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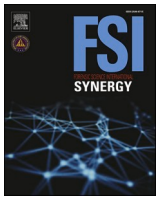

Impact of swabbing solutions on the recovery of biological material from non-porous surfaces

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ABSTRACT

Cotton swabs are one of the most effective methods of retrieving biological evidence. The efficiency of swab-based DNA recovery is impacted by many factors, such as the swabbing technique, source of DNA and volume and type of wetting solution used to moisten the swab head. This study aimed to evaluate a series of different swab-moistening solutions. The types of swabbing solutions included buffers, detergent-based solutions, and chelating agents. The DNA deposits, including cell-free DNA, cellular DNA, blood, and saliva, were collected from three non-porous surfaces: plastic, glass, and metal. The difference in the performance of the swab-wetting solutions was heavily influenced by the type of biological fluid, with the chelating agents, EGTA and EDTA, being the most suitable for recovering DNA from saliva and blood samples. Conversely, water and detergent-based solutions were more appropriate for cell-free and cellular DNA material likely to be found in trace DNA deposits.

1. Introduction

Multiple factors can affect the recovery of DNA from surfaces. These include the method of recovery [1–5], the characteristics of the surface the DNA material is located on [6–10], and the source of the DNA [3,8]. Selection of the appropriate recovery technique can often strongly influence the outcome of DNA recovery [1,2].

Swabbing remains one of the most popular and commonly used methods of DNA recovery [11,12]. This technique is relatively inexpensive, easy to use and suitable for DNA extraction [11–13]. The swabs are particularly effective when collecting biological material from non-porous surfaces [7,14]. However, when swabbing is chosen as the preferred method of collection, several variables need to be taken into consideration to maximise DNA recovery. Those variables include the type of material the swab head is made of [11,12,15–19], duration and pressure [20,21] and swabbing techniques such as single or double swabbing [6,20–22]. The other important factor that requires consideration is how the type of swab-wetting solution may affect DNA recovery. Studies show that the type of wetting agent [23–25] and its volume [16,20,21,25–27] can have an impact on DNA recovery.

The published studies demonstrate that there is no overall optimum swabbing solution [23–25] but still clearly show that detergent-based solutions are more efficient at DNA recovery than water [23,25], which has often been the swabbing solution of choice [25,28]. The amphiphilic detergent-based wetting agents such as sodium dodecyl sulphate (SDS) and non-ionic detergentsTween®20 and Triton X-100 are more efficient at solubilizing cellular components [23,29]. Water, due to its hypotonic nature, can cause cell lysis and lead to the released DNA being trapped within swab fibres, resulting in less efficient DNA recovery [30]. The other alternative to water is also found in the form of isotonic buffers such as phosphate-buffered saline (PBS) [25,26,29,31], which can help maintain cell integrity, resulting in a lower probability of DNA entrapment in fibres and higher DNA recovery [29]. However, because all the current research on swabbing solutions is based on the recovery of trace and touch DNA from various surfaces [22–24], it is still unclear whether the efficiency of a swabbing solution may depend on the type of biological evidence.

This study aimed to compare eleven wetting solutions including deionised water, Tris-HCL (at two different concentrations), chelating agents Ethylenediaminetetraacetic acid (EDTA), Triethyleneglycoldiaminetetraacetic acid (EGTA) and Bicine, Dithiothreitol (DTT) and detergent based agents such as sodium dodecyl sulphate (SDS), Triton X-100 (TX 100), Zwittergent and Tween®20 and their impact on the recovery of DNA from cell-free, cellular, saliva and blood deposits on plastic, glass and stainless steel surfaces. This investigation of the efficiency of wetting agents was carried out with four different sources of DNA deposits, including cell-free trout DNA (cfDNA), mouse cells, bovine blood, and human saliva. The variety of DNA sources allows for

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the use of species-specific primers, eliminating any concerns over cross-contamination. The in-house extraction protocol, although not used in forensic laboratories, has been tested against commercial kits and shown to be as effective and, in some cases, outperforming the commonly used forensic extraction methods [13,32].

2. Materials and methods

2.1. DNA sources

The cell-free trout DNA used for this experiment was extracted from frozen rainbow trout liver with chloroform phenol extraction [33] 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. Before use, the cells were centrifuged and resuspended in Tris-buffered saline (TBS) to the required concentration [13]. Human saliva was collected from a volunteer who signed an informed consent 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, frozen and stored at – 20 °C until required.

2.2. Surface materials

The three non-porous surfaces used in the study were glass slides, polypropylene plastic sheets, and stainless-steel squares. All the items were cleaned with PCR Clean™ (Minerva Biolabs GmbH, Berlin, Germany) and 70 % ethanol.

