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Assessing primary, secondary and tertiary DNA transfer using the Promega ESI-17 Fast PCR chemistry

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ABSTRACT

The current generation of PCR chemistries used in many forensic science laboratories offer improved sensitivity and detection. In a criminal justice setting, it can often be important for the forensic scientists to be able to address the issue of how and when DNA might have been deposited, and not simply the issue of from whom the DNA might have originated [1].

We have used the Promega ESI-17 Fast system to investgiate DNA transfer in a series of three experiments with known donors. The experiments all involved participants gripping a plastic tube, with hand shaking then progressively introduced to precipiate the possibility of secondary and tertiary transfer events. The levels of DNA transfer as a result of direct contact, as well as secondary and tertiary transfer events, were then measured and compared.

Our results show the variable nature of primary transfer and also clear examples of secondary transfer, including occasional examples where the degree of secondary transfer exceeded primary transfer. Unambiguous tertiary transfer was difficult to detect but cannot be ruled out.

1. Introduction

It has been demonstrated that a person's DNA can be deposited onto a surface through direct contact, as well as indirectly via one or more intermediary surfaces, such as another person's hand during hand shaking [2]. There has been considerable research in this area exploring the effect of key variables on the rate of the transfer of an individual's DNA by such mechanisms. These include the type of biological material being transferred and whether it is dry or wet, the substrate surface, the manner of contact and the period of delay between each subsequent transfer stage. The amount of DNA transferred via skin contact is also believed to be dependent on a person's propensity for transfer, sometimes referred to as their 'shedder' status [2].

Much of this research has been carried out using DNA chemistries that have now been superceded by a new generation of multiplexes with increased sensitivity. Newer chemistries are capable of routinely producing full DNA profiles from picogrammes quantities of DNA. However, these improvements also mean that there is an increased chance of detecting the transfer of DNA from individuals not involved in the commissioning of an offence to scenes and/or exhibits and of the cross transfer of DNA between scenes or items [4].

In the UK, casework scenarios presented to scientists can commonly entail hypotheses involving the possibility of indirect transfer of a person's DNA. An improved understanding in levels of DNA transfer that these data offer will support a scientist's ability to evaluate such findings and to provide an opinion for the courts as to whether or not the findings might be more likely given one version over the other.

This research has been carried out using the Promega ESI-17 Fast system. As many variables have been controlled within the experimental design as was practical.

2. Materials, methods studies, techniques

All experiments were conducted one hour after thorough hand washing with water and soap. During the one hour after washing, participants carried out everyday desk duties within an office in which no forensic material was present. When participants shook hands, they did so for 30 s using both hands and then without delay

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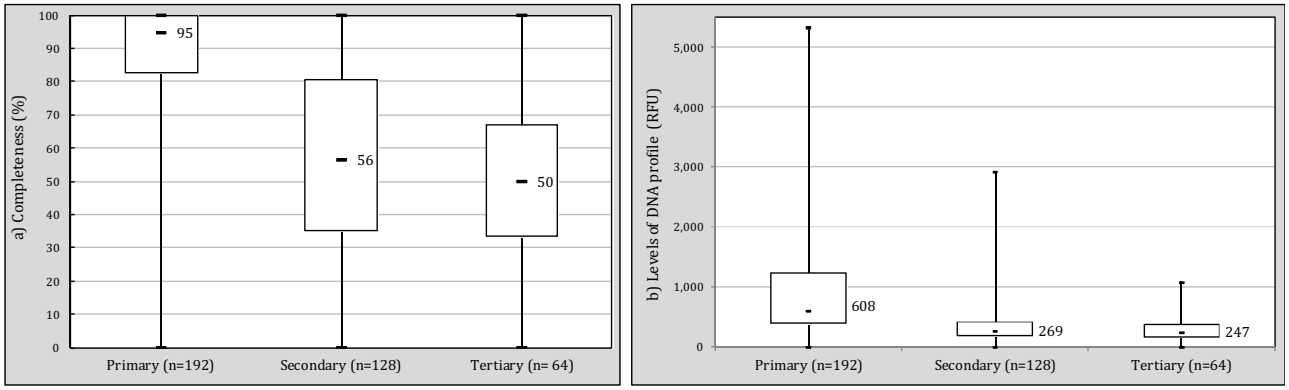

