Blood Specimen Processing

Section:	**Biobank - OHIRC**	**SOP No:**	**B-3-002.01**
Issued By:	**Biobank**	**Supersedes:**	**B-3-002.00**
Approved By:	**Senior Management**	**Release Date:**	**April 9, 2021**
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1. PURPOSE

This SOP applies to UOHI Biobank personnel and outlines standardized procedures for blood processing. This SOP is intended to ensure that blood samples obtained from consented Biobank participants are processed in a safe and efficient manner to obtain products with high integrity and quality. This SOP has been adapted for the UOHI Biobank use from the CTRNet Standard Operating Procedure: Blood Processing and Storage.

2. INTRODUCTION

Blood products are a precious resource in the Biobank and procedures must be followed to obtain products with high integrity and quality. Blood samples will be processed by qualified personnel. This SOP does not cover detailed safety procedures for handling blood; personnel must follow institutional bio-safety guidelines.

3. ROLES AND RESPONSIBILITIES

Personnel	Responsibility / Role
Biobank Laboratory Technician / Biobank Manager / Research Coordinator / Research Assistant	Transport, Process and Store Blood and Derivatives

4. SUPPLIES

Evacuated blood collection tubes for plasma (e.g. Lavender top tube with K2 EDTA such as BD 367863)
Evacuated blood collection tubes for serum (e.g. Red top tube such as BD 367815, with silica clot activator)
Blood Collection and Processing Worksheet (see Appendix A for sample form)
1.4 ml cryovial tubes (1.0 ml working volume) with unique 2D barcode embedded in bottom of each
Caps for cryovial tubes
Cryorack storage boxes for cryovial tubes, with unique 1D barcode permanently affixed to cryorack
Racks to hold tubes while processing

Microtubes (2 to 5 ml) for pooling buffy coat
Centrifuge (swinging bucket rotor with bio containment lid)
Small ice bucket
Pipettors
Transfer pipets
Filter pipet tips
Hamilton Robot (used when volume dictates)
Hamilton scanner/capper/de-capper
PPE (lab coat, gloves, benchtop biohazardous shield)
TRIzol Isolation Reagent (or equivalent)

5. PROCEDURES

5.1 Timing for Blood Processing

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

5.1.2 Responsible personnel transport labelled tubes to the specified Biobank lab area for processing blood samples.

5.1.3 Transport tubes at room temperature. Do not allow the samples to freeze or be exposed to an ambient temperature of greater than 25° C.

5.2 Verification of Information

5.2.1 Assess risk group and apply appropriate containment level precautions according to the medical record of the patient.

5.2.2 Verify participant 1D barcode number on accompanying Blood Collection and Processing Worksheet and ensure that it corresponds with the information on labels on blood collection tubes.

5.3 Separation of Plasma from the Cellular Fraction

5.3.1 In the designated Biobank lab, process whole blood collected in purple top EDTA tubes (EDTA acts to prevent clotting). Tubes will have been inverted 8 times immediately following collection by responsible personnel to ensure proper mixing of EDTA.

5.3.2 Process whole blood as described below to obtain a buffy coat fraction and plasma for cryopreservation.

5.3.3 Store purple top EDTA tube upright at 4°C until centrifugation. Blood samples for plasma and buffy coat should be centrifuged within four hours of collection.

5.3.4 Fractionate the whole blood (blood collected in purple tubes containing EDTA) by centrifuging at 1500 x g for 15 minutes at 4°C. This will separate the blood into three visible layers:

- 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.

5.3.5 Using a disposable transfer pipette, or the Hamilton Robot, aspirate the plasma layer down to approximately 1 mm from the buffy coat layer. Take care not to disturb leukocyte or buffy coat layer.

