Measurement of Maternal Life Experience Study Visit 2: Blood Collection & Processing Protocols

Summary

	TUBE	ANALYTE	VOLUME OF PLASMA	VOLUME OF CRYOVIAL	SITE OF ANALYSIS
1	4.0 mL EDTA +	CRH	1.5mL	2mL	UC Irvine
	APROTININ		remainder	2mL	
2	10mL EDTA	CORTISOL	0.5mL	2mL	UC Irvine
		CRP/EBV	0.5mL	2mL	Northwestern
			0.5mL	2mL	
		CYTOKINES	8 *0.1mL		San Antonio
			(strip tube),	strip tube +	
			0.750mL	2mL	
			(cryovial)		

Cortisol, CRP/EBV and Cytokines (10mL EDTA Purple-Top Tube)

Collect blood and gently invert tube 10 times to adequately mix blood with EDTA anticoagulant.

Processing Sample:

1. Adjust centrifuge to 3000rpm @ 4°C and spin for 15 minutes.

Aliquots (On Ice):

- 1. Pipette 0.5mL of plasma into 3 of the 2mL cryovials.
- 2. Pipette 0.75mL of plasma into the fourth 2mL cryovial.
- 3. Pipette 8 X 0.1mL of plasma into the strip tube.
- 4. Store additional samples in extra 2mL cryovials (store locally).
- 5. Immediately store all vials in -80°C freezer.
- 6. Store strip tubes in a small box with 96 x 0.2mL racks with lids.
- 7. Use extra fine alcohol resistant marker to label the side of <u>each</u> tube (tubes can break off the strip when frozen).
- 8. Place a sturdy rubber band around the lid and box.

CRH (4mL EDTA Purple-Top Tube + Aprotinin)

• Aprotinin aliquots will be prepared in advance. (Aprotinin from SIGMA A1153-100mg \$462.00)

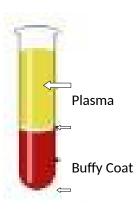
- Make Aprotinin on ice and aliquot on ice. Dilute in the original bottle with 5mls of sterile saline.
 Mix well. Aliquot 35uL into each small 0.6mL tube for storage at -20°C. Make sure to deliver liquid to bottom tube.
- This gives you a concentration of 20mg/mL or 1mg/50ul or 6TIU/50ul. (We need 1TIU/ml blood).
- These aliquots can be stored for up to 1 year.

On day of draw:

- 1. Remove Aprotinin from freezer, put on ice, and take to clinic.
- 2. If aliquot is still frozen when needed, gently roll tube in gloved hands to defrost.
- 3. Collect blood and gently invert tube 10x to mix blood with EDTA anticoagulant.
- 4. Pipette 33uL of Aprotinin protease inhibitor into tube.
- 5. Recap tube and gently invert tube 4x to mix blood with inhibitor.
- 6. Chill on ice until centrifugation.

Processing Sample:

- 1. Sample must be processed within 30 minutes of collection (if needed, sample can sit on ice for up to 1 hour).
- 2. Centrifuge at 3000rpm for 15 minutes at 4°C.
- 3. Carefully remove tube from centrifuge as not to disturb buffy coat and note specimen color (yellow, pink or red).
- 4. Pipette 1.5mL of plasma (carefully so as not to disturb buffy coat) into the first 2mL cryovial and then pipette remainder into the second 2mL cryovial.
- 5. Immediately store all plasma cryovials in -80°C freezer (or -20C if necessary).



Ship frozen specimens on dry ice to:

1) <u>University of California Irvine</u>

Institute for Clinical and Translational Sciences 101 THE CITY DRIVE SOUTH, Building-55-Room-334

Orange, Ca. 92868

Attn: Dr. Frank Zaldivar 714-456-6914

> 714-456-8248 Georgia 714-456-3417 Mila

<u>Aliquots</u>

2X cryovials of plasma + aprotinin

1X cryovial of plasma

2) <u>Northwestern</u>

Thomas McDade

1810 Hinman Avenue

Evanston, IL 60208

p: 847/467-4304

f: 847/467-1778

e: t-mcdade@northwestern.edu

<u>Aliquots</u>

2X cryovials of plasma

3) <u>San Antonio</u>

Joe Cuellar

Biomarkers Laboratory

2.528 McDermott Building

8403 Floyd Curl Drive

San Antonio, TX 78229

p: 210/567-8084

f: 210/567-5507

e: cuellarj4@uthscsa.edu

<u>Aliquots</u>

1X cryovial of plasma

1X strip tube