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An Innovative Solution to Collect Touch DNA for Direct Amplification

Salem K Alketbi[1,2]*

[1]*University of Central Lancashire, UK*

[2]*General Department of Forensic Science and Criminology, UAE*

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***Corresponding author:** Salem K Alketbi, General Department of Forensic Science and Criminology, University of Central Lancashire, Preston, UK, Dubai Police, UAE

Abstract

Touch DNA Profiling is a commonly used tool to link suspects to their crimes, but it is often challenging compared to other types of DNA evidence, such as blood and saliva, because it is usually found in minute quantities. Using direct amplification techniques to avoid sample extraction and quantification during the DNA profiling process can help preserve DNA loss. Therefore, this study explored an innovative solution to collect blood, saliva and Touch DNA using a cotton swab in combination with a microFLOQ® swab (CS+MF) for direct amplification to preserve the collected samples for re-analysis or additional testing. The allele recovery rate was 100% for blood and saliva samples, and 84% for trace samples, with a considerable difference in the average signal (RFU) between the evidence types ($p < 0.001$).

Keywords: Forensic science; Trace DNA; Touch DNA; DNA recovery; Cotton swab; MicroFLOQ™ swabs; DNA direct amplification; GlobalFiler™ PCR Amplification Kit

Introduction

Touch DNA profiling is a commonly used tool to link suspects to their crimes. Touch DNA is present on many crime scene items but is often challenging compared to other types of DNA evidence [1-9], such as blood and saliva because it is usually found in minute quantities. The use of direct amplification techniques to avoid sample extraction and quantification during the DNA profiling process can help preserve DNA loss [1]. Furthermore, the development of new protocols to collect and process different types of DNA samples can lead to good results with DNA direct amplification. The use of microFLOQ® Direct swab (co-developed by the French Gendarmerie Forensic Research Institute, IRCGN™ and Copan) has proven to be a beneficial tool to collect trace DNA and biological samples for direct amplification [10]. Therefore, this study explored an innovative solution to collect blood, saliva and trace DNA using a cotton swab in combination with a microFLOQ® swab for direct amplification to preserve the collected samples for re-analysis or additional testing.

Materials & Methods

Experimental setup

Blood and saliva from three participants were collected in EDTA-treated tubes, serially diluted (20%, 10%, 5%, and 1%) with molecular grade water before 10 µl of each sample was pipetted onto sterilised glass microscope slides (7.5 x 2.5 cm) and allowed to dry overnight. For Touch DNA deposition, the same participants were requested to wash their hands with antibacterial soap, abstain from any activity for 10 minutes, then charge the fingers of both hands with eccrine sweat by touching behind their ears or forehead to load them with DNA [3] before touching the surface of the glass microscope slides using their index, middle, and ring fingers of both hands separately while applying medium pressure for 1min. The same procedure was repeated on all surfaces for equal deposition and left overnight. The glass microscope slides were sterilised with 2% Virkon (viricidal disinfectant) and ultraviolet radiation (UV) for 20min before use.

DNA recovery

Blood, saliva, and trace samples were collected using a Copan cotton swab (150C) (CS) moistened with 100µL of sterile distilled water applied using a plastic spray bottle technique [5]. Then, microFLOQ™ swabs (MF) moistened with 1µL applied using pipette were used to collect the collected samples from the cotton swabs (CS+MF) while they were still wet. The CSs were stored in a freezer for re-analysis or additional testing.

DNA amplification & analysis

The samples collected using microFLOQ™ swabs from cotton swab (CS+MF) were self-broken directly into a PCR tube (0.2ml)

and amplified directly with GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific). The PCR master mix was added directly to the tubes and the volume of sample solution required by the kit manufacturer was replaced with TE, then amplification was performed on an ABI GeneAmp® 9700 PCR System (Life Technologies, Foster City, CA) for 29 cycles. Amplified products were size-separated and detected on an ABI 3500 Genetic Analyzer (Life Technologies) using 1µl PCR product, 9.6µl Hi-Di™ formamide, and 0.4µl GeneScan™ 600 LIZ® Size Standard v2.0 (Thermo Fisher Scientific) following the manufacturer's protocol. Statistical analysis was performed with RStudio using factorial analysis of variance (ANOVA) and Microsoft Excel.

