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1 Comparison of operational DNA recovery methods: Swabs versus tapelifts

2

3 Abstract

4 It is routine among many jurisdictions to recover DNA using tapelifts on porous
5 substrates (e.g. clothing) and swabs on non-porous substrates (e.g. tool handles).
6 Here, we examine this by comparing the efficiency of the NSW jurisdiction's specific
7 swabbing and tapelift techniques on a range of porous and non-porous substrates. To
8 test DNA recovery efficiency, 30ul aliquots of 1:50 and 1:100 saliva dilutions were
9 deposited onto the substrates, left to dry overnight, recovered, extracted, quantified
10 and a subset profiled. Tapelifts recovered more DNA and DNA profiles with more
11 detectable alleles than swabs for both saliva dilutions on porous substrates. For non-
12 porous substrates, similar DNA quantities and profiles were generally recovered with
13 both methods for both saliva dilutions. These data underpin current practices to
14 recover DNA using tapelifts for porous substrates and swabs for non-porous
15 substrates. These data also revealed severe degradation of DNA recovered from
16 brass, supporting the on-going need to improve DNA recovery and analysis methods
17 for brass substrates.

18

19 Keywords

20 Trace DNA; DNA recovery; Swabs; Tapelifts

21

22 1. Introduction

23 For DNA recovery, it is routine among many jurisdictions, particularly in Australia and
24 the UK, for tapelifting to be used on porous substrates (such as clothing [1]) and
25 swabbing on non-porous substrates (such as tool/weapon handles [2]). However,
26 anecdotal evidence and emerging data [3] suggest that a fresh assessment of the
27 collection methods for various substrates is warranted.

28 Here, we examine this by comparing the efficiency of the specific swabbing and tapelift
29 techniques used in the NSW jurisdiction for sampling a range of porous and non-
30 porous substrates. The specifics of the techniques employed are tailored to the
31 automated DNA processing pipeline utilised within the Forensic & Analytical Science

32 Service DNA laboratory. Briefly, a single moist-dry swab is used [4] or a tapelift of
33 specific size to fit a 2ml robot-ready tube when rolled.

34

35 **2. Materials and Methods**

36 Saliva was donated by a consenting participant by spitting into a DNA-free 50ml falcon
37 tube. A preliminary experiment was conducted in which 30µl aliquots of four dilutions
38 of the provided saliva (1:25, 1:50, 1:100 and 1:200) were pipetted on to two pre-
39 cleaned substrates (cotton and tile) and were recovered by swabbing and tapelifting
40 in duplicate per substrate and recovery method. Results from this initial experiment
41 identified the 1:50 and 1:100 dilutions as those to be used in the main experiment. The
42 1:50 dilution resulted in DNA quantities corresponding to those routinely recovered
43 from dilute body fluids, whereas the 1:100 dilution provided DNA quantities similar to
44 those routinely recovered from trace DNA samples.

45

46 As such, 30µl aliquots of each of these two saliva dilutions were deposited onto pre-
47 cleaned DNA-free substrates (5 replicates per dilution per substrate per recovery
48 method: n=120 plus controls). The substrates used were: cotton, denim and polyester
49 for porous substrates, and tile, brass and synthetic leather for non-porous substrates.
50 The deposited saliva was left to dry on the substrates overnight and then sampled
51 using moist-dry rayon swabs (Medical Wire & Equipment, UK) or tapelifts (3M tape
52 cleaned and packaged by Lovell Surgical Solutions Pty. Ltd., Australia) in the manner
53 employed within the NSW jurisdiction.

54

55 All samples were lysed and extracted using the PrepFiler™ Automated Forensic DNA
56 Extraction Kit (Thermo Fisher Scientific) on the Hamilton Microlab® AutoLys STAR
57 and Tecan Freedom EVO® robotic workstations, respectively, with an elution volume
58 of 50µl. Samples were quantified using the Quantifiler™ Trio DNA Quantification Kit
59 (Thermo Fisher Scientific), and a sub-set was profiled using the PowerPlex® 21
60 Amplification System (Thermo Fisher Scientific) with a 0.7ng input amount and 29
61 cycles.

