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Comparison of swabbing and cutting-out DNA collection methods from cotton, paper, and cardboard surfaces

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ABSTRACT

Choosing an inappropriate method of sample collection can often have a detrimental impact on DNA recovery. Multiple studies highlight the importance of selecting the recovery method based on the type of surface the DNA sample is located on. This study aimed to investigate the efficacy of sample collection via the single cotton swabbing method in comparison to recovery directly from the material cut from the surface. The three types of surfaces included cotton, paper, and cardboard. DNA sources comprised cell-free and cellular DNA, as well as blood and saliva as examples of body fluids commonly encountered at crime scenes. The data analysis revealed that the cutting-out method resulted in higher DNA recovery from all but cardboard surfaces, making it the more efficient collection method. Despite its limitations, the cutting-out method should be considered as the DNA recovery method of choice when suitable.

1. Introduction

Biological evidence at a crime scene can be found on many surface types. As those surfaces vary in composition, texture, and porosity, there is no unified method or approach to DNA evidence collection. Each type of surface comes with its DNA recovery challenges. Therefore, selecting the most appropriate collection method is often a crucial first step that can significantly impact the amount of recovered DNA [1,2]. At present, swabs are the most versatile and most commonly used evidence collection method for crime scene and laboratory examination [3]. The swabbing technique has been shown to be the most efficient for smooth surfaces, with decreased efficiency observed on porous and absorbent surfaces [4]. One of the most successful variations of swabbing techniques is collection with a wet swab followed by swabbing of the same area with a dry swab. Co-extraction of both swabs has been shown to yield the highest DNA recovery when compared with a single swab collection [5]. However, as Hedman et al. note, even though the double swab technique may be beneficial, focusing on the correct sampling technique for the single wet swab should be prioritised as it saves time and minimises workload [6]. It is especially relevant when dealing with non-absorbing surfaces, where the wet swab collects most of the material from dried stains, making the application of secondary swabs excessive [6].

Another popular method for the recovery of trace evidence is tape lifting [7], often shown as superior to the swabbing technique [8–11]. However, there is also some evidence indicating that there is not much difference in DNA recovery between adhesive tapes and cotton swabs [12].

Another alternative to these techniques is the FTA paper-scraping method, which shows higher DNA recovery from non-porous surfaces than traditional methods such as double swabbing [13].

Cutting out the area with a biological sample is one of the least popular and least frequently used methods of evidence collection [1], since this results in evidence destruction and not many surfaces are suitable for direct cutting, this technique is mostly applied to soft items [14]. As Tozzo et al. point out in their review article, there is a limited amount of data comparing the performance of the cutting-out technique (direct extraction) method with more common recovery methods [1], therefore its efficacy may not be fully appreciated. Solomon et al. demonstrated that the cutting-out method resulted in the highest amount of recovered DNA when compared to single and double swabbing techniques [15]. However, a different study reported that even though the cutting-out method yielded better results when compared with dry swabbing and tape lifting, the results were not statistically significant [16]. Tozzo et al. also suggest that the poor outcome of the method documented in research papers is most likely caused by its limitations (it can damage evidence and is not suitable for all surfaces) rather than the low efficacy [1].

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The main aim of this study was to assess the efficacy of DNA recovery from three types of surfaces: cotton, paper, and cardboard. Using two different methods of collection: swabbing and cutting out (direct extraction). The cutting-out method will be referred to as direct extraction throughout this paper. The secondary objective was to investigate the impact of potential inhibitors transferred to the sample via these collection methods.

2. Materials and methods

2.1. DNA sources

The cell-free trout DNA (cfDNA)used for this experiment was extracted from frozen rainbow trout liver with chloroform phenol extraction [17] and then sonicated to create 400–600 bp fragments. The sonicated DNA was quantified by Qubit fluorometer (Qiagen, Manchester, UK) and stored at – 20 °C.

Mouse embryonic fibroblasts were removed from culture flasks by trypsinisation and washed in phosphate-buffered saline (PBS) pH 8.0 by centrifugation. The cells were then DAPI stained, and their nuclei were counted in a haemocytometer to determine their concentration. This was followed by cell resuspension at a concentration of 1×10^6/ml in PBS 20 % glycerol. The resuspended cells were stored at – 20 °C until required. Prior to use, the cells were centrifuged and re-suspended in Tris-buffered saline (TBS) to the required concentration [18].

Human saliva was collected from a volunteer and used fresh within 10 min of collection.

Bovine blood was sourced from a local abattoir (ABP Perth, Inveralmond Industrial Estate, Ruthvenfield Road, Perth UK) and treated with 12.5 % (v/v) of anticoagulant ACD immediately after sample collection. The blood was divided into aliquots which were frozen and stored at – 20 °C until required.

2.2. Surface materials

The three materials used in this study were cotton, paper, and cardboard. The cotton surfaces consisted of the following worn and washed clothing items: a dark green, 100 % cotton T-shirt, a red, 100 % cotton knitted jumper and a yellow knitted cotton jumper composed of 65 % recycled cotton and 35 % virgin cotton. The paper surfaces included white (80 g/m^2), multi-purpose copier printer paper (Q-Connect, Belgium), ECO TrendWhite, 100 % recycled, off-white (80 g/m^2) printer paper (Steinbeis, Germany), glossy leaflet paper and Filter Paper Fisherbrand® QL100 (Fisher Scientific, UK).

