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ORIGINAL ARTICLE

Comparison of Isohelix™ and Rayon swabbing systems for touch DNA recovery from metal surfaces

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Abstract

A previous study evaluating two swabbing systems found that DNA was best recovered from sterile metal substrates using an Isohelix™ swab wetted with isopropyl alcohol rather than a Rayon swab with water as the wetting agent. We tested the same swabbing systems on metal (aluminum, brass, and stainless steel) and plastic substrates in a regularly touched environment to simulate the non-deliberate transfer of touch evidence likely seen in a casework scenario, to ascertain the performance of these swabs in an uncontrolled situation. Higher amounts of touch DNA were recovered with Isohelix™ swabs (0.5 – 3.3 ng) compared to Rayon swabs (0.13 – 1.2 ng). The Isohelix™ swabbing system was found to significantly recover more touch DNA ($p = 0.04$) from the metal substrates than the Rayon swabbing system, consistent with the findings of our previous work. The results contribute to our understanding of the impact of sample collection techniques on touch DNA recovery from problematic metal surfaces and suggest that supplemental cleaning of substrates as a precautionary step against the spread of infections may affect touch DNA persistence and the recovery efficiency of swabs.

Keywords Forensic Science · Touch DNA · Isohelix™ swab · Rayon swab · DNA recovery · Short tandem repeat (STR) · Metals

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Introduction

In frontline forensic practice, touch DNA evidence is often scarce, damaged or of low quality [1] and especially difficult to recover from metals compared to plastic and glass substrates [2–5]. The poor recovery of DNA from metal substrates has been partially attributed to the strong metal-DNA interactions that impede the ability to dislodge and recover bound DNA from the substrate [6], and the inefficiencies of existing recovery methods [2]. Moreover, metal ion contaminants in recovered DNA may compromise its integrity and/or act as PCR inhibitors, disrupting the prospect of developing a reliable DNA profile for identification [7]. Efficient sample collection and processing of trace DNA using the most appropriate techniques is therefore critical. Several methods are currently utilized for touch sample collection from metal surfaces, and we have recently reviewed the merits and limitations of these methods [2]. For instance, trace DNA has been noted to get physically trapped and entwined within the fibers of cotton swab devices, resulting in significantly reduced efficiency of DNA recovery [8]. Also, the stickiness of tapes used for lifting trace biomaterial complicates the DNA extraction process [9, 10], and sampling

can be labor intensive [11]. Further, the inability to perform any repeat measurements from the same sample has limited operationalization of direct PCR in most forensic laboratories [2]. Despite claims that it is a more useful approach than conventional swabbing of surfaces [12], the submersion of cartridges, bullets and casings in the soaking technique enhances the leaching of metal ions [13] and contaminants, which are detrimental to nucleic acid integrity and adversely impact achieving interpretable profiles [2]. Sampling methods based on swabs, therefore, remain the most researched and utilized due to the ease of training requirements, adaptability to robotic extraction systems, and their relatively low cost. Notwithstanding this, there is no universally accepted protocol regarding swabs used by forensic laboratories, with the choice of swabs and wetting solutions mostly informed by practicality and in-house assessments.

An ideal swabbing system for trace DNA recovery from metal substrates should have enhanced collection and release efficiencies. We recently evaluated the efficiency of two swabbing systems—Rayon and Isohelix™ swabs, with sterile water and isopropyl alcohol as wetting agents respectively—used in two major Australian laboratories, for DNA recovery from brass, copper and steel using known and consistent amounts of pure acellular DNA applied on each surface [4]. The study demonstrated that DNA samples deposited on metal surfaces were best recovered using Isohelix™ swabs wetted with isopropyl alcohol. However, a relatively high but consistent amount of single source, pure acellular male genomic DNA was applied to the substrates, limiting the real-life applicability of these findings. Further research in uncontrolled, casework-like environments that often present with minute, variable amounts of cellular and acellular touch DNA and contaminants was recommended to demonstrate the applicability of Isohelix™ for enhanced DNA recovery from a broader range of metal surfaces. Moreover, while Rayon swabs have been previously assessed [14–16], excepting our previous research, there had been no published work evaluating the Isohelix™ swab for trace DNA collection from metal substrates. In this study, we tested the swabbing systems utilized in our previous study [7] with the aim of assessing the efficiency of the Isohelix™ and Rayon swab systems for DNA recovery from metal surfaces that are regularly touched to simulate the non-deliberate transfer of touch DNA evidence in a probable casework scenario such as a 'break and enter'.

