Analysis of Circulating miRNA from Dried Blood Spots

Jeanette R. Hill[1], James E. Hill[1], Jason K. Wright[1] and John Repass[2]

*[1]**Spot On Sciences**, Austin, TX [2]**ARQ Genetics**, Bastrop, TX*

Introduction

Circulating microRNA (miRNA) molecules have been implicated in many disease states including cancer, diabetes and cardiovascular disease. Many recent studies suggest that specific miRNA molecules in blood may be excellent, non-invasive biomarkers for predicting and/or detecting disease.

Collection of blood samples by finger stick and preservation by drying on filter paper (dried blood spot, DBS) is a simple and convenient method for sample collection, especially useful for collection from home or other remote locations. In this study, we demonstrate for the first time that circulating miRNA levels can be accurately measured from DBS samples.

To collect DBS samples, a novel collection device was used (HemaSpot) (Figure 1), that simplifies self collection from any location and at any time. HemaSpot contains an innovative "fan" form for the absorbent paper which improves sample consistency and drying rate while simplifying sample removal by plucking a blade as compared to traditional spot punching (Figure 2).

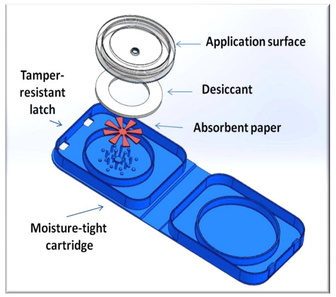

Figure 1. HemaSpot™ blood sampling device

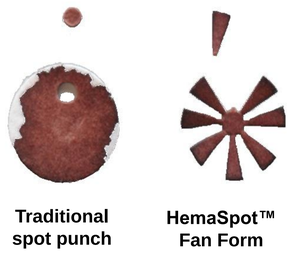

Figure 2. Traditional spot punch and HemaSpot™ fan form

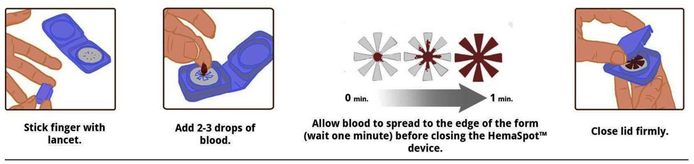

Figure 3. How to use the HemaSpot™ blood collection device

Advantages of DBS & HemaSpot for MDx

- Easy sampling from remote and resource constrained areas
- Minimized contamination
- Stable at ambient temperature
- Low blood volumes
- Simplified shipping, transport & storage
- Reduced biohazard exposure

Materials and methods

Circulating miRNA

Representative miRNA molecules that are detected in human blood at varying levels were chosen for this study (Table 1).

microRNA	Function	Expression level	Causes of abnormal expression	Implicated in condition(s)
miR16	tumor suppressor	high	deletion of gene locus	CLL, prostate cancer, myeloma
miR21	oncomir	high/moderate	chromosomal fusion/translocation	multiple cancers
miR184	oncomir	very low	chromosomal fusion/translocation	squamous cell carcinoma
miR197	oncomir	low	chromosomal fusion/translocation	non-small and small cell lung cancer
miR320	tumor suppressor	moderate	deletion of gene locus	colorectal cancer, sickle cell anemia
let7b	tumor suppressor	high	LIN-28 disregulation (alteration of let7 processing)	germ cell tumors, hepatocellular carcinoma, regulation of IFNg
RNU48	reference gene	moderate		
miR152	reference gene	low		

Table 1. Circulating miRNAs for analysis

Blood Collection:

Wet samples were collected by finger stick into an eppendorf tube or by venipuncture into a vacuum tube in the presence or absence of heparin.

Dried samples were prepared by adding 2 drops directly from a finger stick (capillary) or by applying 70 µL (venous) to the center of the fan shaped filter paper (Fig. 2) in a HemaSpot device (Figure 1). The device was closed and the samples dried within 2 hours under desiccant and stored at ambient temperature.

RNA isolation:

Dried samples were prepared from a single blade (equivalent to ~8 µL of liquid sample) by extraction in TriZol (Invitrogen) overnight at 4°C followed by mechanical disruption using a Kontes Pellet Pestle system (Fischer Scientific).

To wet samples, TriZol was added immediately, mixed and stored on ice.

RNA extraction: The resulting aqueous phase was extracted by phenol:chloroform:isoamyl alcohol and a final chloroform extraction performed to remove organic contaminants, followed by ethanol precipitation.

RNA analysis: RNA concentration and purity were determined using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). RNA integrity was measured using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and the RNA 6000 Nano Chip Kit according to the manufacturer's instructions.