62

63 Statistical testing was conducted using IBM SPSS Statistics, version 28. Of the 24
64 combinations of recovery method, substrate and saliva dilution, five combinations
65 resulted in DNA quantification datasets that were not normally distributed, as
66 determined by the Kolmogorov Smirnov test ($p < 0.05$). As such, to make pairwise
67 comparisons across all the datasets, the non-parametric Mann-Whitney U test was
68 used to identify any significant differences in DNA quantities recovered. Effect size of
69 significant differences was interpreted from r^2 , determined by squaring the value of r ,
70 calculated from $r = Z/\sqrt{N}$, where $N = 10$ for each comparison.

71

72 **3. Results and Discussion**

73 *3.1 Porous substrates*

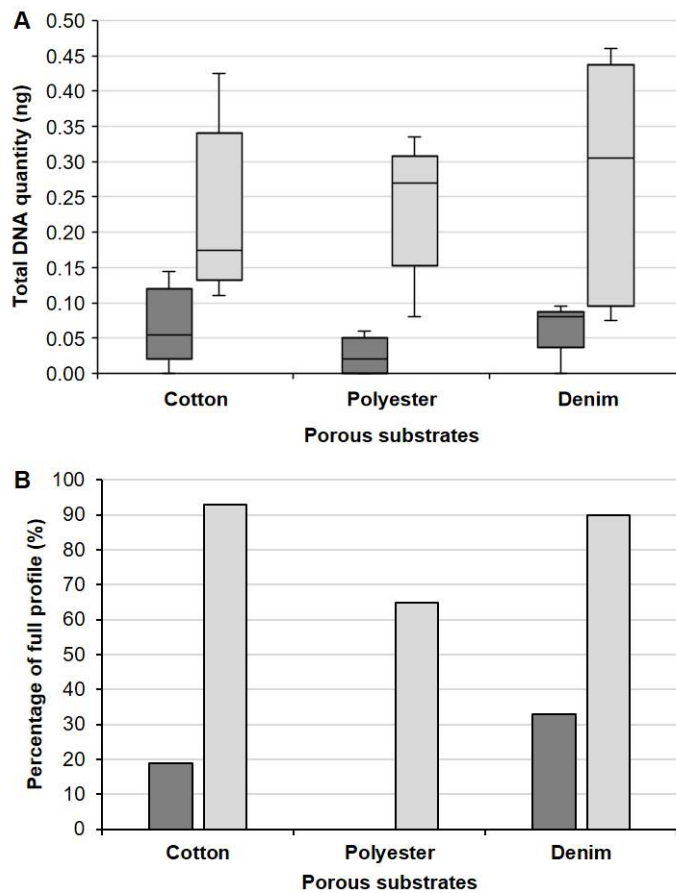
74 For the 1:50 saliva dilution on porous substrates, tapelifts recovered notably more
75 DNA than swabs (Fig. 1A). This was statistically significant for cotton ($Z = -2.402$,
76 $p < 0.05$, $r^2 = 0.58$) and polyester ($Z = -2.619$, $p < 0.05$, $r^2 = 0.69$), indicating that 58% and
77 69% of the variability is accounted for by the recovery method for cotton and polyester,
78 respectively. The difference in DNA quantity for denim was not significant ($Z = -1.892$,
79 $p = 0.056$), presumably due to the large variability in results obtained from denim for
80 this saliva dilution (Fig. 1A). Higher DNA recovery was also observed with tapelifts
81 than swabs for the 1:100 saliva dilution, though this was only significant for polyester
82 and denim (data not shown). Tapelifting also resulted in DNA profiles with more
83 detectable alleles than swabbing for the 1:50 (Fig. 1B) and 1:100 (data not shown)
84 saliva dilutions, which was expected given the quantification data.