Methods

Substrate selection and sample collection

The substrates for the experiments were four metal surfaces including: the handles of the front door (brass), bathroom door (aluminum), an office door (aluminum), bathroom tap (stainless steel) and soap dispenser (plastic, as control) found in the Darling Building on the University of Adelaide's North Terrace campus. The two-story building houses specialized laboratories, offices and seminar rooms and is accessed by at least fifty people daily. Access through these doors is such that an individual must physically hold the door's exterior door handle and push to enter; likewise, hold the same door's interior handle and pull to exit. Therefore, we assumed that a person should have interacted with these surfaces evenly and relied on transfer and accumulation of touch DNA on the surfaces during normal day-to-day activities by building's occupants as may occur in a typical crime scene. However, supplemental and touchpoint cleaning for COVID-19 of door handles at building entrances and toilet (bathroom) doors is currently performed. Therefore, these surfaces could be cleaned up to 3–4 times per day compared to, for instance, the handles of office doors (once daily), the bathroom tap handle and plastic soap dispenser (once daily to once weekly). We anticipated that the increased cleaning frequency would affect touch DNA transfer, persistence, and recovery in this scenario, given that wiping can remove or redistribute biomaterial on surfaces [17] and cleaning agents may render surfaces DNA-free [18]. In contrast to our previous work, where metal and plastic surfaces were pre-sterilized, and a known amount of acellular DNA applied, the surfaces utilized in this study were uncontrolled to mimic real-life scenarios.

Touch samples were collected from the substrates, using a single wet swab procedure across the entire surface for 30 s, with 70µL of isopropyl alcohol and 90µL of sterile (DNA-free) water added to the Isohelix™ and Rayon swab tips respectively, as previously described [7]. We collected touch samples from the exterior handles using the Isohelix™ and the interior handles with the Rayon swab systems with the assumption that a person would have interacted equally with both substrates upon entry and exit as described above. We did not swab the same spot on each surface, as it would have negatively affected the amount of biological material available for the second swab to collect. We chose not to swab adjacent spots on the same surface as this would have required that biological material was deposited evenly over the entire surface. Prior internal validation by two Australian laboratories had established the solvents used with each swab type as optimal for trace DNA recovery. For each door, touch samples were collected from the exterior and interior handles at the same time using the Isohelix ™ and Rayon swab systems, respectively. Negative controls consisted of swabs wetted with 70µL of isopropyl alcohol (Isohelix™) or 90µL sterile water (Rayon). Subsequently, individual swab tips were snapped into 2 mL microfuge tubes and extracted with the Promega DNA IQ™ System into 30µL elution buffer, following the manufacturer's protocol [19]. Touch

DNA extracts were stored at -20 °C before quantification and profiling.

DNA Quantification and STR Profiling

The DNA concentration in the extracts was determined using the Quantifiler™ Trio DNA Quantification kit (Thermo Fisher Scientific) and multiplied by the elution volume to estimate each sample's yield. Quality assessments for each sample's degradation and inhibition were performed using the Internal PCR Control (IPC) and degradation index (DI) data. Short tandem repeats (STR) profiling of touch DNA was performed using the GlobalFiler™ PCR amplification kit (Thermo Fisher Scientific) based on the manufacturer's recommendations. Electropherograms were read under the validated GeneMapper™ ID-X Software v1.6 (Applied Biosystems, Foster City, CA) settings of Forensic Science SA, which includes a 50 Relative Fluorescence Units (RFU) analytical (baseline) threshold. All stutter and artefact peaks were removed, and the number of contributors determined for each profile. The Mann–Whitney test was performed to test the significance of differences between degradation index, RFU and touch DNA recovered with the two swabbing systems using GraphPad prism (version 8.0.0 (224), GraphPad Software, California).

Results

Touch DNA recovery from metal substrates

The Isohelix™ swab showed better recovery of touch DNA than the Rayon swab on the plastic and all but one of the metal substrates (Fig. 1). Among the tested metal surfaces, touch DNA recovery was poorest from brass (front door handle) with both swab systems; notwithstanding, the Isohelix swab collected 0.52 ng compared to 0.13 ng for the Rayon swabs. On the aluminum office door handle however, the Rayon system recovered a slightly higher amount of DNA than the Isohelix™ system (Fig. 1). The highest quantity of touch DNA was recovered from the plastic (soap dispenser) surface, again a higher recovery with the Isohelix™ swab (3.3 ng) than the Rayon swab (0.84 ng). The Isohelix™ swabbing system was found to significantly recover more touch DNA ($p = 0.047$) from the metal substrates than the Rayon swabbing system.