2.3. Sample preparation and collection

The samples were deposited in triplicate for each DNA source directly onto swatches of the materials in 5 µL aliquots and left until dry. The cfDNA and cells were deposited in a synthetic sebum solution as described by Arsenault et al. [19]. This was because resuspending cfDNA and cells in sebum solution has been shown to better imitate components of touch DNA samples than the same material resuspended in a buffer [19]. Approximately 20 ng (as estimated by Qubit) of DNA was deposited for cfDNA samples. The same DNA input was estimated for cellular deposits. However, as cell suspensions are prone to clumping, the input DNA for mouse cell is inherently variable to some extent.

Human saliva was deposited directly on the surface material with no prior preparation.

Bovine blood was left to thaw and then deposited directly on the material swatches.

The triplicate deposits were swabbed with a cotton swab (Technical Service Consultants Ltd. Lancashire, UK) moistened with 50 µL of buffer EB (QIAGEN, Hilden, Germany). The cotton swab was then left to air dry for a few minutes. Once dry, the cotton tip was cut with a clean pair of scissors directly into a 1.5 mL Eppendorf tube and placed at −20 °C until required. For the direct extraction, triplicate 1 cm^2 pieces of each material with DNA deposits were cut out, transferred into a 2 mL Eppendorf tube, and stored at −20 °C until required.

2.4. DNA extraction

The extraction from swabs and cut-out items was undertaken using an in-house extraction method described in detail by Gray et al. [18]. In summary, the components, and their concentration in 250 µL of lysis buffer were as follows: 1 % polyvinylpyrrolidone (PVP), 1 % tween 20 in 20 mM TrisHCl with 20 µg/mL proteinase K in 250 µL. Samples were incubated for 1 h at 56 °C with shaking at 1000 rpm followed by a 10-min incubation at 95 °C shaking at 300 rpm.

2.5. DNA purification by SPRI beads

The purification step was based on the method presented by DeAngelis et al. [20] but with slight modification. Sera-Mag™ Carboxylate-Modified Magnetic Beads (Cytiva, Marlborough, UK)) supplied at 50 mg/mL concentration were diluted 5-fold and washed three times in 0.5 M EDTA pH 8.0 for the final concentration of 10 mg/mL in 0.5 M EDTA. To each extracted sample an equal volume (250 µL) of 20 % PEG 8000 in 2.5 M NaCl was added followed by the addition of 10 µL of magnetic beads (10 mg/mL). The samples were then vortexed and centrifuged briefly and placed in a thermal shaker for 1hr incubation at room temperature shaking at 1000 rpm. The samples were then placed on the magnetic stand for up to 10 min. After the removal of the supernatant, the samples were washed with 750 µL of 70 % ethanol, vortexed to resuspend the pellet, centrifuged briefly and placed back on the magnetic stand. This step was performed twice. After removing all the residual ethanol, the samples were left on the magnetic stand to air dry for about 2–3 min. The DNA was eluted from the beads with 50 µL of 10 mM Tris-HCl pH 8.0 elution buffer with a 10-min incubation at 45 °C shaking at 700 rpm followed by bead removal on the magnetic stand. The eluted DNA was transferred to a clean Eppendorf tube.

2.6. DNA quantitation

The species-specific primers for trout, mouse and bovine DNA were designed with the NCBI genome browser tools [21]. The primers for each species were as follows: trout forward TCAGCAATCAGATGGGGAGG, trout reverse TTTCAATGATGGCCTAGTGGGT with a 110 bp product, mouse forward GACGAGGGGGAGCTTTACTTG, mouse reverse ATTGACTGTCTTGTGGACATGGG with a 231 bp product and bovine forward GATCACCCCGTCCCAGTGCC, bovine reverse TTGACGCCCCGCTCCTTTGT with expected product size 208 bp.

A set of GAPDH primers was used for human DNA samples with AAAGGGCCCTGACAACTCTTT forward and TCAGTCTGAGGAGAACATACCA reverse primer and expected product size of 400 bp.

The primers for trout, mouse and human DNA were obtained from Eurofins Scientific (Lancaster, UK), while the bovine primers were provided by Sigma-Aldrich (Gillingham, Dorset, UK).

The qPCR runs were performed in duplicate for each of the triplicate samples with Luna® Universal qPCR Master Mix (New England Biolabs Hertfordshire, UK) according to the manufacturer's instructions and in 10 µL total reaction volume. The analysis was performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific Inc, Waltham, Massachusetts, United States) with the following cycling mode: 95 °C for 2 min, 40 cycles of 30 s at 95 °C denaturing, 30 s annealing at 56 °C and elongation for 30 s at 70 °C.

2.7. Data analysis

The statistical analysis was carried out with GraphPad Prism. The p-value was calculated using a paired sample T-Test.

3. Results and discussion

3.1. Cell-free and cellular deposits

Cellular and cfDNA and a mixture were deposited on the test samples and allowed to dry. DNA was recovered from the test samples by swabbing and by direct extraction. Duplicate 5 μl samples were taken from the triplicate 250 μL extracts for qPCR quantitation and the remainder was extracted using the SPRI beads. These will be referred to as pre and post-purification samples. This was done to assess the impact of any potential inhibitors from the surface materials on PCR amplification. The experimental results are shown as percentage recovery with direct-to-buffer extraction defined as 100 %. The recovery of DNA was determined for cell-free and cellular deposits.