Results & Discussion

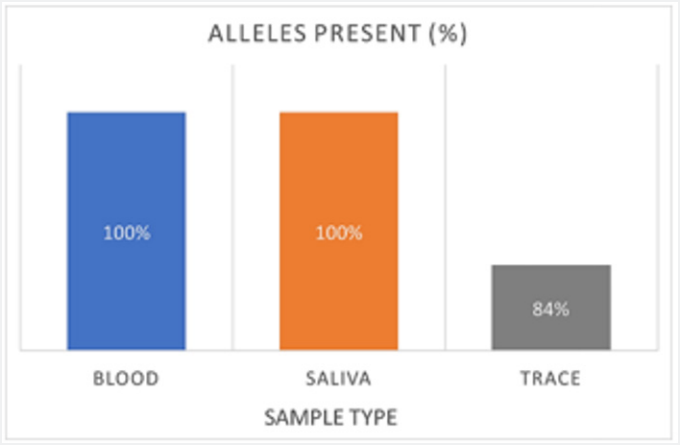

Figure 1: Percentage of alleles recovered by CS+MF from the three sample types: blood (n=24), saliva (n=24), and trace (n=24).

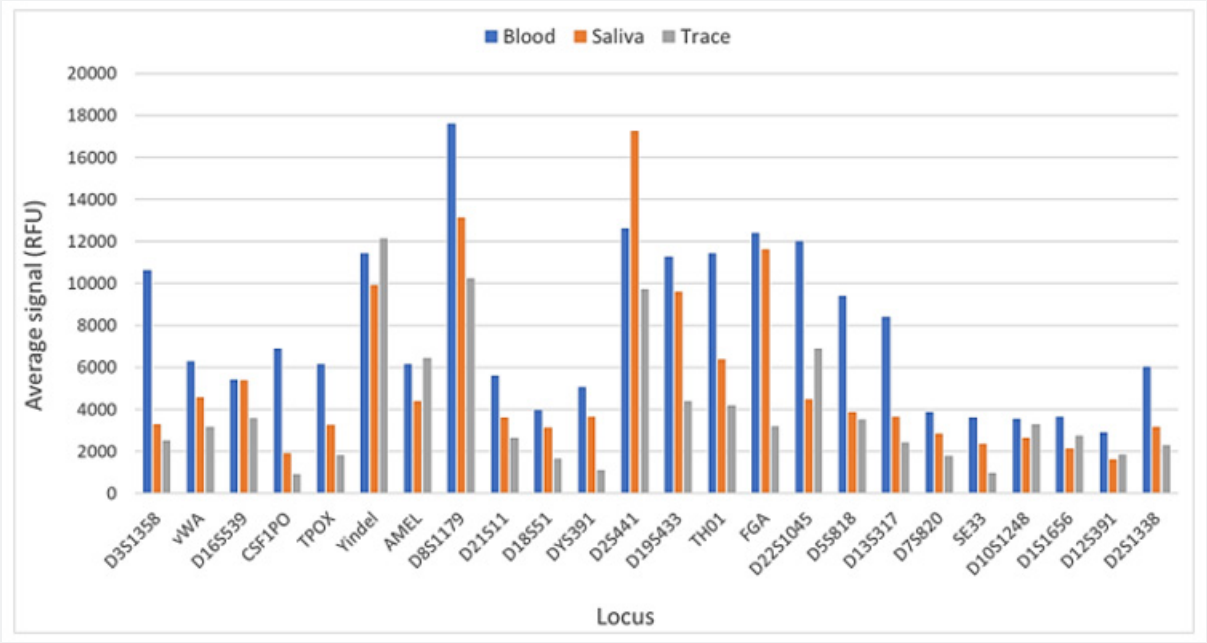

Figure 2: Average signal (RFU) per locus of profiles collected by CS+MF from the three sample types: blood (n=24), saliva (n=24), and trace (n=24).