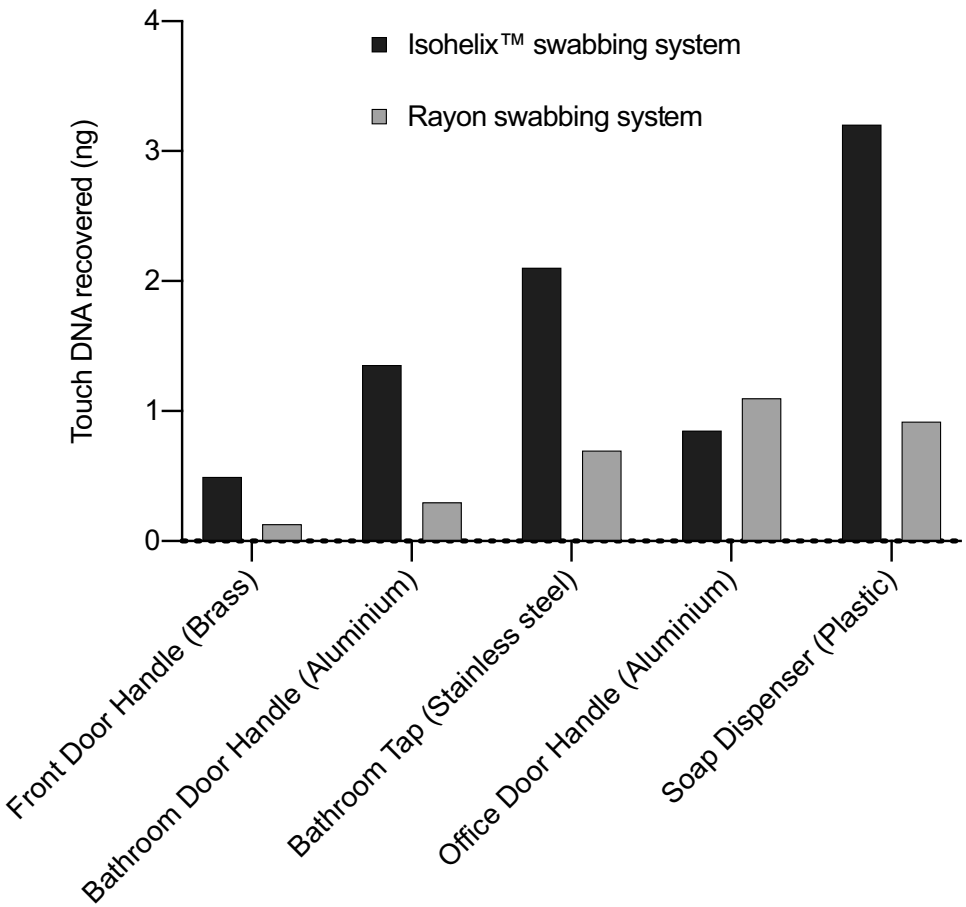

Fig. 1 Total human DNA recovery from five touched surfaces in the Darling Building, University of Adelaide – front door handle, bathroom door handle, bathroom tap, office door handle and plastic soap dispenser. Isohelix™ swab system shows overall better recovery of touch DNA from metal and plastic substrates