2.3. Wetting agents

Tris(hydroxymethyl)aminomethane (Tris) base and Ethylenediaminetetraacetic acid (EDTA) were obtained from ForMedium™, Norfolk, UK. Dihydroxyethylglycine (Bicine), Sodium Dodecylsulphate (SDS), Triethyleneglycoldiaminetetraacetic (EGTA) and Tween 20 were obtained from Sigma-Aldrich Company Ltd, Gillingham, Dorset, UK. The detergents Triton X-100 (Tx-100) and Zwittergent were obtained from VWR International, Leicestershire, UK and Merck KGaA Darmstadt, Germany, respectively. EB buffer 10 mM Tris-Cl, pH 8.5, was obtained from Qiagen, Manchester, UK and Dithiothreitol (DTT) from Fisher Scientific, Leicestershire, UK. Stock solutions of 1M Tris HCl and Bicine at pH 8.0 and DTT were prepared with Milli-Q water and diluted to the required concentrations as detailed in the experimental protocols. Detergents were prepared as 10 % stock solutions in Milli-Q water and diluted as required. Stock solutions of 0.5M EDTA and EGTA at pH 8.0 were also prepared in Milli-Q water and again diluted as required for the wetting agents. Table 1 provides a summary of the working concentrations of the wetting agents used.

2.4. Sample preparation and collection

Approximately 50 ng of cfDNA (by Qubit) and cellular DNA (by cell count) were deposited as triplicate samples in a volume of 5 µl of synthetic sebum solution [34] and left to dry. However, as cell suspensions are prone to clumping, the input DNA for mouse cells is inherently variable to some extent. Human saliva and thawed blood samples were deposited in triplicate directly on the substrates at a volume of 5 µl with no prior preparation. The deposits were swabbed with a cotton swab (Technical Service Consultants Ltd. Lancashire, UK) moistened with 50 µL of the required wetting agent. The tip of the cotton swab was cut with a clean pair of scissors directly into a 1.5 mL Eppendorf tube and placed at −20 °C until the swabs were extracted.

Table 1
Wetting agents and their concentrations used in the study.

Wetting agent	Concentration
Water	NA
EB	10 mM Tris-Cl, pH 8.5
Tris-HCL	50 mM, pH 8.0
EDTA	50 mM, pH 8.0
EGTA	50 mM, pH 8.0
Bicine	50 mM
DTT	1 mM
SDS	0.1 % v/v
Triton X-100	0.1 % v/v
Zwittergent	0.1 % v/v
Tween 20	0.1 % v/v

2.5. DNA extraction

The extraction of DNA from swabs was carried out using an in-house extraction method described in detail by Gray et al. [13]. In summary, the 250 µL of lysis buffer is composed of 1 % polyvinylpyrrolidone (PVP), 1 % tween 20 in 20 mM Tris-HCl and 20 µg/mL proteinase K in 250 mL. Samples were incubated for 1 h at 56 °C with shaking at 1000 rpm, followed by a 10-min incubation at 95 °C with shaking at 300 rpm.

2.6. DNA purification by SPRI beads

The purification step carried out on the extracted DNA is based on the method by DeAngelis et al. [35]. 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 with 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.

2.7. DNA quantitation

The species-specific primers for trout, mouse and bovine DNA were designed with the NCBI genome browser tools [36]. 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 an 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.

2.8. Data analysis

The statistical analysis of variances (ANOVA) and *t*-test were carried out with SigmaPlot (v. 14.5, Grafiti LLC, Palo Alto, California, United States). The Shapiro–Wilk test for normality and Brown-Forsythe test for equal variance were automatically applied to all datasets as part SigmaPlot analysis. Kruskal-Wallis one-way ANOVA on ranks was performed in cases where either of the tests failed. Based on the results of the statistical analysis, the data is presented as means or medians of triplicate results as described in figure legends.

3. Results and discussion

The experimental results for cell-free and cellular DNA are shown as percentage recovery with direct-to-buffer extraction defined as 100 % recovery. The results from saliva and blood deposits are presented as the total amount of recovered DNA in nanograms. Despite the non-porous nature of all three substrates, it was clear that the amount of recovered DNA was impacted not only by the wetting solution but also by the surface type. Therefore, the analyses of wetting solution efficiency were carried out separately for each surface type. The analyses are based on DNA quantification results, with the performance of each wetting agent assessed by the overall recovery. The used PCR primers generate amplicons of 100-400bp, which corresponds to the product sizes of amplicons produced with the most common STR profiling kits [37,38].

