Body fluid mixtures; resolution using forensic microRNA analysis

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Abstract. Body fluid identification is a crucial aspect of forensic biology; particularly in sexual assaults which is usually characterised as a mixture of body fluids. Whilst there are considerable efforts to identify single source body fluids using genetic markers; no substantial research appears to have been carried out on mixed body fluids. This is a potentially complex area and before such genetic based body fluid identification can be utilised, an understanding of the effects of mixtures on the results is required. Can body fluid mixtures be identified and if so, what is the value of the information gained?

Samples of blood and saliva were acquired from volunteers with informed consent. The samples underwent total RNA extraction. A range of mixtures were then prepared in the mixing ratios of 1:1, 5:1, and 10:1 (both with blood and then with saliva as the major contributor). Single source controls were included. All samples then underwent stem-loop reverse transcription and quantitative PCR analysis targeting blood and saliva specific microRNA markers using commercially available kits.

When compared with the single source controls, the mixed body fluid samples could be easily identified. By comparing the samples with the 1:1 blood/saliva mixture, the major and minor contributor for each body fluid mixture could be correctly identified. Finally, when compared with the mixed DNA results, the major body fluid could be correctly associated with the major DNA contributor and vice versa.

Keywords: MicroRNA; body fluid identification; mixtures; qPCR; stem loop reverse transcription; blood; saliva

1. Introduction

In many cases, especially in sexual assaults, stains can be a combination of different body fluids; for example, semen and vaginal material or blood and saliva. Whilst there are many strategies for the successful resolution of DNA mixtures, there are currently no analytical methods for resolving mixtures of body fluids. RNA analysis is becoming increasingly common place, utilising mRNA and microRNA (miRNA) for body fluid identification purposes. Provisional research has been conducted on using RNA analysis to resolve body fluid mixtures; with Harteveld *et al* [1] working on mRNA and Uchimoto *et al* [2] working on miRNA. The aim of this study is to further expand on the Uchimoto study [2] and assess whether the major-minor contributors are maintained post-analysis and whether or not the major body fluid can be associated with the major DNA profile and the same for the minor contributor.

2. Methods and materials

2.1 Sample acquisition, extraction, and mixture preparation

Blood and saliva samples were acquired from a number of volunteers with their informed consent and ethical approval. Blood samples were acquired using a finger-prick method, and saliva samples were collected via buccal swabs. Both sets of samples were extracted using the buccal protocol of the QIAAmp DNA Mini extraction kit (Qiagen, UK) as per the Omelia *et al* study [3]. Blood and saliva extracts were used to prepare mixed sample by volume. Two sets of mixing ratios of 1:1, 5:1, and 10:1 were prepared; one with blood as the major contributor and one with saliva as the major contributor.

2.2 slRT-qPCR

All prepared samples then underwent stem-loop reverse transcription (slRT) [4] before undergoing quantitative PCR (qPCR). A Veriti thermocycler and a Fast 7500 Real-Time PCR machine were used for analysis along with reverse transcription kits, Taqman master mixes and miRNA assays (Life Technologies, UK). Markers miR-451 and miR-205 were targeted for blood and saliva, respectively. The small nuclear RNA molecule RNU-44 was used as the endogenous control.

2.3 Controls

PCR and reverse transcription negative controls were included in this study, along with single source controls. No amplification was detected in the negative controls for this study. A 1:1 mixed body fluid sample was included as a control.

2.4 Data analysis

ΔCt values were derived from Ct_{RNU44}-Ct_{marker}. These values were used for subsequent data analysis. For analysis of mixed samples, a normalising formula was derived from the 1:1 control as per the Uchimoto study. This formula was applied to all samples within the study.

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2.5 DNA profiling

Aliquots of the prepared mixed samples underwent standard STR analysis, using a Quantiplex kit (Qiagen, UK), NGM SElect Kit (Life Technologies, UK) and the ABI 3130 Genetic Analyser (Life Technologies, UK) with the data analysed by GeneMapper V3.2 Software.

3. Results

3.1 qPCR results

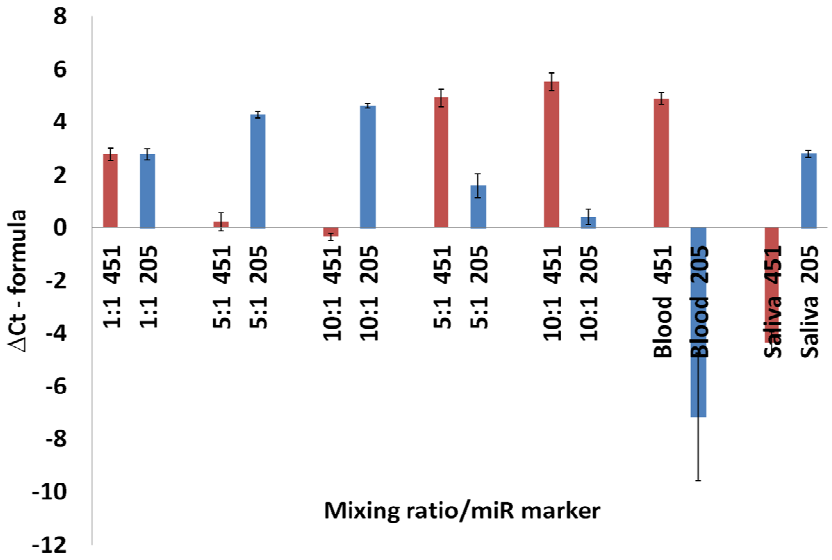

Fig 1; A chart indicating the relative expression of miR-451 and miR-205 in mixed body fluid samples.

The data shown in Figure 1 indicates that the mixing ratios do not appear to be maintained post analysis. For example, the 5:1 and 10:1 mixing ratios appears to indicate that, even though the major and minor contributors are correctly identified, there is no substantial difference between them.

3.2 DNA profiling

The aliquots from the prepared mixed samples underwent standard DNA profiling and in all cases mixed DNA results were obtained that matched the corresponding components of the DNA profiles of the donors. In all cases, the overall mixing ratio of the DNA results were comparatively maintained post analysis.

Where the qPCR data indicated that the major contributor was blood, the DNA profile obtained was also from the blood and the same was true of the saliva. This was true of all samples; thus giving a correct body fluid-DNA attribution rate of 100%.

5. Role of funding and conflict of interest

Research funded internally by the University of Huddersfield. No conflict of interest

6. References

[1] J. Harteveld, A. Lindenbergh, T. Sijen. RNA cell typing and DNA profiling of mixed samples: Can cell types and donors be associated? Science & Justice (2013) In Press

[2] ML. Uchimoto, E. Beasley, N. Coult, et al. Considering the effect of stem-loop reverse transcription and real-time PCR analysis of blood and saliva specific microRNA markers upon mixed body fluid stains. Forensic Science International: Genetics. (2013) 7(4):418-21

[3] EJ. Omelia, ML. Uchimoto, G. Williams. Quantitative PCR analysis of blood-and saliva-specific microRNA markers following solid-phase DNA extraction. Analytical Biochemistry (2013) 435(2):120-2

[4] C. Chen, DA. Ridzon, AJ. Broomer, et al. Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic acids research (2005) 33(20):e179