PROCEDURE	Fetal Blood Collection	
PREPARED BY	GAPPS Staff	
DATE ADOPTED		
REVIEWED BY	SIGNATURE	REVIEWED DATE

REVISED BY	SIGNATURE	REVISED DATE
GAPPS Staff		1Nov2013
GAPPS Staff		22Jan2014

SUMMARY OF CHANGES TO THIS SOP
Version 2.0 1. Maximum time that specimen collection, processing and storage must be completed extended from within 2 hours to 8 hours.
Version 2.5 1. Safety section expanded 2. 6 ml purple to 10 ml purple 3. 6 aliquots to 10 aliquots
Version 3.0 1. Safety section expanded 2. Changed 6 mL purple to 10 mL purple top 4. Processing steps added to allow for plasma and buffy coat collection
Version 3.1 1. Expanded SOP to include collection of fetal blood from the placenta.
Version 3.2 1. Increased the blood collection to 20mL: 6 aliquots whole blood, 4 aliquots plasma, 2 aliquots buffy coat. 2. Added instructions for “short” specimens.
Version 3.3 1. Added DMSO-preserved whole cord blood procedure
Version 3.4 1. Added transfer section for CBMC isolation destined specimens

PURPOSE

This Standard Operating Procedure (**SOP**) describes a procedure for collection of whole Umbilical Cord Blood using EDTA as an anticoagulant.

SCOPE

This procedure covers the collection, processing, and storage of whole Cord Blood. It does not cover 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 (or his/her designee) 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

Site Supplied:

1. 5 mL pipets
2. 1mL blue tip pipets

Supplied in Cord Blood Whole Kit:

1. 2- 10 mL purple top vacutainers
2. 20- 1.8mL GAPPS pre-labeled cryo-vials.

Supplied in Cord Blood-DMSO Kit:

1. 2- 10mL purple top vacutainers
2. 10- 1.8mL GAPPS pre-labeled cryo-vials

Supplied in Cord Blood Spun Kit:

1. 2- 10mL purple top vacutainers
2. 12- 1.8mL GAPPS pre-labeled cryo-vials

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 the any procedures described in any of the GAPPS SOPs.

LIMITATIONS OF THE PROCEDURE

1. Cord Blood collection can occur in utero or less than 30 minutes after delivery of placenta to avoid clotting of blood. Do not collect cord blood greater than 30 minutes after delivery of placenta.
2. Maximum time lapsed from collection to placing aliquots in the freezer can be no greater than 8 hours, but ideally should occur within 2 hours.

SPECIMEN STORAGE AND TRANSFER FOR CBMC ISOLATION

1. Collections designated for CBMC 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 CBMC 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 Collection:

Umbilical cord blood or blood from a large placental vein on the chorionic (fetal) surface can be collected by Site Coordinators or delivery staff who may already be collecting cord blood for clinical purposes. In such cases, delivery staff will be provided with a single 10 mL EDTA vacutainer with the GAPPS “Clinic” label attached and specimen collection documents. Delivery staff may use their phlebotomy SOP’s and collection devices to collect umbilical Cord blood as long as it is not in conflict with GAPPS requirements. Blood is collected from the **fetal or placental vein only** directly into the 10 mL EDTA vacutainer with a GAPPS clinical label. After collection they will hand specimens and specimen collection documents over to study coordinators.

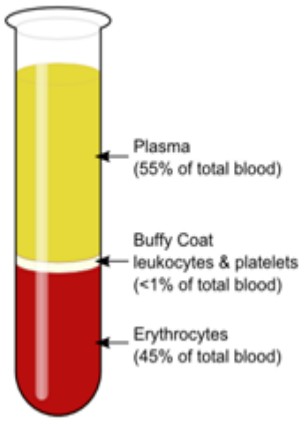
1. **Collecting Whole Cord Blood EDTA tubes (purple top):**

a. Make sure to have gauze, blood collection kit, extra gloves, two 10 mL EDTA vacutainers, labels, and lab requisition form within easy reach.
b. Umbilical or placental blood should be received within 30 minutes after delivery and clamped at both ends by delivery medical staff. Do not collect blood if > 30 minutes has elapsed after delivery of placenta.
c. Upon receipt of cord and/or cord and placenta identify the fetal umbilical or placental vein.

