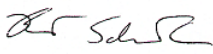


CTRNet Standard Operating Procedure Blood Processing and Storage			
SOP Number:	08.02.002	Version:	e2.0
Supersedes:	8.2.002 e1.0	Category:	Materials Handling and Documentation - Blood
Approved By:	CTRNet Management Group (CMG)		01-May-2012
	Per: Brent Schacter		13-June-2012

## 1.0 PURPOSE

Blood samples are drawn from patients that have been through the informed consent process and agreed to participate in the tumour biobank 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 biobank resource.

## 2.0 SCOPE

This 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 CTRNET SOPS OR POLICIES

*Note: When adopting this SOP for local use please reference CTRNet.*

- 3.1 CTRNet Policy: POL 5 Records and Documentation
- 3.2 CTRNet Policy: POL 2 Ethics
- 3.3 CTRNet Policy: POL 4 Privacy and Security
- 3.4 CTRNet Policy: POL 7 Material and Information Handling
- 3.5 CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material Waste Management
- 3.6 CTRNet Standard Operating Procedure: SOP 08.02.001 Blood Collection
- 3.7 CTRNet Standard Operating Procedure: SOP 06.003 Immunization and Personnel Accident Reports

## 4.0 ROLES AND RESPONSIBILITIES

The SOP applies to all laboratory personnel from CTRNet member biobanks that are responsible for the processing of blood to obtain blood products for storage in the tumour biobank. It also applies to personnel responsible for collection of the blood from the consented participant.

Tumour Biobank Personnel	Responsibility/Role
Laboratory Technician/Technologist	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 Laboratory Technician/Technologist	
Appropriate racks to hold tubes while processing	
Sufficient appropriate labels (see <i>CTRNet SOP 08.01.001</i> ) for collection tubes and Blood Collection/Processing Worksheets	
Blood Collection/Processing Worksheets (see Appendix A for sample form)	Site specific Name of form and version #

## 6.0 DEFINITIONS

See the CTRNet Program Glossary: <http://www.ctrnet.ca/glossary>

## 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

- 7.1.1 Preferably, blood collection should be done pre-operation and as close as possible to the time when the tissue is donated to the biobank or at an alternative time, if appropriate for the research study.

7.1.2 The time requirement for sample processing depends on the intended use and therefore, time to processing should be recorded.

## **7.2 Verification of Identification Information on Tubes**

As applicable, verify the accuracy of patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on blood collection tubes. Ensure that all personnel are trained in the use of the filing system.

## **7.3 Accessioning of Samples**

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

## **7.4 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.

7.4.1 In the area designated by the biobank for processing blood, fractionate the whole blood (blood collected in tubes containing an anticoagulant such as ethylene-diamineteraacetic acid-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):

- a) The upper layer is generally clear and pale yellow in colour.
- b) The second layer is a narrow grayish white interface band representing the "buffy coat" or leukocyte fraction.
- c) The third or bottom layer is dark red and consists of the erythrocytes or red blood cells.

7.4.2 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.

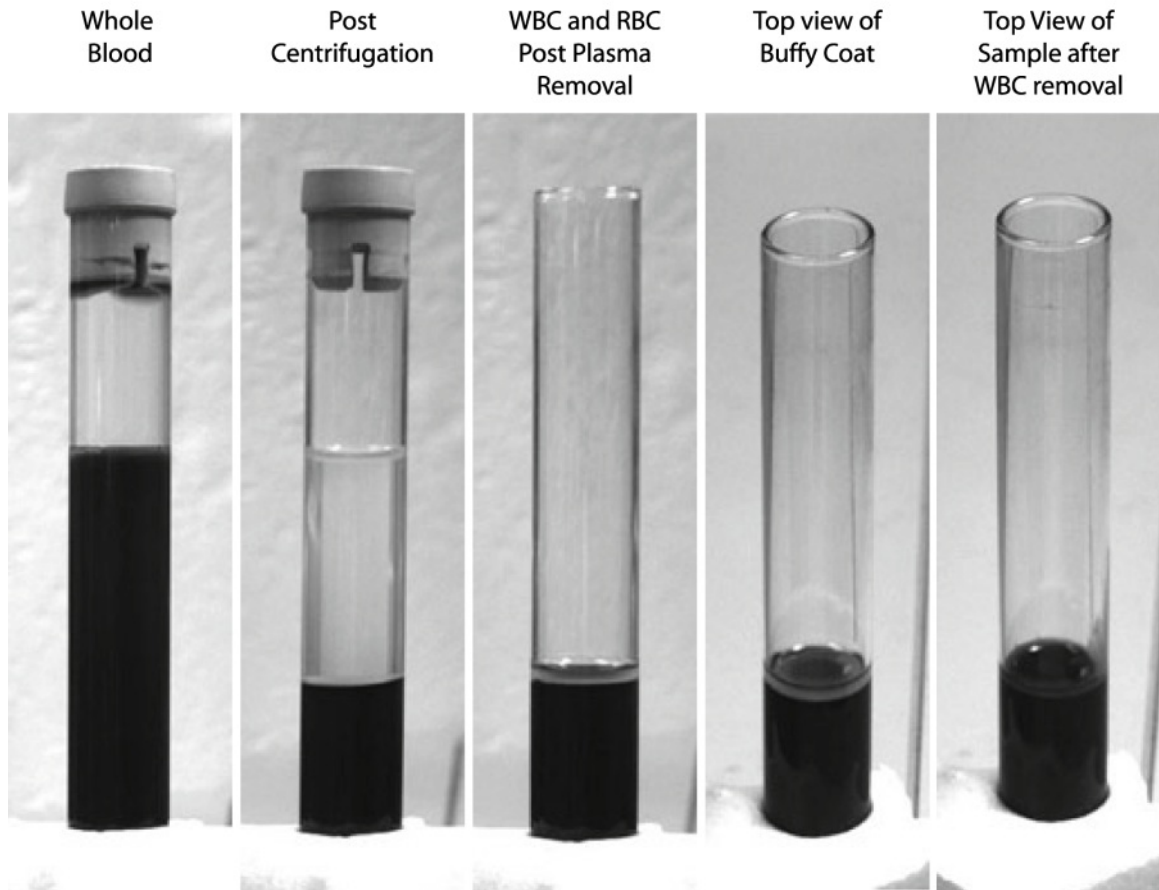
7.4.3 Expel all plasma from the pipette into a plasma collection tube.

