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ORIGINAL ARTICLE

Evaluation of the efficiency of Isohelix™ and Rayon swabs for recovery of DNA from metal surfaces

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Abstract

Purpose We investigated the recovery and extraction efficiency of DNA from three metal surfaces (brass, copper, steel) relevant to forensic casework, and plastic (control) using two different swabbing systems; Rayon and Isohelix™ swabs, with sterile water and isopropyl alcohol respectively, as the wetting solutions.

Methods Twenty nanograms of human genomic DNA were applied directly to Isohelix™ and Rayon swabs; and to the metal and plastic substrates. All substrates were left to dry for 24 h, followed by single wet swabbing and extraction with the DNA IQ™ System. DNA extracts were quantified using real time quantitative PCR assays with SYBR green chemistry.

Results DNA was extracted from directly seeded Isohelix™ swabs with a high efficiency of 98%, indicating effective DNA-release from the swab into the extraction buffer. In contrast, only 58% of input DNA was recovered from seeded Rayon swabs, indicating higher DNA retention by these swabs. Isohelix™ swabs recovered 32 – 53% of DNA from metal surfaces, whilst the Rayon swabs recovered 11—29%. DNA recovery was lowest from copper and highest from brass. Interestingly, Rayon swabs appeared to collect more DNA from the plastic surface than Isohelix™ swabs, however, due to the lower release of DNA from Rayon swabs they returned less DNA overall following extraction than Isohelix™ swabs.

Conclusion These results demonstrate that DNA samples deposited on metal surfaces can be more efficiently recovered using Isohelix™ swabs wetted with isopropyl alcohol than Rayon swabs wetted with sterile water, although recovery is affected by the substrate type.

Keywords Metal surfaces · Isohelix™ swab · Rayon swab · Collection efficiency · Release efficiency · Extraction efficiency

Introduction

Improved recovery of trace evidence from surfaces encountered in frontline practice has been a recurring theme of interest in forensic research. Trace evidence, such as touch DNA, results from the transfer of biological material to the surface of a substrate following human contact or handling. Recently, there has been a global increase in requests for forensic analysis of trace DNA [1, 2], attributed in part to the need to obtain investigative leads in cases presenting with no biological fluid stains [3]. Similarly, cold cases, with potentially degraded samples, are being resubmitted for trace DNA analysis [4]. Trace amounts of genetic material retained on non-porous surfaces are typically recovered speculatively using five main methods [4], including swabbing [5, 6]. Sampling methods based on swabs remain the most utilized due to their relative cost-effectiveness, amenability to robotic extraction techniques, and the ease of training requirements. Notwithstanding, the choice of swabs is primarily determined by practicality (often through in-house validation) and by the surface substrate harboring the evidence sample.

Surface swabbing to collect biological material is a critical technique for crime scene investigations and a range of forensic analyses [7]. An extensive range of simple to use and easily transportable swabs are commercially available for DNA sampling from different substrates [6, 8–10]. Previous forensic casework, method development and validation studies have evaluated nylon flocked [7, 9–15], cotton [5, 9, 10, 12–14, 16–20], foam [10], rayon [10, 14, 21] and polyester (dacron) [10, 19] swabs as collection devices for

