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An Evaluation of the Performance of Two Quantification Methods for Trace DNA Casework Samples

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Abstract

Trace or Touch DNA is one of the most common types of DNA samples found at crime scenes and is often collected in vast quantities from crime scene exhibits. Quantification methods that use real-time PCR (qPCR) technology are highly sensitive compared to traditional quantification methods, therefore, this study evaluated the performance of Quantifiler™ Human and Quantifiler™ Trio quantification kits for the same set of trace DNA samples collected from handled items recovered from crime scenes at the Biology and DNA Section of the General Department of Forensic Science and Criminology of Dubai Police Force. Both methods achieved comparable results indicating that there was no significant difference between the two quantification methods to quantify the collected trace DNA from the handled items ($p > 0.05$).

Keywords: Forensic science; Trace DNA; Touch DNA; DNA quantification; Quantifiler™ Human DNA Quantification Kit; Quantifiler™ Trio DNA Quantification Kit; Global Filer™ PCR Amplification Kit

Introduction

Trace or Touch DNA is one of the most common types of DNA samples found at crime scenes and is often collected in vast quantities from crime scene exhibits. However, it is frequently found in minute quantities and the process of Touch DNA profiling can be impacted by many factors [1-14], which leads to low levels of DNA recovery, such as the efficiency of DNA extraction and quantification methods [4,15]. Quantification methods that use real-time PCR (qPCR) technology are highly sensitive compared to regular quantification methods such as Nano Drop™ or Gel electrophoresis, so they are the preferred technique for the quantification of trace DNA samples. This study evaluated the Quantifiler™ Trio and Quantifiler™ Human (Thermo Fisher Scientific) quantitative real-time PCR DNA kits widely used in forensic labs [1,2] on the same set of trace DNA samples collected from items recovered from crime scenes in the Biology and DNA Section of the General Department of Forensic Science and Criminology of Dubai Police Force.

