Initial evaluation of the stability and persistence of mRNA and miRNA for body fluid identification in forensic samples

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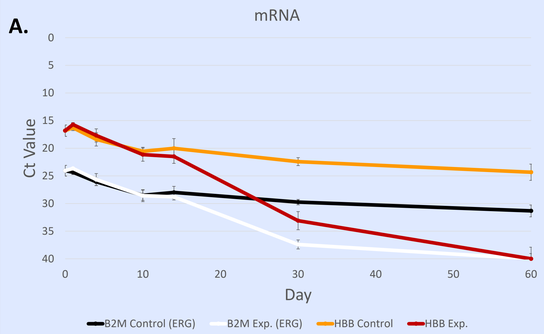
INTRODUCTION

The identification of body fluids in evidentiary stains may provide investigators with probative information during the course of a forensic case. Traditional serological tests currently used in forensic labs are presumptive in nature and have varying levels of specificity and sensitivity. The interrogation of nucleic acids, such as mRNA and miRNA, has the ability to assist analysts in determining the body fluid of origin in forensic samples. These methods allow for co-extraction with DNA, the consumption of less sample, and the potential to identify a greater number of body fluids. While nucleic acid-based methods may lead to the development of a confirmatory approach for body fluid identification, concerns remain regarding the recoverability of mRNA and miRNA in casework type samples over time. Several experiments have been performed regarding the stability and persistence of mRNA in forensic samples [1,2]. Due to their small size (19 – 25 nucleotides) and encapsulation in protein, miRNAs are thought to be resistant to degradation; however, few studies have investigated the persistence of miRNAs in environmentally challenged samples over time.

In this study, mRNA and miRNA targets were investigated in blood and semen samples. Stained swatches were placed in temperature, humidity, and light controlled and uncontrolled conditions for up to 3 months. Laundered cotton swatches stained with blood or semen were also examined.

RESULTS AND DISCUSSION

BLOOD



SEMEN

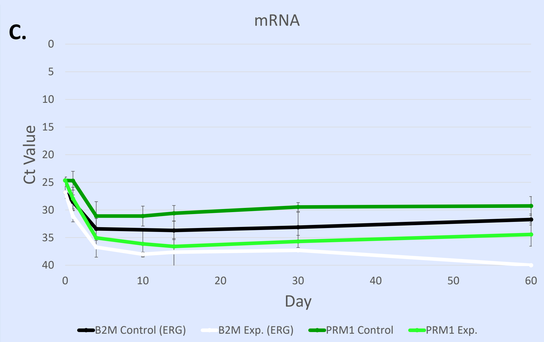


Figure 3A-D. Results of the time course study examining Ct values of ERGs (B2M and let-7g) and body fluid specific targets up to day 60. Data presented as verage (n=3) ± SD. **A.** Blood mRNA targets. **B.** Blood miRNA targets. **C.** Semen mRNA targets. **D.** Semen miRNA targets.

Degradation of Targets (Fold-Change)		
mRNA	Control	~1.7E2
	Exp.	~4.8E6
miRNA	Control	~9.8
	Exp.	~6.2E1

Degradation of Targets (Fold-Change)		
mRNA	Control	~2.5E1
	Exp.	~2.6E4
miRNA	Control	~2.1
	Exp.	~1.7E4

[a] **Table 1.** Approximate fold change of targets compared to control values.

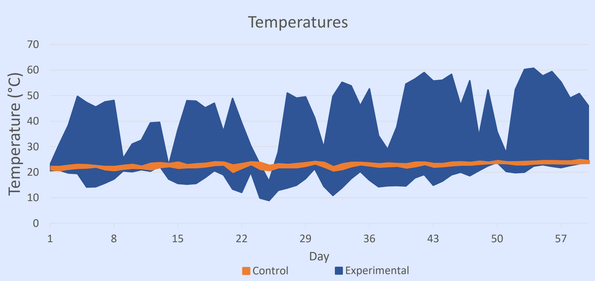


Figure 4. High and low temperatures recorded within the control and experimental environments.