3.1. Cell-free deposits

The results of DNA recovery from cell-free deposits are shown in Fig. 1. Surprisingly, for the plastic surface collection, water-moistened swabs resulted in the highest DNA recovery (~45 %). The swabs treated with Zwittergent recovered less than 10 % of the input DNA, and the other three detergent-based wetting agents, SDS, TX-100 and Tween 20, resulted in under 40 % recovery. The differences in mean recovery from each wetting agent were statistically significant ($p = 0.027$). The better performance of water-moistened swabs over detergent-based agents was not an expected outcome, considering the results from the previous studies where detergents outperformed water in every experiment [23,25]. However, because those results were based on cellular DNA from touch DNA deposits, cell-free DNA may not be affected by those solutions in the same manner. Since we are considering only cell-free DNA in these experiments, the risk of cell lysis no longer needs to be considered.

The most suitable wetting agent for the cell-free deposits on the glass proved to be 50 mM Tris solution (Fig. 1), recovering almost 60 % of the initial DNA input. Three out of four detergent-based solutions (TX-100, Zwittergent, and Tween 20) slightly outperformed water (43 %), with all under 50 % recovery. The least efficient wetting agent turned out to be buffer EB, with less than 16 % of the input DNA recovered, which is quite interesting as buffer EB is essentially a 5-fold dilution of 50 mM Tris solution. It has been previously shown that, in some cases, increasing the concertation of swabbing solutions results in a higher DNA yield [23]. Once again, the differences between mean % recoveries were statistically significant ($p = 0.010$).

In general, for cell-free DNA deposits, collection from metal substrates generates the lowest overall recovery scores. The best-performing swabbing solution, Bicine, recovered ~36 % of the input DNA, while the swabs treated with EGTA led to the lowest observed recovery of under 8 % (Fig. 1.). This time, all four detergent-based treatments outperformed water-moistened swabs, resulting in 10–20 % higher DNA yield. The differences between mean recovery for all swabbing solutions were statistically significant ($p = 0.043$).

Additionally, as previously mentioned, the lowest overall average recoveries were obtained from metal surfaces (23 %). This is not surprising as metal surfaces are known to be challenging substrates for DNA recovery [9,30]. The highest overall average DNA recovery of 42 % was observed for the glass slides. This is in contrast to results reported by Wood et al. [9], where higher DNA recovery was observed for the plastic surface than for the glass slides.

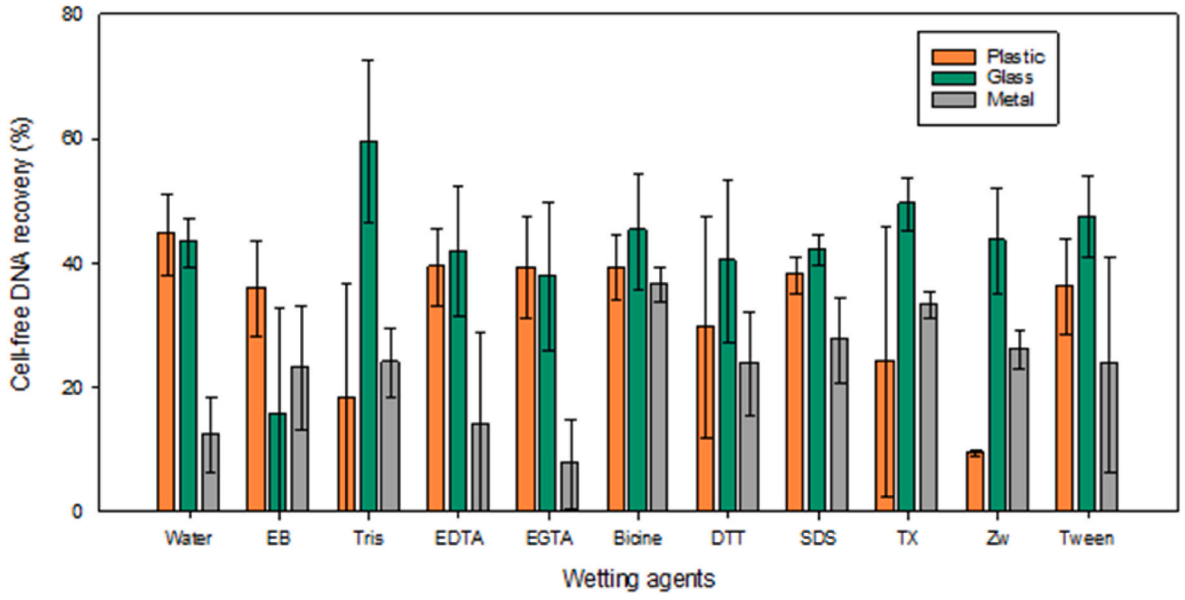

Fig. 1. Percentage recovery of cell-free DNA from plastic, glass, and metal surfaces collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %). All data points are means and standard deviation of triplicate experiments.

