ITN093AI DARE-APS: Preparation of Platelet-Poor Plasma from Sodium Citrate Tubes Standard Operating Procedure

Instructions: Study personnel must be trained and authorized by the site Principal Investigator to perform blood processing. Personnel performing blood processing are required to read and understand the following ITN093AI DARE-APS: Preparation of Platelet-Poor Plasma from Sodium Citrate Tubes Standard Operating Procedure. The following procedures MUST be followed for all specimens.

1. Purpose

To describe the procedure for preparation and cryopreservation of platelet-poor plasma from sodium citrate tubes.

2. Required Supplies

Items Supplied by ITN:
- Sterile 15mL conical centrifuge tubes (x 2)
- Sterile 2.7mL sodium citrate vacutainers (light blue top)
- Sterile 1.8mL cryovials, pre-labeled

Items Supplied by Clinical Sites:
- Pipettes and pipetting system for 1mL volume
- Sterile disposable plastic transfer pipettes (optional)
- Alcohol-proof black marker for labeling centrifuge tubes
- 70% ethanol
- Gloves, goggles, lab coat

Equipment Supplied by Clinical Sites:
- BSL 1-2 Safety Cabinet
- -70 to -80°C Mechanical Freezer (preferably on back-up power and monitored for maintenance of temperature)
- Centrifuge capable of accommodating 15mL conical tubes and 2.7mL vacutainers

3. Procedures

General Precautions:
- Informed consent must be obtained from all participants before any research procedures, including specimen collection, are performed.
- Always follow appropriate precautions for handling of human specimens, including appropriate use of personal protective equipment.
- Perform all processing in a BSL 1-2 Safety Cabinet.
- Always use sterile technique.
- Always prepare materials and workspace by wiping with 70% ethanol.

Procedure for collection of platelet-poor plasma:

1. Collect blood into 2.7mL sodium citrate vacutainers (light blue top), ensuring that sample draw closely matches fill indicator on tube. A complete and accurate draw is critical to testing.
2. Immediately after collection, gently invert tube 3-4 times to mix. DO NOT SHAKE.
3. Store sample at room temperature until processing, and process within 2 hours of collection.
4. Centrifuge vacutainers at room temperature, 2,000 x g, for 15 minutes.
5. Label two sterile 15mL conical centrifuge tubes with PID.
6. Gently remove vacutainers from centrifuge. Slowly and gently transfer the top ¾ of the plasma layer to the first 15mL conical tube. This may be accomplished with a sterile disposable transfer pipette, a serological pipettor, or a micropipettor. Be careful to avoid the buffy coat layer and the red blood cell layer. Approximately 0.5mL of plasma should be left behind.
7. Repeat with other vacutainers, combining all the plasma into the same 15mL conical tube.
8. Being careful to balance the centrifuge, centrifuge the 15mL conical tube of plasma at **room temperature, 2,000 x g, for 15 minutes**.
9. Gently remove the tube from the centrifuge. Transfer the top ¾ of the plasma to a new 15mL conical tube labeled with PID. This may be accomplished with a sterile disposable transfer pipette, a serological pipettor, or a micropipettor. **Be careful to avoid the cell plug at the bottom of the tube.** Approximately 0.5mL of plasma should be left behind. Discard the tube with the cell plug at the bottom.

10. Using either a 1mL serological pipettor or micropipettor, aliquot 500uL of platelet-poor plasma into each pre-labeled 1.8mL cryovial.
11. Immediately transfer cryovials to a -80°C freezer. Store at -80°C until shipment.