Investigating the Simultaneous Extraction of miRNA and DNA from Forensically Relevant Body Fluids

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ABSTRACT

It was recently recognized that microRNAs (miRNA) may serve as potential biomarkers for body fluid identification, thereby providing tissue source information in addition to DNA profiling. This study investigated simultaneous extraction of both DNA and RNA from forensically relevant body fluids.

Using two commercially available kits namely; ZR-Duet™ DNA/RNA MiniPrep kit (ZYMO) and AllPrep™ DNA/RNA Mini Kit (Qiagen), RNA/DNA was extracted from a range of body fluids (n=20). A series of dilutions of each body fluid were co-extracted for their RNA/DNA content. The quality/quantity of each extract was analyzed using a spectrophotometer. RQ-PCR was performed to determine if a miRNA signal was present and STR analysis was performed to obtain DNA profiles.

The results showed that quantifiable amounts of both DNA and RNA were obtained from all body fluids using both kits. The results were variable depending on the body fluid and the kit used. Overall, the Zymo Kit provided higher concentrations of both DNA and RNA when compared to the Qiagen Kit. Finally, miRNA signals and full DNA profiles were obtained from all samples profiled. This research highlights the potential of miRNAs in forensic investigations with the ability to extract both miRNA and DNA from a single sample.

INTRODUCTION

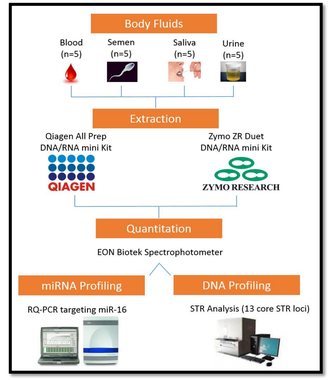
In the field of Forensic Science, it is essential that from biological stains, both the type of tissue and the individual who deposited the stain be identified. Recently, microRNAs (miRNA) have been suggested as potential biomarkers for body fluid identification (Zubakov, 2010). However, depending on the amount of biological evidence left behind, if there are only trace amounts, miRNA analysis will consume the sample and as a result eliminate the possibility of obtaining a DNA profile (Hanson,2012). Therefore, there is a great need for a method, which performs both RNA and DNA extraction simultaneously from one sample. There are only a few methods currently offered for this purpose. Therefore it is crucial that these methods are tested extensively and compared to one another. Recent research has shown that microRNA (miRNA) shows great potential for the identification of body fluids in forensically relevant samples, due to their small size, stability and robustness (Zubakov, 2010). MiRNA does not degrade easily and can be analyzed months, and in some cases, years later (Haas, 2013). However, it is crucial that we can extract both RNA and DNA from the one sample simultaneously, thereby allowing us to identify both the 'who' and 'what' of the sample.

OBJECTIVES

1. To investigate two commercially available co-purification kits for obtain quantifiable amounts of RNA and DNA from a range of human body fluids.
2. To compare the sensitivity of each kit by examination of varying dilutions of each of the body fluids tested.
3. to investigate if RNA/DNA of sufficient quality/quantity can be obtained to provide molecular profiles for both body fluid identification and also person identification

METHODS & MATERIALS

Following ethical approval from the Institutional Review Board and informed volunteer consent, venous blood, semen, saliva and urine were collected from 5 volunteers (n=20). The two commercially available kits that were investigated were the ZR-Duet™ DNA/RNA MiniPrep kit (ZYMO) and the AllPrep™ DNA/RNA Mini Kit (Qiagen). The manufacturers guidelines were followed for each kit. First, neat samples were extracted from 200µl of each body fluid using each kit. Following this, a series of dilutions of each body fluid were created in 1:2, 1:10, 1:25 and 1:50 ratios and then the co-extraction of miRNA and DNA were performed using the same kits. Following RNA/DNA extraction, all eluates were analyzed to determine the RNA/DNA concentration in each sample. This was achieved using Biotek EON Spectrophotometer which measures the full spectrum (220-750nm) for accurate measurement of concentrations (A260) and protein contamination (ratio A260/280). The concentration of each extracted sample was recorded in ng/ul and quality assessed by analysis of the 260/280 ratio. In the final step, RQ-PCR was performed on a selection of the RNA samples targeting miR-16 to determine if a miRNA signal was present within the extract. In parallel STR analysis was performed on a selection of the DNA samples to obtain full human DNA profiles.