3.2. Cellular deposits

Considering the results of swabbing cellular deposits from the plastic surface (Fig. 2), the DNA collection from the swabs moistened with either water, EB, or Tris resulted in the highest observed recoveries of ~30 %. Unlike in the previous experiment comparing Tris and EB buffer (Fig. 1), the concentration of Tris did not have a significant impact on DNA recovery. The lowest recovery (11 %) was recorded for the collection carried out with swabs moistened with EDTA. As stated previously, it is unexpected that the detergent-based swabbing solutions were less efficient than water at recovering cellular DNA from the surface, given the results of previous studies [23,25]. Statistical analysis showed that differences in the median values among the treatment groups are greater than would be expected by chance ($p = 0.013$). In the case of the deposits collected from glass, the detergent-based wetting agent SDS resulted in the highest recovery (~40 %) and the lowest was obtained using Zwittergent ~12 %. However, the differences in the median values among the results were not statistically significant ($p = 0.2$). Tween 20 was the least efficient swabbing solution for the cellular deposits collected from the metal surface (under 20 %), while the swab moistened with Tris resulted in the highest DNA yield of 37 %. Still, as for the case of glass deposits, there was no statistical difference between the results ($p = 0.705$). In contrast to the cell-free deposits (Fig. 1), the highest overall average recovery was observed for the metal substrates. This could be explained by the fact that cellular DNA may not be affected by metals in the same way as cell-free DNA. The cellular material encapsulating the DNA provides a protective barrier for the DNA [39] and, along with tight binding to histones [40], may also shield DNA from the damaging impact of metals [41–43]. There was only a 0.5 % difference in recovery between the plastic and glass surface.

3.3. Biological fluids

For more realistic crime scene imitating scenarios, the efficiency of the swab-wetting solutions was tested during the collection and recovery of DNA from biological fluids. The comparisons of results from the recovery of DNA from saliva and blood deposits on the three substrates are shown in Figs. 3 and 4, respectively. The cotton swabs treated with EGTA recovered the highest amount of DNA for both plastic (27 ng) and glass (33 ng) surfaces (Fig. 3.). Water outperformed every single detergent-based solution for the saliva glass deposits. However, the difference between the outcomes was significant for Zwittergent ($p = <0.001$) and Tween ($p = 0.004$) but not for SDS and Tx100. For the samples collected from the plastic surface, only Zwittergent was marginally better than water, but the difference in results was not statistically significant ($p = 0.930$). Additionally, Zwittergent was shown to be the least effective wetting solution for the deposits collected from the glass surface (Fig. 3.), with a total amount of recovered DNA of under 6 ng. Statistical analysis by ANOVA test showed that the differences in the mean DNA recovery obtained using different wetting agents from the deposits on glass were statistically significant ($p = <0.001$). For the saliva deposits on the metal substrate (Fig. 3), two detergent-based solutions, SDS and Zwittergent, outperformed the collection of DNA with water alone. However, none of the differences between the results was statistically significant ($p = 0.448$ and $p = 0.122$ respectively). The highest average DNA yield was achieved with buffer EB (20 ng), while the lowest result was observed for the saliva deposits collected with cotton swabs moistened with TX-100 (~4 ng). Interestingly, a lower concentration of Tris in the form of buffer EB (10 mM) resulted in higher DNA recovery than the 50 mM solution across all three substrates. However, none of the differences in recovery was statistically significant ($p = >0.05$). The lowest overall recovery was observed for the metal surface (under 12 ng), while less than 1 ng difference was noted between glass and plastic surfaces (~18 ng).

The analysis of the results from blood deposits revealed that the swabs moistened with EDTA resulted in the highest DNA yield (Fig. 4) across all three substrates. The results were particularly high for the glass surface, where the recovered amount of DNA exceeded over 60 ng. For comparison, the swabs treated with Tris, the second most effective wetting solution for blood on the glass surface, resulted in a 50 % lower recovery. For two out of three substates, plastic and glass, the differences in the mean recovery from the different wetting agents were statistically significant $p = <0.001$ and $p = 0.003$, respectively. It is worth noting that in this case, the increased concentration of Tris solution resulted in higher DNA recovery than standard EB buffer concentration, with the differences in recovery outcomes being significantly different for the