Fig. 1. Distributions of a) completeness scores of profiles assumed to originate from each of the donor types and b) levels of DNA profiles detected, with median values shown in each.

moved to the next action. The final action always involved gripping a virgin 100 ml plastic tube which had been pre-demarcated with pen to show the required contact zone. Primary donor DNA transfer was captured by participants gripping the plastic tubes within the zoned area (experiment 1). Secondary donor DNA transfer was captured by paired individuals each shaking hands before each gripping tubes (experiment 2). Participants were put into groups of four in an effort to capture tertiary donor events. Two participants initially shook hands, and each then shook hands with another respective partner. In doing so they performed the role of vector passing on the tertiary donor's DNA to their respective partners as well as potentaily their own DNA (as secondary donor). Each respective partner then gripped their own tubes, in doing so potentially transferring their own DNA via primary contact as well as any from the secondary and/or tertiary donors (experiment 3).

The pre-demarcated contact zones of each plastic tube were swabbed using one wet and one dry fat head cotton swab in precisely the same way. This meant that the same area of substrate was targeted in every experiment and that the DNA recovered in an identical fashion for all the experiments that were conducted. The experiments were carried out at roughly the same time each day using the same selected group of volunteers and operators. The DNA was extracted from the

combined wet and dry swabs using the QIAsymphony SP (Qiagen). Recovered DNA in the extracts was quantified using the Plexor HY Quantification system (Promega) before amplification using the ESI17F kit according to manufacturer's intstructions. One PCR replicate of each sample was produced and a maximum of 500 pg of template was added to each PCR reaction or as near to that quantity as the concentration of DNA in the extract would allow. PCR products were separated by CE using a 3500XL Genetic Analyser (Thermo-Fisher) and the data analysed using the DNA INSIGHT analysis software.

Sixteen participants were selected from a larger panel of volunteers to minimise amounts of allele sharing. All peaks above 40 RFU, clear and distinct of baseline were scored. Shared alleles were discounted for the purposes of data analysis, and where donors were homozygous this was counted as a single allele and the peak height halved.

The completeness of a donor's DNA profile was measured as the percentage of unambiguous alleles actually detected compared to those available for detection. The level of each result was measured by summing of the peak heights of all of the detected unambiguous alleles matching the expected donor, divided by the number of alleles expected.

3. Results

In total, 64 sets of data were produced for each of the three experiments. However, in only 7 of the 192 results was there a sufficient template DNA to allow a profiling attempt using the optimum 500 pg of template. The median quantification measure was 6.9 pg/μl (equivalent to adding 120 pg of template to the PCR). Fig. 1 shows the decline in completeness and magnitude scores as more transfer steps were introduced.

Even in primary transfer experiments, DNA was consistently detected which was not attributable to any of the participating donors (hereafter referred to as non-donor DNA). Control samples (data not shown) suggested that, assuming participants effectively removed DNA on their hands during washing, the majority of this non-donor DNA must have accumulated on the participant's hands during the everyday activities carried out in the hour preceding the experiment. An average of 21 non-donor alleles were detected per result in experiment 1 with an average peak height of 259 RFU.

4. Discussion

Our results show clear examples of both primary and secondary transfer. On average, there was almost three times more DNA detected from primary transfer than from secondary transfer in the same sample. However, in 20% of findings, the degree of secondary transfer exceeded the level of primary transfer in the same sample. However, in those instances, the primary donor was still detectable, at an average 73% completeness.

Unambiguous tertiary transfer was difficult to establish due to the complexity of the final mixtures. Although tertiary results showed an average magnitude of 291 RFU, with at least 67% completeness in 75% of results, this was at a level which could not be distinguished from DNA from non-donors. As such, in most datasets it was not possible to establish if there was a partial DNA profile from a tertiary donor or interference from a low level of non-donor DNA.

The results demonstrated a high degree of both within-person and between-person variability.

Conflict of interest

None

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigss.2015.09.022.

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