James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research

STANDARD OPERATING PROCEDURE

Title:	**Blood Processing**		
Procedure:	**BB.014.01**	**Supercedes:**	**none**
Originator and Date:	**Lise Matzke 22OCT2008**	**Effective Date:**	**22OCT2008**
Review Frequency:	**annually**	**Approved By:**	**The iCAPTURE Centre Privacy Team**
Total Number of Pages: 7			

Revision History		
Date	**Reviewer**	**Summary of revision**
20Apr2009	Crystal Leung	Reformatted to iCAPTURE format

Purpose

Adherence to this SOP will ensure that Biobank blood specimens are processed in a way that is safe, standardized and expedient, thereby guaranteeing that blood products (e.g., DNA, RNA and proteins) are produced reliably and are of the highest possible quality.

Responsibilities

This procedure is applicable to the following teams:

- Biobank personnel who may be responsible for processing blood specimens

Safety

- Treat blood and all materials coming into contact with blood as biohazardous materials: wear personal protective equipment (PPE) throughout the procedure, including lab coat and disposable gloves.
- Cover work area with absorbent underpad.
- Disposal of solid and liquid wastes: refer to the Biohazardous material handling SOP (BB.001.01).

Definitions

Anti-coagulant	**A substance that prevents the clotting or thickening of blood.**
Biospecimen	**All biological material of human origin, including organs, tissues, bodily fluids, teeth, hair and nails, and substances extracted from such material such as DNA and RNA**
Buffy coat	**A thin white layer of white blood cells (leukocytes and platelets) found covering the top of packed erythrocytes (red blood cells) of a blood specimen.**
EDTA	**Ethylenediamine tetra-acetate. The EDTA binds calcium ions thus blocking the coagulation cascade. Erythrocytes, leukocytes and thrombocytes are stable in EDTA anti-coagulated blood for up to 24 hours.**
Plasma	**Blood fraction that is remaining when erythrocytes have been removed from whole blood collected in a tube containing an anticoagulant.**
PPE	**Personal Protective Equipment. The equipment and clothing required to mitigate the risk of injury from or exposure to hazardous conditions encountered during the performance of duty. PPE includes, but is not limited to: face shields, lab coat, goggles and gloves.**
Serum	**Liquid part of whole blood from which red cells and clotting proteins have been removed.**
SOP	**Standard Operating Procedure. Document used to control the methods and requirements by which personnel will perform their activities.**

Materials and Equipment

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products.

Blood collection tubes for plasma (e.g. lavender top tube with EDTA; Becton Dickinson item ID-366643)	**Storage boxes and freezers**
Blood collection tubes for serum (e.g. serum separator tube; Becton Dickinson item ID-367988)	**PPE (including but not limited to gloves, lab coat, safety eye glasses)**
1.2 mL cyrotubes	**Appropriate racks to hold tubes while processing**
Refrigerated centrifuge with rotor capable of accommodating blood collection tubes	**Appropriate labels**

to be employed	
Transfer pipets	**Blood Collection/Processing Worksheets (see Appendix 1 for an example)**

Procedures

1) Handling Conditions

a. While time requirements for specimen processing and cryopreservation are not as stringent as those for tissue specimens, it is recommended that plasma and buffy coat DNA and RNA be processed within 24 hours of removal from the subject. It is useful for downstream proteomic analysis of serum or plasma for the time of blood collection and freezing down of the blood products to be recorded.
b. Verify patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on blood collection tubes.
c. Transport blood collection tubes on ice if possible. Do not allow the blood specimens (whole blood) to freeze or be exposed to an ambient temperature of greater than 25° C.
d. If specimens are coming from a location distant to the Biobank, then ship specimens on ice packs by overnight, express delivery using a qualified courier company.
e. The centrifugation speeds listed here are for those specific blood collection tubes listed. Always check the manufacturer's recommended centrifugation speeds for the blood collection tubes being employed at your site.
f. If blood has been collected into site specific collection tubes not discussed here (e.g., PAXgene DNA or RNA tubes) then proceed with processing of these specimens as per the manufacturer protocol.