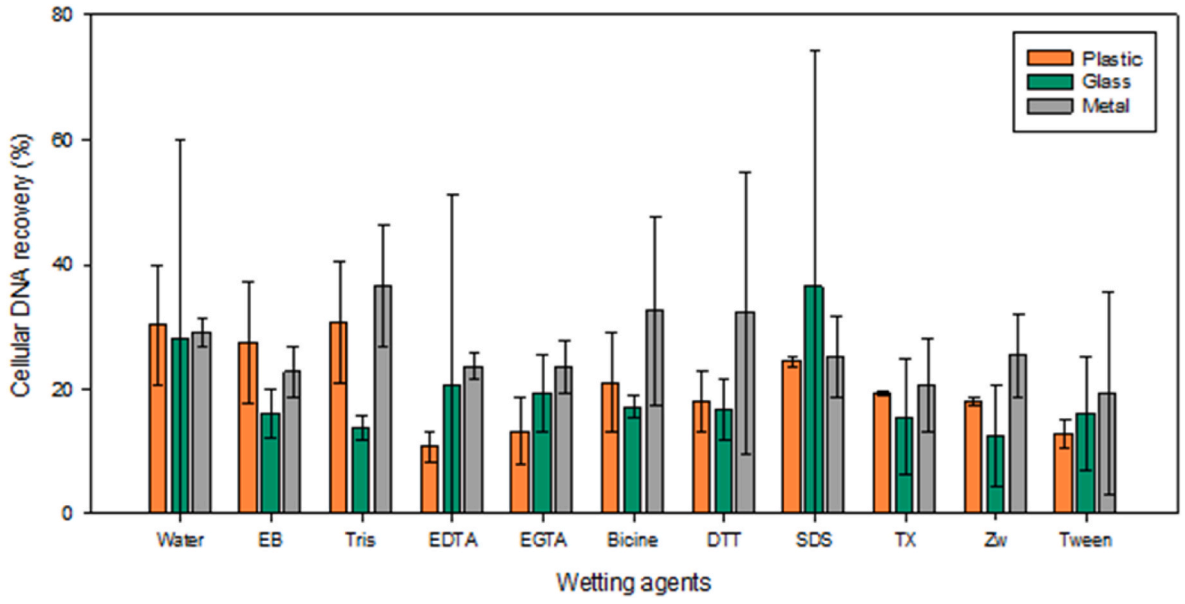

Fig. 2. Percentage recovery of cellular DNA on plastic, glass, and metal surfaces collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %). The data points for plastic and glass surfaces are medians and interquartile ranges while the data points for the metal surface correspond to means and standard deviation of triplicate experiments.

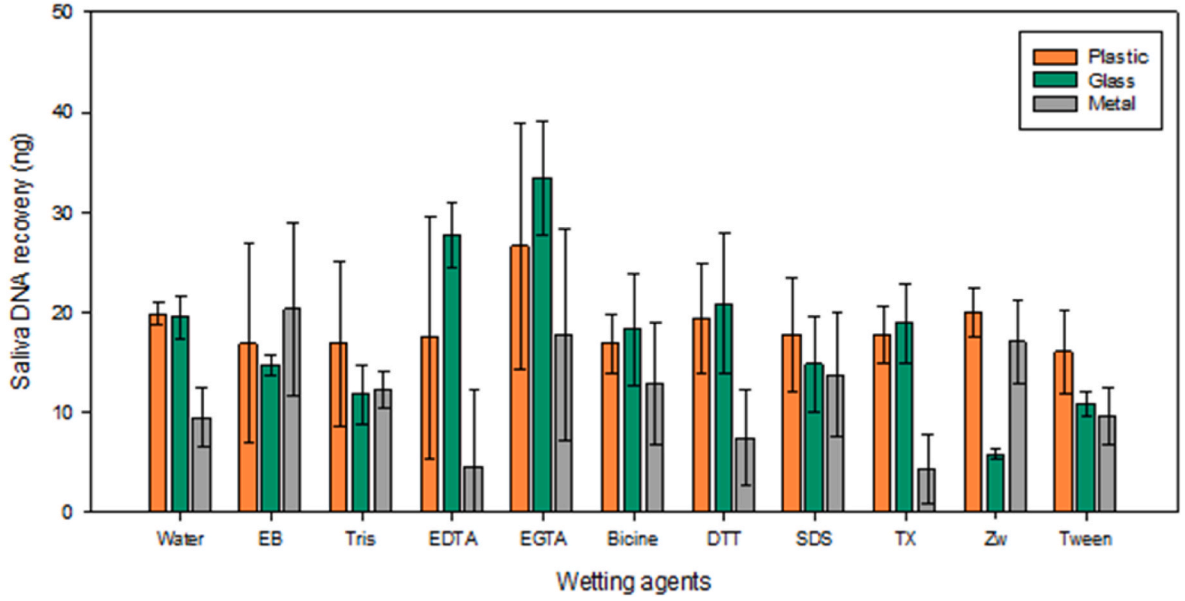

Fig. 3. Recovery of DNA from saliva deposits on plastic, glass, and metal surfaces collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %). All data points are means and standard deviation of triplicate experiments.