Degradation Index and STR Profiling

For all samples recovered from metal and plastic substrates, the threshold cycle (C_T) values for the internal PCR control (IPC) were lower (27.81 ± 0.08) than the C_T of each sample (and within the typical threshold range of 20 – 30 for Quantifiler™ Trio IPC [20]). The degradation index (DI) varied from 0.09 to 1.70 for all samples and averaged 0.98 ± 0.56 and 1.17 ± 0.37 for the Isohelix™ and Rayon swabs, respectively, for the substrates studied (Supplementary Table 1; Fig. 2a). A higher degradation index was observed on the brass front door handles, albeit not significantly different ($p = 0.3651$) between the Rayon (DI = 1.70) and Isohelix™ (DI = 1.62) swabbing systems. For STR profiles generated for all metal substrates, we found a significantly ($p < 0.05$) higher average RFU for touch DNA recovered by the Isohelix™ compared to the Rayon swabbing systems (Supplementary Table 2; Fig. 2b). However, for both swabbing systems, there was no significant difference ($p = 0.2316$) in the average RFU from plastic substrate (Fig. 2b). For touch DNA samples recovered with the Isohelix™ swab, the number of contributors in the mixed STR profiles was highest on plastic (7) and lowest on the office door handle (3) (Supplementary Table 1; Fig. 2c and Fig. 3). Similarly, the most donors were found on plastic (5) and least (1) on the office door handle with the Rayon swabbing system (Supplementary Table 1; Fig. 2b). There was a significant difference ($p = 0.039$) between the number of contributors picked up by the swabbing systems.

Discussion

Touch DNA recovery from metal substrates

We previously tested the Isohelix™ and Rayon swabbing systems on metal surfaces and found that Isohelix™ swabs recovered DNA with greater efficiency than Rayon swabs [7]. However, the foregoing study was conducted within a controlled setting, using relatively large amounts (20 ng) of pre-purified DNA pipetted onto sterilized metal substrates, unlike real life situations where touched exhibits are uncontrolled and often present with minute, variable amounts of cellular biomaterial, including potential contaminants. In the current study, therefore, we sought to explore the performance of the two swabbing systems for trace DNA recovery from regularly touched metal surfaces to simulate the non-deliberate transfer of touch DNA evidence likely in a casework scenario. The Isohelix™ swab showed better recovery of touch DNA than the Rayon swab on the plastic and all but one of the metal substrates (Fig. 1). Plastics are known to be inert surfaces for DNA interactions, improving recovery [3, 7]. However, DNA recovery in this study could also be higher due to less frequent cleaning of the soap dispenser compared to other surfaces. The limited recovery of touch DNA from brass substrates has been previously reported [21, 22], and is likely due to the copper ion content of the alloy; however, the supplemental cleaning of this entrance door handle (3—4 times compared to once daily for office doors), as a precautionary measure against COVID-19, possibly reduced recovery of touch DNA. While the findings of this study are consistent with our previous report [7], there was an interesting discrepancy with the office door handle (aluminum), where a higher recovery was observed for the Rayon swab than for the Isohelix swab system. This deviation from an Isohelix swab exhibiting enhanced touch DNA recovery efficiency from all substrates tested (Fig. 1) is potentially due to a greater quantity of DNA persisting on the inside handle of the door (swabbed with Rayon) than the outer handle (swabbed with Isohelix). As this office door is kept locked, the cleaners have far less access to the inside handle than the outside handle for this particular door.

Surface swabbing to collect DNA traces from previously used items is a critical technique for crime scene investigations [14]. An extensive range of commercially available swabs have been tested on different substrates in controlled and quasi-operational conditions [15, 23–26]. However, as noted by Bonsu et al. [2], there is currently no consistency in swabbing devices used in different forensic laboratories and none is as yet explicitly acclaimed for touch DNA collection from metal substrates.

For instance, cotton swabs have been noted to retain DNA [8, 24, 27], often leaving fibers (especially on rough-textured surfaces and metals [15]) and impurities in extracts which may inhibit PCR [14, 28]. The microporous membrane matrix of the Isohelix™ swabs is, however, quickly and actively dry following sample collection, to stabilize and preserve the integrity of DNA on the swab. The latter ensures maximal DNA yield [29] as shown in this and our previous [7] study; pitching Isohelix™ as an ideal system for touch DNA recovery from problematic metal substrates given its higher collection and release efficiencies [7]. Notwithstanding, the choice of swabs is conventionally based on practicality, cost, efficiency, convenience of use, substrate type and/or amenability to specific instrumentation [2].