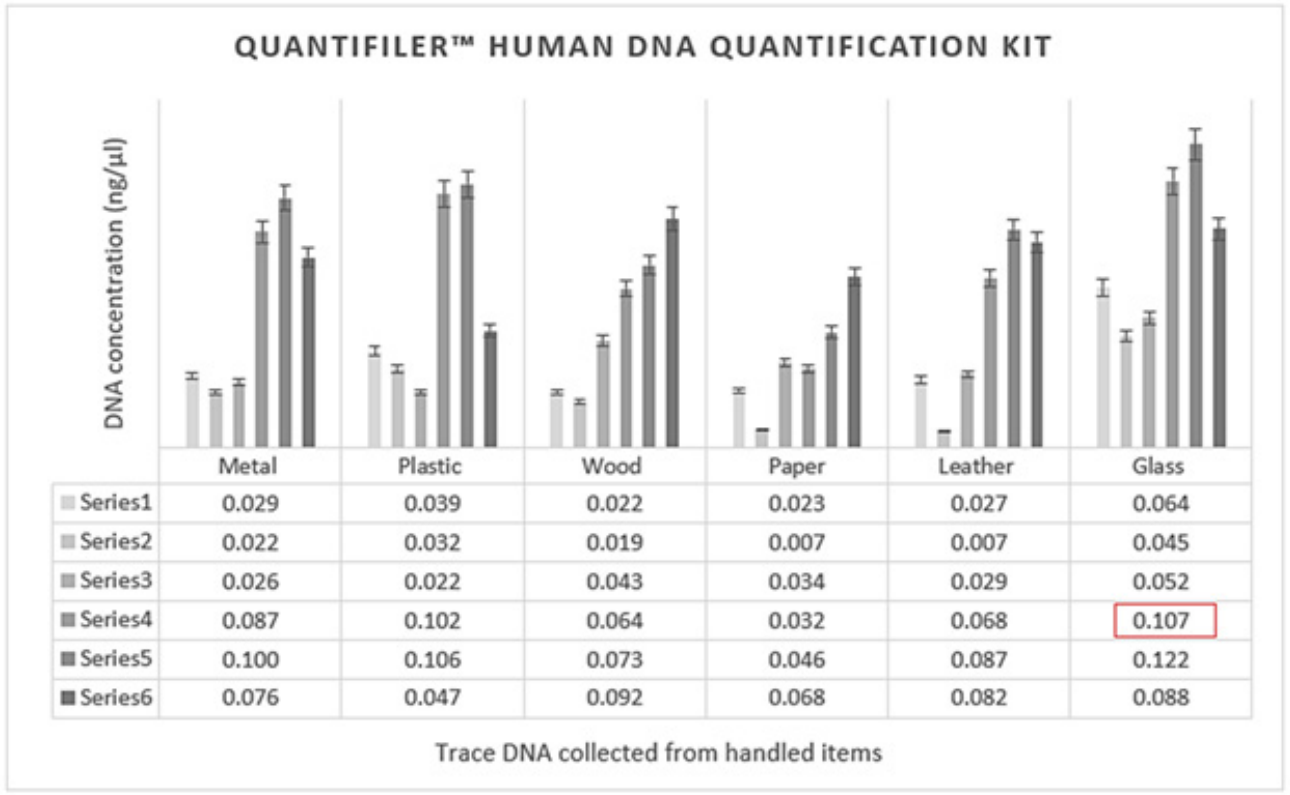
Materials and methods

Trace samples were collected with a Copan cotton swab (150C) moistened with 100 µL of molecular-grade water using a spray bottle [8] and extracted using the Prep Filer Express kit (Thermo Fisher Scientific) and the liquid handling and automation Tecan robot according to the manufacturer's protocols. A full swab head was used with a final sample volume of 50 µL. The samples were then quantified manually using the Quantifiler™ Trio and Quantifiler™ Human, Quant Studio 5 Real-Time PCR (qPCR) and HID Real-Time PCR analysis software v1.3 (Thermo Fisher Scientific).

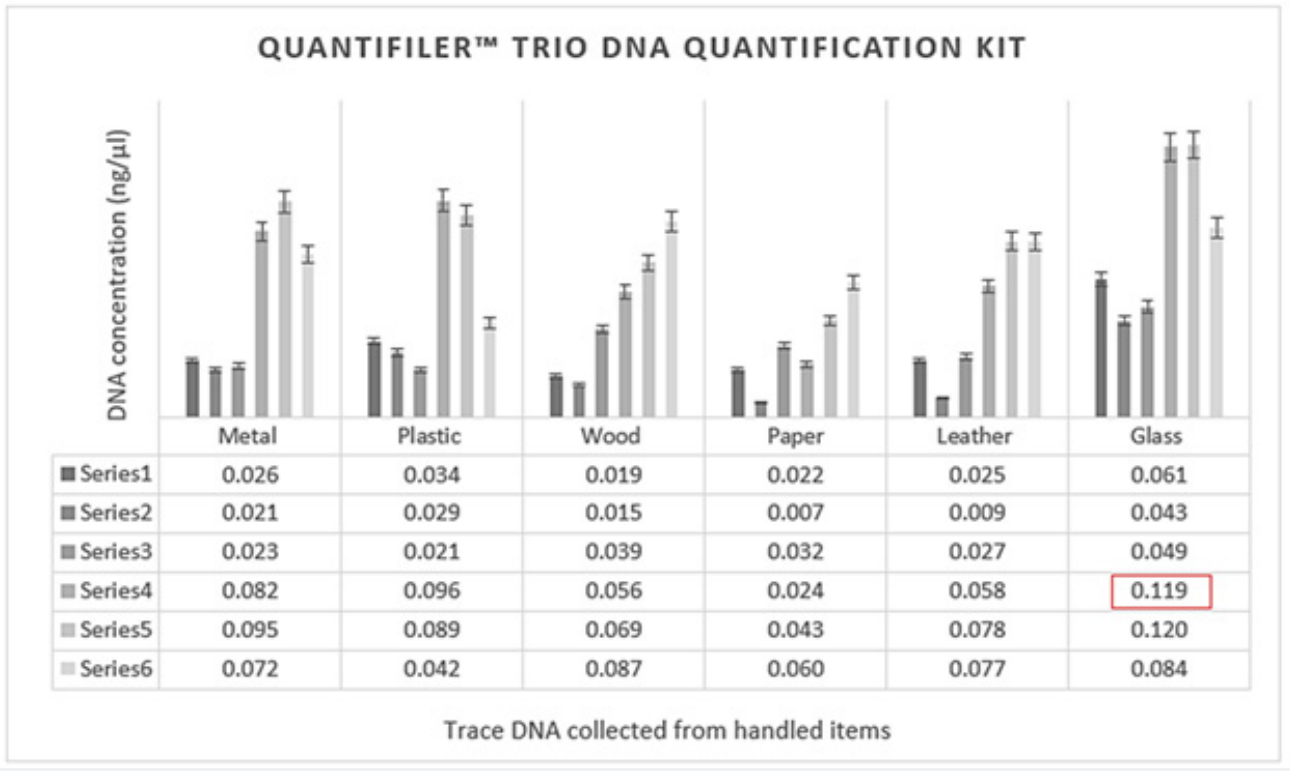
DNA amplification for some of the samples was performed using the Global Filer™ PCR amplification Kit on an ABI GeneAmp® 9700 PCR System (Life Technologies) for 29 cycles. The products of amplification were size-separated and detected on an ABI 3500 Genetic Analyser (Life Technologies) using 1 µl of PCR product, 9.6 µl Hi-Di™ formamide, and 0.4 µl GeneScan™ 600 LIZ® Size Standard v2.0 (Thermo Fisher Scientific). The data were statistically analysed using RStudio and factorial analysis of variance (ANOVA) and Microsoft Excel. All negative controls for DNA collection, extraction and amplification were confirmed DNA-free.