There was a considerable difference in the average signal (RFU) between the evidence types ($p < 0.001$) (mean RFU blood=7765, saliva=5325 and trace=3892). There was also a significant difference in the average signal (RFU) between the different blood dilutions ($p < 0.001$), with the peak height decreasing with decreasing blood dilution (mean RFU 20% – 14622, 10% – 10988, 5% – 3992 and 1% – 1460). Regardless of the decrease in the peak height of the blood samples, the allele recovery rate was 100% with CS+MF. Like the saliva samples, the peak height decreased with the decreasing saliva dilution ($p < 0.001$) (mean RFU 20% – 8443, 10% – 4340, 5% – 4740 and 1% – 3776) and the allele recovery rate was 100% with CS+MF. However, the trace sample allele recovery rate was 84% with CS+MF and the average signal (RFU) was lower than the other biological samples (Figure 1 & 2).

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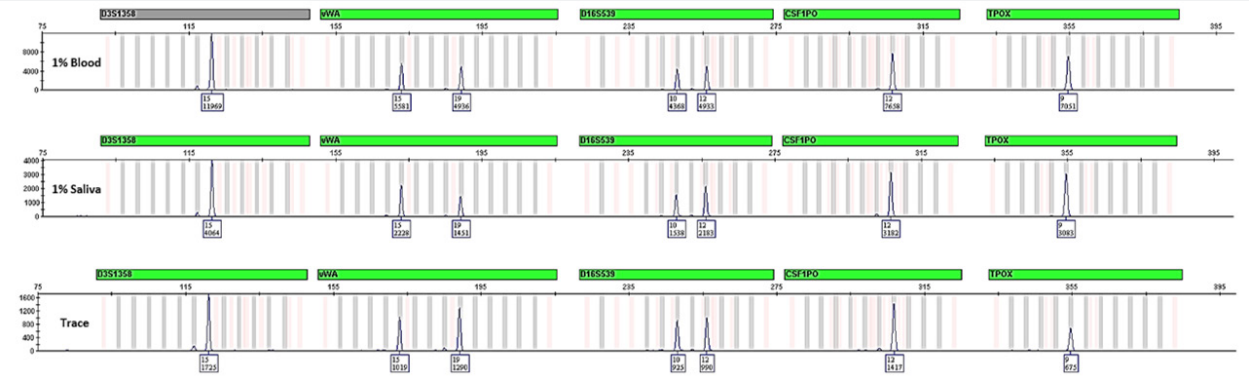

Figure 3: Comparison of the electropherograms at five loci (D3S1358, vWA, D16S539, CSF1PO and TPOX) of 1% blood, 1% saliva and trace samples after direct amplification using CS+MF and the GlobalFiler™ PCR amplification Kit.

The difference in DNA quantity among the samples collected (blood, saliva, and trace) were used to test the sensitivity of the direct amplification process via this innovative sample collection technique (Figure 3). Blood and saliva samples yielded full STR profiles with balanced loci but there were observable patterns of inhibition present, such as split peaks mostly in the concentrated samples (20%) of blood and saliva, which were similar to the results reported previously [2]. Trace samples produced full and partial STR profiles with balanced loci, with no sign of inhibition. Blanks were collected from surfaces after sterilisation and negative controls for the collection and extraction methods, all of which proved negative for DNA when amplified.

Conclusion

The success of the direct amplification from a variety of DNA samples such as blood, saliva and trace are affected by the amount of material available, and the collection techniques used. The present study showed that the use of a cotton swab to collect the DNA sample, then swabbing the cotton swab with a microFLOQ® swab to collect the DNA was a successful technique for direct amplification. The cotton swab helps to concentrate the sample in a small surface area for a more efficient sample collection with the microFLOQ® swab and allows re-analysis or additional testing.

Acknowledgement

This study was approved by the General Department of Forensic Science and Criminology in the Dubai Police and ethical approval was granted by the University of Central Lancashire's Research Ethics Committee (ref. no. STEMH 912). Many thanks to COPAN DIAGNOSTICS INC. for supporting this experiment with free swabs and to ThermoFisher Scientific™ for product discounts.

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