

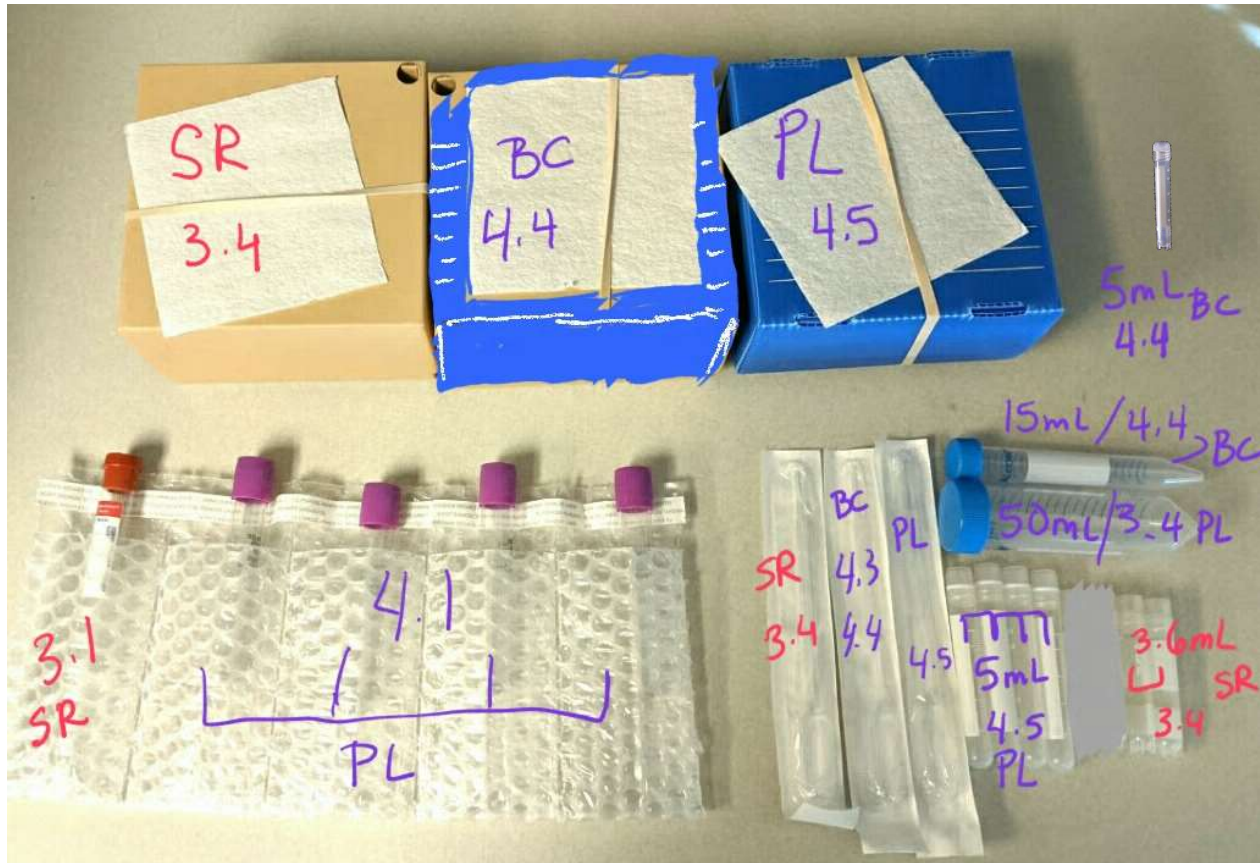
1 UTERINE LAVAGE STANDARD OPERATING PROCEDURE (SOP)

1.1 SOP: Blood Specimen Collection, Processing, Shipping and Storage

1. SUPPLIES AND KITS PROVIDED BY FREDERICK NATIONAL LABORATORY FOR CANCER RESEARCH (FNLCR):

The Frederick National Laboratory for Cancer Research (FNLCR) will send each recruiting site a kit that includes the Participant ID Labels to use on the Consent, Case Report Forms and Specimen Worksheets as well as all the supplies for collection and processing. Sites will associate the Participant ID to the Kit ID in VSIMS (See Manual of Operations, Section 5.5.2).

The institution may collect blood twice if the participant agrees, ideally on two separate days. All blood is collected within 31 days and preoperative. Use one Kit per collection date.



2. Label supplies as follows (see strip of labels provided in the kit, starting at the bottom):

- Serum, 2 x 3.6 mL vials with labels: 10 digit #, SE1 and 10 digit #, SE2
- Plasma, 4 x 5 mL vials with labels: 10 digit #, PL1, 10 digit #, PL2, 10 digit #, PL3, 10 digit #, PL4
- Buffy coat, 1 x 5 mL vial with labels: 10 digit #, BC1

