PROCEDURE | Blood Collection - Whole Blood, Plasma, Buffy Coat & Serum
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PREPARED BY | GAPPS Staff
DATE ADOPTED | 
REVIEWED BY | SIGNATURE | REVIEWED DATE
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 | | 1Nov2013

**SUMMARY OF CHANGES TO THIS SOP**

**Version 2.0**
1. Removed 1, 6ml EDTA tube from blood draw.
2. Reduced the number of plasma aliquots to a maximum of 2, 1ml tubes
3. Reduced whole blood aliquots to 2, 1ml tubes
4. Added 1, 5ml blood tube for serum
5. Added 2, 1ml aliquots of serum
6. Specimen collection, processing and storage must be completed in less than 2 hours rather than 8 hours.

**Version 3.0**
1. 6 ml purple to 10 ml purple
2. 2 aliquots whole blood to 4 aliquots whole blood
3. 1 aliquots plasma to 3 aliquots plasma

**Version 3.5**
1. Safety section expanded

**Version 3.6**
1. Added PBMC tube
2. Added instructions regarding triage for PBMC collection.
3. Added instructions for “short” specimens

**Version 3.7**
1. Added DMSO-preserved whole blood procedure

**Version 3.8**
1. Added additional PBMC processing language
PURPOSE
This Standard Operating Procedure (SOP) describes a procedure for collection of whole blood, serum, plasma and buffy coat using red-top serum tubes and purple-top tubes containing EDTA as an anticoagulant. It also includes instructions for designation and transfer of certain collections to the Central GAPPS laboratory for isolation of Maternal PBMCs.

SCOPE
This procedure covers the collection, processing, and storage of whole blood, plasma, buffy coat and serum. It does not cover how to draw blood from humans or any assays performed with the blood or blood products after processing.

Authority and Responsibility for SOP's
1. The GAPPS Medical Director (or his/her designee) and Laboratory Manager have the authority to establish this procedure.
2. The GAPPS Laboratory and the QA monitors are responsible for the implementation of SOP documentation at participating sites.
3. The site’s PI and Coordinator is responsible for the implementation of this procedure at their site and for ensuring that all appropriate personnel are trained and sign “Acknowledgement of Understanding” document for this SOP.
4. All health care providers and technicians who implement this SOP at study sites are responsible for reading and understanding this SOP prior to performing the procedures described.
5. All health care providers and technicians are expected to be trained and follow the procedures described in any of the GAPPS SOPs and have their signature on file at the collection site.

Supplies
On Site:
1. 5 ml pipettes
2. 1 ml blue tip pipettes

Supplied in Kit:
1. 10.0 ml purple top Vacutainer
2. 5.0 ml red-top serum tube
3. 2 ml brown glass vial w/ 0.5 ml DMSO
4. Urine cup (to hold cryo-vials)
5. 11 cryo-vials, GAPPS labeled.

Safety
1. Required Training for processing
   a. Blood borne pathogens
   b. Standard laboratory practices including centrifuge safety
2. Risks
   a. Sharps hazard
   b. Blood and biofluid exposure
3. Required safety equipment
   a. Lab coats/scrubs
   b. Face shield/safety goggles
   c. Closed toed shoes
   d. Gloves
All health care providers and technicians are expected to be trained and follow universal precautions when handling biological or hazardous materials when performing any procedures described in any of the GAPPS SOPs.

LIMITATIONS OF THE PROCEDURE

To avoid poor quality specimens or blood hemolysis, the **time duration from participant blood draw to completion of specimen processing in the laboratory is not to exceed 2 hours**. Specimens which exceed 2 hours should be discarded and noted as such in the specimen designation record.

Specimen Storage and transfer for PBMC isolation

1. Collections designated for PBMC isolation should have a MINIMUM volume of 10mL of EDTA treated (purple-top tube) blood.
2. Maximum time lapsed from collection to transfer to the central lab can be no greater than 12 hours, but ideally should occur within 2 hours. Collections designated for PBMC isolation should be held within the timeframe of Monday-Friday, 2am-4pm.
3. Gently mix blood in EDTA vacutainer by inverting 5-10 times immediately after drawing from participant and prior to any of the processing steps.
4. After specimen has been collected, hold at room temperature in the Blood Collection Kit bag with the associated kit components until transfer to the GAPPS laboratories.
5. Complete upper portion of the GAPPS Lab Requisition form. Indicate with a check on the lower portion of the form if both a purple and red-top tube were collected. Complete any additional required local site forms for the specimen collection.
6. Consult “Shipping SOP” when specimens are ready to be shipped. Transfer samples to the GAPPS lab via prearranged personal pickup or yellow cab. Provide the cab with a GAPPS supplied cab voucher. See page 85.

