The purpose of this Standard Operating Procedure (SOP) is to describe the instructions for collection, processing, and storage of collected specimens for the PGB Study.

1. Definitions

1. EDTA – An EDTA tube is an evacuated tube used to collect a blood sample by venipuncture. The tube contains ethylenediaminetetraacetic acid, a known anticoagulant.
2. SST – A Serum Separating Tube is an evacuated tube used to collect a blood sample by venipuncture. The tube contains a "yellow gel" and a silica coating to accelerate blood clotting. When centrifuged the "yellow gel" will separate the serum from the cells in the blood.
3. CPT – A Cell Preparation Tube is an evacuated tube used to collect a blood sample by venipuncture. The tube contains a polyester gel and density gradient liquid (FICOLL). When centrifuged the polyester gel forms a barrier between the mononuclear cells from the blood.
4. PAXGene RNA Tube – A PAX RNA tube is an evacuated tube used to collect a blood sample by venipuncture. The blood tube contains a patented additive that that protects RNA molecules from degradation.
5. Urine Collection Cup – The collection cup provided to the participant for collection of urine.
6. LP – A Lumbar Puncture, also knows as a spinal tap, is an optional procedure in this study to collect cerebral spinal fluid (CSF).
7. PBMC – These are peripheral blood mononuclear cells being studied in this protocol.
8. HBSS – Hanks's Buffered Salt Solution, used in preparation of PBMCs.
9. FBS – Fetal Bovine Serum, used in preparation of PBMCs.

2. Sample Collection Guidelines

2.1. General Blood Collection Procedure

1. Blood will be collected in the following order (where applicable):
    a. 3 – EDTA Tubes (purple top)
    b. 3 – SST Tubes (tiger top)
    c. 1 – CPT Tube
    d. 1 – PAX Gene RNA Tube

2. BLOOD DRAW:
    a. Perform venipuncture with patient's arm in a downward position and the tube stopper in the uppermost position.
    b. Pull skin tight over area of blood draw with one hand, then slide needle with bevel up into the skin and blood vessel until flash of blood is seen in the connecting tube.
    c. Hold needle in place while placing EDTA tube into the vacutainer cylinder and advance until blood begins to fill.
    d. **Release tourniquet**.
    e. Remove EDTA tube, gently invert 8-10 times.
    f. Repeat this procedure for second and third EDTA tubes.

g. Fill **first SST tube**, invert 8-10 times and **store upright at room temperature for at least 1 hour up to a maximum of 2 hours.**
h. Repeat the procedure for second and third SST Tubes.
i. Fill CPT tube, gently invert 8-10 times and **store upright at room temperature while protecting tubes from direct light**.
j. Fill PAX Gene RNA tube while keeping it below the veins to prevent backflow, invert 8-10 times and **store upright for a minimum of 2 hours, but no more than 24 hours at room temperature.**
k. Pick up gauze pad and place over blood draw site as you remove needle, ask subject to apply pressure.

2.2. General Cerebral Spinal Fluid (CSF) Collection Procedure

A licensed practitioner designated by the Site Investigator on the Study Delegation Log will collect cerebrospinal fluid (CSF) samples in accordance with the site's institutional policies and guidelines.

1. Use a standard sterile technique during sample collection.
2. Ask the study participant to lie on their side and curl up in the fetal position or to sit upright and round his/her back.
3. Collect up to 16 ml (about 3 teaspoons) of CSF.
4. Apply a sterile bandage after collection.

2.3. General Urine Collection Procedure

1. Give patient a sterile urine collection cup provided in the kit and ask for a sample of urine.
2. Remove the urine collection cup from its plastic wrapping and ensure the cap is not dislodged nor the inside of the cup touched.
3. Ask subject to provide a urine sample following the instructions below:
    a. Wash hands with soap.
    b. Do not open the collection cup until just before collection.
    c. The inside of the container and cup must not be touched or come in contact with any part of the body. Exposure to air should be minimized as much as possible.

