

Title	Blood Processing and Storage for Biorepositories
SOP Code	SOP111_01
Effective Date	01-Sep-2012

Site Approvals

Name and Title (typed or printed)	Signature	Date dd/Mon/yyyy

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures for biorepositories to follow for blood processing and storage. The SOP does not describe detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

This procedure applies to all personnel involved in blood collection, processing, and storage.

3.0 RESPONSIBILITIES

This procedure applies to all biorepository personnel responsible for the processing of blood to obtain blood products for storage in the biorepository. It also applies to personnel responsible for collection of the blood from the consented participant.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 Timing for Blood Collection and Processing

- 5.1.1 Preferably, blood collection should be done pre-operation and as close as possible to the time when the tissue is donated to the biorepositories, or at an alternative time, if appropriate for the research study.
- 5.1.2 It is recommended that plasma and buffy coat RNA be processed within 24 hours of removal from the participant. 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.
- 5.1.3 Communicate with personnel responsible for blood collection to determine whether blood has been collected and needs to be processed.

5.2 Verification of identification information on tubes.

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

5.3 Separation of Plasma from the Cellular Fraction

Note: 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.

- 5.3.1 Process the blood in the designated area. 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.
- 5.3.2 Using a 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.
- 5.3.3 Expel all plasma from the pipette into a plasma collection tube.
- 5.3.4 Aliquot recovered plasma and place into labelled cryovials.
- 5.3.5 Place the cryovials in dry ice until freezer storage.

- 5.3.6 Transfer the cryovials to a labelled freezer storage box and place the box immediately in the -80° C freezer or in liquid nitrogen.
- 5.3.7 Document the date and time of storage, as well as exact location of tubes in freezer.

Appearance of Blood Samples during Recovery of WBCs

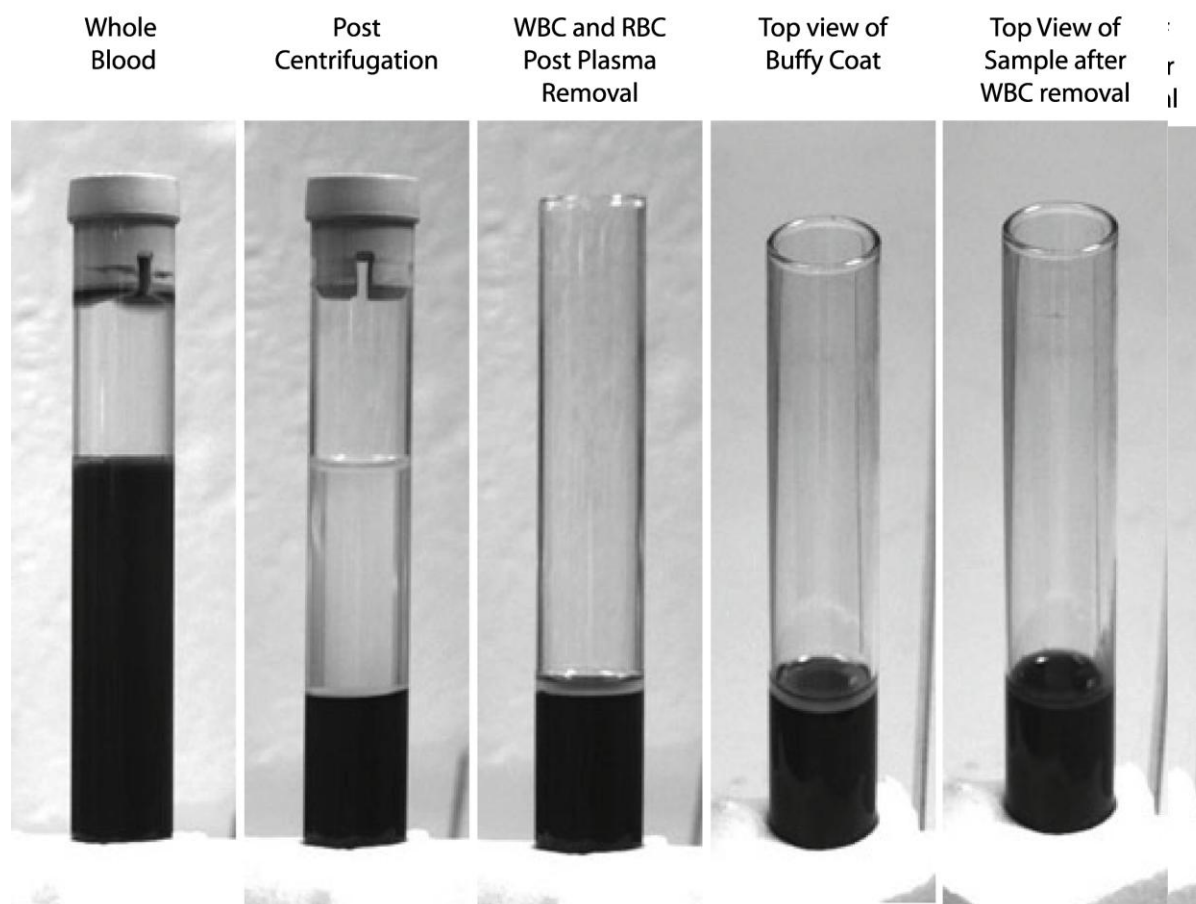


Figure 1: Blood Samples during WBC Recovery

5.4 Recovery of White Blood Cells (Buffy Coat)

- 5.4.1 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 from 10ml of whole blood).
- 5.4.2 Expel the buffy coat into a single labelled cryovial and then take half the sample and store in a separate labelled cryovial.
- 5.4.3 Place the cryovials in dry ice until freezer storage.

- 5.4.4 Transfer the cryovials to a labelled freezer storage box and place the box immediately in the -80° C freezer or in liquid nitrogen.
- 5.4.5 Document the date and time of storage, as well as exact location of tubes in freezer.

5.5 Separation of Serum from Blood Samples

- 5.5.1 Collect the whole blood in serum tubes coated with particles such as silica which act as a clotting activator.
- 5.5.2 Invert the tubes 8 times immediately following collection to ensure proper coagulation.
- 5.5.3 Incubate the mixed serum tubes for 1 hour at room temperature to ensure complete coagulation.
- 5.5.4 Following incubation, centrifuge the serum tubes at 1500 g for 15 minutes.
- 5.5.5 Aspirate the supernatant and transfer directly to the two labelled cryovials.
- 5.5.6 Place the cryovials in dry ice until freezer storage
- 5.5.7 Transfer tubes to a labelled freezer storage box and place the box in a -80° C freezer or in liquid nitrogen.
- 5.5.8 Document the date and time of storage, as well as exact location of tubes in freezer.

5.6 Accessioning of Samples

- 5.6.1 Accession plasma, serum, and white blood cell/buffy coat samples into biorepository inventory database system, as per established procedure for the site-specific inventory system. Affix completed labels on the vials.

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.



2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD.

<http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf>

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research. Cell Preservation Technology 6(1), 3-58, 2008 <http://www.isber.org/Pubs/BestPractices2008.pdf>

CTRNET Standard Operating Procedures, Canadian Tumour Repository Network, <http://www.ctrnet.ca/operating-procedures>



7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP111_01	01-Sep-2012	Original version