Immune Tolerance Network

**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

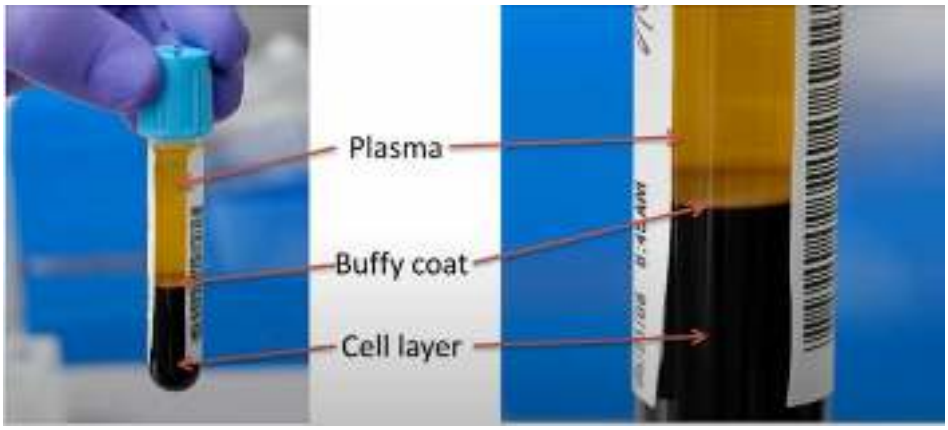
**3. Procedures**

***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.




(Photo credit: Mayo Clinic Laboratories, "Platelet poor plasma update", https://www.youtube.com/watch?v=tNndl656B5A)

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.

(Photo credit: Mayo Clinic Laboratories, "Platelet poor plasma update", https://www.youtube.com/watch?v=tNndl656B5A)

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.