BC Generations Project
Processing EDTA Vacutainer Tubes

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1.0 PURPOSE

The BC Generations Project is collecting blood in Becton Dickenson EDTA vacutainer tubes for the purpose of obtaining plasma, white blood cells (buffy coat) and red blood cells. Plasma samples will be used for measuring a variety of biomarkers (e.g. cytokines, proteins); white blood cells will be used as a DNA source for genetic testing, and the red blood cells are being kept for biomarker analysis. As we can't predict all tests in the next 25 years optimal processing, storing and documenting the sample history is of highest importance. These samples are collected by a certified phlebotomist either during the participant's visit to an assessment centre or at a community laboratory.

2.0 MATERIAL, EQUIPMENT AND FORMS

Officially received EDTA samples	Biological Safety Cabinet Class II type A2
Lab gown & gloves (nitrile)	Ice bucket
P1000 pipette & tips	Nunc Cryovials with purple, pink and orange cap inserts capped

Labelled Storage boxes with 9x9 inserts	Cryovial racks
Biohazard waste bag	Dry ice
Vacutainer racks	Computer with access to Laboratory Information Management System
CoolBox™	4°C Centrifuge
70% ethanol	

3.0 EDTA SAMPLE PROCESSING

3.1 You will receive three EDTA vacutainer tubes per subject. Samples will arrive at 4°C and can be found in the refrigerator if already received. Proceed using officially received EDTA tubes only.

3.2 Process samples in batches of 48 tubes (16 participants) or less, maintaining the order in which they were collected and starting with the oldest. Collection time can be obtained from the lab requisition. Sample processing and storage will be documented using the Laboratory Information Management System (LIMS).

3.3 Gently mix the samples by inverting the tubes 7X.

3.4 Centrifuge EDTA samples inside a sealed bucket at 1300xg for 10 minutes @ 4°C. **After centrifugation the sealed buckets are to be opened in the Biological Safety Cabinet** and placed upright in a rack, careful as to not disrupt the layers**.**

3.5 Keep samples cool while processing.

3.6 Document the following attributes in LIMS for each EDTA sample: presence of hemolysis in the plasma phase, presences of lipidemia, presences of post-centrifugation fibrin clot, processing note.

3.7 Label each cryovial with each own unique label generated by LIMS. *Note: Plasma: purple cap cryovials, WBC (white blood cells): pink cap cryovials, RBC (red blood*

cells): orange cap cryovials – double check to ensure you have the correct sample type on the correct colour cap tube.

3.8 Aliquot samples in the biological safety cabinet (BSC) (Class II type A2).

3.9 Aliquot each EDTA tube individually into its respective cryovials. DO NOT POOL aliquots from the different EDTA tubes.

3.9.1 Uncap the cryovials that you will be pipetting into.

3.9.2 Using a P1000 pipette, transfer 1.0ml (1000µl x 1) of plasma to each of the 2 labelled purple capped cryovials. Pipette the remaining plasma volume (<0.5 mL) to either the 3rd plasma labelled cryovial (if using) or to the 2nd plasma cryovial leaving ~2 mm of plasma above the white blood cell interface layer. *Double check to make sure the correct EDTA tube is being aliquot into the correct cryovials.*

3.9.3 Using the same pipette tip, transfer the buffy coat layer (white blood cells & platelet interface) to the labelled pink cap tube (up to 1 mL). It is acceptable if some plasma and RBC are included while making sure to get the entire buffy coat. *Double check to make sure that correct EDTA tube is being aliquot into the correct cryovial.*

3.9.4 With a new filter pipette tip, transfer 1 mL(1000µl x 1) of the RBC to the labelled orange cap tube. *Double check to make sure the correct EDTA tube is being aliquot into the correct cryovial. This step may not be carried out for a participant's 3rd EDTA tube.*

3.10 Recap the cryovials and place in the 4°C Coolbox.

3.11 Discard the plastic EDTA tube (now only containing a small amount of RBC) into the red biohazard bag.

3.12 Proceed with steps 3.2 To 3.11 until all EDTA tubes have been processed. Document processing in LIMS.

3.13 Proceed with Section 4.0: Filling & Inventory the Storage Box.

3.14 Clean up the biological safety cabinet.

4.0 FILLING & INVENTORY THE STORAGE BOX

4.1 All samples will be kept on dry ice when outside the freezer.

4.2 Select either the last partially filled storage box or a new storage box and place on dry ice.

4.3 Physically transfer the samples from the Coolbox™ location to the storage box. Each sample type (plasma, buffy coat, red blood cells) will be transferred to 3 storage boxes (9 boxes total) to be stored in 3 separate freezers. Maintain the same sample order.

4.4 Inventory the samples in LIMS.

4.5 When the storage box is full or if you are done processing the batch, transfer these samples to the -80^{o}C freezer.

4.6 If and when samples are relocated to another freezer, including -190^{o}C vapor phase freezer, document the move in LIMS.