The results of the DNA recovery from the three different cotton garments can be seen in Fig. 1. From the initial observations, it is clear that the direct method of sample collection is more suitable for the recovery of DNA from cotton garments. The statistical analysis of the results further demonstrated that the direct method was significantly more efficient at DNA recovery, with p-values under 0.05 for all the deposits with the exception of cell-free material on the green cotton surface ($p = 0.1$ (Fig. 1c). It also comes as no surprise that the cellular material was a more recoverable DNA source when compared with the cell-free deposits. The encapsulated form of DNA benefits from a protective barrier of the cell [22] and being bound to histones [23], making it less susceptible to any damage from environmental factors. There are noticeable differences in the percentage recoveries of DNA between the three different garments. The highest values for the direct method were observed for the red jumper (Fig. 1b), while the dark-green T-shirt was the most challenging surface (Fig. 1c). It is important to note that the observed over 100 % recovery from the red garment (Fig. 1b) was caused by the previously mentioned predisposition of cells to clump together, which leads to uneven distribution of cells within each deposit. The recovery of DNA via swabbing was the most successful for the yellow jumper (Fig. 1a), although only for the cellular deposits. The recovery of cfDNA deposits via swabbing resulted in barely detectable values for all three garments. While a noticeable increase in detectable DNA post-purification step suggests the presence of PCR inhibitors in the samples, the addition of the extra step also led to the loss of DNA in some of the analysed samples (Fig. 1a and b) which can often be an unwanted side effect of sample purification [24].

The outcome of the comparison between the two collection methods from cotton surfaces demonstrates that direct extraction yields higher DNA recovery for all cotton samples. These findings are in broad agreement with the results presented by Solomon et al. [15], where the cutting-out method outperformed swabbing techniques. The differences in recoveries between the three pieces of material are most likely due to the texture and composition of each garment rather than its colour, as the chemical structure of fibres has been shown to impact DNA retention and recovery [25]. Nevertheless, the clear impact of PCR inhibitors should not be ignored. In all but three analyses, a higher percentage of recovery was observed in the post-purification samples. This is not surprising, as a negative impact on PCR amplification due to the presence of fabric dyes has been reported previously [26,27]. Additionally, as none of the garments was brand new, it is also possible that the inhibition came from residual laundry detergents [28].

The results of DNA samples collected from various paper types indicated that out of three tested surfaces, white paper was the most challenging to recover DNA from (Fig. 2a). None of the cell-free deposits resulted in even 1 % DNA recovery, regardless of the collection method. A slightly better outcome was observed for the cellular material, possibly due to the previously mentioned protective effects of the cell proteins and nucleus. The poor recovery of DNA from white paper could be caused by the interaction with bleaching and whitening agents used in the manufacturing process. Those agents may either destroy a DNA sample while it is still on the paper or interact with DNA during the extraction incubation [29]. This is supported by the observation for the direct extraction samples there was no increase in amplifiable DNA in the post-purification samples suggesting that the DNA is degraded. There was a marginal improvement in recovery post-purification for the swab samples this may reflect the fact that for the swab samples, the bulk of the paper was not present during the extraction process.

Much higher recovery percentages of DNA were observed for the deposits on the glossy magazine paper (Fig. 2b). This is not unexpected as the non-porous, smooth surface allows for easier and more efficient DNA recovery than any porous paper type [30]. Once again, the direct collection method proved to be more efficient for DNA recovery, especially for the cellular material ($p = 0.03$). However, it must be noted that, as previously observed in the analysis of the cellular deposits collected from the red jumper (Fig. 1b), the overall recovery exceeded 100 %. Nevertheless, there was a noticeable improvement in cellular DNA recovery in post-purification aliquots. The purification step was also beneficial for the cell-free samples, with the post-purification analysis resulting in over two times higher recovery (Fig. 2b). High DNA content was also observed in the cellular material obtained via swabbing. However, this time, the purification step resulted in a loss of DNA material, with DNA yield dropping by 30 %. Poor recovery was once again observed for cfDNA deposits (Fig. 2b).

The deposits collected from the off-white paper surface (Fig. 2c) resulted in much higher DNA content than their counterparts recovered from the white paper (Fig. 2a). This discrepancy could be explained by the lack of whitening and bleaching agents present in this type of printer paper. Once again, the samples collected with the swabbing technique resulted in much lower DNA yields, especially for the cellular DNA material. As in the previous analysis, the purification step increased the amount of amplifiable DNA.

As for the results of DNA recovery from the cotton surfaces, the outcome of DNA collection paper surfaces demonstrates the advantage of the direct, cutting-out method over the swabbing technique. It also confirms that cellular material is much more durable and easier to collect from various surfaces when compared with cell-free DNA.

Swabbing from the brown cardboard surface was the first case where this technique resulted in a higher yield than the direct collection method (Fig. 3a), with a statistically significant difference for the cell-free deposits ($p = 0.042$). This difference between methods was particularly noticeable for the cellular DNA samples, where collection with swabs resulted in over ten times higher values (Fig. 3a). However, this difference was not statistically significant ($p = 0.07$) due to the high variance in the data for the post-clean-up samples. A possible explanation for the poor performance of the direct method could be an interaction of unknown chemicals in the cardboard with DNA during the extraction incubation resulting in DNA degradation. As swabbing only introduced trace amounts of cardboard into the aliquots, it did not impact the recovery as significantly. Another explanation could be due to DNA getting trapped in the cardboard and not being released into the extraction buffer. A similar outcome was observed for the white cardboard surface, with most samples collected via direct method being under detection levels (Fig. 3b). The addition of a purification step almost doubled the amount of detectable DNA from the cellular deposits. The results of these analyses clearly demonstrate that the swab collection method is a more suitable technique for recovering DNA from cardboard surfaces.

