STANDARD OPERATING PROCEDURE	**SOP No. 003** **Blood Collection and Processing**
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Electronic Filename: PCBN.SOP03.v1.3 Blood Collection and Processing	

1. PURPOSE

To describe the procedure for the collection of blood samples and it's processing into derivative blood products, namely plasma, serum and buffy coat. NOTE: This SOP does not cover detailed safety procedures for handling blood and personnel must follow institutional bio-safety guidelines.

The following SOP for RNA extraction from frozen tissue is followed by the JHU, NYU Network Sites of the PCBN.

2. RESPONSIBILITIES

Authorized personnel collecting and processing participant blood must ensure that:

- all procedures are followed correctly
- all samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Calibrated p1000 and p200 micropipettes Centrifuge Counter-balance tubes Electronic pipetteman PPE (gloves, lab coat/gown, eye/face shield) Transport container (eg. Playmate Igloo Thermos)	1.8ml Cryovials / Conical tubes Cryoboxes / Freezer boxes 2.0ml glass pipettes Ethanol / Alcohol wipes Kimwipes p1000 and p200 aerosol pipette tips Pink top vacutainer tubes Purple top vacutainer tubes Red top vacutainer tubes Serum separator Sterile plastic pasteur pipettes

5. PROCEDURES

5.1 Blood Collection

NOTE: The collection of blood must be performed by personnel qualified by training to draw blood.

- Once the participant blood is drawn, the designate will be alerted.

  Designate: the person responsible for obtaining the blood sample - JHU: Prostate Specimen Technician, NYU: Research Coordinator.

- After notification, the designate retrieves the specimen.

Johns Hopkins University Network Site

- Blood specimens are retrieved from:
  - Express Testing (1st floor of the Outpatient Center, 5-1682)
- Each participant should have the following vials of blood drawn:
  - Two (2) red top vacutainer tubes (10ml)
    NOTE: The tube should be inverted 5 times to allow clot activator to mix with blood, and allowed to clot for 30mins at room temperature.
  - One (1) purple top vacutainer tube (6ml)

NOTE: Specimens should be retrieved and processed within 3hrs of blood collection.

New York University Network Site

- Blood is drawn pre-operatively, either at time of consent or pre-admission testing.
- Blood specimens are retrieved from:
  - Smilow Comprehensive Prostate Cancer Center
  - Tisch Hospital Pre-Admission Testing Facility
- Each participant should have the following vial of blood drawn:
  - One (1) pink top vacutainer tube (4.5ml)

NOTE: Specimens should be retrieved immediately after notification (where possible) and processed at earliest convenience.

Ensure to verify patient information (in keeping with privacy and ethical policies) and that it corresponds with the information on the labels on blood collection tubes.

- The designate delivers the specimen to the Biorepository Laboratory.

NOTE: When transporting blood specimen(s), an appropriate transport container should be utilized to contain associated biohazards. Recommend - White Playmate Igloo Thermos.

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 Blood Processing

- Once at the Biorepository Laboratory, the blood specimen(s) is/are handled according to optimized techniques for the respective institution.

  NOTE: Processing is performed by the Prostate Specimen Technician / Research Coordinator unless the responsibility is delegated to a qualified technician.

Separation of serum from blood samples
Johns Hopkins University Network Site

- Confirm that the blood clot inside the red top vacutainer tube is not attached to the underneath of the blood tube lid. If the clot is attached, gently tap the tube bottom on the counter-top to dislodge the clot.
- Holding the vacutainer tube away from your face and over the top of the biohazard box, wrap the lip of vacutainer tube with large kimwipe and gently twist to remove blood tube lid. This will avoid exposure to any aerosols created by opening the vacutainer tubes. Place lid/kimwipe in biohazard box and return the vacutainer tube to the rack.
- Place a serum separator in each red top vacutainer tube. The open end of the separator should be towards the bottom of the tube.
- Centrifuge the serum tubes at **1800 g** for **15mins** using the designated centrifuge (IEC/Centra centrifuge).