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saliva [7, 9, 10, 13, 14, 19, 20], blood [20–22], vaginal [11], epithelial [10, 16] and acellular DNA [7, 15] on various substrates. Cotton swabs have been historically used as reliable collection devices; however, the cotton matrix is known to retain DNA (poor release efficiency) [9, 11, 23] and also leave fibers or impurities in the DNA extract which may result in PCR inhibition [7, 24]. Rayon swabs were mainly designed to improve microbial specimen recovery with better 'moisture-holding and maintenance' capacity to prevent desiccation of microbes [25]; and to curtail the toxicity of conventional cotton swabs to some microbes [26]. Rayon swabs have also been reported to show improved retrieval and release of DNA compared to cotton swabs [7, 27, 28]. Foam based swabs, such as mini-popules, developed as a more efficient alternative, eliminate the drying requirements of cotton and forestall microbial degradation of sampled DNA [4, 29, 30]. These have been deemed to be ideal for sampling of porous surfaces due to the increased surface penetration afforded by the flexibility of the material [7, 10]. The hydrophilic fibers of the nylon flocked swab have been documented to typically improve sample collection and release efficiencies [7, 11] similar to the non-chemisorbing matrix (designed for optimum adsorption and absorption) of dacron [7, 27, 28], but may leave fibers, especially on rough-textured surfaces and metals [10]. The Isohelix™ DNA buccal swabs (Cell Projects, Kent, UK) consist of a microporous membrane designed to quickly and actively dry the swab following sample collection, to stabilize and preserve the integrity of DNA on the swab while ensuring maximal yield [31] as shown in previous works [32, 33]. Swabs are typically used together with various buffer solutions during sample collection. The type of wetting agent used has been reported to be vital to the ability to release and recover DNA bound to a substrate [16, 17]. A double-swab method [34] is sometimes used; however, a recent study found that there was no significant difference between the latter and using a single wet swab [20].

Previous works have reported difficulty in the recovery of DNA from metal surfaces utilizing various swabs and wetting solutions, in contrast to the relative ease of recovery and amplification from other substrates such as glass and plastic [4, 15, 35–37]. Metal substrates like doorknobs, jewelry, knives, firearms, and ammunition are routinely encountered in crime scenes either as part of the built environment, wearable material, and/or weapons used during the commission of a crime [4]. The apparent inability to obtain and amplify sufficient DNA from some metal substrates has been partly attributed to their physicochemical properties [38–40] and the inefficiencies of the available recovery methods [4, 15]. Metals possess a range of ionization energies and affinities that impact their interaction with molecules like DNA that are negatively charged. For instance, the interaction of metal cations with the negatively charged phosphate backbone of DNA, has been noted to facilitate ionic bond formation that may hinder its release from metal surfaces, leading to poor DNA recovery from such substrates [4, 41]. Development and validation of new methods, as well as refinement of existing techniques has been suggested [4] given the current lack of consistency in choice of swabbing devices and wetting solutions for metal substrates across different forensic laboratories.

Rayon swabs have been extensively studied, often with sterile water, for the standard sampling of DNA on non-porous surfaces including metals (for example [7, 10, 29]), and are a common sampling device in many forensic laboratories. In contrast, the Isohelix™ swab, despite its prospects as noted earlier (see [31–33]), has had less extensive study for sample collection from porous or non-absorbent surfaces of forensic interest. Given that DNA recovery from metal surfaces is generally low and unpredictable [4], establishing an efficient DNA collection method is an important step towards improving trace DNA analysis. In this study, we examined the recovery of DNA from selected substrates using two swabbing systems currently employed in two major Australian forensic laboratories for the collection of trace DNA, these being the Rayon and Isohelix™ swabs wetted with sterile water and isopropyl alcohol respectively. This study investigated the collection and release efficiency of these swabs and the respective wetting agents for DNA recovery from three different metal surfaces and a plastic control substrate, including a consideration of the DNA extraction efficiency of the Promega DNA IQ™ System.

Materials and methods

Samples and reagents

Three different metal surfaces were selected for testing (brass, copper, steel) and plastic was used as a control. These metals were chosen as they represent common crime scene items [20]. We purchased small 2 × 2 cm plates of each metal from commercial suppliers. The plastic surface was a sterile petri dish. Each substrate was cleaned with 20% sodium hypochlorite, rinsed with DNA-free ultra-pure water, wiped with ethanol and a Kimwipe™ (Kimtech Science, Australia) followed by UV irradiation to remove any background DNA on the surface. We used commercially available male Human Genomic DNA (2 ng/µL) (Promega, cat#: G1471), two different swabs—Isohelix™ SK-2S DNA/RNA Buccal Swab (Cell Projects Ltd., Harrietsham, United Kingdom) and Rayon swabs (Puritan, Guilford, USA) (Fig. 1) and the DNA IQ™ Extraction System (Promega) for all testing.