Degradation Index and STR Profiling

We reviewed the degradation index (DI) and Internal PCR Control (IPC) data generated with the Quantifiler Trio Quantification Kit, as a quality assessment for degradation and inhibition of the recovered touch DNA samples. The lower C_T values obtained for each sample indicated that the assay worked as expected with nothing impeding the reaction. While the increased DI, indicative of a degradation or inhibition effect, may be attributed to copper-induced damage

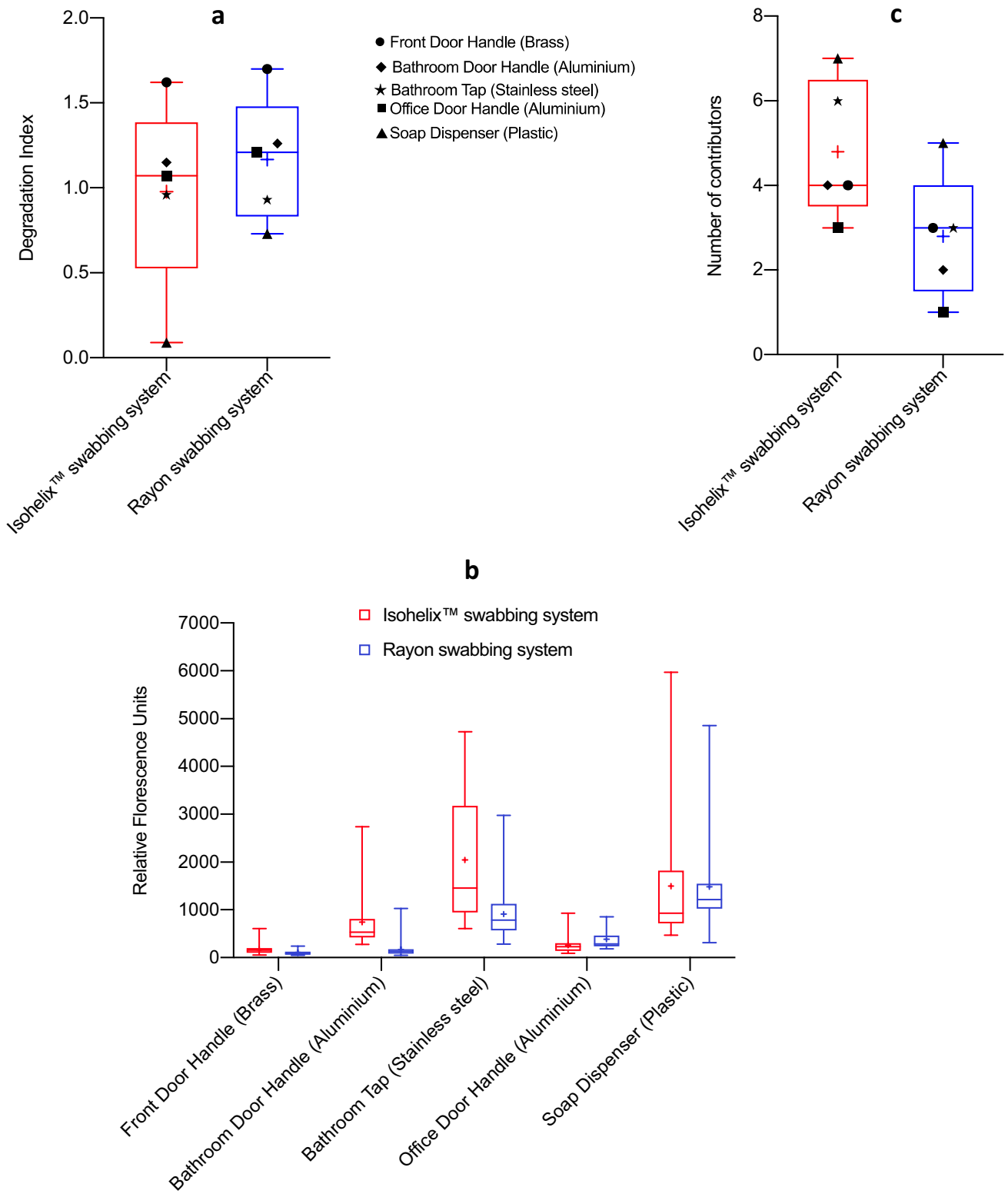

Fig.2 Human touch DNA recovery from four metal and one plastic surface: **a** degradation index of touch DNA recovered with Isohelix™ and Rayon swabbing systems **b** number of contributors to the STR profile for recovered touch DNA on all substrate for Isohelix™ and Rayon swabbing systems, and **c** the average relative fluorescence units of peak heights across the STR profiles for touch DNA recovered from each substrate. Means are shown as '+'

