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.

<table>
<thead>
<tr>
<th>Tumour Biobank Personnel</th>
<th>Responsibility/Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Technician/Technologist</td>
<td>Transport, process and store blood and blood products</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Materials and Equipment</th>
<th>Materials and Equipment (Site Specific)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evacuated blood collection tubes for plasma (e.g. Lavender top tube with EDTA)</td>
<td>Lavender top tube with EDTA</td>
</tr>
<tr>
<td>Evacuated blood collection tubes (e.g. tube for separating serum)</td>
<td></td>
</tr>
<tr>
<td>Tube for extraction of nucleic acids from blood (e.g. Paxgene tube)</td>
<td></td>
</tr>
<tr>
<td>2.0 ml cryotubes</td>
<td></td>
</tr>
<tr>
<td>Centrifuge</td>
<td></td>
</tr>
<tr>
<td>Filter product for easy long term DNA storage (e.g. Whatman FTA Elute)</td>
<td></td>
</tr>
<tr>
<td>Pipettors</td>
<td></td>
</tr>
<tr>
<td>Transfer pipets</td>
<td></td>
</tr>
<tr>
<td>Aerosol-resistant pipet tips</td>
<td></td>
</tr>
<tr>
<td>Storage boxes</td>
<td></td>
</tr>
<tr>
<td>Gloves worn to protect Laboratory Technician/Technologist</td>
<td></td>
</tr>
<tr>
<td>Appropriate racks to hold tubes while processing</td>
<td></td>
</tr>
<tr>
<td>Sufficient appropriate labels (see CTRNet SOP 08.01.001) for collection tubes and Blood Collection/Processing Worksheets</td>
<td>Site specific Name of form and version #</td>
</tr>
<tr>
<td>Blood Collection/Processing Worksheets (see Appendix A for sample form)</td>
<td></td>
</tr>
</tbody>
</table>

6.0 DEFINITIONS

See the CTRNet Program Glossary: [http://www.ctrnet.ca/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° C or colder is recommended.

7.4.6 Record position and location of the tubes.
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°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.

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.

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


8.5 US National Biospecimen Network Blueprint

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

<table>
<thead>
<tr>
<th>SOP Number</th>
<th>Date revised</th>
<th>Author</th>
<th>Summary of Revisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP 001.001</td>
<td>2005</td>
<td>CTRNet</td>
<td>CTRNet Generic SOP for Blood Collection and Processing</td>
</tr>
<tr>
<td>8.2.002</td>
<td>2008</td>
<td></td>
<td>Revised to cover blood processing only.</td>
</tr>
</tbody>
</table>
| 8.2.002 e1.0 | June 2012 | CMG    | • Grammatical and formatting throughout  
• Definitions removed  
• Revision History moved to bottom  
• Reference links updates  
• Updated SOP references  
• Section 1.0: Deleted last sentence.  
• Section 7.1.2: Revised  
• Inserted Section 7.6 after 7.2  
• Sections 7.4 and 7.5 revised procedures |
## 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

<table>
<thead>
<tr>
<th>Collection Site</th>
<th>Date Blood is Drawn</th>
<th>Time Blood is Drawn</th>
<th>Date Sample Received by Processing Laboratory</th>
<th>Time Sample is Received by Processing Laboratory</th>
<th>Name of Person Drawing Blood</th>
<th>Additional Collection Notes:</th>
</tr>
</thead>
</table>

### Sample (tube) Information

<table>
<thead>
<tr>
<th>Tube Label (Unique identifier)</th>
<th>Tube Type</th>
<th>Tube Lot#</th>
<th>Volume (ml)</th>
</tr>
</thead>
</table>

### DNA Filter Card (if used)

<table>
<thead>
<tr>
<th>Unique identifier</th>
<th>Card type</th>
<th>Laboratory Technician/Technologist</th>
<th>Date and Time Created</th>
<th>Card Lot#</th>
<th># of spots</th>
</tr>
</thead>
</table>

### Plasma Processing

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

### Plasma Tubes Obtained:

<table>
<thead>
<tr>
<th>Processed Plasma tube number</th>
<th>Tube 1</th>
<th>Tube 2</th>
<th>Tube 3</th>
<th>Tube 4</th>
<th>Volume</th>
<th>Storage Location</th>
</tr>
</thead>
</table>

### Serum Processing

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

### Serum Tubes Obtained:

<table>
<thead>
<tr>
<th>Processed Serum tube number</th>
<th>Tube 1</th>
<th>Tube 2</th>
<th>Tube 3</th>
<th>Tube 4</th>
<th>Volume</th>
<th>Storage Location</th>
</tr>
</thead>
</table>
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:

<table>
<thead>
<tr>
<th>Buffy Coat tube number</th>
<th>Tube 1</th>
<th>Tube 2</th>
<th>Tube 3</th>
<th>Tube 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage Location</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>