Reverse transcription was performed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's instructions. For reverse transcription reactions, 1 mg and 10ng of total RNA was used for mRNA and miRNA analyses, respectively.

Real-time PCR was performed on the 7900HT Fast Real-Time PCR System (Applied Biosystems). Reaction conditions were as follows: 10 min activation at 95°C, followed by 40 cycles of 15s at 95°C and 1 min at 60°C. TaqMan assays for mature miRNAs and mRNAs were purchased from Applied Biosystems and used according to the manufacturer's instructions. Samples were analyzed for relative gene expression by the dDCt method.

Results

Equal miRNA Levels between Wet and Dried Blood

A strong correlation between dried and wet whole blood samples was seen for levels of 6 representative miRNAs.

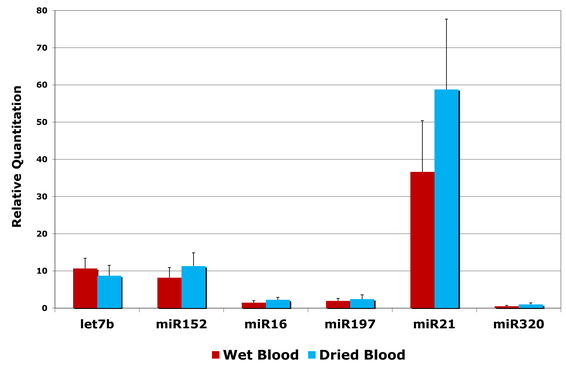

Figure 4. miRNA levels in dried and wet whole blood
Average of 4 separate donors, standard deviation

Equal miRNA Levels Between Venous and Capillary Blood

Equal levels of 2 representative miRNAs were seen between venous and capillary blood. As additional comparison, miRNA levels in serum and clotted cells was also measured.

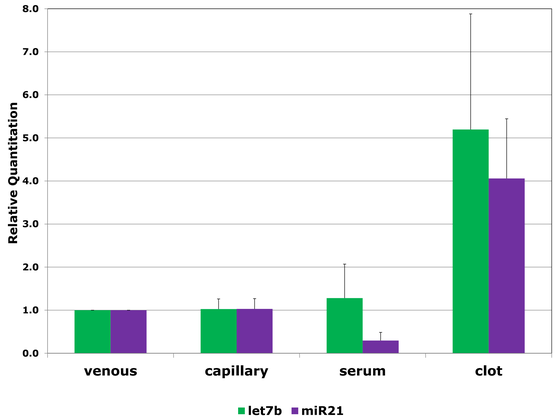

Figure 5. miRNA levels in venous, capillary, serum and clotted blood.
Average of 3 separate donors, standard deviation

Results

Heparin Interferes with miRNA Detection

Use of heparin as anticoagulant for venous blood reduces detection of miRNA by ~100x, likely by inhibition of RT-PCR.

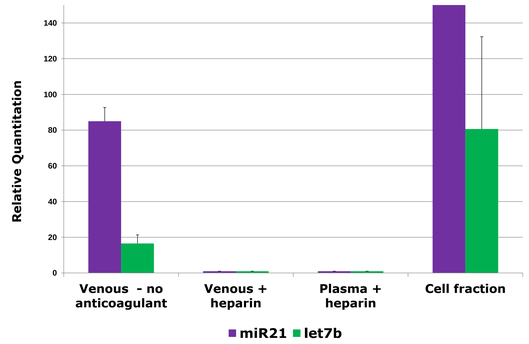

Figure 6. Effect of heparin on miRNA detection.
Average of 3 separate donors, standard deviation.

Circadian Rhythm of Circulating miRNA

Cardiovascular disease-related miRNA show variable levels according to time during a 24 h cycle.

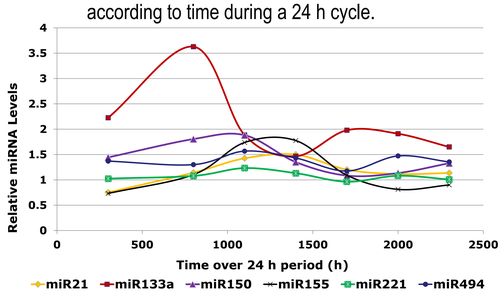

Figure 7. miRNA levels in blood over 24 hours
Average of 3 separate donors.

Conclusions

- miRNA levels were equivalent for dried and wet whole blood.
- Venous and capillary blood showed equal miRNA levels
- Heparin reduces miRNA detection by >10-fold, likely due to heparin inhibition of RT-PCR.
- Levels of circulating miRNA can be accurately determined from finger stick and dried blood spot samples.

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