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Are you collecting all the available DNA from touched objects?

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Abstract

Retrieval of DNA from touched objects, for the purpose of generating genetic profiles, can be improved. The amount of DNA deposited varies depending on parameters that include; the individual, the area of contact, the history of previous touches, the material being touched, moisture levels and the presence of fingerprint powder. Much of what is deposited is not retrieved from an object by a single swabbing. This can be improved by employing multiple swabbing techniques. Furthermore, much of the DNA that is collected by a swab is not retrieved from the swab during the extraction phase. Quantiblot™ quantitation results of the retrieved DNA are not accurate and should be used as a guide only, for further analyses. Further improvement in the collection and extraction techniques of trace amounts of sample should be sought to increase the chance of providing probative value to criminal investigations.

Keywords: Forensic; DNA; Trace

1. Introduction

Since the principle of obtaining genetic profiles from touched objects became known [1], it has been well utilised world wide in police investigations. There is, however, often very little DNA on touched objects and investigators are not always able to generate a genetic profile from them. Here, we investigate issues relating to the collection of material

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from touched objects in order to provide information that may assist improvement in obtaining genetic profiles from them. Three relevant areas include deposit, collection and quantitation.

2. Deposit

It is well known that there are significant differences among individuals in terms of the amount of DNA that they deposit upon touching an object. The reasons for these differences remain unclear and need further investigation. Under experimental conditions, prints of portions of hands on plastic (whereby the DNA was extracted directly from the plastic and all of it used as template for PCR amplification) yielded full profiles from prints from 'one finger' to 'full hand' prints from four of five individuals. One individual only provided partial profiles or none at all. In a separate experiment, all 18 'finger tip' prints only gave partial or no profiles. Results of a comparison of left and right hand prints (eight individuals) indicated that the amounts deposited by the left and right hands of an individual are similar, i.e. far less variation between the left and right hands of an individual than between individuals.

An experiment whereby an individual placed their hand on 30 pieces of plastic for 10 s each (with minimal time in between each) and each third sheet was analysed (cut up, DNA extracted, all DNA amplified for Profiler Plus, peak heights analysed, and repeated for four individuals), revealed that the amount deposited can drop significantly after the initial touch. For some individuals, the observed peak heights from touches 3 to 30 fluctuated from undetectable to a maximum of only a small proportion (17%) of that observed from the initial touch. Others, however, showed more consistent amounts deposited over the 30 touches. With one such individual depositing, on the 24th touch, an amount that produced peak heights that were 40% of that observed from the initial touch. While the first prints gave full profiles, the remainder provided many partial profiles and profiles displaying stochastic effects.

Transfer studies involving all possible combinations of surface material (plastic or sticky tape), pick-up material (plastic or sticky tape) and sample (wet or dry sample of saliva (15 μl) spread over 4 cm^2) whereby a transfer block of known weight and dimensions was placed on the original deposit for 10 s then picked up and placed on a second clean piece of plastic for the same amount of time and repeated till the sixth piece of plastic (this was repeated five or six times for each possible combination) showed: (1) minimal or no pick-up and transfer of dry sample by non-sticky pick-up material, (2) more transfer when the sample is wet and when the pick-up material is stickier (with dry sample), (3) detectable amounts of DNA were retrieved after the fifth transfer in situations involving wet samples.

As the need to swab touched areas that have previously been fingerprinted, for retrieval of genetic profiles, becomes more common place, one needs to be aware of the potential for the collection and transfer of DNA between fingerprinted objects. To this end, we are currently undertaking experiments to determine the extent of pick-up and deposit of DNA using different types of routinely used brushes and powders. The results of such experiments may have impact on the types of brushes used and how they are used.

Previous studies have shown that secondary transfer can occur [1,2]. Even though the latter [2] do not think that it will compromise DNA typing results under typical forensic conditions, one still needs to be wary of, and consider, the possibility when analysing case work.

3. Collection

Earlier studies [3,4] have discussed the benefits of appropriate targeting of samples and shown that multiple swabbing of the same area can significantly improve the recovery of available DNA. Tests comparing a number of different cleaning agents concluded that water was still the preferred moisturising solution [4].

Further studies have shown that a significant proportion (20–76%) of the DNA that is collected by a cotton cloth/swab stick is lost during the extraction phase (Tables 1 and 2). The proportion lost was least with the smaller amounts of DNA from cotton cloth. Some of the losses are due to the extraction process (chelex, organic) and some are due to the type of matrix on which the sample is present (cotton cloth vs. swab stick), the amount of sample present in the matrix and/or the condition of the sample on the collection device (fresh vs. dried). There were some significant differences in retrievability depending on the presentation of sample (dry, wet, cloth, stick) (Table 2). This was most pronounced between wet cotton cloth and dry swab stick, with DNA being more readily retrieved from the former (Tables 1 and 2). Apart from wet cotton cloth, where chelex extraction outperformed organic extraction, there were no significant differences between the chelex and organic extraction methods used.

