Attribution of DNA profiles to body fluid stains

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Abstract. Ultra-sensitive DNA profiling techniques, which now enable a profile to be obtained from a single cell, are greatly advantageous to the forensic community where biological material is often limited. However, it is not always possible to infer the cellular origin of any resulting profile. Here, we assess the likelihood of generating a DNA profile by the standard processing of visible stains that originates from extraneous cells on the substrate rather than the body fluid. Stain size and quality, substrate material and an individual's propensity to deposit DNA were all proven to be significant factors. It was demonstrated that an association between DNA profile and body fluid is not implicit, even where a positive presumptive test is obtained. © 2003 Elsevier B.V. All rights reserved.

1. Introduction

Scientists reporting body fluid cases in court are frequently requested to provide their expert opinion regarding the origin of any DNA profile obtained. It is well documented that, with the recent development of low copy number (LCN) profiling techniques [1,2], DNA profiles can be obtained from minute amounts of biological material [3,4]. Forensic laboratories now routinely analyse samples yielding less than 100 pg DNA [1].

The recovery of genetic profiles from touched objects and the transfer of DNA from one individual to another in the process of shaking hands has been demonstrated [5]. It has also been shown that a full profile can be recovered from secondary transfer of epithelial cells (from one individual to another and subsequently to an object) at 28 cycles [6]. This sensitivity increases the chance of detecting extraneous cells deposited in an event unrelated to a crime by the standard processing of body fluid stains. Precautions taken in the reporting of LCN cases may therefore be applicable to all cases involving low levels of DNA, such as minute blood stains on touched objects, even when amplified using standard 28 cycle profiling techniques.

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2. Methodology

Neat blood and saliva stains were prepared in duplicate from five body fluid donors. 1 μl and 15 μl stains were deposited on cotton and glass surfaces (total of 40 samples). Substrates were briefly handled before or after stain deposition by an individual known to have a high tendency to deposit DNA from epithelial cells (a 'good DNA shedder').

Ten dilutions of blood and saliva were prepared, up to 1/10,000. Substrates were each handled by one of six individuals prior to deposition of diluted samples.

Stains degraded for 0, 3, 5, 7 and 10 months were handled by a good shedder.

Leucomalachite green (LMG) was used for identification of bloodstains [7].

Phadebas tablets were used for detection of salivary amylase in cell harvests, with a blue supernatant indicating a positive result.

DNA extractions were carried out using QIAgen® QIAamp® DNA kit or Chelex 100. PicoGreen® quantification of saliva extracts was performed using Fluoroskan Ascent apparatus and associated software, by comparison with known standards of SHP DNA. The QuantiBlot™ kit (Applied Biosystems) was used to quantify blood samples according to the kit protocol. Template DNA was amplified using the AmpFlSTR® SGM Plus™ kit, electrophoresed and analysed using Genescan® 2.1.1 and Genotyper® 1.1.1 software [8].

The relative contribution of the body fluid and epithelial cells to the resulting DNA profile were calculated as Mx, which is explicitly defined by:

$$Mx = Pa(H)/[Pa(H) + Pa(D)],$$

where Pa(H) is the peak area contributed to the DNA profile by the substrate handler and Pa(D) is the peak area contributed by the body fluid donor.

Mx values were used to calculate the statistical significance of all factors contributing to the resulting profile using Minitab 10.51 Xtra software.

3. Results

A positive presumptive test was obtained from all samples. Processing of large stains produced DNA profiles originating from the body fluid. The substrate handler contributed the major component of profiles recovered from small stains.

In one exception, a large bloodstain on cotton substrate yielded predominantly the substrate handler's profile. This stain had given a positive presumptive test, although the quant value was uncharacteristically low (0.07 ng/μl compared to an average 0.7 ng/μl for this sample type).

Profiles of substrate handlers were only detected from individuals known to have a very high tendency to deposit DNA. These individuals accounted for approximately 13% of the population, according to the study by Lowe et al. [6].

Results from substrates handled by good shedders showed that the more dilute or degraded a stain, the higher the contribution of the substrate handler's DNA to the resulting profile. A positive presumptive test could be obtained from samples when a profile originating from the body fluid was no longer detectable.

Statistical analysis of all contributing factors showed that the most significant were the tendency of an individual to deposit DNA from epithelial cells, substrate type and stain size and quality ($P < 0.001$). The body fluid type was also significant ($P = 0.042$).

4. Discussion

In this study, DNA profiles from epithelial cells were only recovered from substrates handled by good DNA 'shedders'. This corresponds to reports that higher levels of DNA can be recovered from objects touched by certain individuals [5,8]. The mechanism for this is uncertain; it may be that a good DNA shedder sloughs a greater number of epithelial cells, or a higher proportion of nucleated cells, than a poor DNA shedder [4].

Where a quant value obtained is lower than expected for the sample size, then the scientist may not be justified in attributing any DNA profile obtained to the stain. It is suggested that low copy number considerations are applied whether amplification of these samples is carried out at 34 or 28 cycles. The 'hierarchy of propositions' described by Evett et al. [9] and Cook et al. [10] explains levels at which prosecution and defence propositions should be examined. Results from this study suggest that, where samples give unexpectedly low quant values, then the evidence may be best evaluated at 'sub-source level', consistent with LCN guidelines. This addresses whether a DNA profile obtained matches the suspect. Further information, such as the suspect's previous legitimate access to a scene, would be required in order for the scientist to infer the source of a profile. All DNA and non-DNA evidence must be considered and may be analysed using Bayesian networks [9]. These models may be used to calculate a likelihood ratio relating to higher level propositions, defined as 'activity' or 'offence' level, which the scientist can use to advise the courts on the probability that a suspect or an unrelated person has been involved in an activity or committed a crime.

References

[1] P. Gill, et al, An investigation of the rigor of interpretation rules for STRs derived from less then 100 pg of DNA, Forensic Sci. Int. 112 (2000) 17–40.

[2] J.P. Whitaker, E.A. Cotton, P. Gill, A comparison of the characteristics of profiles produced with the AMPFlSTR® SGM Plus™ multiplex system for both standard and low copy number (LCN) STR DNA analysis, Forensic Sci. Int. 123 (2001) 215–223.

[3] R.A.H. Van Oorschot, M.K. Jones, DNA fingerprints from fingerprints, Nature 387 (1997) 787.

[4] R.A. Wickenheiser, Trace DNA: a review, discussion of theory and application of the transfer of trace quantities of DNA through skin contact, J. Forensic Sci. 47 (2002) 442–450.

[5] R.A.H Van Oorschot, M.K. Jones, DNA fingerprints from fingerprints, Nature 387 (1997 June) 6635–6767.

[6] A. Lowe, C. Murray, J. Whitaker, G. Tully, P. Gill, The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces, Forensic Sci. Int. 129 (2002) 25–34.

[7] M. Cox, A study of the sensitivity and specificity of four presumptive tests for blood, J. Forensic Sci. 36 (1991) 1503–1511.

[8] E.A. Cotton, et al, Validation of the AMPFlSTR® SGM Plus™ system for use in forensic casework, Forensic Sci. Int. 112 (2000) 151–161.

[9] I.W. Evett, P.D. Gill, G. Jackson, J. Whitaker, C. Champod, Interpreting samm quantities of DNA: the hierarchy of propositions and the use of Bayesian networks, J. Forensic Sci. 47 (2002) 520–530.

[10] R. Cook, et al, A hierarchy of propositions: deciding which level to address in casework, Sci. Justice 38 (1998) 231–239.