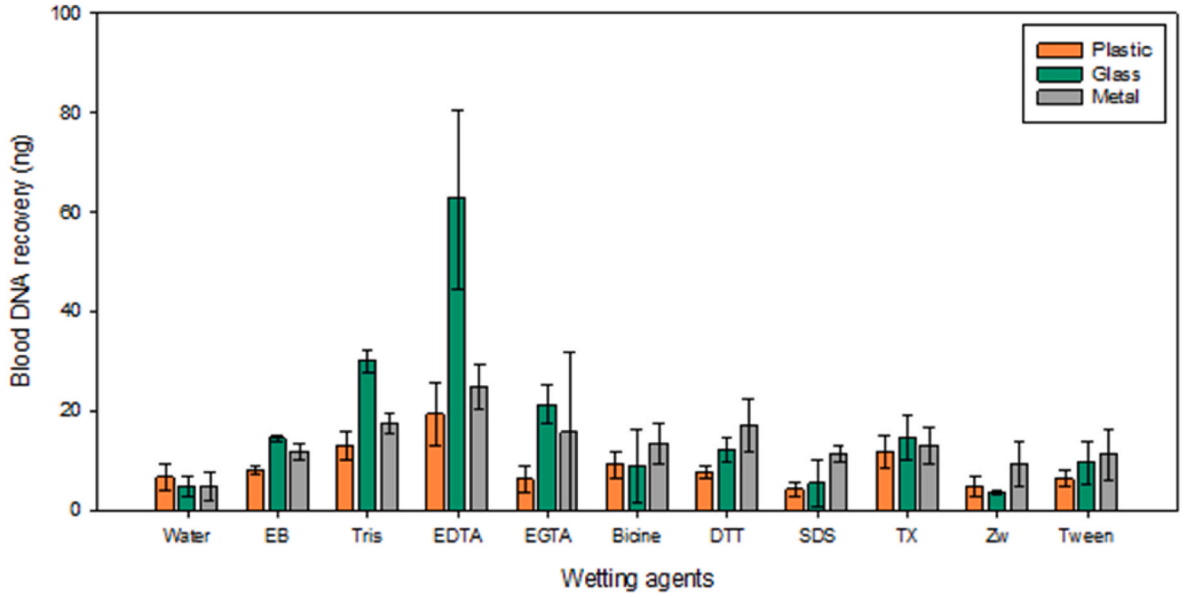

Fig. 4. Recovery of DNA from blood deposits from plastic, glass, and metal surfaces collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %). All data points are means and standard deviation of triplicate experiments.

glass ($p = <0.001$) and metal substrate ($p = 0.024$). Zwittergent was the least efficient swabbing solution for the deposits on plastic and glass, with, on average, under 5 ng of DNA recovered.

All four detergent-based swabbing solutions outperformed water in recovering DNA from blood deposits from the metal surface (Fig. 4). However, only the differences in recovered DNA amount between water and SDS ($p = 0.0242$) and water and TX-100 ($p = 0.037$) were statistically significant. The recovery of DNA from blood deposits was the only instance where the same swab-wetting solution (EDTA) was the most effective for recovering biological material from all three substrates. It has also been shown that EDTA can ease the dislodgement of dried blood off the surface [44]. Since EDTA is an efficient metal chelator [45], it will effectively bind any free iron released from haemoglobin, which may have a detrimental effect on DNA integrity. Both of these factors could contribute to EDTA being the most effective swab-wetting agent for the collection of blood from non-porous surfaces.

3.4. Overall average recovery

The results of this study reveal that for most of the samples, the performance of the wetting agents depended not only on the type of sample (cfDNA, cellular DNA or body fluids) but also on the surface from which the deposits were collected. Apart from the recovery of DNA from blood deposits, where EDTA was shown to be the most effective across all three substrates (Fig. 4), none of the other sets of results allowed for a clear identification of the most suitable swabbing solution for any of the

type of biological evidence tested. To evaluate the performance of each swabbing solution for all DNA deposits and without considering the substrate type, the overall average recovery for each wetting agent is summarised in Figs. 5–8.