5.3.6 Aliquot recovered plasma and place into 2D labelled cryovials.

5.3.7 Discard visibly hemolyzed plasma (pink or red tint).

5.3.8 Apply purple screw caps manually or with capper/decapper. Check that all cryovial caps are secure.

5.3.9 Place the cryovials on ice until freezer storage.

5.3.10 Transfer the cryovials to a 1D labelled freezer storage box and place the box immediately in the designated -80° C freezer or in liquid nitrogen.

5.3.11 Store plasma cryovials from multiple participants in cryovial rack for plasma with purple caps.

5.4 Recovery of White Blood Cells (Buffy Coat)

5.4.1 After removing the plasma layer, use a transfer pipette to aspirate all of buffy coat layer (usually a volume of 1.0 mL or less).

5.4.2 Expel the buffy coat into a single labelled microtube. Triturate the sample.

5.4.3 Aliquot recovered buffy coat into 2D labelled cryovials as follows:

- Two plain cryovials.
- Two cryovials containing TRIzol or equivalent 3:1.

5.4.4 Manually apply or utilize capper/decapper (when volume dictates) to apply screw caps as follows:

- White screw caps to plain cryovials with buffy coat
- Blue screw caps to cryovials with buffy coat containing TRIzol or equivalent

5.4.5 Check that all cryovial caps are secure.

5.4.6 Place the cryovials on ice until freezer storage.

5.4.7 Transfer the cryovials to a 1D labelled freezer storage box and place the box immediately in the designated -80° C freezer or in liquid nitrogen.

5.4.8 Buffy coat cryovials from multiple participants will be stored in cryovial racks as follows:

- Plain buffy coat with white caps (for later DNA retrieval) and for
- Buffy coat with TRIzol with light blue caps (for later RNA retrieval).

5.5 Separation of Serum from Blood Samples

5.5.1 Process whole blood collected in serum tubes (red tubes coated with silica which acts as a clotting activator). Tubes will have been inverted 8 times immediately following collection by responsible personnel to ensure proper coagulation.

5.5.2 Incubate the mixed serum tubes for 1 hour at room temperature to ensure complete coagulation. 90 minutes may be required for samples from participants on anticoagulant therapy.

5.5.3 Following incubation, centrifuge the serum tubes at 1500 x g for 15 minutes at 4°C.

5.5.4 Aspirate the supernatant and transfer directly to the 2D labelled cryovials.

5.5.5 Apply red screw caps manually or with capper/decapper. Check that all cryovial caps are secure.

5.5.6 Place the cryovials on ice until freezer storage.

5.5.7 Transfer tubes to a 1D labelled freezer storage box and place the box in the designated -80° C freezer or in liquid nitrogen.

5.5.8 Store serum cryovials from multiple participants in cryovial rack for serum with red caps.

5.6 Accessioning of Samples

5.6.1 Complete the Blood Collection and Processing Worksheet with the date and time of plasma, buffy coat and serum processing and storage. Document the exact location of tubes in freezer.

5.6.2 Accession plasma, serum, and white blood cell/buffy coat samples into biorepository inventory database system. Utilize scanner (Hamilton or hand scanner) to accession the 2D labels embedded in bottom of cryovials and 1D labels permanently affixed to cryoracks.

5.6.3 Store respective cryovial racks with serum, plasma, buffy coat and buffy coat with TRIzol (or equivalent) in the designated -80 °C freezer at UOHI equipped with audible or remote alarm, for eventual transfer to LN2 vapour phase tanks at OHRI (refer to SOP B-3-003 for details).

