A74 An Evaluation of MicroRNA Stability and Internal Standard Selection for Forensic Body Fluid Identification

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After attending this presentation, attendees will gain an understanding of microRNA expression in biological fluids, specifically, the advantages of microRNA analysis as a potential method of forensic body fluid identification.

This presentation will impact the forensic science community by familiarizing scientists with the concept of microRNA analysis for body fluid identification purposes, a relatively new area of research in forensic science, focusing on the stability of microRNA targets after being subjected to contamination and physical treatment conditions similar to those seen in crime scene evidence.

While forensic DNA analysis has reached a level of maturity in the forensic science field with regards to the sophistication of the techniques and confidence in the results, the equally important question of body fluid identification has lagged behind, and could still be considered to be in a primitive state. While there is widespread confidence in the DNA profile generated, there is often significantly less assurance of the identity of the body fluid from which the DNA profile was developed. It is common during trials for attorneys to categorically accept the STR analysis, but probe the forensic scientist on the source of the DNA that generated the profile. Because of this dichotomy, significant efforts have been made over the past ten years in order to develop forensic serological techniques of a more discriminatory nature.

Recently, there has been some work in the forensic science field in regards to exploring microRNAs (miRs) for a molecular-based, forensic body fluid identification method. MiRs are small structures that specifically repress protein expression through binding to messenger RNA (mRNA) in the cytosol. There are no known postprocessing modifications, and thus miRs are simpler, and potentially less problematic for detection than proteins and mRNAs.

Because of their small size and lack of a poly-A tail, miRs are inherently less susceptible to degradation than mRNA. Additionally, miRs are very hardy, and have been recovered from highly compromised samples including Formalin-Fixed Paraffin Embedded (FFPE) tissue. In serum, miRs have been shown to survive harsh conditions such as boiling, low or high pH, cycles of freeze-thaw, and extended storage. Moreover, there is considerable evidence that many miRs are encapsulated in an exosome, which, depending on the microRNA and the secretion process, could be membrane-based or protein-based. Because of this, recent studies have shown that samples can even be treated with RNase enzymes and the encapsulated miRs are still detectable. This implies a high degree of stability of the species, and therefore, a very good possibility that if body-fluid specific miRs are found and described, a very robust miR method for forensic body fluid identification could be developed.

Biological evidence is naturally compromised when it is left or deposited under nonsterile conditions of a variety of environmental factors and chemical contamination. This study evaluated microRNAs under environmental and treatment conditions that forensic evidence could be subjected to. Blood, urine, semen, and saliva were collected and samples exposed to chemical treatment, prolonged high temperatures, and multiple freeze/thaw cycles. No differences were observed in microRNA levels using qRT-PCR, regardless of the number of times the sample had been frozen and thawed. Similar results were observed with other treatment methods. Detection of various internal reference controls typically used as standards (RNU6B, SNORD48, etc.) were found to vary between body fluids, which could potentially complicate studies attempting to compare relative expression levels of target miRs between biological fluids. Evaluation of additional forensically relevant body fluids, as well as more candidate internal control miRs, should be evaluated before target miRs specific for each biological fluid can be chosen and evaluated for implementation.

MicroRNA, Semen, Blood

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