7.4.4 Aliquot recovered plasma and place into labelled cryovials.

7.4.5 Place the cryovials in appropriate storage units. For long-term storage,  $-80^{\circ}\text{C}$  or colder is recommended.

7.4.6 Record position and location of the tubes.

## Appearance of Blood Samples during Recovery of WBCs



**Figure 1: Blood Samples during WBC Recovery**

### 7.5 Recovery of White Blood Cells

7.5.1 After removing the plasma layer, use a transfer pipette to aspirate all of the buffy coat layer (usually a volume of 0.5 mL or less).

7.5.2 Expel the buffy coat into a single cryovial. Triturate the sample, and then take half the sample and store in a separate cryovial.

7.5.3 Place the cryovials in appropriate storage units. For long-term storage,  $-80^{\circ}\text{C}$  or colder is recommended.

7.5.4 Record position and location of the tubes.

### 7.6 Separation of Serum from Blood Samples

7.6.1 If serum is to be obtained from the blood samples, collect the blood in appropriate tubes.

7.6.2 For non-gel serum tubes, invert the tubes 8 times immediately following collection to ensure proper coagulation.

7.6.3 Incubate to ensure complete coagulation. It is recommended to process within 1 hour.

- 7.6.4 Prepare the required amount of 2 ml cryovials to be used for storage of the serum with the relevant labels on each tube.
- 7.6.5 Following incubation, centrifuge the serum tubes at 1500 g for 15 minutes.
- 7.6.6 Aspirate the supernatant and transfer directly to the labelled cryovials.
- 7.6.7 Place the cryovials in appropriate storage units. For long-term storage, -80° C or colder is recommended.
- 7.6.8 Record the position and location of the vials in the storage container.

### **7.7 Alternate Processing of Blood Samples**

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.

## **8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES**

- 8.1 Declaration of Helsinki.  
<http://www.wma.net/en/30publications/10policies/b3/index.html>
- 8.2 Tri-Council Policy Statement 2; 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, December 2010.  
<http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/>
- 8.3 Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics  
<http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420>
- 8.4 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/Search/search.asp?zoom\\_query=best+practices+for+repositories](http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories)
- 8.5 US National Biospecimen Network Blueprint  
<http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp>
- 8.6 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>
- 8.7 Blood Collection: Routine Venipuncture and Specimen Handling.  
<http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html>

## **9.0 APPENDICES**

- 9.1 Appendix A – Blood Collection and Processing Worksheets

## 10.0 REVISION HISTORY

SOP Number	Date revised	Author	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.
8.2.002 e1.0	June 2012	CMG	<ul style="list-style-type: none"> <li>• Grammatical and formatting throughout</li> <li>• Definitions removed</li> <li>• Revision History moved to bottom</li> <li>• Reference links updates</li> <li>• Updated SOP references</li> <li>• Section 1.0: Deleted last sentence.</li> <li>• Section 7.1.2: Revised</li> <li>• Inserted Section 7.6 after 7.2</li> <li>• Sections 7.4 and 7.5 revised procedures</li> </ul>

## Blood Collection and Processing Worksheets

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 Laboratory	
Time Sample is Received by Processing Laboratory	
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	Laboratory Technician/Technologist	Date and Time Created	Card Lot#	# of spots

### Plasma Processing

**Processed by:** Technician/Technologist 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:** Technician/Technologist 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:** Technician/Technologist name

**Centrifugation:** Duration of spin, G Force, Temperature

**Time stored in Transporter:**

**Buffy Coat (White Blood Cell) Tubes Obtained:**

<b>Buffy Coat tube number</b>	<b>Tube 1</b>	<b>Tube 2</b>	<b>Tube 3</b>	<b>Tube 4</b>
<b>Volume</b>				
<b>Storage Location</b>				