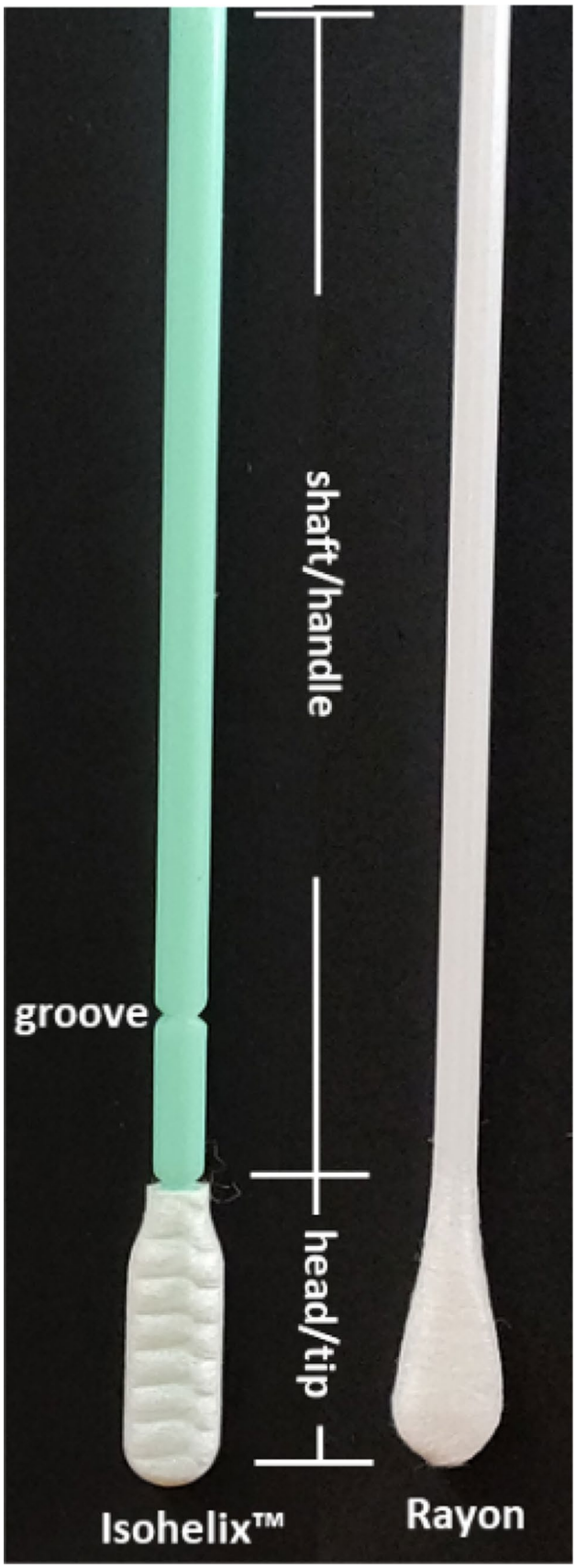

Fig. 1 Isohelix™ and Rayon swabs

DNA extraction efficiency of DNA IQ™

To examine extraction efficiency of the DNA IQ™ extraction kit, 20 ng (10 µL of 2 ng/µL) of male Human Genomic DNA was added directly to the Lysis/DTT buffer (n = 5 replicates). To examine the DNA release and extraction efficiency from the two swabs, aliquots of 20 ng (10 µL of 2 ng/µL) of male Human Genomic DNA were directly applied to the Isohelix™ (n = 5) and Rayon (n = 5) swabs pre-wet with 70 µL of isopropyl alcohol and 90 µL of sterile water respectively, and left to dry for 24 h.

