



H29 Investigating Commercially Available MicroRNA Extraction Kits for Use With Forensically Relevant Body Fluids

*Autumn T. Muise**, West Haven, CT 06516; *Karly L. Johannsen*, University of New Haven, West Haven, CT 06516; *Claire Glynn, PhD*, Forensic Science Department, West Haven, CT 06516

Learning Overview: The goal of this presentation is to inform attendees of a variety of commercially available microRNA (miRNA) extraction kits and their use with forensically relevant body fluids.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing insight into the various methods available for miRNA extraction, a variety of methods for quantifying miRNA extracts, and the optimal method for use with forensically relevant body fluids from those tested in this study.

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 more than a dozen commercially available miRNA/RNA extraction kits, with new kits regularly being released onto the market. However, all miRNA extraction kits currently available 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. Therefore, an investigation of some of the currently available, and widely reported, miRNA extraction kits is warranted for use with forensically relevant body fluids. The goal 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. Following miRNA isolation, the extracts were stored at -20°C until quantitation was performed. The extracts were quantified using the NanoDrop™ One UV/Vis spectrophotometer (Thermo Scientific™), Qubit® 3.0 fluorometer (Invitrogen™), with the RNA HS assay kit, and the Agilent® Bioanalyzer 2100 (Applied Biosystems®) with the small RNA chips. 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 results generated using the NanoDrop™ One UV/Vis Spectrophotometer showed the miRNeasy kit to be the optimal kit in the majority of the bodily fluids, with yields ranging from 2,844-6,279ng total, except for 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. Agilent's® Bioanalyzer 2100 quantified the miRNA concentration in extracts that held promising results from the Qubit®. Qiagen's® miRNeasy Mini Kit's miRNA concentration values were present for all five of the bodily fluids. miRNeasy's miRNA concentration ranged from 487-12,749ng total. The use of three separate quantification methods did not consistently give the same amounts.

Qiagen's® miRNeasy Mini Kit was overall the optimal kit in extracting miRNA from forensically relevant bodily fluids, with the exception of High Pure miRNA Isolation Kit, yielding higher results in the extraction of vaginal material. Although the miRNeasy is slightly more expensive than the High Pure, the quantity of miRNA eluted is greater, thus offsetting the costs.

These results in the extraction of miRNA using commercially available kits were successful and will aid the forensic science community in future casework.

MicroRNA, Extraction, Body Fluids