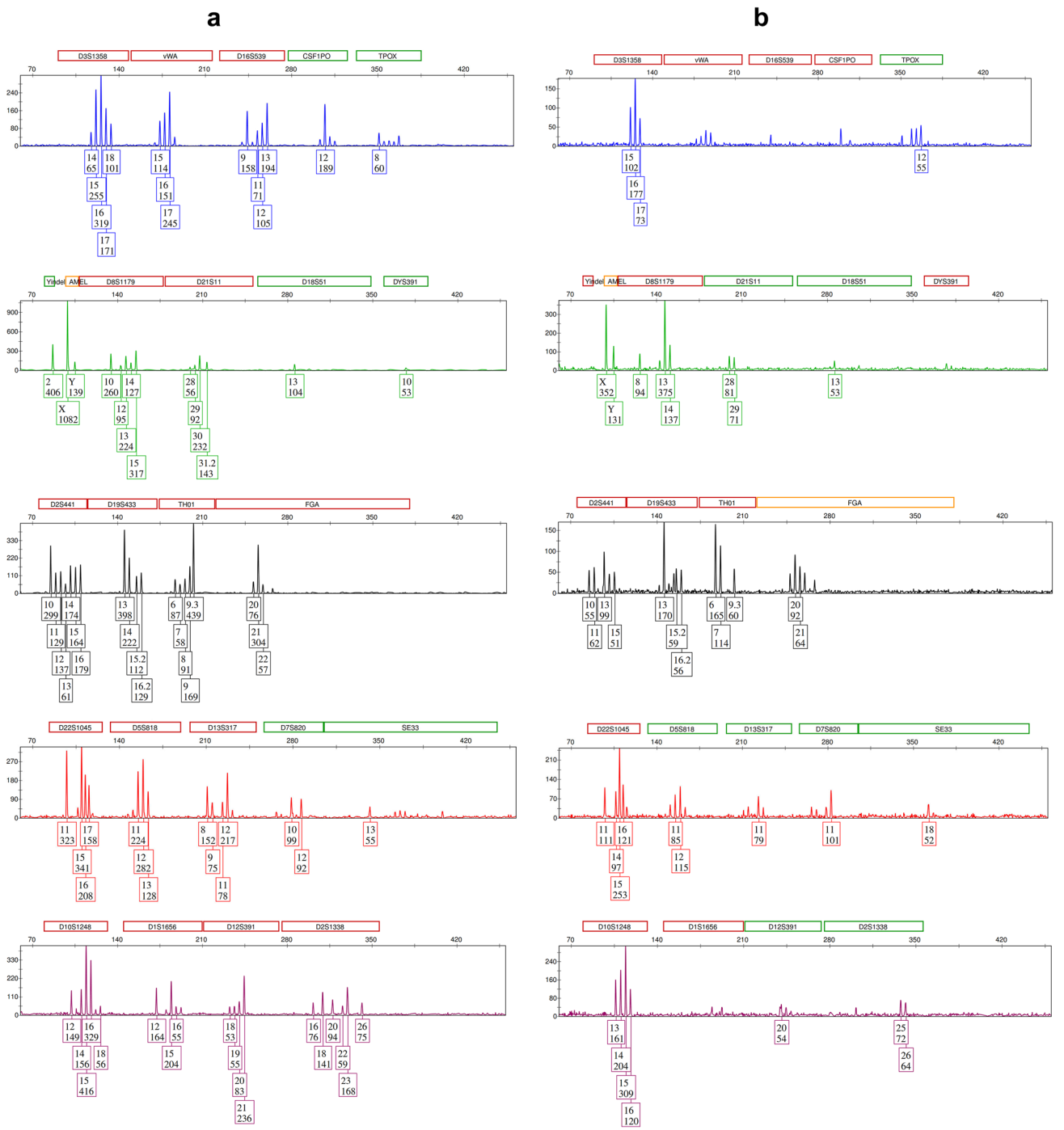

Fig. 3 Example GlobalFiler STR Profile of touch DNA recovered from front door handle (brass) with **a** Isohelix™ and **b** Rayon swabbing systems. The average RFU of peaks across the profile was higher for the Isohelix™ swab compared to the Rayon swab. The mixed profile showed four contributors for the Isohelix swab and three contributors for the Rayon swab

of DNA [2], it may be exacerbated by the repeated cleaning of the substrate and the impact of active ingredients of the cleaning agents (detergents) [18]. The Safety Data Sheets of the two cleaning agents; Tango Disinfectant (Agar Cleaning Systems Pty. Ltd, Australia) and Shield Citrus (Diversey Australia Pty. Ltd., Australia), used during routine cleaning as well as the supplemental and touchpoint cleaning for COVID-19, shows one main active ingredient: benzalkonium chloride (BAC) also known as alkyldimethylbenzylammonium chloride (ADBAC). This cationic surfactant has

