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

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Background

MicroRNAs (miRNAs) are small noncoding RNA molecules that are 18-25 nucleotides in length. They were previously considered to be junk evolutionary debris, and to serve no function at all. However, they were later to be found to actually play a role in crucial biological processes. Their main purpose is for translation repression, mRNA cleavage, and deacetylation which all play a role in gene translation where it was described in 1993 by Lee et al[1].

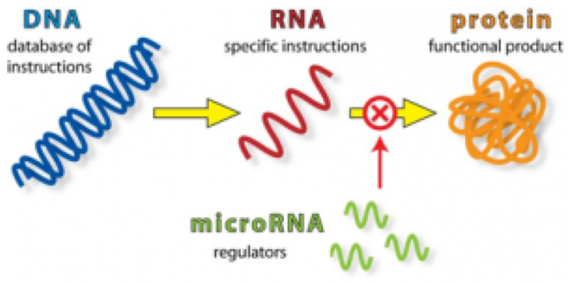

Figure 1: The schematic of the generation of microRNA

Through biomedical and cancer research studies, it has been reported that some miRNAs can be tissue specific. This sparked the interest of forensic scientists where it has been suggested that miRNAs could be used as a new tool to identify certain body fluids typically encountered in forensic investigations. Due to the small size of miRNAs, they are remarkably stable, and have been shown to withstand degradation from environmental conditions[2]. This makes them ideal candidates for forensic investigations, as crime scene samples are frequently exposed to harsh conditions. 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-, nor has an industry standard been created.

There are over a dozen commercially available miRNA/RNA extractions kits available to purchase. These kits, however, have been designed for use with pristine clinical laboratory samples, such as cell cultures, primary tissues, etc. There has yet to be designed a kit specifically for use with forensic samples (venous blood, semen, saliva, menstrual blood, and vaginal material) that may not be in ideal condition.

Therefore, the **aims of this research** was to:

1. Choose a selection of the commercially available miRNA extraction kits reported most frequently in the literature
2. Investigate the use of these kits with 5 forensically relevant body fluids from a set of donors
3. Compare and contrast the kits based on quantity and quality of miRNA extracted, and also ease of use and cost of kit.

Materials and Methods

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.

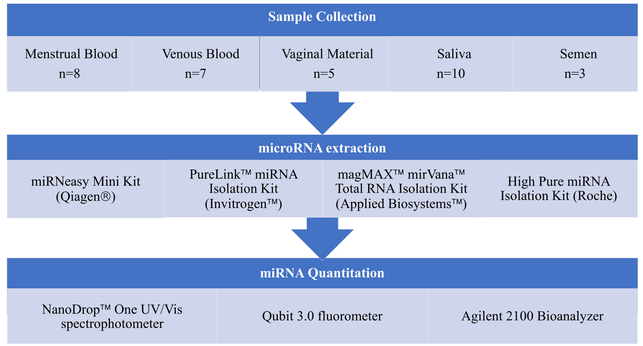

Figure 2: Research Methods Flow Chart

Results

NanoDrop™ One UV/Vis spectrophotometer

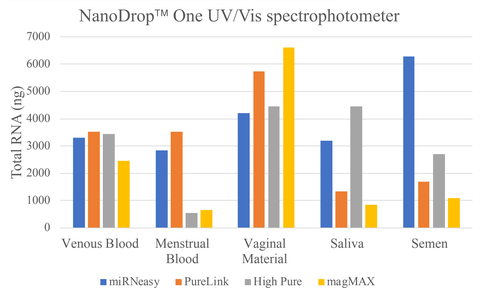

Figure 3. NanoDrop™ One UV/Vis spectrophotometer using four miRNA kits

Qubit 3.0 Fluorometer

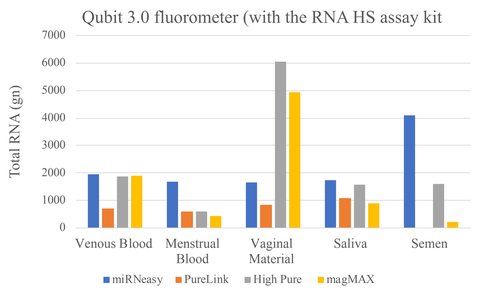

Figure 4: Qubit 3.0 fluorometer with the RNA HS assay kit using four miRNA kits

Agilent 2100 Bioanalyzer

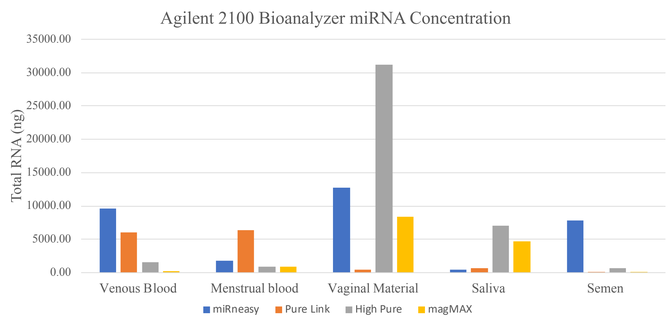

Figure 5. Agilent 2100 Bioanalyzer miRNA concentration using four miRNA kits

Discussion

Samples that were extracted using the four miRNA extractions kits all yielded quantifiable amounts. When using the miRNeasy Mini Kit it was observed that this kit constantly produced higher yields than the other selected kits. The findings in this study are supported by other research that have examined the miRNeasy along with other varying miRNA extraction methods. Furthermore, in the literature, it is commonly used across both forensic and biomedical research.

The PureLink™ miRNA isolation Kit and High Pure miRNA Isolation Kit were two kits that were comparable to each other in the extraction methodology. However, PureLink™ was at a higher cost than the other kits and could only perform 25 reactions, where as High Pure performed 50 reactions. The magMAX™ mirVana™ Total RNA Isolation Kit quantifiable results were on average lower than the other kits. These extracts were performed using the manual extraction procedure, however samples can be extracted using an automated protocol.

The results using miRNeasy had yields ranging from 2844-6279 ng total RNA, with the exception of vaginal material in which magMAX™ yielded higher results. The results were verified using the Qubit 3.0 Fluorometer. Theses results were verified using the Qubit 3.0 Fluorometer, and had yields ranging from 1665-4103 ng total RNA, with the exception of vaginal material using the High Pure. This can be accounted for as the Qubit 3.0 Fluorometer HS assay kit is human specific- which resulted in lower total RNA yields. Samples extracted using the miRNeasy Mini Kit were further quantified using the Agilent 2100 Bioanalyzer with the small RNA chip. 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.

Cost Per Kit

Kit	Elution Volume	Total Cost	# of reactions	Price Per Sample
miRNeasy Mini Kit	40 uL	$368.00	50	$7.36
Pure Link miRNA Isolation Kit	75 uL	$226.00	25	$9.04
High Pure miRNA Isolation Kit	100 uL	$345.00	50	$6.90
Mag Max mirVana Total RNA Isolation Kit	75 uL	$455.00	96	$4.74

Figure 6. Cost per kit of the 4 miRNA extraction kits

Conclusion

The results of this study shows the miRNeasy Mini Kit yielded consistent with the five forensically relevant body fluids, verified using the three separate quantification methods. The results of this study contribute to the growing understanding of the potential of microRNAs as a novel tool for forensic scientists.

References

- [1] Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, *116*(2), 281-297.
- [2] Zubakov, D., Boersma, A. M., Ying, C., van Kuijk, P. F., Wiemer, E. C., & Kayser, M. (2010). MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. *International Journal Of Legal Medicine*, *124*(3), 217-226. doi:10.1007/s00414-009-0402-3

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