85

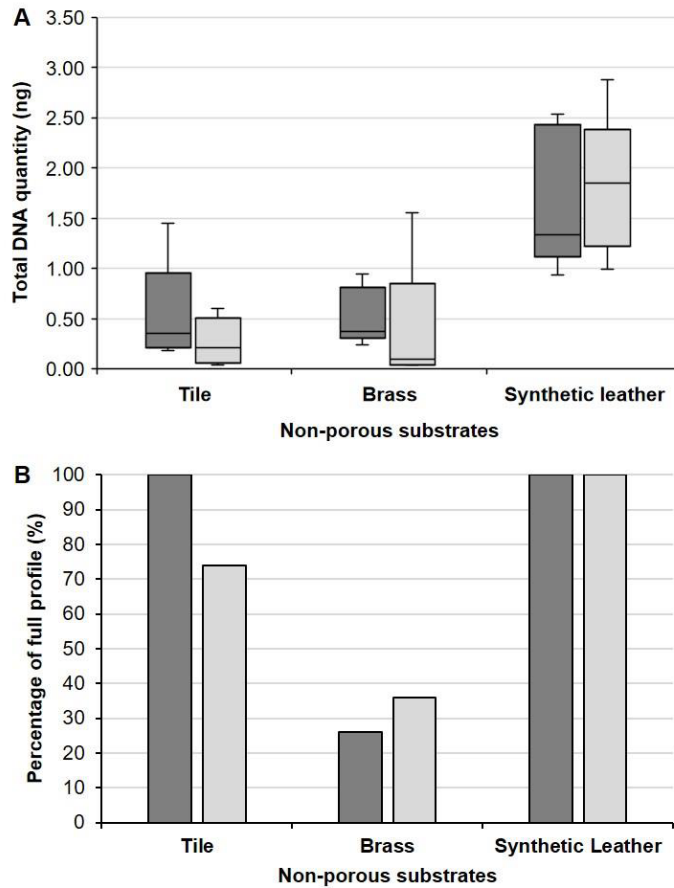
86 *3.2 Non-porous substrates*

87 For the 1:50 saliva dilution, there was no significant difference in DNA quantity
88 recovered between tapelifting and swabbing on tile ($Z = -0.940$, $p = 0.347$), brass ($Z = -$
89 1.571 , $p = 0.116$) and synthetic leather ($Z = -0.522$, $p = 0.602$) (Fig. 2A). Whilst similar
90 quantities of DNA were recovered from both tile and brass, poorer quality DNA profiles
91 were recovered from brass than tile (Fig. 2), with 'ski-slope' degradation observed in
92 the DNA profiles from brass. This supports prior findings that DNA profiling from metal

93 substrates is challenging and may be attributed to the physicochemical properties of
94 brass, in particular the copper within brass, causing DNA degradation [5]. For the
95 1:100 saliva dilution, similar DNA quantities were recovered with both methods on
96 brass and synthetic leather, but swabbing recovered notably more DNA than tapelifting
97 on tile (data not shown). This increase was statistically significant ($Z=-2.095$, $p<0.05$,
98 $r^2=0.44$), indicating that 44% of the variability is accounted for by the recovery method.
99 The DNA profile percentages obtained for the non-porous substrates reflected the
100 DNA quantity findings for the 1:50 (Fig. 2B) and 1:100 (data not shown) saliva dilutions.
101



102
103 **Fig. 1.** DNA quantities (A) and profile percentages (B) recovered from 1:50 saliva dilution on porous
104 substrates by swabbing (dark grey) and tapelifting (light grey).
105



106

107 **Fig. 2.** DNA quantities (A) and profile percentages (B) recovered from 1:50 saliva dilution on non-porous
 108 substrates by swabbing (dark grey) and tapelifting (light grey).

109

110 **4. Conclusions**

111 The results in this study underpin the current NSW practices to recover DNA using
 112 tapelifts for porous substrates and swabs for non-porous substrates. These data also
 113 revealed severe degradation of DNA recovered from brass, supporting the on-going
 114 need to improve DNA recovery and analysis methods for brass and other copper-
 115 based substrates.

116

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122

123 **Conflict of interest statement**

124 None.

125

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