- miRNA markers appear more stable than mRNA
- Both mRNA and miRNA are stable at room temperature
- Most degradation takes place during the first 4 days
- Blood
  - Similar time frame for degradation as reported by Setzer et al. [1]
- Semen
  - Endogenous reference genes (ERGs) appear to be degrading more quickly
  - May be due to low expression levels of ERGs in persistent spermatozoa

MATERIALS AND METHODS

Sample Preparation Venous blood and semen (n=3) were collected according to Sam Houston State University IRB approval (#2015-09-26124). Cotton swatches were stained with 50 µL of each fluid.

Swatches were placed in an outdoor glasshouse (Fig. 1) exposed to heat, humidity, and UV exposure as well as controlled conditions in a lab cabinet (room temperature, low humidity, and darkness). Samples were collected at days: 0, 1, 4, 7, 10, 14, 30, and 60.

Figure 1. Location of experimental samples

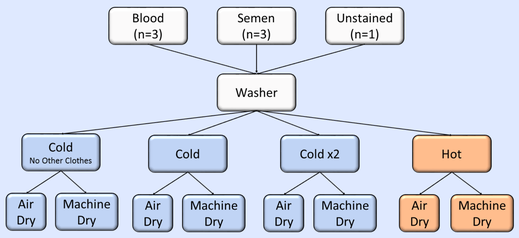


Figure 2. Experimental conditions of laundered items

Laundered swatches were prepared as seen in Fig. 2

RNA Extraction Samples were extracted with the miRNeasy RNA Mini Kit (Qiagen) according to manufacturer's recommendations with the addition of a centrifuge step with spin basket (5 min at 2000g) to remove liquid from fabric.

Reverse Transcription Reverse transcription was performed using the QuantiTect® Reverse Transcription Kit and the miScript II Reverse Transcription Kits (Qiagen) according to manufacturer's protocols. A 10 µL volume of extract was used for each kit.

Quantitative PCR qPCR amplification was performed using the miScript SYBR® Green PCR kit with miScript Primer Assays (let-7g, miR-451, and miR-891) and QuantiTect® Primer Assays (B2M, HBB, and PRM1) (Qiagen). Amplification was detected using a 7500 Real-Time PCR System (Applied Biosystems) with HID Real-Time Analysis Software v1.2. A threshold of 0.08 was applied for all markers.

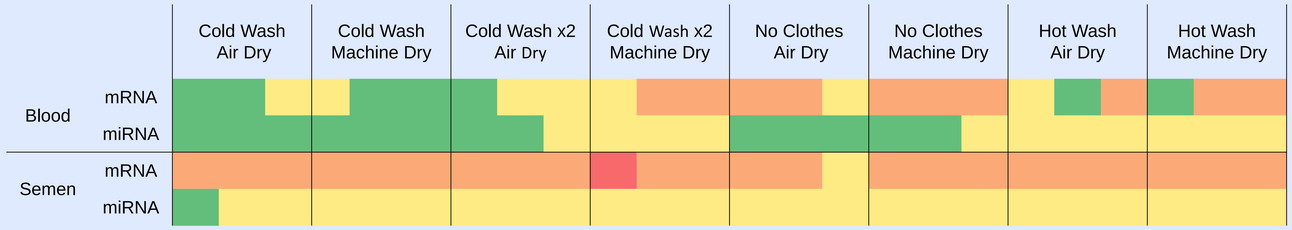


Figure 5. Heat map denoting the presence of mRNA or miRNA targets after being laundered (n=3). Unstained sample data not shown.

- Both miRNA targets were observed in all laundered samples
- Body fluid specific mRNA targets were observed in most laundered samples
  - No body fluid specific mRNA targets were observed when swatches were washed with no other clothes (also observed by Kulstein & Wiegand [3])
  - Results from laundered items should be interpreted carefully as body fluid specific markers can persist from normal interaction with items

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ACKNOWLEDGEMENTS

This work was supported by Award #2016-DN-BS-001 (National Institute of Justice, Office of Justice Programs, U.S. Department of Justice). The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the author(s) and do not necessarily reflect those of the Department of Justice.