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Preliminary investigation of differential tapelifting for sampling forensically relevant layered deposits

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ABSTRACT

The analysis of DNA mixtures can be problematic, especially when in trace quantities such as when a biological sample is deposited onto a substrate which contains background DNA (for example, in the case of touch DNA deposited onto a garment containing the wearer's DNA). We conducted a preliminary investigation into the possibility of removing such multi-donor deposits layer by layer using a differential tape-lifting method. Two types of tape were tested using two different numbers of applications for sampling layered deposits of touch DNA/touch DNA and touch DNA/saliva, both on the same polyester–cotton plain woven material. The data showed that there was no significant increase in the ratio of secondary to primary deposit when sampled in this manner, compared to direct extraction from cuttings of the touched fabric. A similar result was also obtained even when the deposits were on opposing surfaces of the fabric and the sampling was carried out on the secondary deposit side. These findings indicate that biological material, whether touch DNA or saliva, does not predominantly remain on the side of the fabric on which it is deposited (at least for plain-woven polyester–cotton). They also highlight the importance of considering substrate properties when making assumptions as to the resulting location of biological materials from a deposition event, and the necessity to conduct further research on the interactions between substrates and deposits.

1. Introduction

DNA originating from skin and deposited/shed at crime scenes, herein defined as touch DNA, can be an asset in many forensic investigations [1–8]. There are, however, difficulties associated with sampling touch DNA. Firstly, there is generally a smaller quantity in a deposit of touch DNA than in deposits of other biological materials [2], although this can vary greatly [9]. Secondly, deposits of touch DNA are not usually visible to the naked eye (apart from when fingerprinting methods have been able to identify a touched area, a disturbance is noted in the substrate, or in the case of a fingerprint in blood) and, therefore, choosing an area to target is generally based on inference and previous experience of sampling similar objects.

When touch DNA is deposited on an item such as clothing or bedding which, as a result of prolonged contact with a person, contains an embedded background profile of the frequent user, any profile originating from the latest touch deposit can mix with this background profile and complicate interpretation [10]. Furthermore, in situations where touch DNA is co-localised with a deposit containing a high concentration of DNA, especially comprising blood, saliva, or semen, DNA from the non-touch portion may completely overpower the touch DNA sample during amplification, leading to a profile which mostly comprises peaks matching the non-touch donor. As the sensitivity of STR kits has improved, the probability of obtaining mixed samples has increased [11].

It is often thought that the wearer's DNA is deposited mostly on the interior side of a garment through rubbing against the skin, with a minimal amount applied on the external surface through occasional touch events. Therefore, it is often assumed by forensic scientists that this pre-deposited biological material may not physically interact with an external deposit, potentially enabling separate sampling of the deposits. Although this hypothesis is commonly acted upon during sample collection, this notion of targeting the outside surface of a garment for touch in the hope that wearer DNA will not be collected has not been examined in detail.

Many variables can affect the efficiency of sampling DNA, especially when in trace quantities. These include the type of device used which, in itself, has a number of sub-variables including composition, size, and extraction efficiency [12–19]; the manner of

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application of the sampling device during collection, including the amount of pressure used, choice of substrate area to target, and how many times the device is applied [12,13,20]; and the substrate on which the material is deposited [13,21]. Previous research has shown that certain sampling devices are more efficient at collecting DNA than others [13]. In the case of sampling from fabric, evidence suggests that a strong tapelift collects more DNA than either a weak tapelift or a swab [12]. Direct extraction from excised fabric, as a method with minimal loss, is expected to collect more DNA than either of these methods.

Given that some sampling devices disrupt the structure of a deposit less than others, we hypothesised that it is possible to remove the uppermost deposit with a weak collection device such as a tapelift which causes minimal disturbance to the deposit, and then sample the underlying deposit by direct extraction. This approach would, at best, lead to generation of two separate profiles rather than one complex mixed profile and, at least, change the ratio between donor DNA peak heights in the mixture, potentially improving its probative value. The choice of which of these two deposits one would give priority to during further analysis would be case-dependent. The major assumption underlying this hypothesis is that there is minimal mixing of both samples on the substrate after, or during, the last deposition step, potentially due to a 'fixing' of the first deposit on the substrate. Similarly, it is assumed the external deposit, especially for non-liquid samples, will not migrate through the material to the opposing side, meaning that sampling DNA from the non-wearer side may lead to a single source profile of the external depositor. The extent of this migration may be dependent on the substrate used, although little work has been published on this issue.

