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Collection Protocols for the Recovery of Biological Samples

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ABSTRACT

The main focus in forensic genetics for two decades has been to improve the extraction of DNA from a wide variety of evidence and to make the profiling technology more sensitive and robust. In contrast, the recovery methods for biological material have seen little development. This study aims to improve the efficacy of the collection and storage processes, from crime scene to receipt at the laboratory. This study compared the use of ultrapure water as a wetting agent when collecting biological evidence using swabs with a detergent-based buffer. The results show that the stability post-collection greatly improved by using a newly developed buffer. When ultrapure water is used, DNA degradation was seen after 6 h at room temperature. However, the detergent-based buffer stabilized DNA for up to 48 h, even when the temperature was increased to 50 °C. The impact of these findings may be limited where crime scene evidence can be refrigerated until it reaches the laboratory. However, there are many situations/contexts where sample refrigeration is not possible and there is scope to improve the preservation of the genetic forensic evidence before it reaches the laboratory.

KEYWORDS: Forensic biology, DNA collection, double swabbing.

1.0 INTRODUCTION

When analyzing biological evidence the precursors to extraction and analysis are the sample collection, handling and storage. Collection, preservation and storage of biological evidence have a fundamental impact on the quality of the sample and the resultant DNA profile. If the sample quality is damaged by poor practices it can undermine the potential for it to be used as evidence [1]. These initial steps must be undertaken carefully, and the most reliable and reproducible protocols should be used for the crime scene collection and preservation of the biological materials before they reach the laboratory. DNA collection procedures can vary, but often start at the crime scene where biological samples such as blood, semen and saliva are identified, collected, and then transferred to the forensic laboratory. Many samples are collected at crime scenes using DNA-free water as a wetting agent with cotton swabs – this basic system does not have any preservative properties.

2.0 MATERIAL and METHOD

2.1 Saliva samples were deposited on a plastic, metal and glass substrates and allowed to dry overnight and then recovered using the double swab technique [2,3] using Ultrapure water and a detergent-based wetting agent (PresGene, UK). PureGene extraction (Qiagen) was used after the samples had been exposed (post-collection) to various environmental conditions (-20 °C, room temperature, 37 °C and 50 °C).The quantity of extracted DNA present in samples was measured using real-time PCR, whereas the quality of the extracted DNA present was determined using in-house multiplex PCR.

2.2 DNA Quantification: All extracted DNA samples from saliva were quantified using the Quantifiler™ Human DNA Quantification kit on the ABI7500 real-time PCR machine (Applied Biosystems, UK). Amplification reactions and amounts were carried as recommended by the manufacturer.

2.3 DNA Amplification: DNA (1 µL) was amplified using the polymerase chain reaction (PCR) using an in-house assay that amplifies four amplicons ranging between 50 bp and 154 bp.

2.4 DNA Analysis: DNA fragment analysis was carried out on ABI 3500 Prism® Genetic Analyzer.

2.5 Data analysis: The data obtained from the capillary electrophoresis (CE) were analysed using an ABI3500 with GeneMapper® ID-X Software Version 1.2 (Life Technologies™, UK). The peak heights (RFU) of the samples were used to perform the statistical analysis. Excel 2010 was used to calculate averages and standard deviations. R-Studio software was used for statistical analyses such as the analysis of variances (ANOVA).

3.0 RESULTS AND DISCUSSION

Samples were recovered in triplicates from glass, plastic and metal, results below are of plastic only using the double swab technique [2,3] using ultrapure water and a detergent-based buffer and stored post-collection for up to 48 h at different temperatures (-20 °C, room temperature, 37 °C and 50 °C). The quantitation data is shown in Figure 1 and the profiles generated using an in-house multiplex are shown in Figure 2. At 48 hours there is a statistically significant difference between the amounts of DNA using the two collection methods.

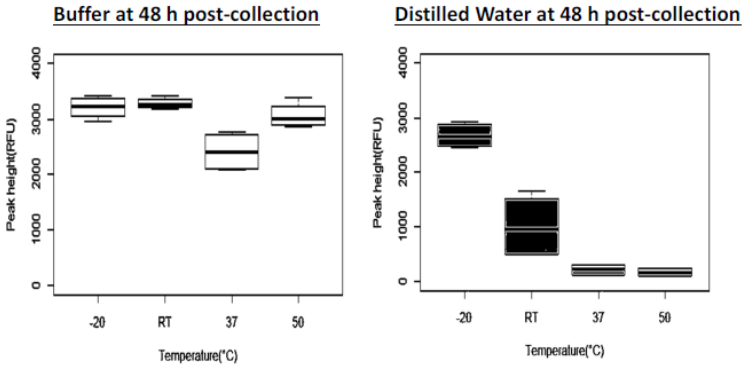

Fig. 1: Boxplots demonstrate the peaks heights of Saliva samples collected by distilled water and buffer separately, at four different temperatures (-20 ^{O}C , room temperature (RT), 37 ^{O}C and 50 ^{O}C) for 48 h post-collection, and amplified by mini 4-plex multiplex PCR.

The data presented demonstrates that post-collection degradation of biological material could be a significant problem when working in environments were optimum storage of the swabbed material is not possible. Using a detergent-based buffer stabilized the DNA on the swabs, as illustrated both by the quantitation and the quality of the DNA profiles, both in terms of peak height and balance.

4.0 CONCLUSION

Further work need to be done to examine the efficacy of the detergent-based buffer when collecting biological evidence such as touch DNA. Gaining a better understanding of the key factors in crime scene sample collection and post-collection handling that impact on DNA recovery should give a clear indication of best practices in post-collection sample handling whilst in transit to the laboratory.

5.0 CONFLICT OF INTEREST:

None.

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