CTRNet Standard Operating Procedure Blood Processing and Storage			
SOP Number:	8.2.002	Version	e1.0
Supersedes:	SR 001.001	Effective Date	09 Jan 08
Subject:	Blood Processing and Storage	Category	Materials Handling and Documentation

Prepared By:		Jean de Sousa-Hitzler		
	Signature	Name	Title	ddMmmyy
Approved By:		Peter Geary	CEO	09 Jan 08
	Signature	Name	Title	ddMmmyy
Approved By:				
	Signature	Name	Title	ddMmmyy

REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
LP 001.001	2005		CTRNet Generic SOP for Blood Collection and Processing
8.2.002	2008		Revised to cover blood processing only.

1.0 PURPOSE

Blood samples are drawn from patients that have been through the informed consent process and agreed to participate in the tumour repository program. Blood samples are obtained by personnel qualified to draw blood from patients in the cancer centre, the hospital or in the physician's office. Processed blood products are an important tumour repository resource. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow for blood processing.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how blood should be processed, accessioned and stored. The SOP does not cover detailed safety procedures for handling blood and it is recommended that personnel follow institutional biosafety guidelines.

3.0 REFERENCE TO OTHER POLICIES AND SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 002.001 Ethics
3. CTRNet Policy: POL 004.001 Privacy and Security
4. CTRNet Policy: POL 007.001 Material and Information Handling Policy
5. CTRNet SOP: 8.1.002 Biohazardous Material Waste Management

4.0 RESPONSIBILITY

The policy applies to all laboratory personnel from CTRNet member repositories that are responsible for the processing of blood to obtain blood products for storage in the tissue bank. It also applies to personnel responsible for collection of the blood from the consented participant.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	Transport, Process and store blood and blood products	

5.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	**Materials and Equipment (Site Specific)**
Evacuated blood collection tubes for Plasma (e.g. Lavender top tube with EDTA)	Lavender top tube with EDTA)
Evacuated blood collection tubes (e.g. Tube for separating serum)	
Tube for extraction of nucleic acids from Blood (e.g. Paxgene tube)	
2.0 ml cryotubes	
Centrifuge	
Filter product for easy long term DNA storage (e.g. Whatman FTA Elute)	
Pipettors	
Transfer pipets	
Aerosol-resistant pipet tips	
Storage boxes	
Gloves worn to protect lab technician	

Appropriate racks to hold tubes while processing	
Sufficient appropriate labels (see SOP # 8.1.001) for collection tubes and Blood Collection/Processing Worksheets	
Blood Collection/Processing Worksheets (see Appendix 1 for sample form)	Site specific Name of form and version #

6.0 DEFINITIONS

Accessioning: The process of recording additions to the tissue bank collection in an inventory system or database.

Anticoagulant: A substance that prevents the clotting or thickening of blood.

EDTA: ethylenediamine tetra-acetate. The EDTA binds calcium ions thus blocking the coagulation cascade.

Plasma: Blood fraction that is remaining when erythrocytes have been removed from whole blood. Blood collection tube contains anticoagulant.

Buffy Coat: A thin grayish white layer of white blood cells (leukocytes and platelets) found covering the top of packed erythrocytes (red blood cells) of a hematocrit.

Serum: Liquid part of whole blood from which red cells and clotting proteins have been removed.

7.0 PROCEDURES

This procedure is intended to ensure that blood samples obtained from consented participants are processed in a safe and efficient manner while eliminating the risks of contamination and loss.

7.1 Timing for Blood Collection and Processing

1. Preferably, blood collection should be done pre-operation and as close as possible to the time when the tissue is donated to the repositories or at an alternative time, if appropriate for the research study
2. While time requirements for sample processing and cryopreservation are not as stringent as those for tumour tissue samples, it is recommended that plasma and buffy coat RNA be processed within 24 hours of removal from the patient. It is useful for downstream proteomic analysis of serum or plasma for the time of blood collection and aliquot freezing to be recorded in the database.

3. Communicate with personnel responsible for blood collection to determine whether blood has been collected and needs to be processed.

7.2 Verification of identification information on tubes.

Verify patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on blood collection tubes.