Collection and release efficiency of Isohelix™ and Rayon swabs

To examine the sample collection efficiency of swabs and the efficiency of the overall process of extraction and recovery, 20 ng (10 µL of 2 ng/µL) of male Human Genomic DNA was applied to each substrate type (n = 5, for each swab type). Substrates were allowed to dry in a clean and sterile hood for 24 h before DNA was recovered using the Isohelix™ SK-2S DNA/RNA Buccal Swab and Rayon swabs.

DNA sampling and extraction

For metal and plastic substrates, a single wet swab protocol was employed, with 70 µL of isopropyl alcohol and 90 µL of sterile (DNA-free) water added to the Isohelix™ and Rayon swab tips respectively. The volumes of wetting solutions used was determined in preliminary experiments to suitably moisten the respective swab heads for stain rehydration and material transfer to the collection devices. The DNA on each substrate was sampled for 30 s. For each substrate, extract negatives (n = 2) were obtained by swabbing areas on the substrate where DNA had not been applied. Individual swab tips were subsequently snapped into 2 mL microfuge tubes and extracted with the Promega DNA IQ™ System into 30 µL elution buffer, following the manufacturer's protocol [42]. DNA extracts were stored at -20 °C prior to quantification. A single swab protocol was used because it is the casework protocol used by two Australian forensic laboratories for surface swabbing. Also, a recent study found that there was no significant difference between a double swabbing method and a single wet swab [20]. The 'Isohelix swab with isopropyl alcohol' and 'Rayon swab with sterile water' were selected based on their current use in forensic casework by two Australian forensic laboratories.

DNA quantification

DNA was quantified using real time quantitative PCR (qPCR) assays with SYBR green chemistry. The qPCR assays targeted a smaller (67 bp) and a larger (156 bp)

human-specific nuclear DNA amplicon. These regions were selected because their primers have been thoroughly validated for use in previous human DNA quantification in forensic casework [43–45] (Table 1). The reactions were carried out in 10 µL volumes consisting of: 1 × Brilliant III Ultra-Fast SYBR Green Low ROX qPCR Master Mix (Agilent Technologies, USA), 0.15 µM forward primer, 0.15 µM reverse primer, 16 ng/µL Rabbit Serum Albumin, and 1 µL extract or standard DNA. All samples, including negative and positive controls, were run in triplicate. Thermal cycling conditions were 95° C denaturation step for 4 min, followed by 45 cycles of 95° C for 10 s, 58° C for 20 s, and 72° C for 15 s. The specificity of primers to a single binding site was assessed using a post qPCR melt curve to visualize the dissociation kinetics.

The real time PCR was performed on a QuantStudio™ 6 Flex Real-Time PCR System (Thermofisher Scientific). DNA concentration was determined using the comparative C_T method by comparing unknown samples to a standard curve using the QuantStudio™ 6 Flex Real-Time PCR Software v1.3 and applying ROX as the passive reference. The standard curves comprised an eight-point, 3 × dilution series from 50 ng/µL to 0.023 ng/µL for each primer set, with each dilution level performed in five replicates. The resulting DNA concentration was multiplied by elution volume to estimate the yield for each sample.

Data analysis

DNA recovery data were analyzed using GraphPad Prism (version 8.0.0 (224)), presented as percentage DNA recovery ± one standard deviations (SD), and visualized with interleaved bar graphs. Mann–Whitney tests were performed to test the significance of differences between collection and release efficiencies of the Isohelix™ and Rayon swabs on each substrate, and the degradation index (DI). The DI was determined as a ratio of the absolute quantity values (in ng) of the two quantified targets. The collection/release data of each swab was normalized on the DNA-IQ™ extraction efficiency to determine their specific percentage efficiencies. We also calculated the percentage of DNA lost and retained for each of the three stages namely: DNA swabbing from substrates, release of DNA directly applied to swab and DNA extraction.

Table 1 Primers used for quantification of DNA.

