H25 Small RNA Sequencing and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Validation of Forensically Relevant Body Fluids

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Learning Overview: The goal of this presentation is to inform attendees of several microRNAs (miRNAs) discovered via next generation sequencing that have the potential to be discriminatory for the identification of forensically relevant body fluids, including venous blood, semen, saliva, vaginal fluid, and menstrual blood.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing insight into the methodology of using next generation sequencing to discover known and potentially novel miRNAs that are specific to forensically relevant body fluids. Additionally, the forensic science community will have the opportunity to learn more about the validation of next generation sequencing results via RT-qPCR.

Body fluid identification is an important aspect of forensic investigations as it can assist with the reconstruction of a crime scene and can refute and/or support witness statements. Currently, there is no universal method for body fluid identification. Each body fluid has several tests, both presumptive and confirmatory. Additionally, there are no reliable confirmatory tests for the identification of menstrual blood and vaginal fluid. This causes body fluid identification to be a time-consuming process that can be highly destructive to an unknown body fluid sample. A universal method for body fluid identification that is sensitive, specific, efficient, and minimally destructive is necessary.

In recent years, miRNAs have been heralded as novel biomarkers for the identification of body fluids. They represent an ideal candidate for this purpose as they are remarkably stable, require minimal starting material, and can be co-extracted with DNA, thereby providing both body fluid identification and person identification simultaneously. Several published studies have reported various lists of miRNAs that show potential for the identification of particular body fluids. However, there is little agreement across the studies of which miRNAs are suitable, and few studies included menstrual blood and vaginal material. In addition, all miRNAs reported to date are shown to be differentially expressed across all body fluids, with none yet reported to be uniquely expressed in one body fluid. With the advent of Next Generation Sequencing (NGS), it is now possible to sequence all forensically relevant body fluids for both known and novel miRNAs, with the expressed interest to identify panels of miRNAs for each body fluid, ideally with several that are uniquely expressed in a particular body fluid.

The first goal of this study was to sequence a range of forensically relevant body fluids to identify known miRNAs and potentially discover novel miRNAs that are specific to venous blood, semen, saliva, menstrual blood, and vaginal fluid. The second goal of this study was to select panels of miRNAs, based upon the sequencing data, for RT-qPCR validation, that show promise for body fluid identification.

Following Institutional Review Board (IRB) approval, venous blood (n=10), semen (n=5), saliva (n=10), menstrual blood (n=10), and vaginal fluid (n=10) was collected from volunteers with informed written consent. Each body fluid was extracted using the miRNeasy® miRNA isolation kit, following the manufacturer's protocol. Liquid fluids (venous blood, semen, saliva) were extracted using 500µL of each. Menstrual blood and vaginal material were collected on sterile cotton swabs and were therefore extracted directly from the swabs. Following miRNA isolation, the extracts were stored at -20°C until required. Four quantification methods were utilized, including the NanoDrop™ One^{C} UV-Vis Spectrophotometer, the Qubit® 3 Fluorometer using the RNA HS Assay kit, and the Agilent® 2100 Bioanlayzer using both the Small RNA kit and the Nano 6000 kit. The concentration of each sample was obtained, and the integrity of the samples were analyzed in the form of the RNA Integrity Number (RIN). Samples were then prepared for sequencing using the TruSeq® Small RNA library preparation protocol. The prepared samples were then sequenced using the MiSeq® FGx platform. The sequencing data was analyzed using the Illumina® BaseSpace small RNA app. Several miRNAs were chosen for further RT-qPCR validation, based on their potential applicability for body fluid identification. The results of this study contribute greatly to the growing body of knowledge for the eventual implementation of miRNAs for body fluid identification.

microRNAs, Body Fluid Identification, Next Generation Sequencing