STANDARD OPERATING PROCEDURE	**SOP No. 009** **Processing of Blood and Cytology Samples in Cancer Biomarkers Team ICR**
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Electronic Filename: PCBN.SOP09.v1.0 Blood and Cytology Samples	

1. PURPOSE

To describe the procedures for processing of blood, buccal swab, saliva and urine samples by the Cancer Biomarker (CB) Team at the Institute of Cancer Research. This procedure applies to all the blood, buccal swab, saliva and urine samples received by the CB team for further processing.

The following SOP for Blood and Cytology Sample processing is followed by the Institute of Cancer Research Network Site of the PCBN ONLY.

2. RESPONSIBILITIES

Authorized personnel processing blood and cytology samples must:

- Comply with the procedure
- Sample manipulation must be carried out in a class II cabinet, or in sealed tubes when using the centrifuges
- Follow all other precautions required for the handling of human blood and tissue samples
- Obey Team Leader who has overall responsibility of the procedure
- All blood and cytology samples received are logged into the Progeny LIMS database. A unique number (Progeny Number) generated shall be used for documentation purposes.

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
Class II Cabinet Centrifuge	DNA Streck Tubes RNA Streck Tubes 1 mL Microtubes 15 mL conical Tubes PAXgene™ Tubes SST Vacutainer EDTA Vacutainer Buccal Swabs

5. PROCEDURES

5.1 Blood Samples- DNA Streck Tube

- DNA streck tube is spun using a swinging bucket centrifuge at 1800 RCF for 15 minutes at room temperature with no brake.
- For trials that started before the start of July 2016:
    - Plasma is aliquoted into 1 mL micro tubes (2 mL plasma per tube). Care must be taken to avoid the white blood cells located closest to the blood/plasma barrier.
    - After the plasma is removed, draw up the white blood cell layer and place into a fresh 2 mL micro tube and cap with a yellow/white cap. Label this DNA WBC.
- For trials that started after the start of July 2016:
    - The whole volume of plasma is drawn and aliquoted into a 15 mL conical tube. Care must be taken to avoid the white blood cells located closest to the blood/plasma barrier.
    - The 15 mL conical tube is spun at 3000 RCF for 10 minutes (with brake) at room temperature. Aliquot the supernatant into fresh 2 mL micro tubes (cap with clear/white caps) (1 mL plasma per tube).
    - After the plasma is removed draw up with white blood cell layer and place this into a fresh 2 mL micro tube and cap with a yellow/white cap. Label this DNA WBC.
- All tubes are labeled and transferred to a -80°C freezer for storage.

5.2 Blood Samples- RNA Streck Tube

- The RNA Streck tube is spun using a swinging bucket centrifuge at 300 RCF for 20 minutes at room temperature with no brake.
- The whole volume of plasma is drawn and aliquoted into a 15 mL conical tube. Care must be taken to avoid the white blood cells located closest to the blood/plasma barrier.
- The 15 mL conical tube is spun at 3000 RCF for 10 minutes (with brake) at room temperature. Aliquot the supernatant into fresh 1 mL micro tubes (cap with green/white caps) (2 mL plasma per tube), label and transfer to -80°C freezer for storage.
- After the plasma is removed, draw up the white blood cell layer and place this into a fresh 2 mL micro tube and cap with a yellow/white cap. Label this RNA WBC and store -80°C.

5.3 Serum Specimens (Collected at pre-treatment)- SST Tube

- Serum is obtained from whole blood collected in vacutainer SST tubes with no additives.
- Allow the blood specimen to clot for 45 minutes (minimum of 30 minutes and maximum of 60 minutes is acceptable) at room temperature and then place at 4°C (on ice).
- Sample should remain on ice no longer than 4 hours after blood draw, if not processed immediately. Record the time the sample was placed on ice on the Blood requisition form.
- Centrifuge sample 15 minutes at 1600 RCF, 4°C.
- Using sterile pipette, aliquot 1 mL of serum into each of 2 mL micro tubes (cap with red/white caps). Use red caps for serum and label the tubes. Freeze at -80°C.
- If specimens are not frozen within 4 hours of collection, record samples as defective on the Blood.

