Article

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Validating Touch DNA Collection Techniques Using Cotton Swabs

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Abstract

Touch or trace DNA analysis has become an important routine of the forensic laboratory workload and a useful tool for investigators. Most samples, such as touch DNA, are collected using cotton swabs and choosing the right collection technique when using a cotton swab can improve DNA recovery from the surfaces. Therefore, this paper investigates three recovery techniques commonly used with cotton swabs and validate different conditions on the collected swabs such as drying prior freezing or direct freezing to see how they affect the amount of DNA recovered. The results show that there is a significant difference between the three recovery techniques used to recover touch DNA with cotton swab ($F_{2,21}$ = 39.504, p<0.001), similarly with the cotton swab tested conditions prior extraction ($F_{2,21}$ = 68.328, p<0.001).

Keywords: Forensic science; Trace DNA; Touch DNA; DNA recovery; Cotton swab; Copan 150C Cotton swab; DNA extraction; QIAamp DNA investigator kit; Quantifiler ™ Human DNA Quantification Kit

Introduction

Touch or trace DNA analysis has become an important routine of the forensic laboratory workload and a useful tool for investigators since it was first reported in 1997 [1]. It has unlocked new possibilities and led to the collection of DNA from a broad range of surfaces, such as tools, knives, clothing, firearms, etc. [2-4].

Most trace samples, such as touch DNA, are collected using cotton swabs. Swabbing an object often requires a moistened swab with some pressure and rotation of the swab head applied to the target area for DNA collection. However, this is not always the case, as a moist cotton swab may pick up less than half of the available sample, leaving some biological material on the surface [5]. Choosing the right collection technique when using a cotton swab can improve trace DNA recovery from the surfaces, such as using the appropriate amount of reagent to moisten the swab or using a double swab technique (wet and dry) [5].

After collection some of the cotton swabs are extracted immediately or freeze while moist until extracted. However, some labs practice dries the swabs at room temperature or use swab drying cabinet's prior extraction or freezing. Some of these practises are not advisable when collecting Touch DNA because if the swab is allowed to dry before extraction that usually leads to loss in some of the collected DNA [6].

Therefore, this paper consists of two experiments, the aim of first experiment was to validate three recovery techniques commonly used with cotton swabs: single swab technique (half wet and half dry), single swab technique with the use of a plastic spray bottle to moisten the swab (developed in the Dubai police forensic DNA lab) and a double swab technique (wet and dry). Moreover, the aim of the second experiment was to validate different conditions to study the influence of immediate extraction, drying and freezing of collected touch DNA using moisten cotton swabs.

Material and Methods

Experimental set up and deposition

A participant, previously confirmed as a high shedder, was asked to wash his hands with antibacterial soap, refrain from any activity for 5 min, and then charge the fingers of both hands with eccrine sweat from behind his ears to load the finger with enough DNA [7]. After a further 5 min, the participant was asked to touch a glass surface using his index, middle and ring fingers of both hands separately, by applying medium pressure on a 5 x 7 cm area of the surfaces for 1 min (Figure 1). The participant was asked to repeat the same process 48 times. The surfaces were sterilised before use by 2% virkon and ultraviolet radiation (UV) for 15 min.

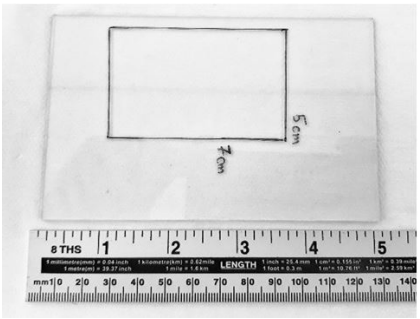

Figure 1: A 5 x 7 cm area of a glass plate used for Touch DNA deposition.

Experiment one

After deposition, 24 samples were collected immediately using a Copan 150C Cotton swab (Copan, Brescia, Italy), with distilled water, a common moistening agent used by forensic labs, used to wet the swabs [5]. Three techniques were used to wet the cotton swab before sample collection using 100 µl of distilled water as follows:

- Half the cotton swab head using a pipette (n=8)
- A cotton swab head using spray bottle (n=8), with each single spray containing approximately 50 µL
- A cotton swab head using a pipette, followed by a dry swab (double swab technique) (n=8)

Swabs heads were extracted immediately after collection using the QIAamp® DNA Investigator Kit (Qiagen) according to the manufacturers' instructions, with a final elution volume of 50 µL.

Experiment two

After deposition, 24 samples were collected immediately using a Copan 150C Cotton swab (Copan, Brescia, Italy), and 100 µl of distilled water was used to moisten the swabs by plastic spray bottle technique (developed in Dubai police forensic DNA lab). After collection three conditions were tested on the swabs;

- Immediate extraction after collection (n=8).
- Freeze at -20 C for a week (n=8).
- Dried for 24 hours at room temperature then Freeze at -20 C for 6 days (n=8).

Swabs heads were extracted by QIAamp® DNA Investigator Kit (Qiagen) according to the manufacturer's instructions, and the final extracted sample elution was 50 µL.

