**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 + 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 |
|  |  |  |  |  |  |  |

# 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 @ 40C 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 -80ᵒC 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 each 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 -20ᵒC. 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.

Plasma

Buffy Coat

Red Cells

1. Pipette 33uL of Aprotinin protease inhibitor into tube.
2. Recap tube and gently invert tube 4x to mix blood with inhibitor.
3. 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 4ᵒC.
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 -80ᵒC 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