Even though cfDNA and cellular DNA individually are not the type of biological evidence encountered at crime scenes, they are both components of touch DNA [46,47]. The evaluation of results from both sources analysed separately indicates that SDS or water may be the most suitable wetting solution of choice to maximise the recovery of both components. It is important to consider the possibility that increasing the concentration of SDS from 0.1 % could lead to a higher DNA yield. Thomasma and Foran [23] demonstrated that increasing the concentration of SDS to 1 or 2 % can result in higher DNA recovery. However, their analysis also showed that increasing the concentration of SDS from 0.1 % to 0.5 % concentration led to poorer recovery. Additionally, there are several studies demonstrating a successful recovery of trace DNA from surfaces with a swab moistened with 0.01 % SDS solution [48,49]. Moreover, SDS has been shown to inhibit DNA polymerase [50], even at concentrations as low as 0.01 % [51]. However, in the case of our study, the purification step was part of the extraction process, minimalizing the chances of any potential PCR inhibition. The purification step was required not only due to the inhibitors present in biological samples [52–54] but also due to the inhibitory effects on PCR of some wetting agents. Apart from the already mentioned potential inhibition from SDS, DTT can inhibit signal detection through fluorescence quenching [55]. By quenching the passive reference signal, the presence of DTT can lead to an overestimation of DNA content in the analysed sample. Despite the published studies indicating that detergent-based swabbing solutions outperform water in recovery from touch DNA samples [23,25], our experiments did not demonstrate any significant advantage for selecting detergent-based moistening agents over water (Figs. 5 and 6).

The calcium chelator EGTA, one of the three tested chelating agents, proved to be the most effective swab moistening solution for the recovery of DNA from the saliva deposits across the three tested substrates (Fig. 7). With just under 30 ng of total DNA recovered, EGTA significantly ($p = 0.032$) outperformed water (17 ng) and the most effective detergent-based solution for this group, SDS (~15 ng, $Pp = 0.011$). The overall average recovery of DNA with water was once again higher for water than for the detergent-based agent. However, the difference between the results was not statistically different ($p = 0.504$).

Similarly to the saliva deposit, a chelating agent, EDTA also proved to be the most efficient swabbing solution for the recovery of DNA from blood samples deposited on plastic, glass, and metal substrates (Fig. 8). The swabs treated with EDTA resulted in the highest average DNA yield (37 ng), significantly surpassing the amount recovered with water (5 ng) and the most efficient detergent-based solution for this type of biological evidence, Tx-100 (13 ng), with p-values of <0.001 and 0.003, respectively.

The outcome of this study demonstrates the importance of considering more than the commonly used swabbing solutions, such as deionised water and detergent-based agents. This is exemplified by the results demonstrating the very efficient recovery of DNA from saliva and blood by the chelating agents EGTA and EDTA, respectively. The performance of the swab moistening solution seems to be heavily dependent on the type of biological evidence, and as highlighted by Phetpeng et al. [24], there is no best-for-all wetting solution and method validations are always needed to maximise recovery. Moreover, as shown here in many examples, wetting agents seem to be not only DNA source specific but also dependent on the surface where the DNA sample is located. However, to avoid overcomplicating the DNA recovery process, it may be necessary to limit the choice based on the best suitability for a particular type of biological fluid. Nevertheless, in selected cases where the efficiency of any given agent is also heavily substrate-dependent, it

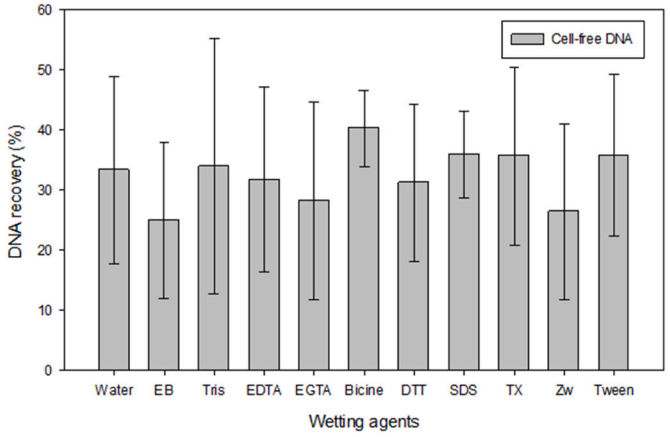

Fig. 5. Fig. 5. The average percentage recovery of cell-free DNA from all surfaces combined collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %).

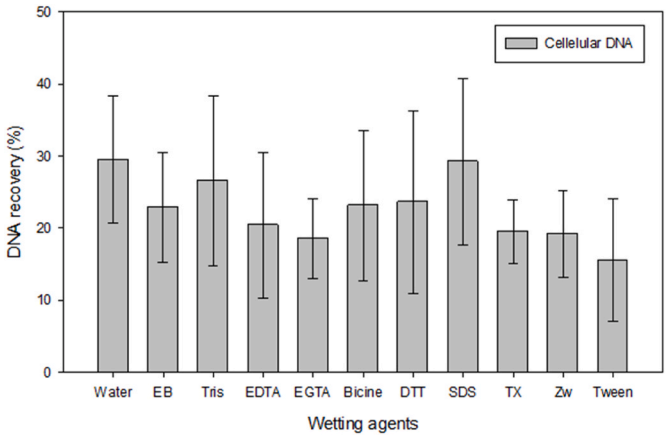

Fig. 6. The average percentage recovery of cellular DNA from all surfaces combined collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %).

