, N.H. Hassan, B. Albarzinji, W. Goodwin, Improving recovery and stability of touch DNA, *Forensic Sci. Int.: Genet. Suppl. Ser.* 6 (2017) e390–e392, <https://doi.org/10.1016/j.fsigs.2017.09.166>.
- [70] S.M. Thomasma, D.R. Foran, The influence of swabbing solutions on DNA recovery from touch samples, *J. Forensic Sci.* 58 (2) (2013) 465–469, <https://doi.org/10.1111/1556-4029.12036>.
- [71] H. Dong, J. Wang, T. Zhang, J.Y. Ge, Y.Q. Dong, Q.F. Sun, C. Liu, C.X. Li, Comparison of preprocessing methods and storage times for touch DNA samples, *Croat. Med J.* 58 (1) (2017) 4–13, <https://doi.org/10.3325/cmj.2017.58.4>.
- [72] M. Poetsch, H. Konrad, J. Helmus, T. Bajanowski, N. von Wurmb-Schwark, Does zero really mean nothing?—first experiences with the new PowerQuant(TM) system in comparison to established real-time quantification kits, *Int J. Leg. Med* 130 (4) (2016) 935–940, <https://doi.org/10.1007/s00414-016-1352-1>.
- [73] Gill P., Bleka Ø., Fonneløp A.E. Limitations of qPCR to estimate DNA quantity: An RFU method to facilitate inter-laboratory comparisons for activity level, and general applicability. *bioRxiv*. 2022;10.1101/2021.06.30.450487: 2021.2006.2030.450487. DOI 10.1101/2021.06.30.450487.
- [74] D.J. Daly, C. Murphy, S.D. McDermott, The transfer of touch DNA from hands to glass, fabric and wood, *Forensic Sci. Int Genet* 6 (1) (2012) 41–46, <https://doi.org/10.1016/j.fsigen.2010.12.016>.
- [75] S. Hartless, L. Walton-Williams, G. Williams, Critical evaluation of touch DNA recovery methods for forensic purposes, *Forensic Sci. Int.: Genet. Suppl. Ser.* 7 (1) (2019) 379–380, <https://doi.org/10.1016/j.fsigs.2019.10.020>.
- [76] Hopkins C. Further evaluation of a dry vacuuming technique for recovery of DNA from handwritten documents [Thesis: Master of Science]: City University of New York (CUNY) 2020.