The controlled sources of input DNA allowed us to assess the efficacy of the two collection methods for different surface types and investigate the potential transfer of PCR inhibitors from surfaces into the lysis buffer. Without interference from any inhibitors that are usually part of biological fluids, any sign of inhibition could be attributed to the surface material.

3.2. Blood and saliva deposits

To better mimic a crime scene scenario and the biological samples

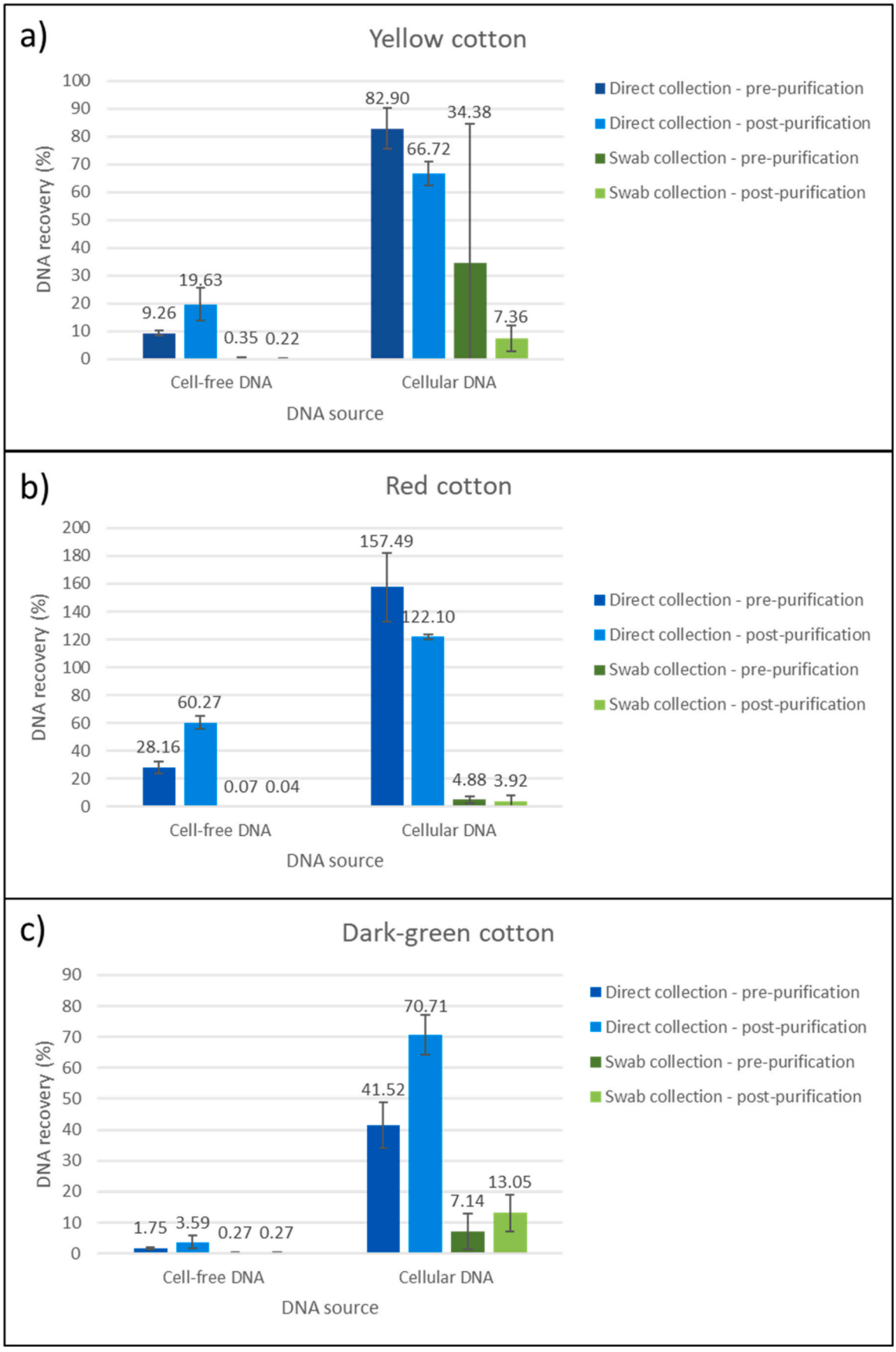

Fig. 1. Pre- and post-purification percentage recovery of cell-free and cellular DNA from cotton surfaces collected via swabbing and direct method. All data points are means and standard deviation of triplicate experiments.