Fragment length	Primer name	Primer Sequence 5' – 3'
Nuclear DNA 67 bp	HomoSap_CSF STR_F/ HomoSap_CSF STR_R	GGGCAGTGTTCCAAC CTGAGGAAAACT GAGACACAGGGT GGTTA
Nuclear DNA 156 bp	HomoSap DQARB1_105F/ HomoSap DQARB1_214R	AGGTTGCTAACTATG AAACACTGGCTG GTTTAGGAGGGT TGCTTCC

Results

The real time quantitative PCR assays were evaluated according to the MIQE guidelines [46]. Reliable quantification was established from 50 ng (15,625 copies) to 0.023 ng (7 copies) of input DNA per reaction, with an acceptable linear range ($R^2 = 0.0999$). For both nuclear DNA targets the amplification was repeatable and reproducible over the five replicates analyses.

DNA extraction efficiency of DNA IQ™ kit

The efficiency of extraction of genomic DNA directly applied to the extraction medium (Lysis/DTT buffer) using the Promega DNA IQ™ was 88.7 ± 1.4% and 87.4 ± 2.4% for the 67 bp and 156 bp fragments, respectively (Supplementary Table 1). There was no significant difference ($p = 0.4603$) in the percentage recoveries of the two targets.

Collection and release efficiency of Isohelix™ and Rayon swabs

Excluding the ~ 12% loss of DNA during the extraction process, DNA recovery from the Isohelix™ swabs yielded high efficiency 97.9 ± 1.8% but this was reduced for the Rayon swabs to 57.7 ± 1.2% (Fig. 2, Supplementary Tables 2 and 3). This percentage release of directly applied DNA was significantly higher ($p = 0.0079$) with Isohelix™ than the Rayon swabs. While levels of recovery were reduced with both swab types when the genomic DNA was applied to the substrates, the Isohelix™ swab still showed better collection efficiency on metals than the Rayon swab (Fig. 2). More than 74% of the DNA applied to the plastic surface was recovered by the Isohelix™ with isopropyl alcohol compared to 50% recovery for the Rayon device with water (Fig. 2, Supplementary Tables 2 and 3). In contrast, DNA collection efficiency from the three metal substrates was only 32–53% with the Isohelix™ system, and 11–29% with the Rayon system (Fig. 2, Supplementary Tables 2 and 3). DNA recovery was poorest from copper surfaces with both swab systems (32% for Isohelix™ and 11% for Rayon). For all substrates tested, recovery efficiency was significantly higher ($p = 0.0079$) for Isohelix™ swabs with isopropyl alcohol than Rayon with sterile water.

Loss of DNA varied between the two swabbing systems for (i) recovery of DNA from the different substrates and (ii) release of DNA into the extraction buffers. For the Isohelix™ system, DNA recovery from metal surfaces was low

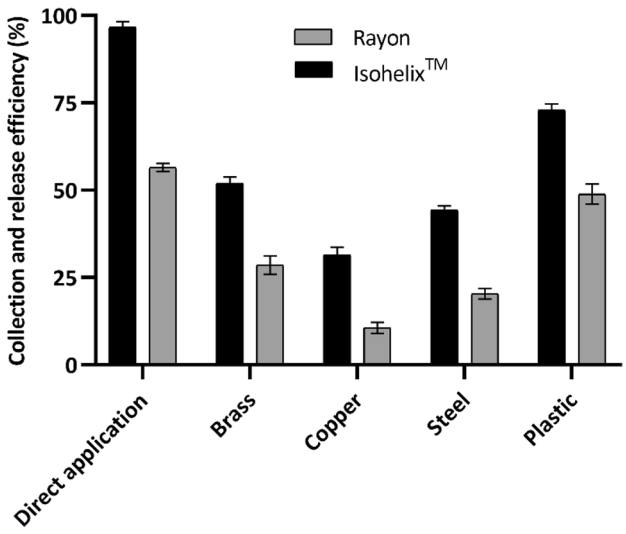

Fig. 2 Collection and release efficiency of Rayon and Isohelix swabs where 20 ng human genomic DNA was either applied directly to the swab or swabbed from metal and plastic surfaces. These values are based on the quantification results of the 156 bp target and are normalized for the percentage loss of DNA for each swab during the DNA IQ extraction process

(29% for copper to 48% for brass) compared to plastic (67%), with most collected DNA (98%) released into the extraction buffers (Fig. 3, Supplementary Tables 2 and 3). For the Rayon system, DNA recovery from metal surfaces was also low (19% for copper to 49% for brass) but much higher for plastic (84%), but a high percentage of DNA (42%) was not released into the extraction buffers (Fig. 3, Supplementary Tables 2 and 3), resulting in low overall DNA yields.

