AN EVALUATION OF THE DIFFERENTIAL STABILITY OF NUCLEIC ACIDS IN BIOLOGICAL FLUIDS COMPROMISED BY ENVIRONMENTAL EXPOSURE

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The visualization, presumptive, and confirmatory tests used for body fluid identifications in forensic casework have remained static for many years; this is problematic because many of these tests are known to yield false positive results with other biological fluids, foods, or chemicals. Recent work in the forensic science field has explored RNAs for a molecular-based, forensic body fluid identification method. Messenger RNA has been assessed to identify body fluids by many researchers. While these research studies provide good evidence for messenger RNA as being useful in body fluid identification, some research has provided unsupportive evidence and thus mRNA analysis has been slow to catch on for casework. MiRNAs are small RNAs that have the ability to suppress translation of mRNA into proteins and are shorter in length than mRNAs. Because of these advantages and the remarkable stability observed by the species, they are also being considered as markers for body fluid identification. This study evaluated the relative stability of DNA, RNA, and miRNAs in the same samples under conditions mimicking an outside crime scene.

Samples of blood, urine, semen and saliva were placed in an environmental chamber for defined periods of time corresponding from 24 hours to 14 days in a Virginia summer. The environmental chamber manages the irradiance, air temperature, and humidity for a more controlled assessment of sample degradation. DNA and RNA were isolated from each stain, and qPCR (RT-qPCR for RNA and miRNAs) was performed. Expression levels were calculated relative to the positive untreated control samples. DNA stability was evaluated using both standard TPOX and "mini" length primers for the STR locus TPOX. Messenger RNA and miRNA expression were evaluated using GAPDH and/or ACTB, and Let-7g respectively.

MicroRNA expression was not significantly impacted by treatment in the environmental chamber, unlike the impacted levels of DNA and messenger RNA. When measuring microRNA stability, Let-7g levels were not significantly different from the untreated control levels for all four body fluid samples. The DNA and mRNA data showed how environmental effects can greatly degrade biological material after exposure. Based on these date, samples from the same donation were placed in an open woodland for an extended period of time, and the same methods used to evaluate miRNA, mRNA and DNA stability as compared to the untreated control as well as the environmental chamber samples. The conclusions drawn from this project drives home the environmental effects on biological material, clarifies differential stability of the nucleic acids, and consequently, can provide the practitioner with options for analysis workflows in compromised samples.