The concept of differential sampling from the same substrate area of layered deposits has not been explored but, if shown to be successful, could lead to a paradigm shift in sampling some types of items for touch DNA. This study presents a preliminary assessment of the feasibility of differential sampling as a method for collecting recently deposited touch DNA when biological material (either touch or saliva) is pre-deposited on a substrate, both on the same side of the substrate as the secondary deposit and when deposited on the reverse. As tapelifting is most commonly used on fabrics, and fabrics often contain a background profile of the wearer, it was decided that a fabric substrate would be an appropriate focus for this study. Two tape types previously used in tapelifting studies [12,15], one strongly (Scenesafe FAST™) and the other weakly adhesive (Scotch® Magic™ tape), were applied to cover an area once or multiple times. This allowed comparison of tapes to assess whether or not strength of tapelifter had an impact on differential sampling, as well as a way to assess the effect of variable taping applications on sampling layered deposits.

2. Materials and methods

2.1. Sample preparation

2.1.1. Substrate preparation

Pieces of polyester–cotton plain woven fabric (comprising 65% polyester and 35% cotton fibres woven at a density of 47 × 30 yarns per centimetre), approximately 20 cm × 20 cm, were irradiated with UV light in a PCR hood for 30 min on each side and fixed over A4 templates, each covered with a plastic transparency to prevent contamination. Templates outlined fifteen 3.8 cm × 2.5 cm sampling areas, separated from each other by 0.5 cm on all sides. One of these areas of each fabric was excised before deposition of biological material as a negative substrate control; none of which revealed any alleles. As detailed in Section 2.2, two additional areas were cut after deposition as positive sample controls, and the remaining twelve areas were sampled as described in Section 2.2.

2.1.2. Experiment 1 – touch/touch

Fig. 1a illustrates the preparation of samples for Experiment 1. Touch DNA was deposited over all sampling areas on two pieces of fabric by rubbing evenly for 60 s and repeated after two and four hours by the primary donor (Donor A) to create a relatively high level of background DNA. This rubbing protocol was also performed on two plastic transparencies by a secondary donor (Donor B). After 24 h, one of the transparencies touched by Donor B was applied to one piece of fabric touched by Donor A such that the deposit sides of each contacted each other. The other piece of fabric was flipped upside-down and re-secured to the template, before the deposit side of the other plastic substrate was applied to the non-deposit side of the fabric. A roller was used to apply medium pressure evenly across the back of each transparency for 60 s. This test was then repeated but having Donor A deposit on the plastic transparencies and Donor B deposit onto the fabrics.

2.1.3. Experiment 2 – saliva/touch

Fig. 1b illustrates the preparation of samples for Experiment 2. Aliquots of 10 μL of saliva from a third donor (Donor C) were applied by pipette to each sampling area of two other pieces of fabric. Donor A evenly rubbed two plastic transparencies multiple times as per the first experiment. Deposits were left in a clean, closed PCR hood for 24 h to dry completely. After drying, one fabric was flipped upside-down and resecured to the template. The deposit side of the plastic transparencies containing Donor A's touch DNA were then applied to either the deposit side or the non-deposit side of the non-flipped or flipped fabric, respectively, with medium pressure for 60 s as described in the previous experiment.

2.1.4. Experiment 3 – touch/saliva

Fig. 1c illustrates the preparation of samples for Experiment 3. Donor A applied a background comprising touch DNA to two pieces of fabric as per Experiment 1. 10 μL of saliva from Donor C was then applied by pipette onto each sampling area of these pieces of fabric, on the same side as the touch deposit on one piece, and on the non-touch side of the other touched piece of fabric. These saliva deposits were allowed to dry for 24 h under the conditions described in Section 2.1.3 before sampling.

2.2. Sample collection

Two different tapelift types employed in casework were used to collect DNA from the substrates: Scenesafe FAST™, a known good collector of touch DNA from fabrics, and Scotch® Magic™ tape, a less sticky tape than the former. Each tape was applied to cover the sampling area either once, to represent a light taping event, or sixteen times, the suggested number of tapings to achieve maximum collection [12]. Three replicates of each method per fabric, i.e. twelve taping events, were performed per fabric/sample combination.

Tapes were placed into 2 mL tubes (Treff, Switzerland) by rolling with clean gloves, handling only the non-sticky side, with the adhesive side facing the centre of the tube. All sampling areas of the substrate, as well as two un-sampled positive control areas, were subsequently excised, cut into eight pieces with a sterile scalpel (Swann-Morton, UK), and placed into spin baskets (Promega, USA) suspended in 2 mL tubes (Treff, Switzerland).