Degradation index

The degradation index (DI) varied from 1.01 to 1.10 for all samples and averaged 1.02 ± 0.01 and 1.04 ± 0.04 for the Isohelix™ and Rayon swabs respectively for the substrates studied (Supplementary Table 4). There was no significant difference ($p = 0.5476$) between the DI of the two swabbing systems, indicating no difference in DNA degradation between the 156 bp and 67 bp qPCR targets.

Discussion

This study sought to explore the relative efficiencies of two swabbing systems currently used in Australian forensic laboratories, particularly with respect to recovery of DNA from metal surfaces. Our results indicate that Isohelix™ swabs, wetted with isopropyl alcohol, perform significantly better at recovering DNA from brass, copper, and steel, than Rayon swabs wetted with water. However, DNA recovery efficiency using Isohelix™ was still only 32–53%, depending on the type of metal. Overall DNA recovery efficiency from swabbed surfaces is dependent on three main variables – the efficiency of DNA collection by the swab from the surface, the efficiency of DNA release from the swab to the extraction buffer and the efficiency of the DNA extraction system. The DNA IQ™ DNA extraction system recovered 88% of input DNA (Fig. 3), which is comparable with previous studies [47, 48] indicating high overall extraction efficiency. When DNA was extracted from directly seeded Isohelix™ swabs, similarly high efficiency was obtained, indicating effective DNA-release (98%, Fig. 3(A)) from the swab into the extraction buffer. In contrast, only 58% of input DNA was recovered from the seeded Rayon swabs, indicating 42% DNA retention by the swabs (Fig. 3(B)). Using the DNA IQ™ kit, a previous study [7] showed similarly low recovery of DNA from seeded Rayon swabs. In contrast, Frippiat and Noel [21] found significantly more DNA from Rayon swabs seeded with diluted solutions of biological fluids. The discrepancy in the minimal release efficiency in this study and the latter ([21]) may be accounted for by the differences in the DNA source (acellular DNA versus blood) and wait time between sample application and extraction (drying for 24 h versus immediate extraction). Thus, for acellular DNA, Isohelix™ swabs have a significantly higher release efficiency than Rayon swabs, 98% compared to 58%.

Previous work [10, 15, 37] has reported a higher recovery of trace DNA from plastic substrates in contrast to metallic ones. The latter is partly attributed to the strong metal-DNA interaction that impedes the ability to dislodge and recover bound DNA from the substrate. Plastics, on the other hand, are inert for substrate ion -nucleic acid interactions permitting expedited sample retrieval [15]. In this study therefore, plastic surfaces were used as a control substrate to examine the relative sampling/collection efficiencies of the two swabs. Approximately 73% and 49% of the DNA applied to the plastic surface was recovered by the Isohelix™ and Rayon swabs respectively, following DNA extraction (Fig. 2). Considering DNA losses due to retention by the swab and the extraction process, the Isohelix™ swabs recovered 29—48% of DNA from metal surfaces, whilst the Rayon swabs recovered 19—49% (Fig. 3). Interestingly, Rayon swabs appear to collect more DNA from the plastic surface than Isohelix™ swabs (84% compared to 67%, Fig. 3), but due to the higher retention of DNA by Rayon swabs they return overall less DNA following extraction (49% versus 73%). However, in all instances and for all the metal substrates, there was a significantly higher amount of DNA collected with Isohelix™ swab than with Rayon (Fig. 3). For both swab types, the lowest recovery was observed on copper substrates, consistent with the findings of Bille et al. [38] and Holland et al. [49], presumably due to copper-induced DNA damage [4].