3. Sample Processing Procedure Post-Specimen Collection

This section describes processing procedures for the plasma, serum, DNA, PBMCs, and CSF samples.

3.1. DNA

3.1.1 Equipment and Materials

1. EDTA Tube x1
2. 49-slot freezer box
3. Biospecimen Collection Form for documentation purposes

3.1.2 DNA Processing Procedure from an EDTA Tube

1. Do NOT Centrifuge.
2. **Store at room temperature for no more than 24 hours.**
3. Transfer tubes to a 49-slot freezer box
4. Document time of EDTA (for DNA) collection and time placed in -80°C.

3.2. Plasma and Buffy Coat (Plasma = blue & Buffy = orange)

3.2.1. Equipment and Materials

1. Centrifuge capable of holding 10mL blood tubes
2. EDTA Tubes x2
3. **Blue top storage vials** for plasma
4. **Orange storage vials** for buffy coat
5. Transfer Pipettes
6. Biospecimen Collection Form
7. 81-slot freezer storage box
8. Dry Ice

3.2.2. Plasma and Buffy Coat Processing Procedure from EDTA Tubes

1. Utilize two EDTA blood tubes for plasma and buffy coat collection.
2. Centrifuge immediately if possible, but no later than 2 hours from blood draw at 1750 x g for 10 minutes at 4°C.
3. Using a transfer Pipette, transfer 1.0mL of plasma into each 2.0mL **blue top storage vial.** Add any remaining plasma to the storage vials, up to 2mL. Any unused storage vials should be discarded.
4. Then, using a transfer Pipette, transfer 1mL buffy coat from each blood tube into separate **orange top storage vials**.
5. Immediately place the vials into an 81-slot freezer storage box.
6. **Immediately** place the freezer storage boxes on dry ice or in a -80°C freezer, **within 15 minutes of aliquoting**.
7. Document time of plasma collection, buffy coat collection, and time placed in the freezer.

3.3. Serum (Serum = red)

3.3.1. Equipment and Materials

1. Centrifuge capable of holding 10mL blood tubes
2. SST Tubes x3
3. **Red top storage vials**
4. Transfer Pipettes
5. Biospecimen Collection Form
6. 81-slot freezer storage box

3.3.2. Serum Processing Procedure from SST Tubes

1. Obtain the three SST blood tubes to be used for serum preparation.
2. **Let blood sit upright at room temperature for a minimum of one hour** from the blood draw, but not more than two hours, to allow for proper clotting. It is important to keep tube in an upright position.
3. Centrifuge SST blood tube at 1750 x g for 10 minutes at 4°C.
4. Using a transfer Pipette, place **at least** 1.0mL of serum into the 2.0mL vials. Add any remaining serum to the storage vials, up to 2mL. Any unused storage vials should be discarded.
5. **Immediately** place the freezer storage boxes on dry ice or -80°C freezer, **within 15 minutes of aliquoting**.
6. Document time of serum collection and time placed in the freezer.

3.4. PBMC (PBMC = clear top)

3.4.1. Equipment and Materials

1. Biological Safety Hood
2. Centrifuge with horizontal rotor
3. Nitrogen Tank, vapor cooled
4. Hanks Balanced Salt Solution (HBSS)
5. Flash Freezing Media
6. Fetal Bovine Serum (FBS)
7. 15mL Conical Centrifuge Tube
8. "Mr. Frosty" – style freezing container
9. CPT Tube x1
10. Clear top cryovial
11. Pipette and Pipette tips
12. Transfer Pipette

3.4.2. PBMC Processing Procedure from a CPT Tube

1. PBMC processing **must be completed within 2 hours** of sample collection.
2. Store CPT tube(s) at room temperature if they cannot be processed immediately; cell degradation will occur if tubes are stored for **more than two hours**.
3. Obtain the CPT blood tube to be used for PBMC collection.
4. Remix samples immediately prior to centrifugation by gently inverting the tube 8-10 times.
5. Obtain the two clear top storage cryovials.
6. Centrifuge CPT Tube at 1500-1800 x g for 15-30 minutes at room temperature. Keep the brake OFF.
7. **All the following steps should be carried out in a biological safety hood.**
8. After centrifugation, bring the CPT tube(s) to a biological safety cabinet and carefully open the tops.
9. Add 5mL HBSS to a 15mL conical centrifuge tube.