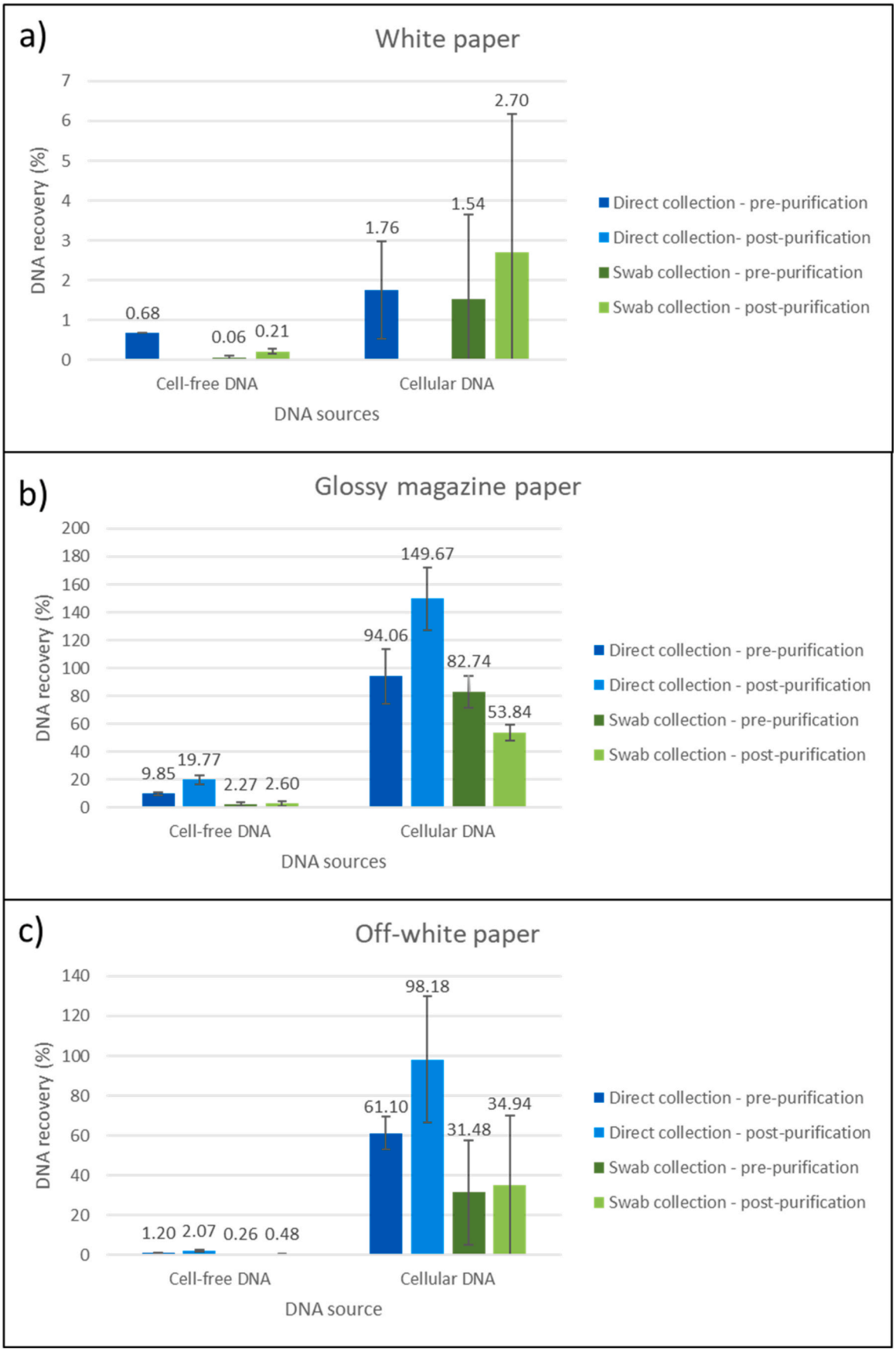

Fig. 2. Pre- and post-purification percentage recovery of cell-free and cellular DNA from paper surfaces collected via swabbing and direct method. All data points are means and standard deviation of triplicate experiments.

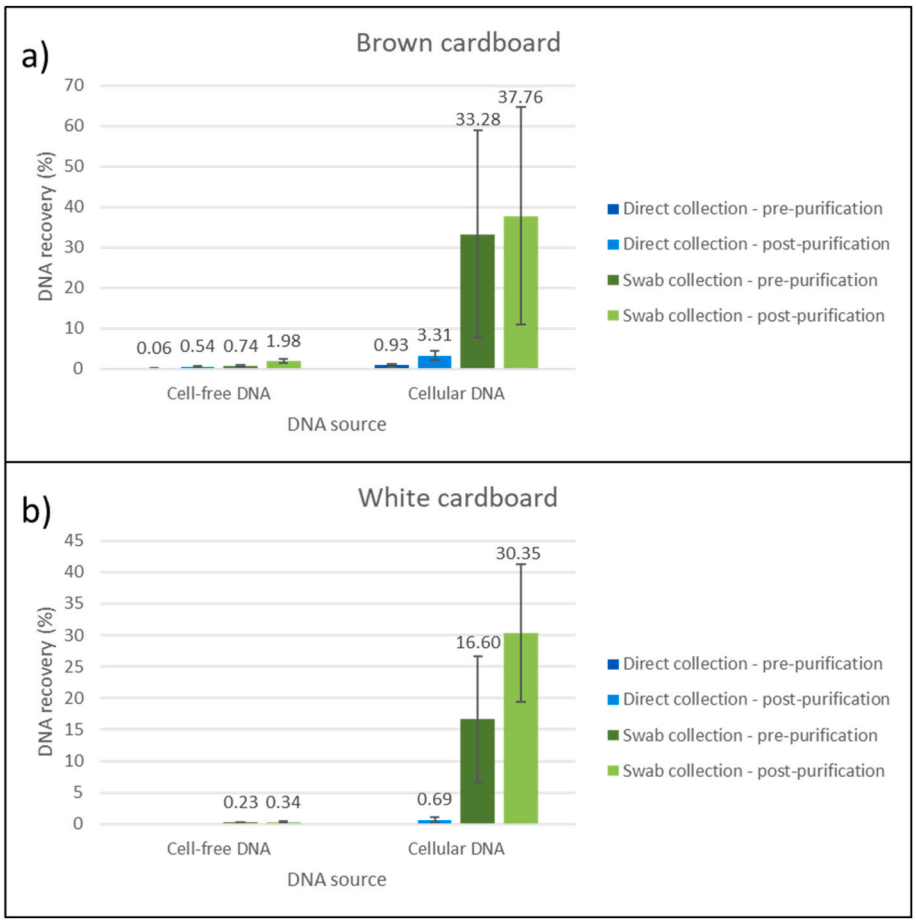

Fig. 3. Pre- and post-purification percentage recovery of cell-free and cellular DNA from cardboard surfaces collected via swabbing and direct method. All data points are means and standard deviation of triplicate experiments.