7.3 Separation of Plasma from the cellular fraction

The whole blood can be processed directly for DNA or RNA, or processed as described below to obtain a buffy coat fraction and plasma for cryopreservation.

1. In the area designated by the repository for processing blood, fractionate the whole blood (blood collected in tubes containing an anticoagulant such as EDTA or Heparin) by centrifuging at 1500-2000 x g for 15 minutes at room temperature. This will separate the blood into three visible layers (see Figure 1):
    - The upper layer is generally clear and pale yellow in colour.
    - The second layer is a narrow grayish white interface band representing the “buffy coat” or leukocyte fraction.
    - The third or bottom layer is dark red and consists of the erythrocytes or red blood cells.
2. 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 leukocyte or buffy coat layer.
3. Expel all plasma from the pipette into a plasma collection tube.
4. Aliquot recovered plasma and place into labelled cryovials.
5. Place the cryovials in dry ice or liquid nitrogen for freezer storage.
6. Transfer the cryovials to a freezer storage box and place the box immediately in the -80° C freezer or in liquid nitrogen.
7. Record position and location of the tubes.

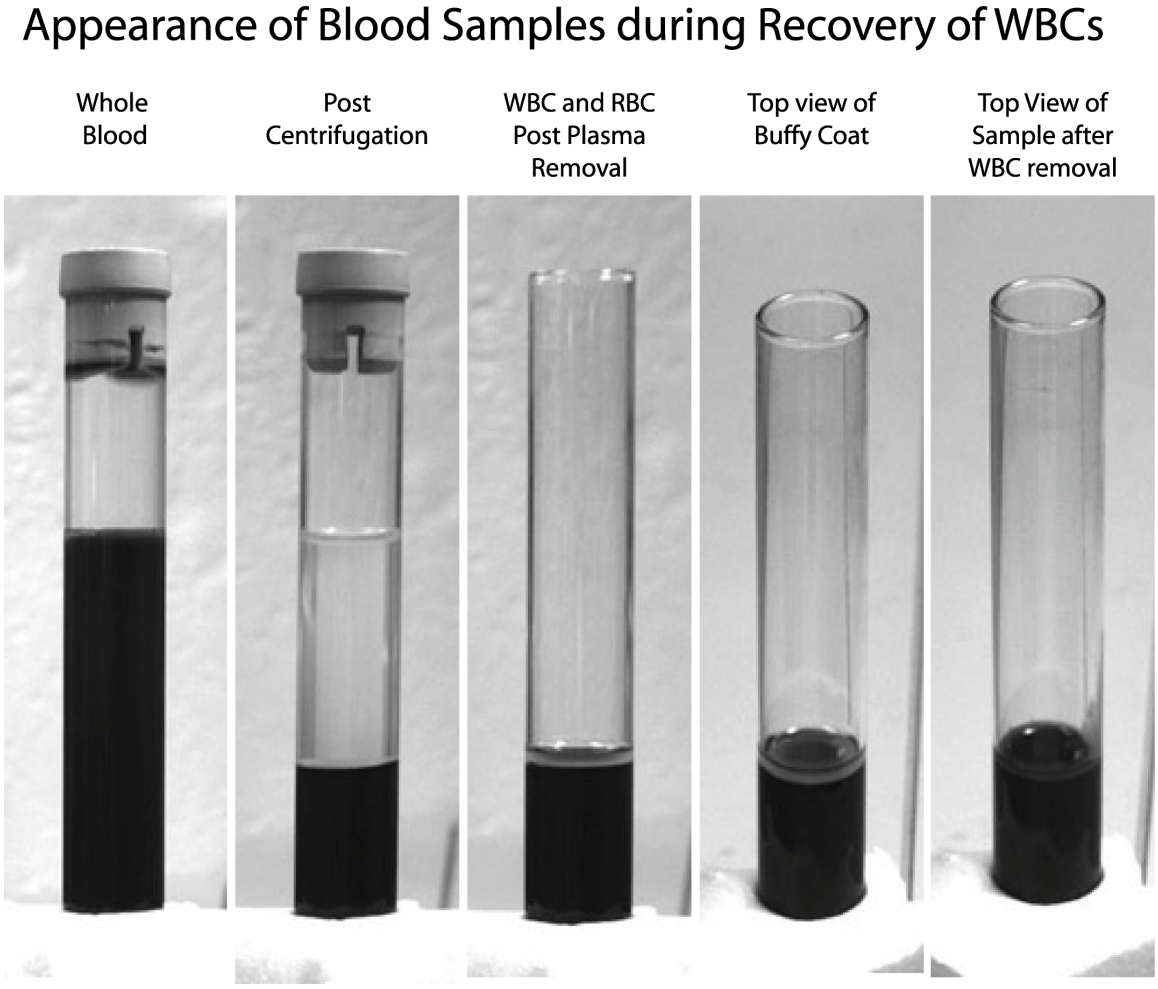

Figure 1: Blood Samples during WBC Recovery

7.4 Recovery of White Blood Cells

The cellular fraction can be processed directly for DNA or RNA, or processed as described below to obtain a buffy coat fraction and plasma for cryopreservation.

1. After removing the plasma layer, use a transfer pipette used to aspirate all of the buffy coat layer (usually a volume of 0.5 mL or less).
2. Expel the buffy coat into a single cryovial. Triturate the sample, and then take half the sample and store in a separate cryovial.
3. Place the cryovials in dry ice or liquid nitrogen for freezer storage.
4. Transfer the cryovials to a freezer storage box and place the box immediately in the -80° C freezer or in liquid nitrogen.
5. Record position and location of the tubes.

6. If blood has been collected in specific collection tubes for extraction of DNA or RNA then proceed with processing of these collection tubes as per established procedures for DNA and RNA processing and extraction).

7.5 Separation of serum from blood samples

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

2. Invert the tubes 8 times immediately following collection to ensure proper coagulation.

3. Incubate the mixed serum tubes for 1 hour at room temperature to ensure complete coagulation.

