Obafemi Awolowo University Teaching Hospitals Complex – ARGO Biobank Center
P.M.B. 5538
Ile-Ife, Osun State
Nigeria

Standard Operating Procedure Manual for Blood Processing and Storage

1.0 PURPOSE

Blood samples are drawn from patients that have agreed to submit their samples for research purposes. Blood samples are obtained by personnel qualified to draw blood from patients at Obafemi Awolowo University Teaching Hospitals Complex or any of the ARGO sites. Processed blood products are an important resource for future research studies.

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 and our safety manual.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to personnel at all ARGO member sites that are responsible for the processing of blood to obtain blood components for storage in the tissue biobank.

Biobank Personnel	**Responsibility/Role**
Research Assistants	Receive, process, and store blood and blood products.

4.0 MATERIALS, EQUIPMENT AND FORMS

Materials and Equipment
Evacuated blood collection tubes for plasma (e.g. lavender top tube with EDTA)
Evacuated blood collection tubes for serum (e.g. red top tube)
2.0 ml cryovials
Centrifuge

Transfer pipettes or micropipettes
Storage boxes
Personal protective equipment
Appropriate racks to hold tubes while processing
Appropriate labels for collection tubes and blood collection/processing worksheets
Blood collection/processing Log Book

5.0 PROCEDURES

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

5.1 Timing for Blood Collection and Processing

5.1.1 Blood collection can be done before the surgical procedure.

5.1.2 The time requirement for sample processing depends on the intended use and therefore, amount of time to process the sample should be recorded.

5.1.3 All red top tubes should stand at room temperature for a minimum of 2 hours to allow for proper coagulation.

5.1.4 All blood tubes containing anticoagulant (blue, purple, etc) should be rocked gently before processing to ensure thorough mixing of the blood with the anticoagulant.

5.2 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 and requisitions.

5.3 Accessioning of Samples in the Blood Log Book

5.3.1 Log in the plasma, serum, and buffy coat samples generated along with information about the patient and time of collection into the **Biobank Blood Log Book**.

5.3.2 Assign a unique blood number BLD that will be used to de-identify the sample and label all the aliquots.

5.4 Accessioning in REDCAP:

5.4.1 Enter patient **MRN** and under **Accession Number** and save the information.

5.4.2 Under **Part Information,** select the appropriate blood evacuated tube type. For example, “Purple Top/EDTA”.

5.4.3 If multiple tubes of the same type are drawn (for instance, 2 red tops), the two red tops will have the same part number but will have different subpart numbers to distinguish the two.

5.4.4 If different types of tubes are drawn (red, blue, purple, etc) then each type will have a distinct part number.

5.4.5 Under **Site Specify,** enter whether the sample is “pre-op” or “post-op”.

5.4.6 The order in which samples will be accessioned and filed away should be in this order: **Serum, Buffy coat, and Plasma**

5.4.7 Under **Biobank Details**, select the appropriate type of aliquot generated from each tube. For example, “plasma”, “serum” or “buffy coat”.

5.4.8 Enter the **box name** and **number** in which the blood sample would be filed in permanent storage. For example, CRC-14, Brst-10, HCC-3. Enter the slot in which individual aliquots will be stored.

5.4.9 Bloods / blood products will be stored in -80°C freezer for long term storage.

5.5 Separation of Plasma from the Cellular Fraction

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

5.5.1 In the area designated by the surgical laboratory for processing blood, fractionate the whole blood (blood collected in tubes containing an anticoagulant such as Ethylene-Diamine Tetraacetic Acid-EDTA or Heparin) by centrifuging at 2500 RPM for 10 minutes at a temperature of 4°C. This will separate the blood into three visible layers:

a. The upper layer or the plasma is generally clear and pale yellow in color.
b. The second layer is a narrow grayish white interface band representing the “buffy coat” or leukocyte/platelet fraction.
c. The third or bottom layer is dark red and consists of the erythrocytes or red blood cells.

5.5.2 Use the refrigerated centrifuges **Beckman Coulter Allegra X22R or Allegra X30R** centrifuges for separation of both plasma and serum at 4°C.

5.5.3 Using an appropriate transfer pipette or micropipette, aspirate the plasma layer down to approximately 1mm from the buffy coat layer. Take care not to disturb the leukocyte or buffy coat layer.

5.5.4 Expel all plasma from the pipette into a smaller Eppendorf tube.

5.5.5 Centrifuge the smaller Eppendorf tubes using the Beckman Coulter Microfuge 20R for 10 minutes, 18000 g, at a temperature of 4°C.

5.5.6 Aliquot recovered plasma and place into multiple labelled cryovials. About 1 ml should be placed in each cryovial.

5.5.7 Place the cryovials in a NALGENE Cryo Freezing Container. This container is used to freeze down biological samples at a slower and controlled rate.

- Fill the freezing container with 250 ml of 100% isopropyl alcohol. Do not overfill the container.
- Add the foam insert in the container and place the cryovials into the vial holder of the container.

5.5.8 Place the NALGENE Cryo Container into the -80°C Freezer Revco for a minimum of 4 hours.

5.5.9 The cryovials will remain in the NALGENE Cryo until the following weekday. The samples will then be stored in their final storage box in the freezer.

5.5.10 Record the box number and location of the tubes in RedCap.

5.6 Recovery of White Blood Cells

5.6.1 After removing the plasma layer, use a pipette to aspirate the entire buffy coat layer (usually a volume of 0.5ml or less).

5.6.2 Transfer the buffy coat into a single cryovial.

5.6.3 Place the cryovials and store overnight in the NALGENE Cryo Container and place the cryovials for final long-term storage in the -80°C freezer the following day.

5.6.4 Record the box number and location of the tubes.

5.7 Separation of Serum from Blood Samples

5.7.1 If serum is to be obtained from the blood samples, collect the blood in appropriate tubes, for example, red top tubes.

5.7.2 For serum blood tubes, invert the tubes 8 times immediately following collection to ensure proper coagulation.

5.7.3 If immediately received after collection, incubate at room temperature and it is recommended to process after 2 hour of collection to ensure proper coagulation.

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

5.7.5 Centrifuge the serum tubes at 2500 RPM for 10 minutes at a temperature of 4°C.

5.7.6 Use the refrigerated centrifuges Beckman Coulter Allegra X22R or Allegra X30R Centrifuges for separation serum from blood cells.

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

5.7.8 The cryovials will remain in the NALGENE Cryo Container until the following weekday. The samples will then be stored in their final storage box in the -80°C freezer.

5.7.9 Record box number and location of the tubes.

5.8 Blood Sample Records

5.8.1 Blood sample information for a specific study, in addition to being documented in the Blood Log Book, should be documented in the appropriate excel file in the Shared Drive.

7.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

7.1 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

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

7.3 Blood collection: Routine Venipuncture and Specimen Handling. http://library.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html

8.0 REVISION HISTORY

SOP Number	Date Reviewed	Reviewed By	Comments