Results and Discussion

There was no significant difference between the results of the two quantification methods tested (Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Trio DNA Quantification Kit) to quantify the collected trace DNA from the handled items ($p > 0.05$) (Figure 1). Similar quantities of DNA were recovered by both quantification methods. All samples were diluted in the same volume (10 µL from the sample with 5 µL of molecular grade water) for amplification with the Global Filer™ PCR Amplification Kit.



	Metal	Plastic	Wood	Paper	Leather	Glass
Series1	0.029	0.039	0.022	0.023	0.027	0.064
Series2	0.022	0.032	0.019	0.007	0.007	0.045
Series3	0.026	0.022	0.043	0.034	0.029	0.052
Series4	0.087	0.102	0.064	0.032	0.068	0.107
Series5	0.100	0.106	0.073	0.046	0.087	0.122
Series6	0.076	0.047	0.092	0.068	0.082	0.088



	Metal	Plastic	Wood	Paper	Leather	Glass
Series1	0.026	0.034	0.019	0.022	0.025	0.061
Series2	0.021	0.029	0.015	0.007	0.009	0.043
Series3	0.023	0.021	0.039	0.032	0.027	0.049
Series4	0.082	0.096	0.056	0.024	0.058	0.119
Series5	0.095	0.089	0.069	0.043	0.078	0.120
Series6	0.072	0.042	0.087	0.060	0.077	0.084

Figure 1: DNA samples collected from handled items (n= 36) which were extracted by the Prep Filer Express kit, and then quantified using the Quantifiler™ Human and Quantifiler™ Trio quantification kits. Error bars represent 95% confidence intervals.

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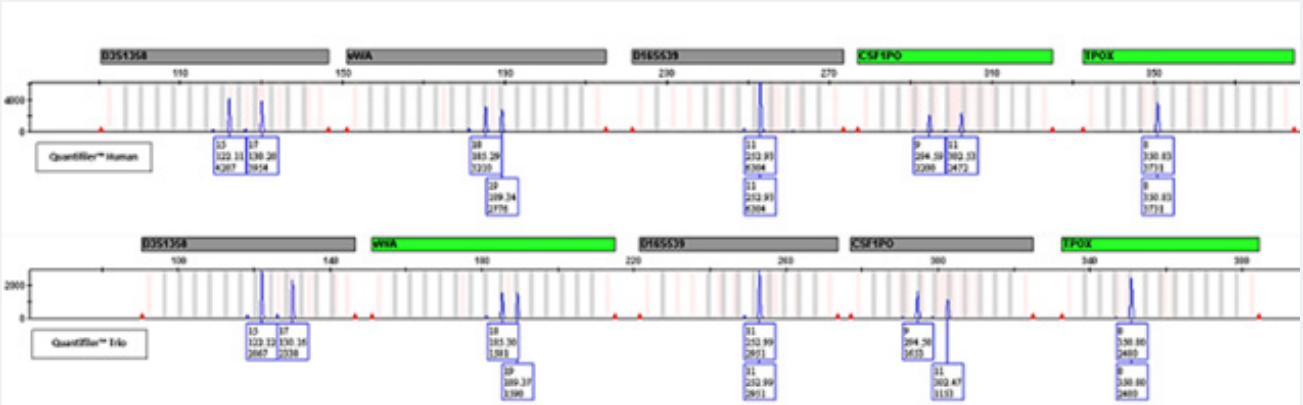

Figure 2: An example of the electropherograms obtained by the Quantifiler™ Human and Quantifiler™ Trio quantification kits. There was a difference in the peak height of the DNA profiles at five autosomal STR loci (D3S1358, VWA, D16S539, CSF1PO, TPOX).

However, different volumes were required for one sample (circled in Figure 1), for which, 9.5 µL of the sample was diluted with 5.5 µL of molecular grade water for the Quantifiler™ Human kit and 8.6 µL of the sample was diluted with 6.4 µL of molecular grade water for the Quantifiler™ Trio kit. Full DNA profiles were obtained by both kits, but the peak heights (RFU) produced using the Quantifiler™ Human kit were relatively higher than the peaks obtained by the Quantifiler™ Trio kit (Figure 2). However, this small difference may be related to a human pipetting error or using an uncalibrated pipette, therefore establishing regular calibration protocols (e.g., every 3 months) or considering automated solutions to process samples for quantification can be beneficial.

Conclusion

The Quantifiler™ Human and Quantifiler™ Trio Quantification Kits are equally effective for the quantification of trace DNA but the Quantifiler™ Trio Quantification Kit provides more information about DNA degradation or human male DNA when using the large autosomal (LA) target and Y chromosome target in the same run.

Acknowledgements

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