

**Autumn Muise**

**Class of 2020**

**Forensic Science conc. Biology and Biology conc. general**

**Investigating Commercially Available microRNA Extraction Kits for Use with Forensically Relevant Body Fluids**

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While current research is highlighting the potential of miRNAs for body fluid identification, little research has been performed to investigate the best method for extracting the miRNA content from forensically relevant body fluids. There are over a dozen commercially available miRNA/RNA extraction kits, with new kits regularly being released onto the market. All miRNA extraction kits currently available, however, have been designed for use with pristine clinical laboratory samples, such as cell cultures, primary tissues, plasma/serum, etc. A kit designed specifically for use with forensic samples –venous blood, semen, saliva, menstrual blood, and vaginal material – is not yet commercially available. The aim of this research was to select four commercially available miRNA extraction kits, and to assess their ability to extract the miRNA content from forensically relevant body fluids in sufficient quantity and quality for downstream analyses.

Following Institutional Review Board (IRB) approval, body fluids were collected from volunteers with written informed consent. Venous blood was collected by a licensed phlebotomist into EDTA vials. Semen and saliva were collected into sterile conical tubes. Menstrual blood and vaginal material were collected using sterile cotton swabs. All samples were stored at -20°C until extractions were performed. The miRNeasy Mini Kit (Qiagen®), PureLink™ miRNA Isolation Kit (Invitrogen™), magMAX™ mirVana™ Total RNA Isolation Kit (Applied Biosystems™) and High Pure miRNA Isolation Kit (Roche) were the chosen kits in this study. Each miRNA isolation was performed according to the manufacturer's protocol. The extracts were quantified using the NanoDrop™ One UV/Vis spectrophotometer (Thermo Scientific™), and the Qubit 3.0 fluorometer (Invitrogen™), with the RNA HS assay kit. As each kit has a different final elution volume, all results were converted from ng/μL to total RNA (ng) obtained.

Quantifiable amounts of miRNA were collected from all samples. The quantitation results generated using the NanoDrop™ One UV/ Vis Spectrophotometer showed the miRNeasy kit to be the optimal kit in the majority of the body fluids with yields ranging from 2844-6279 ng total, with the exception of vaginal material, in which magMAX™ mirVana™ Total RNA isolation Kit yielded higher results. The results were verified using the Qubit 3.0 Fluorometer. This quantification method further confirmed that the miRNeasy was the superior kit, however the Qubit showed that the High Pure Isolation Kit was preferred for the extraction of vaginal material. While some kits resulted in extracts that were too low to produce quantitation values, every sample extracted using the miRNeasy kit resulted in quantifiable yields. miRNeasy's miRNA concentrations ranged from 487-12749 ng total RNA across all body fluids. When comparing the cost per sample, ease of use, and additional resources required, per kit, the miRNeasy kit was found to be the most user friendly, least time consuming, and required minimal additional resources. The cost per sample was in the mid-range of all 4 kits. The results of this study contribute to the growing understanding of the potential of microRNAs as a novel tool for forensic scientists.