RESULTS

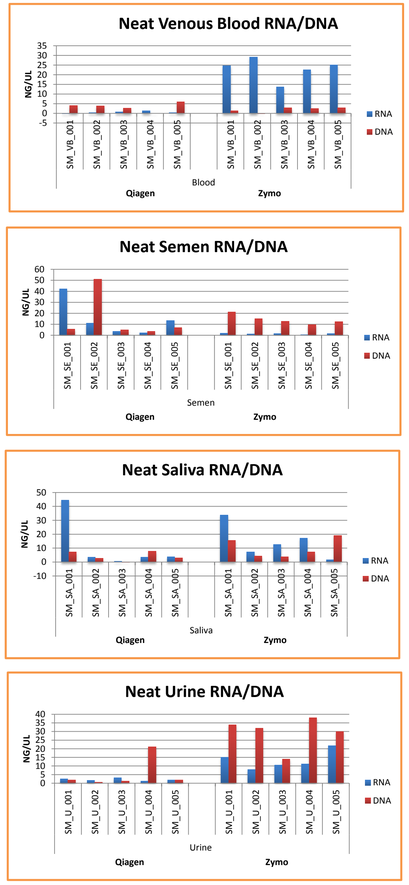


Figure 1: Results obtained from extractions performed on neat samples of venous blood, semen, saliva, and urine, comparing the RNA/DNA content extracted using both kits.

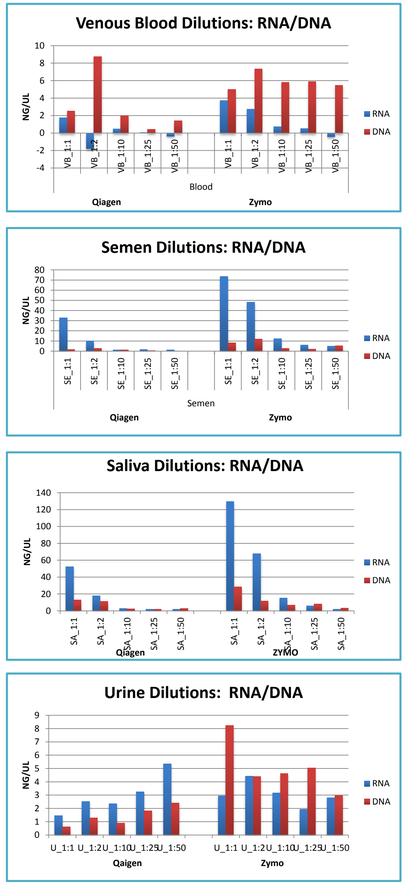


Figure 2: Results obtained from extractions performed on diluted samples of venous blood, semen, saliva, and urine, comparing the RNA/DNA content extracted using both kits.

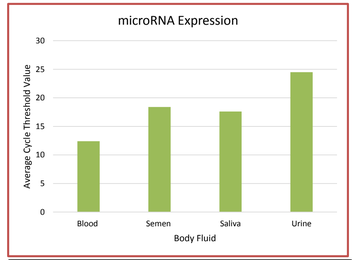


Figure 3: Average CT values for each body fluid obtained using RQ-PCR targeting miR-16

STR Loci	Blood	Semen	Saliva	Urine
D8S1179	12, 15	13, 14	12, 15	Undet
D21S11	29, 31.2	29	29, 31.2	Undet
D7S820	10, 12	10	10, 12	Undet
CSF1PO	9, 12	10, 12	9, 12	Undet
D3S1358	16	16, 17	16	Undet
TH01	9.3	6, 9.3	9.3	Undet
D13S317	11, 13	8, 13	11, 13	Undet
D16S539	12, 13	11, 12	12, 13	Undet
D1S1338	17, 24	19, 20	17, 24	Undet
D19S433	13, 15	13, 16	13, 15	Undet
Vwa	18, 19	14, 19	18, 19	Undet
TPOX	11	8, 11	11	Undet
D18S51	12, 14	14, 16	12, 14	Undet
Amel	x	x, y	x	Undet
D5S818	13	11, 12	13	Undet
FGA	20, 26	21, 23	20, 26	Undet

Figure 4: DNA profiles obtained using STR analysis on each body fluid.