broad-spectrum antimicrobial properties [30] and is known to induce DNA damage (single-strand and double-strand breaks) [31] via oxidative stress [32]. Consequently, the effect of the cleaning agent on touch DNA was also observed on the bathroom door handle (DI = 1.26 and 1.15 for Isohelix and Rayon respectively) which undergoes similar supplemental cleaning as the brass substrate.

We performed short tandem repeat (STR) profiling of all sample extracts to ascertain whether the quality of the profiles obtained was as expected, given the quantification and degradation index data for each swabbing system. The bathroom tap (stainless steel), office door handle (aluminum) and soap dispenser (plastic) showed more complete profiles with a higher average RFU compared to that of the brass substrate (Supplementary Table 3; Fig. 2c and Fig. 3) for Isohelix™ swabbing system. Given that at least 50 people access the building, with the potential for accumulation of biomaterial on the metal surfaces, especially the front door handle (brass substrate) during normal day-to-day activities by building occupants, the number of contributors determined from the mixed DNA profiles was very low, and could be ascribed in part to the frequent cleaning of the surfaces. Also, as shown in Fig. 2b, the Isohelix™ swab picking up more contributors than the Rayon swab is an indicator of its higher recovery efficiency. Touch DNA recovery from all but one substrate (office door handle) showed consistently more complete profiles and higher RFU values for Isohelix™ swabbing systems than Rayon (see Fig. 1).

Research with touch samples is challenging because an unknown (and unknowable) amount of cellular and acellular DNA is deposited with each human touch, making it difficult to estimate the amount of DNA recovered. This presents some practical difficulty in interpreting our results, especially when quantifying the relative performances of two sampling systems. However, it is significant that we found consistent differences in DNA recovery from three different types of surfaces, all of which would have been touched by many people every day, therefore mimicking real-life scenarios, as opposed to similar works that often utilize non-trace quantities of DNA with several controlled variables. Overall, the findings of this study support our previous proof-of-concept of improved trace DNA recovery efficiency from metal surfaces utilizing the Isohelix swabbing system [7]. Notwithstanding, further research to ascertain the impact of precautionary protocols against the spread of infections in a pandemic scenario (i.e. frequent surface cleaning and, handwashing and application of hand sanitizers) on the transfer, persistence, and recovery of touch DNA from metal substrates would be valuable to forensic investigations.

Conclusion

The current study reinforces our previous finding of improved efficiency of trace DNA recovery from problematic metal surfaces utilizing the Isohelix™ swab moistened with isopropyl alcohol in contrast to a rayon swab moistened with water. The results add to our understanding of the impact of substrate type and sample collection technique on touch DNA recovered from metal substrates at the scene of crime. Further, this work provides a basis for further research pertaining to the effect of cautionary measures taken against the spread of infections in a pandemic situation on touch DNA transfer and persistence; the recovery efficiency of swabbing systems as well as the integrity of recovered DNA and STR profiles generated.

Key points

1. Touch DNA evidence is often scarce, damaged or of low quality.
2. Metals are problematic substrates for touch DNA recovery and amplification.
3. Touch DNA is better recovered from metal surfaces with Isohelix™ swabs moistened with isopropyl alcohol rather than Rayon swabs moistened with water.
4. Supplemental and touchpoint cleaning, as seen during a pandemic, may impact touch DNA persistence and recovery.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12024-021-00423-8.

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Author contributions Conceptualization: Jeremy Austin (JA), Denice Higgins (DH) and Dan Bonsu (DB); Methodology: DB, Matthew Rodie (MR) and Julianne Henry (JH). Formal analysis and investigation: DB, MR and JH; Writing—original draft preparation: DB and MR; Writing—review and editing: DB, JA, DH and JH; Supervision: JH, DH and JA.

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Declarations

Ethics approval The study was approved by the Human Research Ethics Committee of the University of Adelaide (Ethics approval code: H-2016–218), consistent with the National Statement on Ethical Conduct in Human Research 2007 (Updated 2018) of Australia.

Conflicts of interest The authors have no relevant financial or non-financial interests to disclose.

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