often encountered at crime scenes, the same comparison of surfaces and collection methods was conducted with human saliva and bovine blood deposits. This time, however, the samples were not analysed before purification due to the obvious presence of inhibitors in biological fluids [31–33]. Also, to further assess whether bleaching agents were contributing to the poor recovery from white paper surfaces, we tested DNA recovery from qualitative filter paper. Since the filter paper is not treated by any whitening agents, there was no risk of inhibitory interference from the surface material. The results of these analyses are reported as the total amount of recovered DNA in nanograms.

It is also important to note that the body fluids were much easier to collect via swabbing as they were still clearly visible, unlike the cell-free and cellular deposits, especially on the cotton surfaces.

The results of DNA recovery from blood and saliva deposited on cotton surfaces can be seen in Fig. 4. As in the previous set of experiments, the direct method of sample collection proved to be more effective in recovering DNA from cotton surfaces. This was particularly noticeable in blood deposits on green cotton ($p = 0.006$) (Fig. 4c) and saliva samples collected from the red jumper ($p = 0.002$) (Fig. 4b). Furthermore, the results of the analyses tally with the outcome of the experiments with cell-free and cellular DNA. The direct sample collection from the red jumper (Fig. 4b) yielded the highest amount of DNA out of the three tested cotton garments. This was particularly noticeable for the blood deposits. Although, DNA recovery from saliva deposits proved to be more challenging compared to blood regardless of the garment and method used. This may be due to the very high protein levels in blood compared to saliva, 202 μg/μl and 0.51 μg/μl respectively [34], acting as a blocking agent preventing strong interaction of the DNA in blood with the fibres of the garment swatches.

The differences in recovery between the cotton garments for each biological fluid are most likely caused by the differences in the fibre structure and composition and, therefore, the ease with which the DNA sample is released into the lysis buffer. It is possible that for some types of material, the incubation period should be longer to increase the amount of biological material transferred into the solution. Longer incubation time for blood sample collection to maximise recovery has been previously suggested [35].

Unsurprisingly, the most challenging type of paper surface for DNA recovery once again proved to be the white printer paper (Fig. 5a), with the swab method proving to be more efficient than the direct collection from blood deposits. The poor results of the direct collection from blood could once again be explained by the interaction between whitening agents and DNA during incubation. A different outcome was observed for saliva deposits, where the recovery via direct method resulted in a five times higher amount of DNA than the swab collection. However, none of the differences between results was statistically significant. It is possible that the proteins and mucus present in saliva [36] may protect the DNA by simple competition for the bleaching agents. Additionally, salivary peroxidase [37] may offer some protection from the whitening/bleaching agents leaching from the paper by the destruction of harmful oxidisers. There was not much difference between the amount of DNA extracted from blood via the direct method between the filter and off-white paper ($p = 0.8$). However, for the swab collection of blood, ten times more DNA was recovered from the filter paper (Fig. 5d) than from the off-white paper (Fig. 5c) although the difference was not statistically significant ($p = 0.18$). The same scenario was observed for the saliva deposits, with almost four times higher recovery for the swab collection of samples from the filter paper (Fig. 5d) than from the