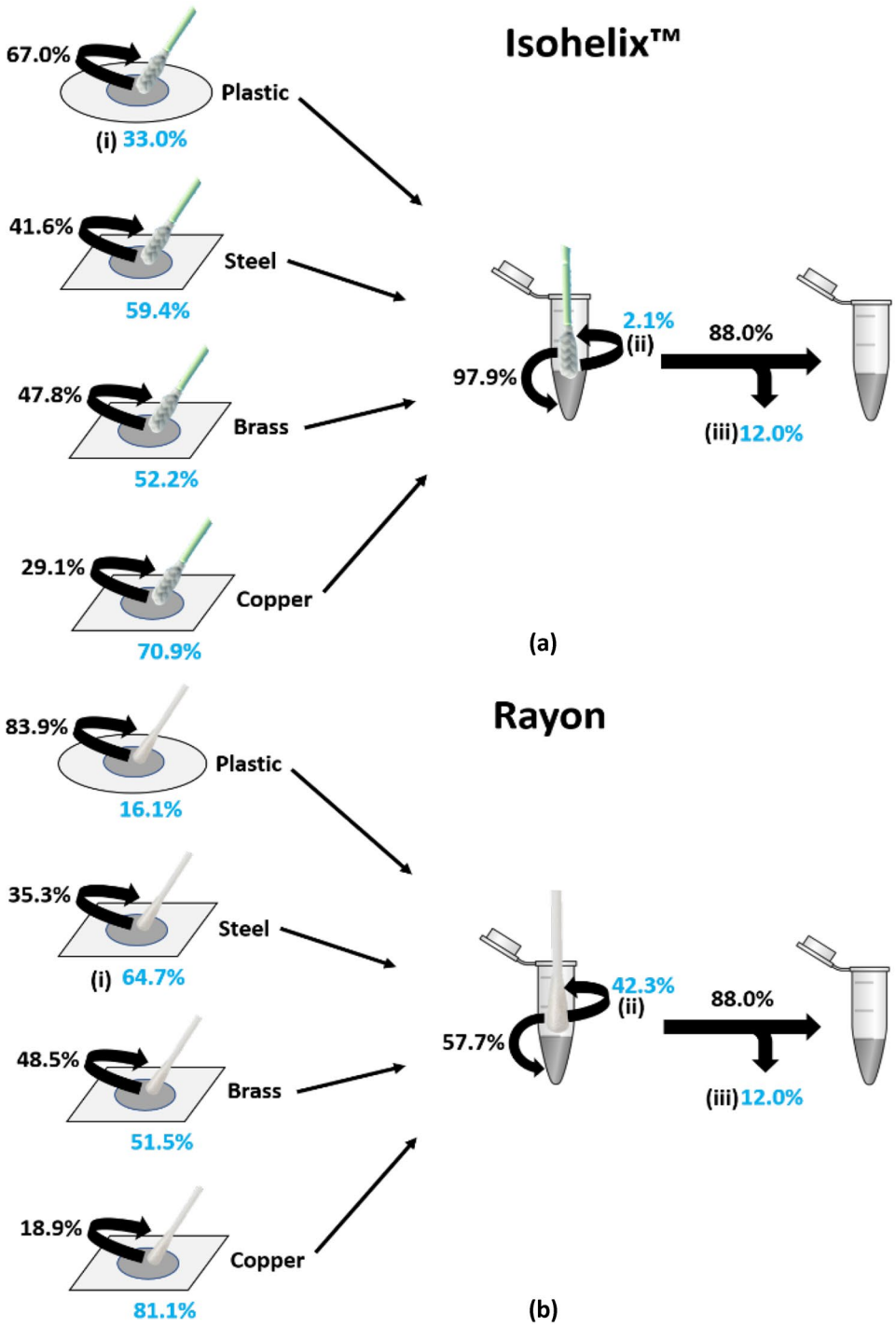

Fig. 3 DNA loss and retention on metal and plastic substrates for (A) Isohelix™ swab pre-wet with isopropyl alcohol and (B) Rayon swab pre-wet with sterile water. The percentages in blue represent DNA loss due to (i) not being picked up by the swabs, (ii) not being released by the swabs and (iii) DNA extraction