DISCUSSION

In this study quantifiable amounts of miRNA and DNA were found in both the Zymo and the Qiagen kits. The concentrations obtained however, were highly variable depending on the particular body fluid and the particular kit used. The results obtained from the diluted samples produced varying concentrations at much lower levels, as expected. Overall, the Zymo Kit proved to obtain higher concentrations of both DNA and RNA when compared to the Qiagen Kit. Finally, miRNA signals and full DNA profiles were obtained from all samples selected for miRNA/DNA profiling.

Due to the trace amounts of samples available in forensic investigations, it is often difficult if not impossible to extract both the RNA and DNA portions (Haas, 2015). Scientists faced with this challenge have therefore developed commercial kits that analyze both RNA and DNA simultaneously within one sample. It was necessary that the sensitivity and accuracy be analyzed using different kits.

CONCLUSIONS

This study reveals the ability to successfully co-extract both RNA and DNA from forensically relevant body fluids, suggesting the Zymo kit as a superior method for this purpose. This research highlights the potential of miRNAs for the identification of forensically relevant body fluids as it has shown possible the ability to extract both miRNA profiles and DNA profiles from a single sample, which could prove crucial to a forensic investigation.

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REFERENCES

. 1. Zubakov, D., Boersma, A. W., M, Choi, Y., van Kuijk, P. F., Wiemer, E. A., ... Kayser. (2010). MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. International Journal of Legal Medicine, 124(3), 217–26. http://doi.org/http://dx.doi.org.unh-proxy01.newhaven.edu:2048/10.1007/s00414-009-0402-3

2. Haas, C., Ballantyne, J. (2012). RNA/DNA co-analysis from blood stains--results of a second collaborative EDNAP exercise. Forensic Science International. Genetics, 6(1), 70–80. http://doi.org/10.1016/j.fsigen.2011.02.004

3.. Haas, C., Hanson, E., Anjos, M. J., Banemann, R., Berti, A., Borges, E., ... Ballantyne, J. (2013). RNA/DNA co-analysis from human saliva and semen stains--results of a third collaborative EDNAP exercise. Forensic Science International. Genetics, 7(2), 230–239. http://doi.org/10.1016/j.fsigen.2012.10.011

4. Haas, C., Hanson, E., Banemann, R., Bento, A. M., Berti, A., Carracedo, Á., ... Ballantyne, J. (2015). RNA/DNA co-analysis from human skin and contact traces--results of a sixth collaborative EDNAP exercise. Forensic Science International. Genetics, 16, 139–147. http://doi.org/10.1016/j.fsigen.2015.01.002

5. Van der Meer, D., Uchimoto, M. L., & Williams, G. (2013). Simultaneous analysis of micro-RNA and DNA for determining the body fluid origin of DNA profiles. Journal of Forensic Sciences, 58(4), 967–971. http://doi.org/10.1111/1556-4029.12160

6. Kruhøffer, M., Dyrskjøt, L., Voss, T., Lindberg, R. L. P., Wyrich, R., Thykjaer, T., & Orntoft, T. F. (2007). Isolation of Microarray-Grade Total RNA, MicroRNA, and DNA from a Single PAXgene Blood RNA Tube. The Journal of Molecular Diagnostics : JMD, 9(4), 452–458. http://doi.org/10.2353/jmoldx.2007.060175

7. Hanson, E., Anjos, M. J., Bär, W., Banemann, R., Berti, A., ... 24. Kotorashvili, A., Ramnauth, A., Liu, C., Lin, J., Ye, K., Kim, R., ... Loudig, O. (2012). Effective DNA/RNA Co-Extraction for Analysis of MicroRNAs, mRNAs, and Genomic DNA from Formalin-Fixed Paraffin-Embedded Specimens. PLoS ONE, 7(4), e34683. http://doi.org/10.1371/journal.pone.0034683