d. Using clean dry gauze, wipe excess blood and fluids from a small 2-3 cm area of the umbilical cord or fetal placental vein for a clear puncture site.
e. Uncap the insertion side of the blood collection holder.
f. Holding the collection set at a downward angle with the bevel side up; insert the needle in clean portion of fetal vein, following the direction of the vein. Avoid puncturing through the vein.
g. Holding blood collection kit firmly in one hand, insert the purple rubber top of the 10mL EDTA vacutainer into the tube holder end of collector until the rubber end of the tube stops and blood begins to flow. Allow tube to fill.
h. Once blood flow has stopped, remove tube from collector and invert it 5-10 times to assure mixing of anticoagulant with blood.
i. Remove collection needle from vein and dispose of in sharps container.
j. Complete lab requisition form and place documents and specimen in the specimen transport bag at room temperature.

If you collect less than 6ml, just aliquot it as whole blood. DO NOT PROCESS. Proceed with processing if you collect 6ml or more.

2. **Processing Cord Blood from EDTA – Tubes**
   a. Whole blood specimens should ideally be processed in less than 2 hours after collection is complete, but may be processed up to 8 hours after collection.
   b. Upon arrival in the process lab, appropriate portions of the lab requisition form should be completed.
   c. **DMSO preserved whole cord blood**: Transfer 110µl DMSO from the provided 2ml brown glass vial to the green capped 2ml cryovials labeled “Cord Blood + DMSO”. Pipet *accurately* 1.000 ml of whole cord blood into each of the 2 the green capped cryovials, 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 24hrs, transfer the vials to long-term storage in vapor-phase LN_2 (-160°C).
   d. **Non-DMSO preserved whole cord blood**: Pipet **four**, 1mL aliquots of whole blood into the three, pre-labelled **red-capped** whole cord blood cryo-vials. The remainder of the specimen will be used to obtain plasma and buffy coat.
      1) Avoid any clots or debris when pipeting. Note any volume discrepancies or excessive clotting on specimen collection documents.
   e. Centrifuge the remainder of blood in the EDTA tubes in a swinging bucket rotor at 2500RPM for 10 minutes at room temperature
   f. After centrifugation 3 layers should be visible in the vacutainer tubes:



1) Top golden layer = plasma: the plasma layer is usually semi-transparent and golden in color. Transfer **four,** 1mL aliquots of plasma into the two GAPPS supplied and labeled **blue-capped** 2 mL cryo-vials. Avoid the incorporation of the lower buffy coat and erythrocyte layers into the plasma fraction.

- If it appears pink or red then it is hemolyzed and should be noted in the lab requisition forms and aliquots should still be made.
- If it has an opaque white color or white layer floating on top it is lipemic (contains fatty lipids) and should be noted in the lab requisition forms. Avoid inclusion of the lipid layer at the top of plasma and aliquots should still be made.