10. Aspirate and discard ½ of the plasma without disturbing the mononuclear cellular layer under the plasma layer.
11. Immediately collect the cell layer with a pipette and transfer to the 15ml conical centrifuge tube containing HBSS.
12. Add HBSS to bring to a volume of 15mL and mix cells by gently inverting tube 5 times.
13. Centrifuge for 15 minutes at 300 x g at room temperature.
14. Aspirate as much of the supernatant as possible without disturbing the cell pellet.
15. Very gently re-suspend cell by adding 500uL 100% FBS.
16. Add 500uL of Flash Freezing Media.
17. Immediately aliquot 500uL of cell suspension into each, labeled, 2.0mL cryovial.
18. Place the cryovials in a Mr. Frosty-style freezing container that has been pre-cooled to 4°C and place in a -80°C freezer for at least 12 hours. After at least 12 hours (Ideal range is 12-48 hours but can be extended to 72 hours if specimens are collected on a Friday), transfer tubes to labeled 81-slot freezer box and place in liquid nitrogen.
19. Document time of PBMC collection and time placed in a -80°C freezer and time placed in vapor cooled Nitrogen Tank.

3.5. RNA

3.5.1. Equipment and Materials

1. PAXGene RNA Tube x1
2. 49-slot freezer box
3. Biospecimen Collection Form

3.5.2. RNA Processing Procedure from a PAX RNA Tube

1. Do NOT Centrifuge
2. Store upright for a minimum of 2 hours (no more than 24 hours) at room temperature.
3. Transfer tubes to a 49-slot freezer box
4. Freeze at 20°C for a minimum of 24 hours & maximum of 72 hours, then transfer tubes to a -80°C freezer.
5. Document time of RNA collection and time placed at -20°C and time placed at -80°C on Biospecimen Collection Form.

3.6. CSF (CSF = green)

3.6.1. Equipment and Materials

1. Centrifuge
2. CSF Collection Tubes x3
3. **Green storage vials** (5 per CSF Collection Tube)
4. STERILE Pipette and tips
5. Dry Ice

6. 81-slot freezer box
7. Biospecimen Collection Form

3.6.2. CSF Processing Procedure from CSF Collection Tubes

1. **CSF processing** must be completed **within 15 minutes** of sample collection.
2. Immediately after collection, centrifuge CSF samples at 1750 x g for 10 minutes at 4°C.
3. Using a sterile pipette, **immediately aliquot**, 5, 1mL aliquots, into each **green top storage vial** per CSF collection tube.
4. **Immediately place vials** into an 81-slot freezer storage box and place **in a -80°C freezer within 15 minutes** of aliquoting.
5. Document time of CSF collection and time placed in the freezer on Biospecimen Collection Form.

3.7. Urine (Urine = yellow)

3.7.1. Equipment and Materials

1. Urine Collection Tube x1
2. Yellow top storage tubes x 5
3. STERILE pipette and tips
4. Dry Ice
5. 81-slot freezer box
6. Biospecimen Collection Form

3.7.2. Processing of Urine Collection

1. Transfer 12-15mls of urine to a 15ml falcon tube.
2. Centrifuge at 1500 x g for 10 minutes at room temperature to remove cell debris
    a. Without disturbing the pellet transfer 2mL aliquots of urine to each of the 5 yellow top storage tubes.
3. Discard remaining urine.
4. **Immediately** place vials into an 81-slot freezer storage box.
5. Place the freezer storage boxes in a -80°C freezer.
6. Document time of urine collection and time placed in the freezer on Biospecimen Collection Form.