Characterisation of body fluid specific microRNA markers by capillary electrophoresis

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Introduction

RNA-based body fluid identification has developed to the stage where it is almost ready for implementation in case work. Messenger RNA work has successfully developed a system where it can be characterised by capillary electrophoresis [1]. Work is also being carried out on microRNA, but currently the analyses are being carried out by quantitative PCR. This study is looking at the validity of conducting CE upon amplified miRNA products. This builds upon the work of van der Meer *et al* [2] and explores further body fluid specific markers. The main benefit of CE analysis is the multiplexing with STR kits to create a much more useful electropherogram for the reporting practitioner.

Sample acquisition and extraction

Blood, saliva, seminal fluid and vaginal material samples were collected from 5 volunteers with informed consent and ethical approval. Blood was recovered by the finger prick method and spotted on to filter paper. All other body fluid samples were collected by swabbing/using sterile swabs. Sections of the stained filter paper and the swabs then underwent extraction using the QIAAmp DNA mini kit (Qiagen, UK) as per the Omelia *et al* study [3].

sIRT-qPCR

Each sample extract then underwent stem loop reverse transcription [4] and quantitative PCR targeting miR-451 (blood), miR-205 (saliva), miR-891a (seminal fluid) and miR-617 (vaginal material). sIRT-qPCR was carried out using the miRNA assay kits and Taqman master mix on a Veriti thermocycler and Fast 7500 Real-Time PCR Machine (Life Technologies) as per manufacturer's instructions. 40 PCR cycles were utilised during each experiment.

Capillary electrophoresis

All samples, following sIRT-qPCR, then underwent CE on the AB3130 Genetic Analyser (Life Technologies, UK), using the same run conditions as the NGM SElect STR kit. The bins and panels were left in, for reference purposes.

Presentation of data

Although multiplexing of the probes have yet to take place, a 'virtual multiplex' was created by overlaying the EPGs for each marker on the same body fluid.

BLOOD		
hsa-miR-203	46.78 bp	± 0.09
hsa-miR-205	No Expression	
hsa-miR-224	58.75 bp	± 0.40
mmu-miR-451	59.52 bp	± 0.55
hsa-miR-891a	58.30 bp	± 0.23

SALIVA		
hsa-miR-203	46.99 bp	±0.18
hsa-miR-205	48.13 bp	±0.06
hsa-miR-224	54.03 bp	±0.38
mmu-miR-451	No Expression	
hsa-miR-891a	No Expression	

SEMINAL FLUID		
hsa-miR-203	62.35bp	±0.88
hsa-miR-205	59.65 bp	±0.39
hsa-miR-224	60.73 bp	±0.84
mmu-miR-451	60.38 bp	±1.46
hsa-miR-891a	59.44 bp	±0.42

VAGINAL MATERIAL		
hsa-miR-203	64.31 bp	±1.35
hsa-miR-205	61.77 bp	±0.99
hsa-miR-224	60.23 bp	±0.21
mmu-miR-451	54.44 bp	±0.35
hsa-miR-891a	61.78 bp	±1.53

The results in Figure 1 are the EPGs associated with the body fluids where the indicated markers were targeted and amplified prior to fragment analysis. The amplicons highlighted in blue demonstrates the base pair sizes, typically between 45 to 65 bp in length. Since the targeted markers amplify to create products of differing base pair lengths for each body fluid and shows little variation between individuals, CE could be used to differentiate between types of body fluid. The peaks visible before the microRNA peak are usually half the size and can be identified as primer dimers. There are some indications of cross-reactivity as demonstrated by similar peaks in different body fluids; however, this is expected given that 40 PCR cycles was used to amplify them along with relatively high primer concentrations.

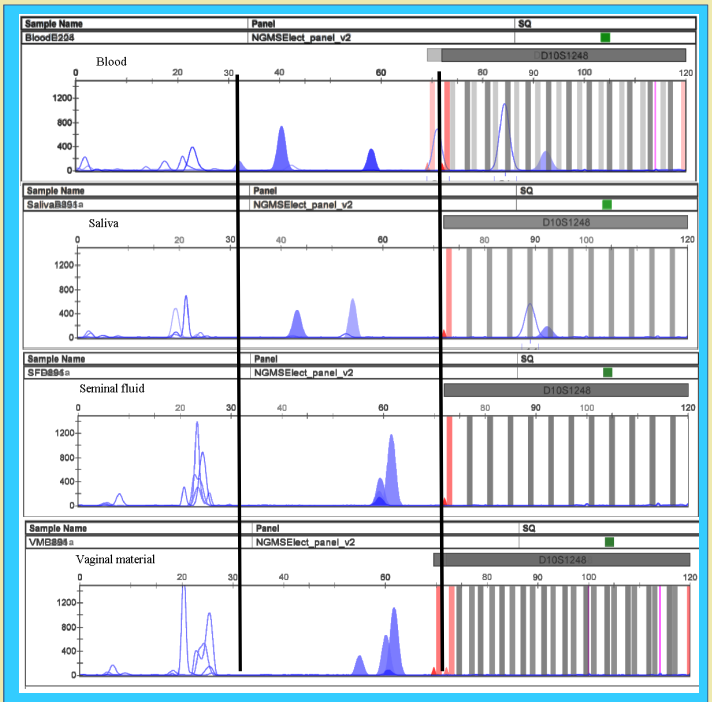

Figure 2: Composite electropherogram derived from an overlapping of 4 different markers (miR-451, miR-205, miR-224 and miR-891a) in four different body fluids (blood, saliva, seminal fluid and vaginal material). Peaks below 30bp are low molecular weight artefacts. The peak variation between individuals is ~±0.7bp. The data presented is from one individual, but is representative of five different individuals.

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As indicated in Figure 1, most of the four markers are being detected in the four different body fluids. However, the amplicon sizes of each of the markers vary (as well as peak height) between body fluids. For example, a peak at 62bp is only present in seminal fluid and vaginal material. Each different body fluid has a different arrangement of peaks (or different 'profile'); thus indicating that CE analysis of microRNA for body fluid identification is possible. Concerns relating to its proximity to the low molecular weight artefacts and the bins and panels of the NGM SElect kit are real. However, it can be observed that there is a range of peaks between 40bp and 65bp which can be utilised.

Further work will include the CE analysis of additional body fluid specific markers and PCR optimisation studies. Initial work relating to the optimisation of PCR cycle number have indicated that miRNA markers can be detected by CE after 30 PCR cycles; in line with the majority of STR kits PCR conditions.

References – **[1]** A. Lindenbergh, M. de Pagter, G. Ramdayal, *et al*. A multiplex (m)RNA-profiling system for the forensic identification of body fluids and contact traces. Forensic Science International: Genetics. (2012) 6(5):565-77. [2] D. van der Meer, M.L. Uchimoto, G.A. Williams,. Simultaneous analysis of microRNA and DNA for determining the body fluid origin of DNA profiles. Journal of Forensic Sciences. (2013) 58(4);967-971. [3] E.J. Omelia, M.L. Uchimoto ML, 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, D.A. Ridzon, A.J. Broomer, *et al.* Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic acids research. (2005) 33(20):e179.