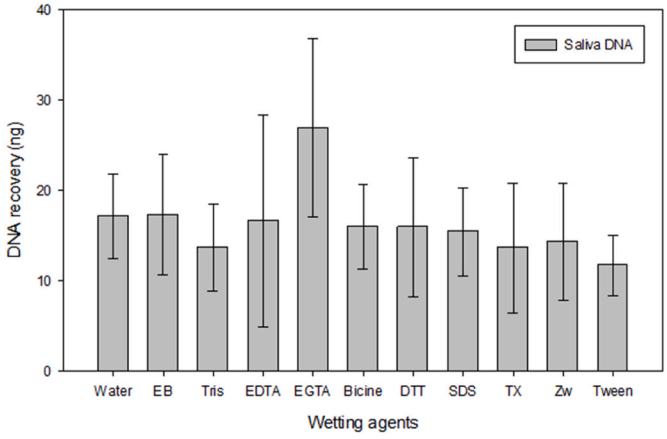

Fig. 7. The average recovery of DNA from saliva deposits from all surfaces combined collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %).

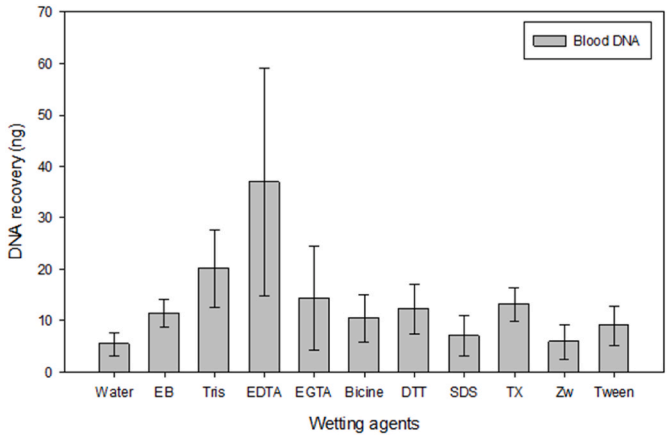

Fig. 8. The average recovery of DNA from blood deposits from all surfaces combined collected with a cotton swab moistened with one of the following wetting agents: water, EB, Tris (50 mM), EDTA (50 mM), EGTA (50 mM), Bicine (50 mM), DTT (Dithiothreitol, 1 mM), SDS (Sodium dodecyl sulphate, 0.1 %), TX (Triton X-100, 0.1 %), Zw (Zwittergent, 0.1 %), Tween (Tween 20, 0.1 %).

may be beneficial to consider both variables. There is also a possible issue of mixed biological evidence samples containing DNA from more than one type of body fluid. However, as demonstrated in this study, not a single tested swabbing solution resulted in unsuccessful recovery. For that reason, not choosing the most suitable wetting agent may impact DNA recovery, but it should not lead to a failed analysis.

4. Conclusions

The cotton swab as a method of collecting DNA-based evidence remains one of the most commonly applied recovery techniques at crime scenes and in laboratory-based examinations. The impact of the swabbing solution, however, is often overlooked. This study aimed to evaluate a series of frequently used and less common swab-wetting solutions. The results of the analysis revealed that the efficacy of moistening agents is dependent on the type of biological evidence, but it can also be affected by the type of surface from which the sample is collected.

Even though this study investigates a substantial number of swab-wetting solutions, there is a limited number of repeats per collection scenario. More extensive tests and validations are required to fully assess the performance of wetting solutions and their impact on the recovery of DNA from various body fluids and surfaces. Additionally, as all DNA is identical at a chemical level, with the only differences found in the sequence of bases, and both bovine and human blood contain the same type of PCR inhibitors, it was decided that, at this point, the use of all human DNA sources would be costly and unnecessary. However, for any crime lab application, validation with all human samples would naturally be required and involve additional verification methods, such as DNA profiling.

Nonetheless, this study provides a good base for further research and brings to attention swabbing solutions (such as EDTA and EGTA) that may require more in-depth investigation. In addition, our study also highlights the importance of using alternative solutions, such as chelating agents, which proved to be much more efficient at DNA recovery from saliva and blood samples than the commonly used water or more favoured detergent-based solutions.

Ethics statement

This project has been approved by the University of Dundee ethics committee. The volunteer signed an informed consent prior to donating their saliva sample for this study.

CRediT authorship contribution statement

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

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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