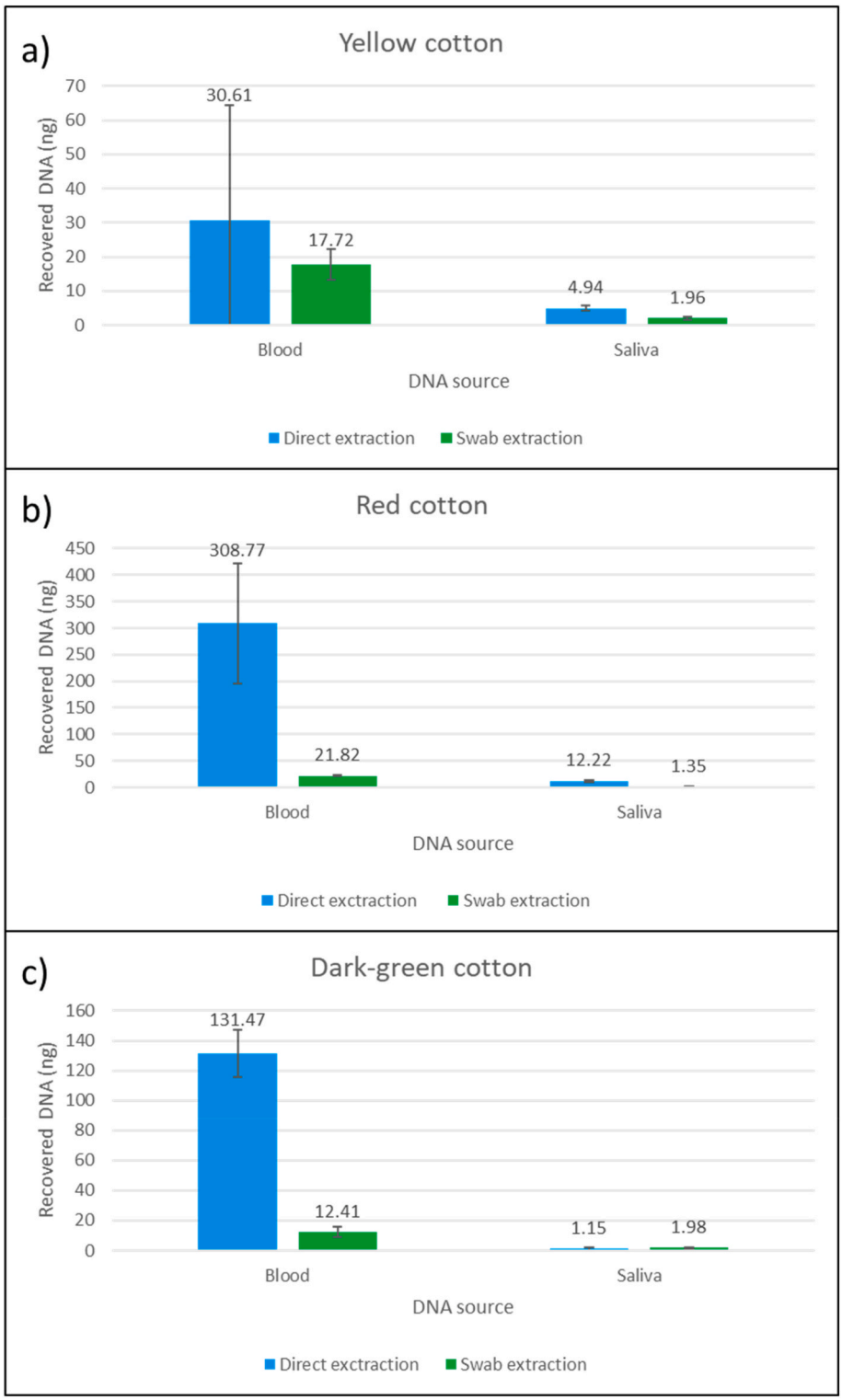

Fig. 4. Total DNA recovery from blood and saliva deposited on cotton surfaces and collected via the direct and swabbing method. All data points are means and standard deviation of triplicate experiments.

off-white paper (Fig. 5c), but with the p-value >0.05. The recovery of material from the glossy magazine once again did not pose any challenges, with the second highest DNA yield for both saliva and blood swab collection out of all tested surfaces and significantly more material recovered with the direct method for both blood ($p = 4.55E-04$) and saliva deposits ($p = 0.049$).

As expected, the outcome of sample collection from the cardboard surfaces (Fig. 6) once again proved challenging. Out of the four collection attempts from the white cardboard surface (Fig. 6b), only the direct collection of blood deposits resulted in the amount of DNA above the limit of detection. As previously observed with cellular and cell-free deposits, the swab method was the more efficient technique for recovering DNA material from brown cardboard (Fig. 6a), however, it is important to note that the p-values were higher than 0.05 Additionally,

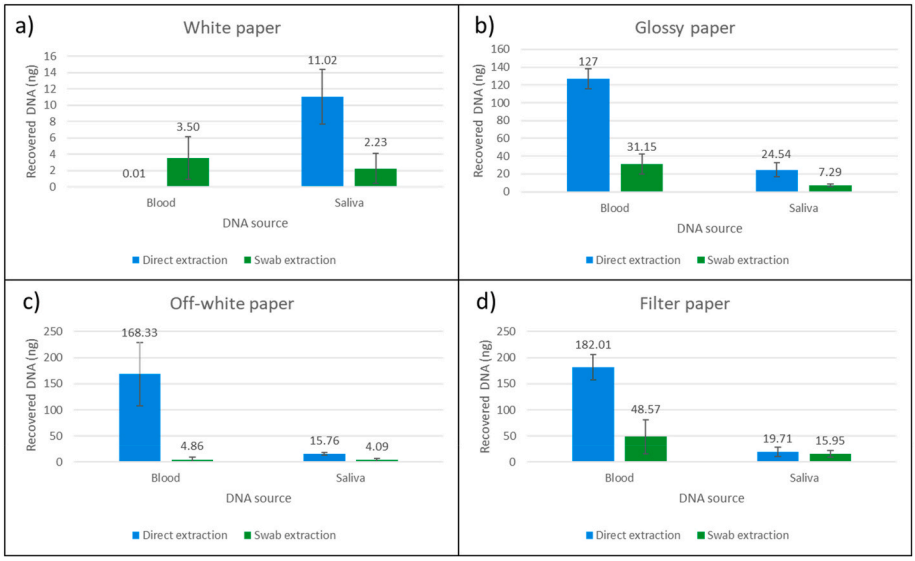

Fig. 5. Total DNA recovery from blood and saliva deposited on paper surfaces and collected via the direct and swabbing method. All data points are means and standard deviation of triplicate experiments.