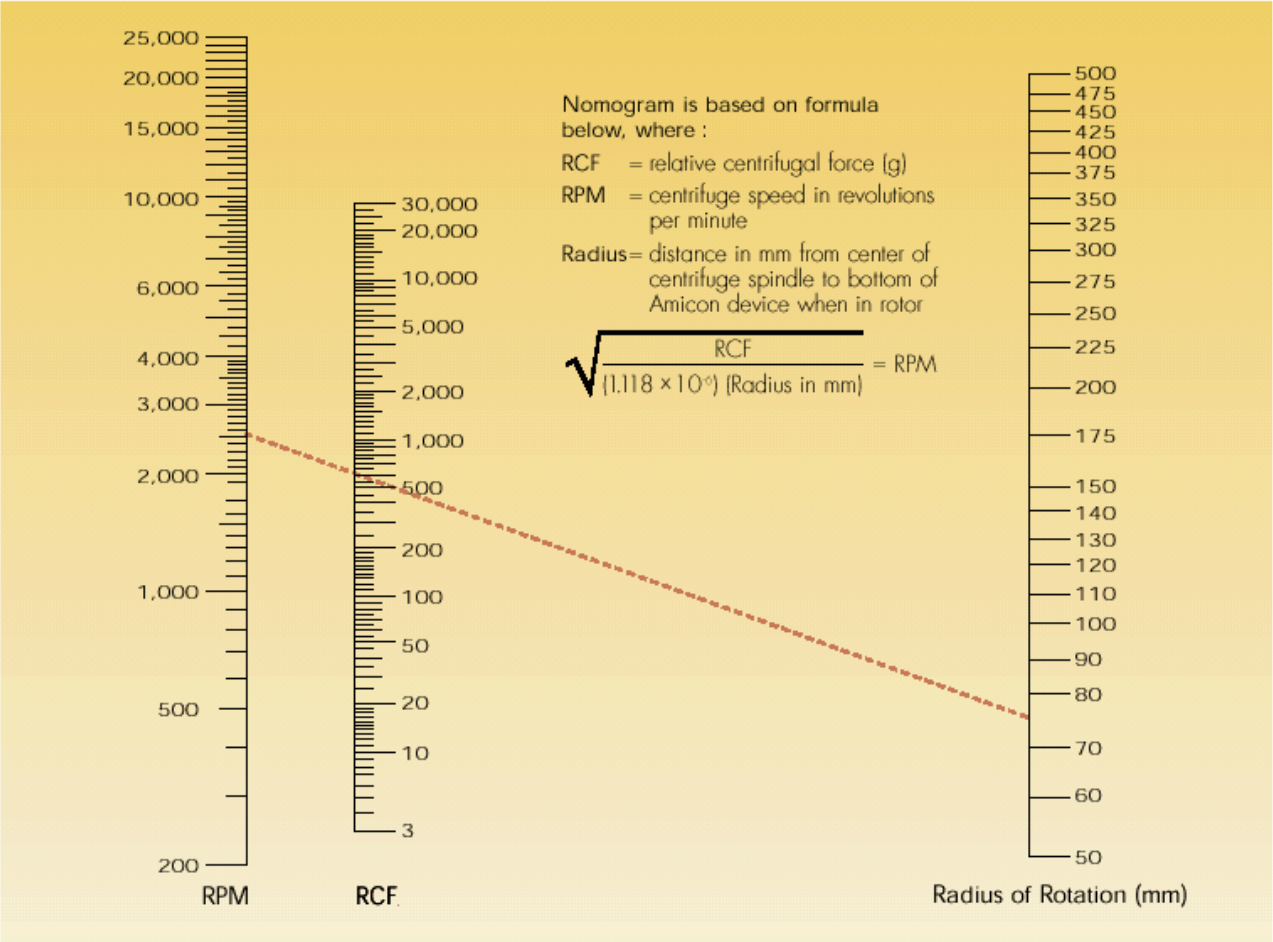
2) Thin middle layer = buffy coat: the buffy coat is a thin layer of white blood cells (~1mm thick) situated between the red blood cells and the plasma. It may not be visible or difficult to see. When pipetting use a circular motion to collect the buffy coat. Avoid the RBC layer below as best as possible or excessive inclusion of plasma. All of the buffy coat material from the two vacutainers (approximately 300µL each) will go into **two to five** GAPPS supplied and labeled **purple-capped** 2mL cryo-vials.
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.

g. Cap vials and complete lab requisition forms, record specimen in specimen tracking data base then store specimen at -80. If a vial contains a “short” specimen, i.e., less than 1.0 ml for whole blood or plasma, put a black dot on the lid with a sharpie.

Specimen Storage

1) After specimen has been processed, aliquotted and labeled all of the aliquots should be recorded into the data systems. Store DMSO preserved blood as detailed above. Store all other specimens at a minimum of -20°C for short-term storage (< 30 days), and 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: Nomogram for conversion of 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 column at left.