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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 miRNA, 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].

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.

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 miRNA 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