B5 Investigating Simultaneous Extraction of RNA and DNA From Forensically Relevant Body Fluids

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After attending this presentation, attendees will be aware of the potential of the simultaneous extraction of microRNA (miRNA) and DNA from within a single sample for forensically relevant body fluids. This includes the investigation and comparison of two commercially available co-extraction kits for the yields obtained from each kit, the sensitivity of each kit, and the ability to obtain both miRNA and DNA profiles.

This presentation will impact the forensic science community by highlighting the usefulness of co-extracting RNA and DNA from a single sample. This method could lead to both confirmatory body fluid identification and human DNA profiling.

MiRNAs are a class of short non-coding RNA molecules which are approximately 22 nucleotides in length and regulate the post-transcriptional gene expression in many eukaryotes. It has recently been suggested that miRNAs could serve as potential biomarkers for body fluid identification in forensic investigations. Previously, messengerRNA (mRNA) was proposed as a useful tool for body fluid identification; however, due to their instability and susceptibility to degradation, they have not shown great promise in the forensic field. In contrast, miRNAs are remarkably robust and infer great stability due to their small size; however, depending on the volume of sample available, the miRNA analysis could consume the sample and potentially eliminate the ability to obtain a DNA profile. Therefore, there is a great need for a method which performs both RNA and DNA extraction simultaneously from one sample. There have been limited advancements in this area with only a few commercial methods currently offered for this purpose. Little research has been performed on this topic in the forensic setting. The goal of this study was to investigate two commercially available co-extraction RNA/DNA kits by examining the quality and quantity of RNA/DNA extracted, followed by a series of dilutions to test sensitivity, and finally by the ability to obtain a miRNA signal and DNA profile from a range of forensically relevant body fluids.

Following ethical approval from the Institutional Review Board and informed volunteer consent, venous blood, semen, saliva, and urine were collected from five volunteers (n=20). The two commercially available kits that were investigated were the Zymo Research ZR-Duet™ DNA/RNA MiniPrep kit and the QIAGEN® AllPrep™ DNA/RNA Mini Kit. The manufacturer guidelines were followed for each kit. First, neat samples were extracted from 200µl of each body fluid using each kit. Following this, a series of dilutions of each body fluid were created in 1:2, 1:10, 1:25, and 1:50 ratios and then the co-extraction of miRNA and DNA were performed using the same kits. Following RNA/DNA extraction, all eluates were analyzed to determine the RNA/DNA concentration in each sample. This was achieved using Biotek Eon™ spectrophotometer which measures the full spectrum (220nm-750nm) for accurate measurement of concentrations (A260), protein contamination (ratio A260/280), and contamination with buffer components or organic compounds (ratio A260/A230). The concentration of each extracted sample was recorded in ng/µl and quality assessed by analysis of the 260/280 ratio. In the final step, Relative Quantitative Polymerase Chain Reaction (RQ-PCR) was performed on a selection of the RNA samples targeting miR-16 to determine if a miRNA signal was present within the extract. In parallel, STR analysis was performed on a selection of the DNA samples to obtain full human DNA profiles.

The results showed that quantifiable amounts of both DNA and RNA were obtained in the neat samples of all the body fluids using both kits; however, the concentrations obtained were highly variable depending on the particular body fluid and the particular kit used. The results obtained from the diluted samples produced varying concentrations at much lower levels, as expected. Overall, the Zymo Research kit proved to obtain higher concentrations of both DNA and RNA when compared to the QIAGEN® kit. Finally, miRNA signals and full DNA profiles were obtained from all samples selected for miRNA/DNA profiling.

In conclusion, this study reveals the ability to successfully co-extract both RNA and DNA from forensically relevant body fluids, suggesting the Zymo Research kit as a superior method for this purpose. This research highlights the potential of miRNAs for the identification of forensically relevant body fluids as it has shown to be possible the ability to extract both miRNA profiles and DNA profiles from a single sample, which could prove crucial to a forensic investigation.

MicroRNA, DNA, Co-Extraction

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