2) Labeling Of Blood Products

Labeling will be project specific and will never include patient names or other identifiable data.

3) Processing of anti-coagulated blood

Anti-coagulated blood may be separated into the cellular fraction (which can be processed directly for DNA or RNA) and the acellular, plasma fraction.

a. Prior to processing blood samples, run the empty, refrigerated centrifuge for 15 minutes at 2,500 X g, 4°C.
b. Leave the blood samples on ice or at 4°C until processing is commenced.

c. Centrifuge the blood collection tube for 10 min at 2,500 X g, 22°C. This will result in the separation of three visible layers:
    i. The upper layer is the acellular plasma. It is generally clear and pale yellow in colour.
    ii. The second layer is a thin white layer between the plasma and the underlying erythrocyte layer. This is the "buffy coat" or leukocyte fraction.
    iii. The bottom layer is dark red and consists of the erythrocytes, or red blood cells.

d. After centrifugation, place the collection tubes in an ice bath until the plasma and buffy coat are aspirated from the blood collection tube.
e. Using an appropriate disposable transfer pipette, aspirate off the plasma layer down to approximately 1 mm from the buffy coat layer. Take care not to disturb the buffy coat layer.
f. Expel the plasma into a 12 X 75 mm plastic tube and mix by drawing the plasma up and down in the tube several times.
g. Aliqout recovered plasma and place into labeled cryovials.
h. Using a non-sterile transfer pipette, remove the buffy coat from the surface of the red blood cells in the collection tube and transfer into a labeled 1.2ml cryotube. Discard the erythrocyte layer.
i. Cap all plasma and buffy coat aliquots and immediately transfer to dry ice or to long-term freezer storage at -80°C or in liquid nitrogen.
j. Record position and location of the tubes.

4) Processing of serum

If serum is to be obtained from the blood specimens, collect the blood in serum tubes. Serum tubes are coated with particles such as silica which act as a clotting activator.

If upon receipt the red top serum tube is not clotted, allow it to sit at room temperature until a solid clot has formed. Processing may then be commenced.

a. Centrifuge the serum tube at 2,000 X g for 15 minutes, 22°C. This will result in the separation of two visible layers:
    i. The upper layer is the serum. It is generally clear and yellow in colour.
    ii. The bottom layer is dark red and consists of the erythrocytes, or red blood cells.

b. After centrifugation, place the collection tube in an ice bath until the serum is aspirated from the collection tube.
c. Using an appropriate disposable transfer pipette, aspirate off the serum down to approximately 1 mm from the erythrocyte layer.
d. Expel the serum into a 12 X 75 mm plastic tube and mix by drawing the serum up and down in the tube several times.
e. Aliqout recovered serum and place into labeled cryovials.
f. Cap all aliquots and immediately transfer to dry ice or to long-term freezer storage at -80°C or in liquid nitrogen.
g. Record position and location of the tubes.

Appendix I - Blood Collection/Processing Worksheet

The Blood Collection/Processing Worksheet may be customized by users to capture relevant information. The following may be used as a guide for relevant sets of information to record.

Blood Collection

Collection Site	
Date Blood is Drawn	
Time Blood is Drawn	
Date Specimen Received by Biobank	
Time Specimen is Received by biobank	
Name of Person Drawing Blood	
Additional Collection Notes:	

Specimen (blood collection tube) Information

Tube Label	Tube Type	Tube Lot#	Volume (mL)

Plasma tube Processing

Processed by (tech's name)	
Centrifugation speed	
Centrifugation temperature	
Centrifugation duration	
Time products frozen down	

Processed Plasma tube number	Tube 1	Tube 2	Tube 3	Tube 4
Volume				
Storage Location				

Buffy Coat tube number	Tube 1	Tube 2	Tube 3	Tube 4
Volume				
Storage Location				

Serum tube Processing

Processed by (tech's name)	
Centrifugation speed	
Centrifugation temperature	
Centrifugation duration	
Time products frozen down	

Processed Serum tube number	Tube 1	Tube 2	Tube 3	Tube 4
Volume				
Storage Location				