5.6.4 Observe colour coding system to identify type of blood derivative as follows:

Derivative	Cryovial Cap Colour	Anticipated number of aliquots
Plasma	Purple	6
Buffy coat	White	2
Buffy coat with TRIzol	Light blue	2
Serum	Red	4

6. ACRONYMS

OHIRC - Ottawa Heart Institute Research Corporation
SOP- Standard Operating Procedure
UOHI - University of Ottawa Heart Institute
PPE – Personal Protective Equipment

7. RELATED REGULATIONS AND/OR POLICIES

References:

7.1 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/

7.2 ICH Guidance E6: Good Clinical Practice: Consolidated guideline http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/ich/efficac/e6-eng.php

7.3 Declaration of Helsinki. http://www.wma.net/en/30publications/10policies/b3/index.html

7.4 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

7.5 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

7.6 US National Biospecimen Network Blueprint. http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

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

7.8 OTRN OICR Standard Operating Procedures for Biorepositories, Ontario Tumor Repository Network/Ontario Institute for Cancer Research http://oicr.on.ca/search/apachesolr_search/SOPs%20for%20Biorepositories

7.9 Elliot, P., and Peakman, T., The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International Journal of Epidemiology 2008:37:234-244

7.10 Standard Operating Procedures for Serum and Plasma Collection: Early Detection Research Network Consensus Statement Standard Operating Procedure Integration Working Group Melissa K. Tuck,*† Daniel W. Chan http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2655764/#R9

7.11 UOHI Laboratory Safety and Procedures Manual https://hearthub.ottawaheart.ca/departments-services/occupational-health-safety-and-biosafety/laboratory-safety-and-procedures-manual

Associated OHIRC SOPs:

UOHI, Occupational Health and Safety SOP-2-11 - Transporting Biological Substances within the Heart Institute Including between Laboratories to the Animal Care Services

8. APPENDICES

8.1 Appendix A – Blood Collection and Processing Worksheet Example

9. HISTORY

SOP #	Effective Date	Review Date	Summary of Changes
B-3-002.00	16 Oct, 2015		Elaborate on PPE

Appendix A – Blood Collection and Processing Worksheet Example

Phlebotomy Date: __ __ /__ __ __ / __ __ __ __ Time: __ __ __ __
Day Month Year hh:mm

Last consumed calories Date: __ __ /__ __ __ / __ __ __ __ Time: __ __ __ __
Day Month Year hh:mm

Last Moderate/Vigorous Exercise, Sustained ≥ 30 minutes: ☐ None in last 7 days or Date: __ __ /__ __ __ / __ __ __ __Time: __ __ __ __
Day Month Year hh:mm

Order of draw:	1) ☐ 6 ml red top #1	2) ☐ 6 ml red top #2	3) ☐ 6 ml EDTA #1	4) ☐ 6 ml EDTA #2	5) ☐ 6 ml EDTA #3
	☐ hemolyzed	☐ hemolyzed	☐ hemolyzed	☐ hemolyzed	☐ hemolyzed

Phlebotomist: ☐ Biobank staff initials: _____ ☐ UOHI Phlebotomist initials: _____ ☐ UOHI research coordinator initials: _____

Needle Gauge: ☐ 21G ☐ 23G Site: ☐ superficial vein, antecubital fossa ☐ other venous site_____

CRF Completed by: _____

Samples Received in Laboratory: __ __ /__ __ __ / __ __ __ __ Time: __ __ __ __
Day Month Year hh:mm

Purple top tube		
Centrifugation Time	Speed	Temperature
__ __ __ __ hh:mm	☐ 1500 x 15 minutes	☐ 4°C

Plasma and Buffy Coat		
Aliquoting Time		Stored in – 80° C freezer Time
__ __ __ __ hh:mm		__ __ __ __ hh:mm
Number of Plasma Aliquots (purple) _____	Number of Buffy Coat Aliquots (white/DNA) _____	Number of Buffy Coat & TRIzol Aliquots (light blue/RNA) _____

Red top tube		
Stand room temperature	☐ 60 minutes	☐ 90 minutes (participant on anticoagulant)
Centrifugation Time	Speed	Temperature
__ __ __ __ hh:mm	☐ 1500 g x 15 minutes	☐ 4°C

Serum		
Aliquoting Time	Stored in – 80° C freezer Time	Number of Serum Aliquots (red)
__ __ __ __ hh:mm	__ __ __ __ hh:mm	_____

Completed by: _____