Blood processing for non-PBMC destined samples

1. **Collecting Serum from Red top tubes**
   a. Qualified personnel should draw a 6 ml red top tube of blood from a participant, with a label designating date and time of collection.
   b. Allow blood to clot for at least 30 minutes at room temperature
   c. After the blood has clotted, centrifuge tube in a swinging bucket rotor at 2500RPM at room temperature for 10 minutes.
   d. Avoiding the bottom dark red layer, transfer 2, 1ml aliquots of the serum (top layer) into the two GAPPS supplied and labeled 2 ml cryovials. The bottom red blood cell layer is not collected and should be destroyed as per lab protocol for biological materials. Aliquots must be frozen at a minimum of -20°C within 2 hours from patient collection.

2. **Collecting Whole Blood in EDTA tubes (purple top):**
   a. A certified phlebotomy technician should draw a 10ml EDTA tube of blood from a participant, with a label designating date and time of collection.
   b. Gently mix blood in EDTA vacutainer by inverting 5-10 times immediately after drawing from participant and prior to any of the processing steps.
c. Blood can be stored at room temperature until processed.
d. Complete GAPPS Lab Requisition forms and any required local site forms for the specimen collection.

3. **Processing Whole Blood from EDTA tubes**
   a. It is important to track labels, specimens and documents of individual participants while processing.
   b. Avoid all clots. Do not include blood clots in whole blood collection or centrifugation.
   c. Dividing up and processing the specimen:
      - **DMSO preserved whole blood**: Transfer 110μl DMSO from the provided 2ml brown glass vial to the green capped 2ml cryovials labeled “Whole Blood + DMSO”. Pipet accurately 1.000 ml of whole blood into each of the 2 GAPPS supplied, green capped, 2ml cryovials labeled “Whole Blood + DMSO”. Cap and mix by inversion 6 times and place in a “Mr. Frosty” that has been pre-chilled to 4°C. Within 30 minutes transfer “Mr. Frosty” to -80°C. After 24hr. transfer the vials to long-term storage in vapor-phase LN₂ (-160°C).
      - **Non DMSO-preserved whole blood**: Pipet two 1.0 ml aliquots of whole blood into GAPPS supplied, red capped 2 ml cryovials labeled “Whole Blood”. If a vial contains a “short” specimen, i.e., less than 1.0 ml, put a black dot on the lid with a sharpie. The remainder of the specimen will be used to obtain plasma and buffy coat. If a vial contains a “short” specimen, i.e., less than 1.0 ml, put a black dot on the lid with a sharpie.

4. **Processing Plasma & Buffy Coat from EDTA tube**
   a. Centrifuge the remainder of blood in the EDTA tube in a swinging bucket rotor at 2500RPM for 10 minutes at room temperature
   b. After centrifugation 3 layers should be visible in the vacutainer tube;
      1) **Top golden layer = plasma**: the plasma layer is usually semi-transparent and golden in color. Aliquot plasma into 3 GAPPS supplied and labeled 2ml cryovials in 1ml aliquots.
         - If it appears pink or red it is hemolyzed. Note on the lab requisition form; aliquots should still be made.
         - If it has an opaque white color or white layer floating on top it is lipemic (contains fatty lipids). Note on the lab requisition form. Avoid inclusion of the lipid layer at the top of plasma; aliquots should still be made. If a vial contains a “short” specimen (less than 1.0 ml), put a black dot on the lid with a sharpie.
      2) **Thin middle layer = Buffy coat**: the Buffy coat is a thin layer of white blood cells (~1mm thick) situated between the red blood cells and the plasma. It may not be visible or difficult to see. When pipetting the Buffy coat it is important to avoid the RBC layer below as best as possible or excessive inclusion of plasma. All of the Buffy coat material will go into 1 GAPPS supplied 2ml cryovial labeled “Buffy Coat”.
      3) **Bottom dark red layer = RBC (red blood cells)**: the bottom layer is not collected and should be destroyed as per lab protocol for biological materials.
Specimen Storage

1. After blood, plasma, buffy coat and serum specimens have been processed, aliquotted and labeled date and time as well as number of aliquots should be recorded. Except for DMSO-preserved whole blood where storage is detailed above, specimens must be stored, within 2 hours of collection, at a minimum of -20°C for short term storage (< 30 days), but preferably at -80°C until shipped to the core repository.
2. Consult “Shipping SOP” when specimens are ready to be shipped.

Converting RCF (g) to RPM:

To convert maximum relative centrifugal force (RCF) to RPM: Determine centrifuge’s radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor. Lay a ruler or draw a line from radius value in right-hand column value that corresponds to the device’s maximum rated g-force. Then read the maximum value from the column labeled “RCF”.

Nomogram for conversion of g to RPM