4. Prepare the required amount of 2 ml cryovials to be used for storage of the serum with the relevant labels on each tube.

5. Following incubation, centrifuge the serum tubes at 1500 g for 15 minutes.

6. Aspirate the supernatant and transfer directly to the labelled cryovials.

7. Transfer tubes to a freezer storage box and place the box in a -80° C freezer or in liquid nitrogen.

8. Record the position and location of the vials in the storage container.

7.6 Accessioning of samples

1. Accession plasma, serum and buffy coat samples into repository inventory database system as per established procedure for the site-specific inventory system and affix appropriate labels on the vials.

8.0 APPLICABLE REFERENCES, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3
http://www.wma.net/e/policy/b3.htm
2. Tri-Council Policy Statement; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series.
http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf

4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org

5. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999. http://bioethics.georgetown.edu/nbac/hbm.pdf

6. US National Biospecimen Network Blueprint http://www.ndoc.org/about_ndc/reports/NBN_comment.asp

7. Blood Collection: Routine Venipuncture and Specimen Handling. http://medlib.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html

Appendix 1. Forms (Blood Collection/Processing Worksheet)

The Blood Collection/Processing Worksheet can be customized by specific sites to capture information relevant to the site.

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 Sample Received by Processing Lab	
Time Sample is Received by Processing Lab	
Name of Person Drawing Blood	
Additional Collection Notes:	

Sample (tube) Information

Tube Label (Unique identifier)	**Tube Type**	**Tube Lot#**	**Volume (ml)**

DNA Filter Card (if used)

Unique identifier	**Card type**	**Lab Technician**	**Date and Time Created**	**Card Lot#**	**# of spots**

Plasma Processing

Processed by: Technicians name
Centrifugation: Duration of spin, G Force, Temperature
Time stored in Transporter:
Plasma Tubes Obtained:

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

Serum Processing

Processed by: Technicians name
Centrifugation: Duration of spin, G Force, Temperature
Time stored in Transporter:

Serum Tubes Obtained:

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

Buffy Coat Processing

Processed by: Technicians name
Centrifugation: Duration of spin, G Force, Temperature
Time stored in Transporter:
Buffy Coat (White Blood Cell) Tubes Obtained:

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