  NOTE: Ensure the centrifuged is loaded correctly (evenly displaced on each side) and is balanced (mirroring each side). Examine the volume of blood between pairs of tubes – ensure they are roughly equivalent. A balance tube must be created if there is an odd number tubes.

- Using a sterile 2.0 ml glass pipette and electronic pipetteman, aspirate the supernatant (serum) and dispense the supernatant (serum) into labeled 1.8 ml plastic conical vials in 1.0 – 1.5ml aliquots.
- Transfer tubes to their respective freezer storage box and store at -80°C.
- Complete all documentation.

  Naming Convention for Serum: (There are five components to the ID number for serum)

  1. Site: JHU, NYU, etc. (Use three letters for site.)
  2. Patient consecutive number: 001, 002, etc
  3. S for serum
  4. Aliquot: a = 1.0 mL aliquot b = 1.5 mL aliquot
  5. Visit: 0 = baseline 1 = 1st post-Tx follow-up 2 = 2nd post-Tx follow-up

Fractionation of plasma and buffy coat from blood samples

Johns Hopkins University Network Site

- Centrifuge the EDTA tube at **1800 g** for **15mins** using the designated centrifuge (IEC/Centra centrifuge).

  NOTE: Ensure the centrifuged is loaded correctly (evenly displaced on each side) and is balanced (mirroring each side). Examine the volume of blood between pairs of tubes – ensure they are roughly equivalent. A balance tube must be created if there is an odd number tubes.

- Using a sterile 2.0 ml glass pipette and electronic pipetteman, aspirate the supernatant (plasma) and dispense the supernatant (serum) into labeled 1.8 ml plastic conical vials in 0.5 – 1.0ml aliquots.

  Naming Convention for Plasma:

  1. Site: JHU, NYU, etc. Just use three letters for site.
  2. Patient consecutive number: 001, 002, etc
  3. P for plasma
  4. Visit: 0 = baseline 1 = 1st post-Tx follow-up 2 = 2nd post-Tx follow-up

- Using a non-sterile transfer pipette, dispense the buffy coat cells (~200ul) into a labeled 1.8 ml conical vial.

  Naming Convention for Buffy Coat:

  1. Site: JHU, NYU, etc. Just use three letters for site.
  2. Patient consecutive number: 001, 002, etc
  3. B for buffy coat
  4. Visit: 0 = baseline 1 = 1st post-Tx follow-up 2 = 2nd post-Tx follow-up

- Transfer tubes to their respective freezer storage box and store at -80°C.
- Complete all documentation.

New York University Network Site

- Centrifuge the EDTA tube at **1300 x g (3000rpm)** for **10mins** using the designated centrifuge (accuSpin centrifuge).

  NOTE: Ensure the centrifuged is loaded correctly (evenly displaced on each side) and is balanced (mirroring each side). A balance tube must be created if there is a single/odd number tubes. Pre-prepared balance tubes are stored beside the designated centrifuge. Examine the volume between pairs of tubes – ensure they are roughly equivalent.

- Wipe the top and outside of the blood tube with alcohol before opening.

- Using a calibrated p1000 micropipette with an aerosol tip or sterile disposable plastic Pasteur pipette, dispense the supernatant (plasma) into labeled 1.8 ml plastic cryovials in 100ul aliquots.

  NOTE: Care must be taken to ensure that the buffy coat layer is not disturbed when dispensing the plasma aliquots.

- Using a calibrated p200 micropipette with an aerosol tip or sterile disposable plastic Pasteur pipette, dispense the buffy coat cells (~200ul) into a labeled 1.8 ml plastic cryovial.
- Transfer tubes to their respective freezer storage box and store at -80°C\
- Complete all documentation.

  NOTE: Irrespective of the procedure, care must be taken to ensure processing uses aseptic techniques.