Title: Isolation of PBMCs from whole blood for Mass Spectrometry with Mononuclear Cell Preparation Tubes.

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Purpose: Isolate peripheral blood mononuclear cells (PBMCs) from whole blood for analysis by Mass Spectrometry (MS).

Revision History

Revision date	Revision Author	Revision notes

I. Reagents and Supplies:
1. BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube - Sodium Citrate tubes (BD Vacutainer #362761)
2. Falcon 15mL Conical Centrifuge Tubes (Fisher #352096 or similar)
3. 1.5mL Nalgene System 100 Cryogenic Tubes (Thermo #5000-1020 or similar)
4. Transfer pipettes, sterile (Fisher #13-711-9BM or similar)
5. Gibco DPBS (Thermo #14190-144 or similar)

II. Equipment:
1. Centrifuge (Eppendorf 5810R or similar)

III. Preparation for PBMC Isolation:
1. Prepare two BD Vacutainer® CPT™ Tubes with Sodium Citrate: tubes should be at room temperature (18-25ºC) and labeled with patient identification (ID).
2. Prepare one Falcon 15mL Conical Centrifuge Tube: label with patient ID.
3. Optional: Prepare one Falcon 15mL Conical Centrifuge Tube for plasma collection.
4. Prepare one Nalgene 1.5mL cryovial: labeled with patient ID and date.

IV. PBMC Isolation
1. Collect blood into the tubes using standard technique for BD Vacutainer® Evacuated Blood Collection Tubes.
2. Gently invert tubes 8 to 10 times to mix anticoagulant additive with blood. DO NOT SHAKE to avoid hemolysis.
3. After collection, store tube upright at room temperature until centrifugation. Centrifuge the blood samples as soon as possible and within one hour of blood collection.
4. Immediately prior to centrifugation remix the blood sample by gently inverting the tube 8 to 10 times and centrifuge tube/blood sample at room temperature (18-25ºC) in a horizontal rotor (swing-out head) for 30 minutes at 1500 RCF (Relative Centrifugal Force).

5. After centrifugation, lymphocytes and monocytes will be in a whitish layer just under the plasma layer (see Figure 1 below). Transfer half the plasma to a separate 15mL tube and set aside on ice if collecting plasma or discard if not collecting plasma.
6. Collect the entire lymphocyte/monocyte layer with a sterile transfer pipette by gently placing the pipette within the layer of cells and transfer to a different 15mL size conical centrifuge tube with cap. The lymphocyte/monocyte from the two blood tubes can be pooled into a single 15mL tube.
7. Add DPBS to the lymphocyte/monocyte tube to bring volume to 14mL. Cap tube and mix gently by inverting tube 5 times.
8. Centrifuge for 15 minutes at 300 RCF at room temperature.
9. Aspirate off the supernatant leaving a volume of approximately 500 µL above the pellet.
10. Add DPBS to the cells to bring volume to 10mL. Cap the tube and mix gently by inverting tube 5 times.
11. Centrifuge for 15 minutes at 300 RCF at room temperature.
12. Aspirate off as much supernatant as possible without disturbing cell pellet.
13. Gently resuspend cell pellet in residual DPBS (50 to 100 µL) and transfer cells to the cryovial.
14. Snap freeze the sample in an upright position either in liquid nitrogen or in a -70 oC (or colder) freezer.
15. Store sample in vapor phase of a liquid Nitrogen tank or in a -70 oC (or colder) freezer.
16. Ship samples on dry ice.

Figure 1. Empty CPT (left), after blood draw (middle), and after centrifugation (right).