DNA quantification and analysis

Extracted samples were quantified using the Quantifiler® Human DNA Quantification Kit, QuantStudio 5 Real-Time PCR (qPCR) and HID Real-Time PCR analysis software v1.3 (Thermo Fisher Scientific) according to the manufacturers' instructions. Statistical analysis was performed with RStudio using factorial analysis of variance (ANOVA). The blanks from surfaces after sterilisation, and negative controls for the collection and extraction methods were all negative for DNA when quantified.

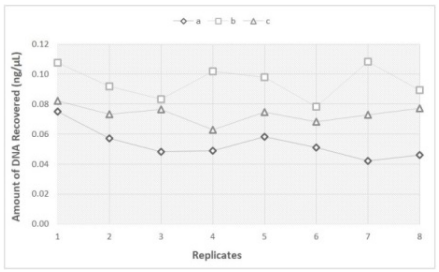

Figure 2: The amount of DNA recovered from eight replicates (n=24) by each technique: (a) single swab, (b) spray bottle and (c) double swab.

Results and Discussion

There is a significant difference between the three recovery techniques (Figure 2) used to recover touch DNA with a cotton swab ($F_{2,21}$ = 39.504, p<0.001). The spray bottle technique to wet the cotton swab (b), or the use of double swab technique (c) were more efficient to collect touch DNA. The single swab technique using the pipette to wet the cotton swab (a) resulted in trace DNA being uncollected on the surface (mean: a–0.05, b–0.09, c–0.07 all in ng/µL) (Figure 3).

Also, there is a significant difference in the amount of DNA collected amongst the cotton swab tested conditions (Figure 4) prior extraction ($F_{2,21}$ = 68.328, p<0.001). All the three conditions show differences (p<0.001). Immediate extraction (a) or direct freezing of cotton swabs after collection (b) is more suitable to retain the collected touch DNA, while drying the samples before freezing (c) can lead to loss of some of the trace DNA collected (means: a–0.09, b–0.08, c–0.05 all in ng/µL) (Figure 5).

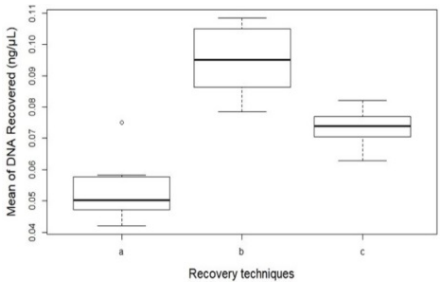

Figure 3: The mean DNA recovered (n=24) by each technique: (a) single swab, (b) spray bottle and (c) double swab.

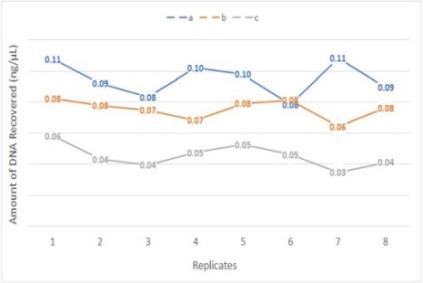

Figure 4: The amount of DNA recovered from eight replicates (n=24) by each condition: (a) Immediate extraction of swabs, (b) swabs were only freezed before extraction and (c) swabs were dried and freezed before extraction.

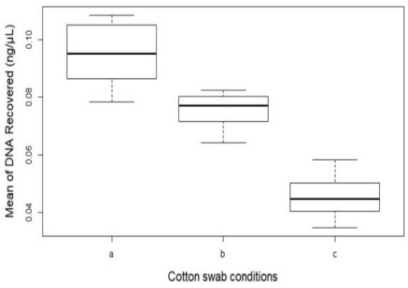

Figure 5: The mean DNA recovered (n=24) by each condition: (a) Immediate extraction of swabs, (b) swabs were only freezed before extraction and (c) swabs were dried and freezed before extraction.

Conclusion

The use of cotton swabs to collect trace DNA is common, even though a significant amount of DNA is wasted as the cotton swab retains some DNA depending on the extraction method used. Therefore, an appropriate collection technique can help to improve the DNA recovery efficiency from the cotton swab. The double swab (wet and dry) technique is better than a single swab (half wet and half dry) technique but is depended on the size of area from which the sample is collected. Furthermore, DNA extraction can be more challenging with the double swab technique. The plastic spray bottle method is a better than using a pipette to moisten the swab because it spreads the distilled water, evenly covering the swab without soaking. In addition, there is less risk of contamination when compared to the use of a pipette.

Drying cotton swab before freezing can be useful for long time storage for some of biological materials such body fluids. This is not the case with Touch DNA, if swab were allowed to dry before DNA extraction less DNA is recovered than if the moist swab was used immediately. Freezing the swab after collection while it is moist rather than drying it before extraction can results in DNA recovery similar to the immediate extraction after collection. It is a better routine to freeze cotton swabs immediately after Touch DNA collection to improve DNA recovery.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

This paper is a part of a PhD study and was approved by the Dubai Police General Department of Forensic Science and Criminology. Ethical approval was granted by the School of Forensic and Applied Sciences, and the University of Central Lancashire's Research Ethics Committee (ref. no. STEMH 912).

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