Measurement of Maternal Life Experience Study
Visit 1: Blood Collection & Processing Protocols

Summary

	TUBE	ANALYTE	VOLUME OF PLASMA	VOLUME OF CRYOVIAL	SITE OF ANALYSIS
1	4.0 mL EDTA + APROTININ	CRH	1.5mL	2mL	UC Irvine
			remainder	2mL	
2	10mL EDTA	CORTISOL	0.5mL	2mL	UC Irvine
		CRP/EBV	0.5mL	2mL	Northwestern
			0.5mL	2mL	
		CYTOKINES	8 *0.1mL (strip tube), 0.750mL (cryovial)	strip tube + 2mL	San Antonio
3	8.5 mL Paxgene tube	DNA	N/A	8.5mL tube (do not aliquot)	

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

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

Processing Sample:

1. Adjust centrifuge to 3000rpm @ 4^{0}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.

<u>DNA (8.5mL Paxgene Tube)</u>

- Collect blood and gently invert tube 10 times.
- No processing or aliquots necessary.

Storage of Aliquots:

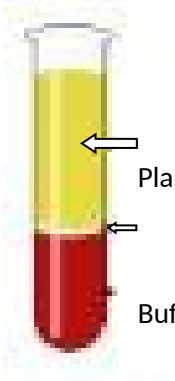
1. Store Paxgene tubes using the styrofoam inserts and box in which they came.
2. Place at ~-20°C for 24 hours and then store tubes in -80°C freezer.
3. IMPORTANT: Place the box on its side when placed inside the freezer. Tubes may crack if upright.

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).

Shipping

Ship frozen specimens on dry ice to:

1) University of California Irvine
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

Aliquots
2X cryovials of plasma + aprotinin
1X cryovial of plasma

2) Northwestern
Thomas McDade
1810 Hinman Avenue
Evanston, IL 60208
p: 847/467-4304
f: 847/467-1778
e: t-mcdade@northwestern.edu

Aliquots
2X cryovials of plasma

3) San Antonio
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

Aliquots
1X cryovial of plasma
1X strip tube
1x Paxgene tube