B127 The Development of a Universal Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Protocol for Differentiating a Mixture of Forensically Relevant Body Fluids Using microRNA (miRNA)

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Learning Overview: After attending this presentation, attendees will understand the advantages of utilizing microRNA as a means of identifying forensically relevant body fluids.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a simple and cost-efficient protocol that can not only be incorporated into the workflow of current forensic laboratories, but provide concrete serological results.

The protocol overall will be comprised of two parts. The first part demonstrates a proof of concept regarding individual miRNA extraction and visualization.[1,2,4,5] Extraction will be carried out with the GeneAll Ribospin II RNA purification kit under a polymerase chain reaction (PCR) hood that has been designated for handling RNA samples.[1] Samples will be pre-screened using the Nanodrop for the presence of sufficient amounts of RNA. A two-step assay will then be performed. The first step will use a universal stem-loop reverse transcription to form complementary DNA (cDNA) of mature miRNAs using the RevertAid H Minus First Strand cDNA Synthesis Kit from Applied Biosystems. Real-time PCR, or qPCR, will be implemented with hsa-miR-16a-5p- and hsa-miR-135a-5p-specific assays, found in blood and seminal fluid, respectively, through a universal PCR approach. Using a universal common reverse primer based off the stem-loop primer introduces a common probe that targets the 3' overhang.[6] Differentiation between the two miRNA sequences will depend on miRNA-specific forward primers. The results of the qRT-PCR (real-time reverse transcription PCR) of the samples will be analyzed in comparison to a house-keeping miRNA, U6 snRNA, to normalize the data via a C_T comparative method. The two miRNA markers chosen will exhibit different percentages of guanines and cytosines within various sequences, or %GC content, and lengths, which allow differentiation through melt analysis. Initially, samples will be exposed to a 1st-derivative analysis through the Rotor-Gene Q.[3] Samples will then be visualized using the Rotor-Gene Screenclust High-Resolution Melt (HRM) Software to identify the specified markers based on their melting temperatures from their differing %GC contents and lengths.

After having proven that the miRNA markers can successfully differentiate the two body fluids, the second part of the protocol will continue. A mixture study will be performed using the same assay to determine if they both can be identified in the same sample in the following various aliquots (blood to seminal fluid): 1 to 1, 2 to 1, and 1 to 2. The aim is to be able to visualize differentiation of the miRNA markers using the Rotor-Gene Screenclust HRM Software, given that the markers have separate clusters. Subsequent research should investigate other miRNA markers for other forensically relevant body fluids, such as saliva.

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