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

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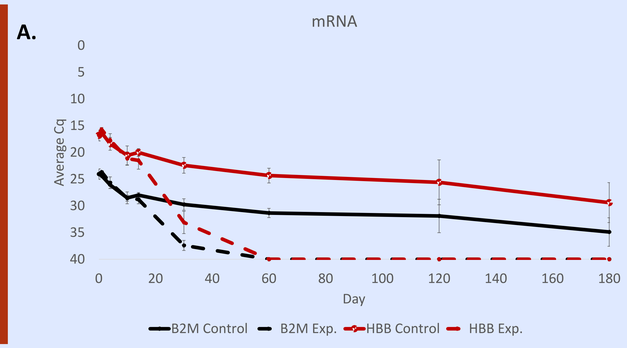
INTRODUCTION

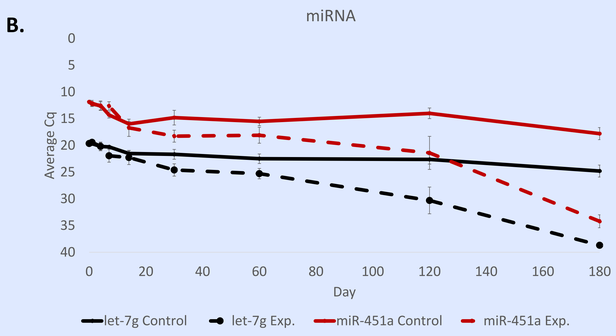
The identification of body fluids in evidentiary stains may provide investigators with probative information during the course of a forensic case. Traditional serological tests currently used in forensic labs are presumptive in nature and have varying levels of specificity and sensitivity. The interrogation 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 6 months. Laundered cotton swatches stained with blood or semen were also examined.

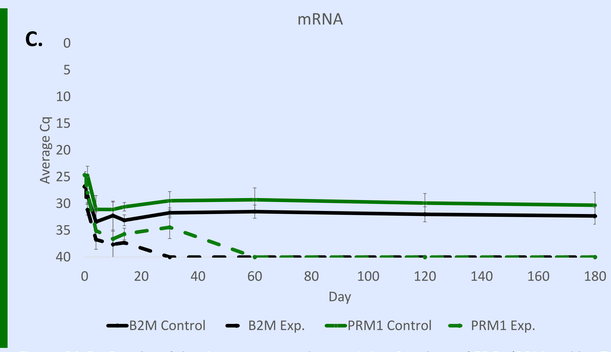
RESULTS AND DISCUSSION

BLOOD





SEMEN



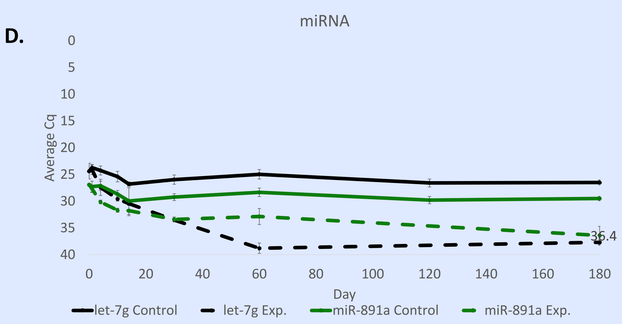


Figure 3A-D. Results of the time course study examining Ct values of ERGs (B2M and let-7g) and body fluid specific targets under room temperature, dark control conditions (control) and experimental conditions exposed to the sunlight and heat (Exp.) for up to 180 days. Data presented as average (n=3) ± SD. **A.** Blood mRNA targets. **B.** Blood miRNA targets. **C.** Semen mRNA targets. **D.** Semen miRNA targets.

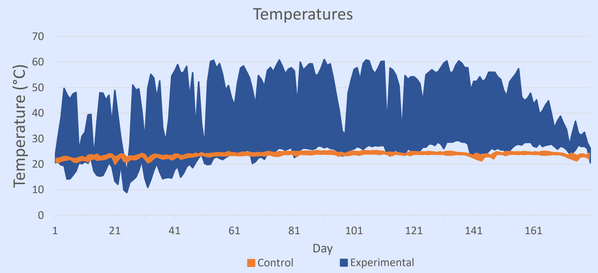


Figure 4. High and low temperatures recorded within the control and experimental environments.

- Both mRNA and miRNA were stable at room temperature
- miRNA markers appear more stable than mRNA when exposed to sunlight and hot, humid conditions (Fig. 3)
- Blood
  - Time frame for degradation consistent with previous reports by Setzer et al. [1]
- Semen
  - Endogenous reference genes (ERGs) appear to be degrading more quickly than body fluid specific targets
  - May be due to robust cell membranes of spermatozoa and/or 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, 60, 180.

Figure 1. Location of experimental samples

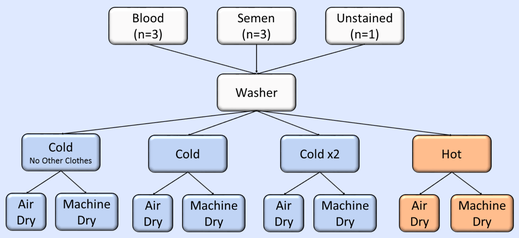


Figure 2. Experimental conditions of laundered items

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.

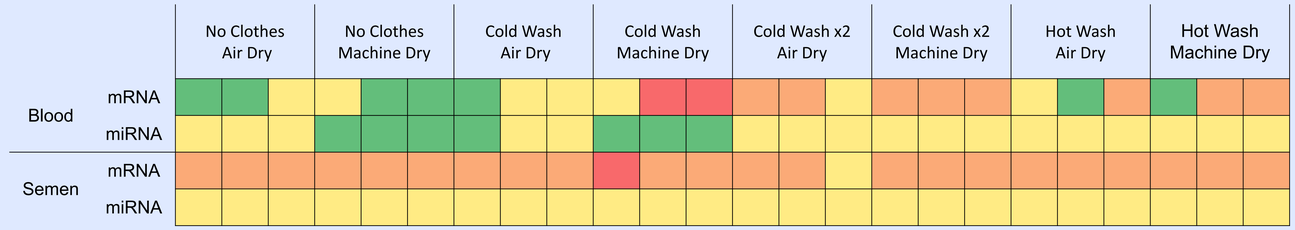


Figure 5. Heat map denoting the presence of mRNA or miRNA targets after being laundered (n=3). Unstained sample data not shown. Each cell represents one sample.

- Both miRNA targets were observed in all laundered samples (Fig. 5)
- 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 in the machine (also observed by Kulstein & Wiegand [3])
  - Transfer of mRNA (3 of 8 samples) and miRNA (8 of 8 samples) was detected
  - Results from laundered items should be interpreted carefully as body fluid specific markers can persist from normal interaction with clothing items

Expected ΔC_q Values (within ΔCq ±SD of control samples)
Greater than Expected ΔCq Values (outside of ΔCq ±SD of control samples)
Fluid Specific Marker Only
No Results

REFERENCES

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