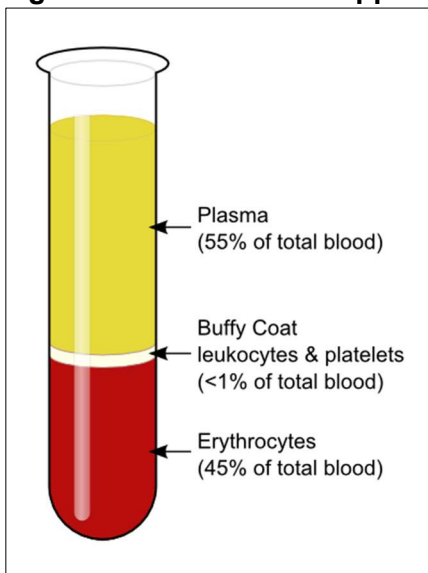
3. SERUM SOP

- Step 1.** Collect one 10 mL red top blood collection tube (BD Vacutainer cat# 366430). Be sure to fill completely. Label with *Kit #NN Serum Tube*. Sit upright at room temperature for 30-60 minutes after blood is drawn to allow clot to form. If the blood is not centrifuged immediately after the clotting time, the tubes should be refrigerated (4°C) for no longer than **4 hours**.
- Step 2.** After clotting time, centrifuge tubes at **1200 RCF for 20 min @Room Temperature** (18-25°C) with brake off. **Note:** Centrifugation speeds are provided in g-force. If your centrifuge measures speed in revolutions per minute, you can calculate your speed setting by measuring your rotor radius and using the calculator provided by your centrifuge manual, or using the calculator on this link: <https://www.sciencegateway.org/tools/rotor.htm> NOTE: if you need to use a refrigerated centrifuge make sure it is set to 18-25°C and allow time for the centrifuge to rise to room temp prior to use.
- Step 3.** Using a sterile pipet, aliquot 2 x 2.5 mL of serum into two 3.6 mL labeled cryovials and freeze at -80°C in a 9x9 (3" tall) storage box labeled with Box ID# generated by the site in VSIMS and write Serum on the box.
- Step 4.** Ship quarterly on dry ice to the FNLCR in VSIMS. Contact FNLCR (NClatFrederickCentralRepositoryOperations@mail.nih.gov) prior to shipping to discuss what size shipper is needed and to coordinate prepaid shipping costs.

4. PLASMA AND BUFFY COAT SOP

- Step 1.** Collect four lavender/purple top 10 mL EDTA blood collection tubes (BD Vacutainer cat# 366450); be sure to fill completely. Immediately mix by gently inverting 8-10 times after each tube is collected to thoroughly mix the blood with the anticoagulant. Label with *Kit #NN Plasma Tube 1-4*.
- If transporting between a collection lab and a processing lab, the tubes can be kept at room temperature. Blood samples should **be centrifuged within 2 hours of collection**. If centrifuging more than 2 hours after collection, please refrigerate (4°C) for no longer than **2 additional hours**. **Processing must be completed within 4 hours**.
- Step 2.** Centrifuge tubes at **1200 RCF for 20 min @ Room Temperature** (18-25°C) with brake off to avoid remixing the plasma and buffy coat layers. Observe the tube for any unusual appearance such as hemolysis.

Figure 1: K2-EDTA tube appearance after centrifugation



Source: https://en.wikipedia.org/wiki/ Buffy_coat

Step 3. Using a sterile transfer pipet, remove the plasma layer from each of the four tubes and combine in a 50 mL centrifuge tube, being careful not to disturb the buffy coat (thin white) layer. Leave about 0.5 mL of plasma above the buffy coat layer to avoid aspirating cells into the plasma. *If processing multiple specimens, label with a Kit # label to identify the sample.* Create a homogenous mixture in the 50 mL centrifuge tube by pipetting up and down two times. Recap 50 mL tube and proceed with removing the buffy coat layer.

Step 4. Buffy coat: Using the same transfer pipet, carefully aspirate the remaining plasma, buffy coat layer, and a little of the RBC layer from each blood collection tube (1-1.5 mL) into a 15 mL centrifuge tube. *If processing multiple specimens, label with a Kit # label to identify the sample.* Mix the pooled “buffy coat” to create a homogenous mixture.

Using the same transfer pipet, aliquot 5 mL buffy coat into one pre-labeled 5mL cryovial. Freeze at -80°C in a BLUE 8x8 (4” tall) storage box labeled with Box ID# generated by the site in VSIMS and write “Buffy Coat” on the box.

Step 5. Plasma: Using a new transfer pipet, aliquot 5 ml plasma into four pre-labeled 5mL cryovials. Freeze at -80°C in a BLUE 8x8 (4” tall) storage box labeled with Box ID# generated by the site in VSIMS and write “Plasma” on the box.

Step 6. Ship plasma and buffy coat quarterly to the FNLCR using the VSIMS database. Contact FNLCR (NClatFrederickCentralRepositoryOperations@mail.nih.gov) prior to shipping to discuss what size shipper is needed and to coordinate prepaid shipping costs..

5. FNLCR PROCESSING

1. Samples will be received and processed, distributed, or stored as follows:

- a. Plasma samples will be thawed to make smaller volume aliquots, refrozen at -80°C, then transferred into the repository for long-term storage.
- b. Serum samples will be thawed to make smaller volume aliquots and refrozen at -80°C. One aliquot will be sent to each of Johns Hopkins and MD Anderson for analysis. The remainder will be transferred into the repository for long-term storage.
- c. Buffy coat samples will be thawed to make smaller volume aliquots and refrozen at -80°C. One aliquot will be used to extract genomic DNA. The remainder will be transferred into the repository for long-term storage.
 - i. Genomic DNA will be isolated using the Qiagen Blood and Tissue kit (#69506).
 - ii. Genomic DNA from the buffy coat will be quantified by Nanodrop and Qbit and aliquoted for analysis or long-term storage. Upon creation of the aliquot, the DNA will be frozen and stored at -80°C. One aliquot of 2µg will be sent to McGill University and TwinStrand for analysis. The remainder will be transferred into the repository for long-term storage.