Despite finding no significant differences in the percentage recoveries of the 67 bp and 156 bp fragments, we determined the DI of the different swabbing systems and surfaces to ascertain the potential impact of degradation. If the concentrations of the small and large amplicons are approximately equal, a DI of ~ 1 indicates intact DNA [50]. Analysis of the DI showed no degradation of the DNA for the two swabbing systems for each substrate, and is consistent with the result obtained by Frippiat and Noel [21].

The difficulty in collecting sufficient DNA from metal surfaces like copper has been well documented [38, 49, 51] with a presently inconsistent success rate of at most 26% [4, 52]. The Isohelix™ swabs were found to exhibit a higher recovery and extraction efficiency (32%) from copper surfaces compared to Rayon (11%) (Fig. 2), suggesting that

the former may be the ideal device for sampling DNA from problematic metal substrates. The efficiency of DNA recovery using Rayon swabs has been studied for porous and non-porous surfaces of forensic interest (for example [7, 10, 21]). However there is no previously published research evaluating the Isohelix™ and associated buffer systems for DNA sampling from porous or non-porous surfaces, especially metal surfaces which have been noted to be problematic for DNA recovery [4]. The manufacturer of Isohelix™ swabs claims that they have significant advantages over other sampling devices in terms of the "efficiency of cell collection by the unique swab matrix, which combines with a quick release surface, to maximize yields of DNA" [53]. Moreover, the swabs are supplied DNA-free due to treatment with ethylene oxide (EtO), in contrast to the Rayon swabs. The specific impact of metal surfaces characteristics on the limited recovery efficiencies observed in this study is currently being further investigated.

A primary consideration for a laboratory's choice of swab is its practicality [4] as related to the ease of use, fragility of swab stick and head (tip), as well as the ease of snapping off the tip following sample collection. The Isohelix™ swab was found to have a strong handle that allows firm and solid pressure to be applied during sample collection, as compared to the Rayon swab. Further, the Isohelix™ swab is designed to allow easier removal of the head for further processing via a manufactured breakpoint (groove) (Fig. 1) at the base of the head or by removing the sampling matrix from the shaft into the collection tube.

This study demonstrates that DNA samples deposited on metal surfaces can be efficiently recovered using the Isohelix™ swab with isopropyl alcohol as the wetting agent. The resulting collection and release efficiencies are significantly better than using the Rayon swab with sterile water. However, further studies on 'real-world' cellular samples (for example saliva, blood, touch) are required to demonstrate the applicability of Isohelix™ for enhanced DNA recovery from a broader range of metal surfaces. The DNA used in this study is a pure genomic one for which a relatively high but consistent amount was applied to the substrates. It is therefore important to ascertain via further research regarding the performance of the Isohelix™ swab on lower amounts of both cellular and acellular DNA, as well as casework samples, which likely contain variable amounts of DNA and contaminants.

Conclusion

This study has shown that two types of swabs and wetting solutions, and the substrate type from which samples are collected, can have a direct effect on the amount of DNA ultimately recovered from an evidence item. Isohelix™ swabs used together with isopropyl alcohol have demonstrated the ability to efficiently recover more DNA from metal surfaces than Rayon swabs moistened with sterile water. The application of the Isohelix™ swabs for copper surfaces, in particular, gives credence to its effectiveness in retrieving DNA from a problematic metal substrate.

Key Points

1 Metals surfaces are problematic substrates for trace DNA recovery.
2 Isohelix™ swab has 98% DNA release efficiency compared to 58% from Rayon swabs.
3 On metal substrates, Isohelix™ and Rayon swabs collect up to 53% and 29% of surface DNA respectively.
4 DNA samples on metal surfaces are best recovered using Isohelix™ swab system.

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