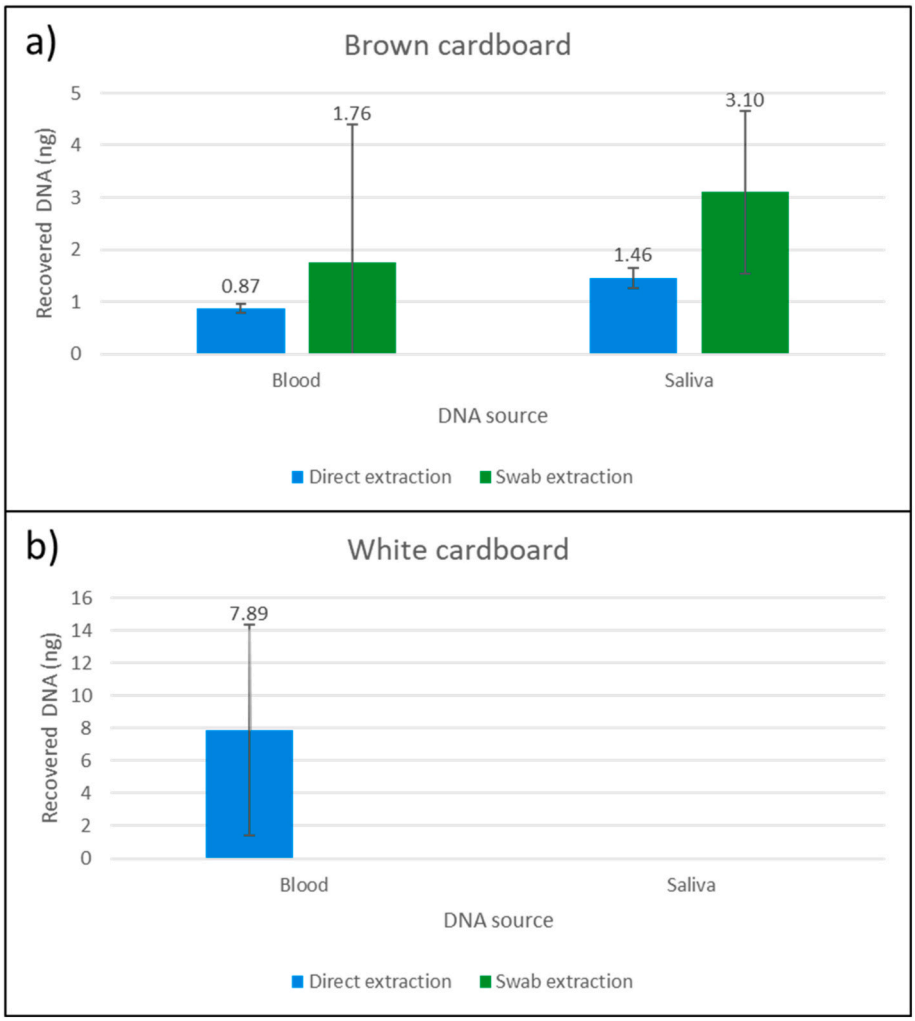

Fig. 6. Total DNA recovery from blood and saliva deposited on cardboard surfaces and collected via the direct and swabbing method. All data points are means and standard deviation of triplicate experiments.

with the highest recorded value of 3 ng, those results were still low compared with the DNA amounts recovered from the other two types of surfaces.

The outcome of this study reinforces the importance of selecting the appropriate collection method for the recovery of DNA material as documented in previous studies [1,15]. The direct collection technique proved to be the more efficient way of DNA recovery when compared to a single-swab technique. It is important to note that the selection of surface materials was biased and based on their suitability for the cutting-out method. In real-life scenarios, cutting out may not be appropriate for many surfaces. Additionally, in some cases, the forensic evidence must be preserved, while this method irreversibly destroys the item. The experiments confirmed that the cutting technique is the most appropriate for fabrics [28]. However, at the same time, it also poses a greater risk of introducing inhibitors into the solution. This was particularly noticeable during the analysis of deposits from the brown cardboard and white paper surfaces. Swabbing introduces fewer potential contaminants than direct extraction, during which the surface material is incubated with the biological sample and may be leaching substances which may interfere with further analysis. This was also noted in a study by Gunnarsson et al. [38], where the authors observed a lower level of inhibition in samples recovered with adhesive tapes than in samples that were extracted directly. Despite its limitations, direct collection is a much more straightforward and cheaper collection technique. In addition, it is not impacted by the variables present in the swabbing method. There are many aspects of the swabbing method that must be taken into consideration to maximise sample recovery. The features that may affect evidence collection include the material the swab head is made of [3,39,40], its length, thickness, shape and design [1]. Another set of variables that need to be considered is the pressure and duration of swabbing [41,42] and the swabbing technique of examiners [42]. The volume [41,42] and the type of the wetting agent [40, 43,44] have also been shown to affect DNA yield. With so many variables to consider, the cutting-out method seems to be a less unpredictable and more effective way of DNA recovery. However, as pointed out by Wahrer et al. [2], there is no bad or good method, as the studies show their efficiency is always based on the type of surface the sample is recovered from. In their review of existing literature, these authors [2] also demonstrate that the limitations of collection methods are often due to the surface DNA material is located on rather than the faults of the method itself.

Another significant observation from our study was how easily DNA or biological fluid is transferred into the solution from the surface material during sample incubation. This was particularly noticeable during sample collection from different cotton garments. Some surfaces are more efficient at retaining biological material, impacting the efficiency of DNA recovery.

4. Conclusions

The outcome of this short study adds to the existing body of research, highlighting the importance of selecting the correct method of DNA recovery. The selection process should be based on the type of surface material and the nature of DNA deposits. Currently, there is no unified and standardised approach to sample collection. Despite the limited amount of data on the performance of the cutting-out method, we believe that it should be considered a viable addition to DNA evidence collection.

CRediT authorship contribution statement

Agnieszka Kuffel: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Niamh Nic Daeid:** Funding acquisition, Writing – review & editing. **Alexander Gray:** Conceptualization, Methodology, Validation, Visualization, Supervision, Project administration, Writing – review & editing.

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.

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