5.4 Plasma and Buffy Coat Specimens (Collected at pre-treatment)- EDTA Tube

- EDTA Plasma is obtained from whole blood collected in a 10 mL purple top plastic vacutainer tube containing EDTA.
- The blood specimen should be placed immediately on ice or 4 °C. Sample should remain on ice no longer than 4 hours after blood draw.
- Centrifuge sample for 15 minutes at 1600 RCF, 4°C.
- Using a sterile pipette, aliquot 1 mL of plasma into each of 2 mL micro tubes. Remove plasma slowly and carefully so as not to disrupt the layer of white blood cells (see Figure 1.). Do not place the pipette tip near the buffy coat. Leave at least 1000 uL of residual plasma at the buffy coat interface.

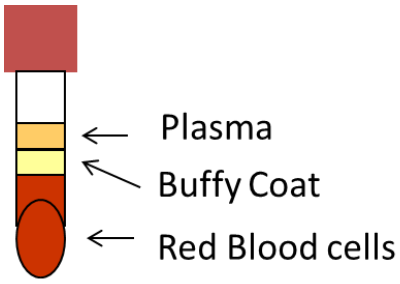

Figure 1: Appearance of buffy coat and plasma layer above the red blood cells

- Cap the micro tube with purple/white caps.
- Use a transfer pipette to harvest the buffy coat by removing the buffy colored layer between the plasma and the cell pellet. The red blood cells remaining in the EDTA tube may be discarded.
- Aliquot into one micro tube and close a clear/natural cap.
- Label the micro tubes and freeze at -80°C. Perform this step as rapidly as possible to ensure that specimens remain cool.
- If samples are not frozen within 4 hours of collection, record the specimens as defective on the Blood requisition form.

5.5 Blood Samples for Immunophenotyping- EDTA Tubes

- Add 30 mL of 1x lysing solution to a 50 mL tube containing 3000 µL of whole blood.
- Gently invert the tube 5 times immediately after adding the lysing solution.
- Wrap the 50 mL tube in foil to protect the sample from light and incubate at room temperature for 10 minutes.
- After the 10-minute incubation, centrifuge at 200 RCF for 5 minutes.
- After centrifugation, carefully aspirate the supernatant, without disturbing the pellet.
- Re-suspend the pellet in 1.5 mL of cold Cryostor CS5 medium.
- Aliquot the sample into 3 x labelled 2 mL microcentrifuge tubes (500 µL per tube) and cap with purple/white caps.
- Place the tubes at -20°C in a MR FROSTY/ Isopropanol freezing container.
- Transfer the tubes to -80°C after 24 hours.

5.6 Procedure for Processing Cytology Samples

- Buccal Swab
  - Attach one label to the sample and another to the requisition form.
  - Store the buccal swab in the -80°C freezer.
- Saliva
  - Attach one label to the sample and another to the requisition form.
  - Store the saliva tube in a labeled box at ambient temperature.
- Urine (Bone Markers- 2nd void of the day)
  - The sample is received and immediately placed on ice.
  - Switch on the centrifuge and cool to 4°C.
  - Measure and record the volume of urine.
  - Add and dissolve 1 Roche Mini protease inhibitor cocktail tablet (stored in the fridge) per 25 mL urine. These are toxic and should be added to the urine pots in the Class II Safety Cabinet in the lab.
  - Check and record the urine pH using pH test strips once the tablet has fully dissolved.
  - Adjust the pH to 7.0 using M HCl of M NaOH as appropriate.
  - Remove particulate matter by filtering the urine through a 100 micrometer cell strainer into a fresh 50 mL centrifuge tube.
  - Centrifuge the sieved urine at 2000g (3000 rpm) at 4°C for 10 minutes.
  - Aliquot 1 mL of the centrifuged urine into each of the 10 labelled microcentrifuge tubes. Split the rest between the two 15 mL centrifuge tubes (1-2 x 15 mL) taking care not to disturb any pellet. Freeze all samples at -80°C.
- Urine (Metabolomics- 24hr urine collection)
  - Switch on the centrifuge and cool to 4°C.
  - Measure and record the volume of urine.
  - Centrifuge the sample at 1500 RCF at 4°C for 15 minutes.
  - Divide the supernatant into roughly 10 mL aliquots in 1-4 15 mL tubes, label and store at -80°C.