B38 Classification of Body Fluid Source in Dried Samples Using a Panel of MicroRNAs (miRNAs)

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Learning Overview: After attending this presentation, attendees will understand how miRNAs can be of significant value for body fluid identification in forensic casework. Attendees will understand that specific miRNA markers can distinguish different body fluids, and that miRNAs may be a better molecular-based method for the identification of body fluids than the use of current serological tests, which are based on enzymatic activity and are often prone to false positives.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that the evidence for miRNAs as molecular markers for body fluid identification continues to build strength.

MiRNAs are small non-coding RNAs, 18–25 nucleotides in length, that have been identified and evaluated as potential markers for the identification of forensically relevant body fluids. There is significant interest in the use of miRNAs for forensic casework because of their short length and high resistance to degradation. They have also been shown to co-extract and be detectable in DNA extracts, which could make the use of miRNAs a more streamlined and easily implementable molecular body fluid identification method than other described methods.

Candidate miRNAs were identified through high-throughput sequencing of the miRNome and quantitative Polymerase Chain Reaction (qPCR) panel analyses of differential expression patterns in venous and menstrual blood, vaginal secretions, saliva, feces, urine, perspiration, and semen. Candidate miRNAs were identified and subsequently further validated using population sample sets from each biological fluid, ultimately identifying seven miRNAs to identify blood, semen, menstrual secretions, saliva, feces, and urine. This panel of miRNAs includes a pair of endogenous reference markers that provide normalization of miRNA expression without evaluation of the RNA quality or known input quantity. miRNA expression is detected using Reverse-Transcription quantitative PCR (RT-qPCR) to identify and differentiate dried body fluids of a volume and type as may be collected from the scene of a crime.

Each of the seven miRNA markers in the miRNA panel was analyzed in 50 samples of blood, saliva, vaginal fluid, urine, and semen samples and, together with previous data, were used to construct a classification regression tree that provides likely sample classification based on its relative expression of each candidate. Identification of the biological fluids was found to be reliable across population samples of mixed ages, ethnicities, and gender, with 80–100% of the unknown samples classified correctly, depending on the body fluid in question.

In conclusion, the classification tree developed in this work demonstrated high accuracy in identifying the biological source of unknown samples. This complements previous research confirming consistent miRNA expression in compromised samples, and a low limit of detection of 10^4–10^5 copies. Consequently, the miRNA panel can provide robust, accurate identification of six biological fluids, with demonstrated utility for implementation into forensic casework.

Body Fluid Identification, MiRNA, MicroRNA

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