Centricons® are used to help clean up and concentrate DNA samples. It is routinely used as part of the organic DNA extraction method employed at our laboratory. Significant proportions of DNA can be lost while concentrating the DNA using Centricons®

Table 1
Average[a] percentage amounts of DNA retrieved from different presentations using different extraction methods

Sample extraction	100 ng in 100 μl Chelex	100 ng in 100 μl Organic	12.5 ng in 100 μl Chelex	12.5 ng in 100 μl Organic
Substrate[b]				
Dry cotton cloth	33 (19)	39 (18)	80 (36)	57 (18)
Wet cotton cloth	58 (26)	36 (10)	76 (17)	63 (18)
Dry swab stick	24 (14)	25 (13)	49 (20)	52 (17)
Wet swab stick	52 (45)	28 (15)	53 (22)	44 (13)
DNA control[2]	82 (31)	55 (30)	99 (26)	70 (28)

Note: aliquots of sample of the DNA utilised in these experiments were re-quantitated along with the samples generated from these experiments after extraction. Data were only included if these standard samples were quantitated as expected.

[a] The averages of 100 ng chelex, 100 ng organic, 12.5 ng chelex and 12.5 organic are from 9, 7, 9 and 6 repeats, respectively. The standard deviations are shown in parentheses.

[b] DNA placed on substrate and extracted from it while still wet or after drying. The DNA control represents DNA placed directly into an eppendorf tube.

Table 2
Significance of differences depending on presentation of sample and mode of extraction

Sample extraction	100 ng in 100 μl Chelex	100 ng in 100 μl Organic	12.5 ng in 100 μl Chelex	12.5 ng in 100 μl Organic
Substrate[a]				
DCC vs. WCC	***	–	–	–
DCC vs. DSS	–	–	**	–
DCC vs. WSS	–	–	*	–
DCC vs. DNA C	***	–	–	–
WCC vs. DSS	***	*	**	*
WCC vs. WSS	–	–	*	**
WCC vs. DNA C	*	–	***	–
DSS vs. WSS	–	–	–	–
DSS vs. DNA C	***	**	***	–
WSS vs. DNA C	*	*	***	*

– = not significant.

[a] DCC = dried cotton cloth, WCC = wet cotton cloth, DSS = dried swab stick, WSS = wet swab stick, DNA C = DNA control.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

subsequent to extraction, especially when dealing with very small amounts of DNA (approximately 14% and 19% with samples of 100 and 12.5 ng, respectively). Much of what is lost can be found in the top plastic section, membrane and rubber ring of the Centricon device.

Others have indicated that there is no negative effect on the typeability of biological samples exposed to various fingerprinting reagents [5]. Preliminary experiments whereby handprints on plastic were extracted and typed either; directly, after being swabbed, after being dusted or after being dusted and then swabbed, indicated a progressive decline in the ability to generate genetic profiles. This lead to an experiment to determine if commonly used fingerprinting powders (white and black powder) effected collection and extraction. Saliva samples (40 μl spread over 2 cm^2 on 48 sheets of plastic) and allowed to dry (overnight, 7, 14 or 21 days) were fingerprinted using either black or white powder (12 repeats each) or not exposed to powder at all. There was a significant decline of approximately 25% in the amount of DNA retrieved from samples powdered with black or white powder compared to those not powdered. Inhibition studies showed that even when only 5μg of black or white powder is present in the amplification mix, it will totally inhibit amplification of template DNA. As currently employed DNA extraction methods remove the powder elements from the DNA, this is not a problem. Further analyses using 1 ng of extracted DNA from samples exposed to powders vs. those that had not revealed that there was no significant difference in the ability to generate genetic profiles or the peak heights, thus confirming previous studies [5].

Sample collection devices need to be sought from which the DNA is more readily retrieved after collection. As there is an interaction between the methods of collection and extraction, the various combinations of the new elements will need to be tested.

4. Quantitation

One must also be wary of quantitation results of trace amounts of DNA. While methods such as Quantiblot™ are an improvement over some other methods, they are still only a guide. Their accuracy is limited. This was illustrated by three tests conducted on regular experienced users of Quantiblot. Test 1: blind trial kits, each containing five repeats of six DNA samples distributed randomly and instructions on what volume to load, were issued to nine individuals (amounts ranged from 0.2 to 5 ng). There were significant variations among the results of duplicates of the same samples tested by individual testees, and among testees. All samples were underestimated by the majority of testees. Several testees reported a negative result for samples of 0.2 and 0.5 ng. Test 2: the photo of the Quantiblot produced in test 1 was presented to the same testees approximately 4 and 8 weeks later for reanalysis. Significant differences were observed in the results reported for the same samples. Test 3: a fresh blot was analysed independently by 10 experienced individuals. Testees, in general, underestimated the results and again reported a wide range of results for the same sample. It is to be noted that useful genetic profiles can be obtained from samples that give negative Quantiblot results [6].

5. Concluding remarks

Through improved collection, extraction and quantitation techniques, we can make more of the available trace amounts of material from touched objects available for genetic profiling, thus increasing the chances of providing probative value to criminal investigations.

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