

Standard Operating Procedure II: Blood Sample Collection and Processing - PLASMA

**** NOTE:** The following procedure is to be performed wearing laboratory coat, gloves, eye protection, and mask.

PRINCIPLE

Arterial or mixed venous blood will be collected from patients at the indicated time points.

The collection of blood should be obtained from an existing arterial or venous line, or by venipuncture, and should be performed by someone experienced in the technique and familiar with infectious precautions.

Specimen Collection & Handling

1. Phlebotomist/nurse is to draw blood samples at the same time as those for diagnostic purposes. If drawing from a line, blood will be drawn into a syringe first then can be transferred to the appropriate vacuum tube.
2. At patient area, collect blood into a labeled sodium citrate tube (light blue top). Whenever possible, ensure the tube is filled completely. Multiple tubes may be used; volumes available are 1.8 ml or 2.7 ml. Invert completely, 3 times. Label should include PIN and time of draw.
3. Place on wet ice immediately and transport to laboratory for processing
4. In laboratory, centrifuge at 1500 x g for 15 minutes at 4 °C with brake off, in a horizontal, swinging bucket centrifuge (centrifuge program 1).
5. While centrifuging, label cryogenic freezing vials as in protocol I. Require one vial for each 250 µL aliquot, so for each 2.7 ml tube, label 8 vials, and 1.8 ml tube, 6 vials (approximate). Also label one vial for each tube of blood for the buffy coat. (SOP I)
6. Carefully collect the plasma using a disposable plastic pipette from the sodium citrate tube and transfer into a new 15 ml conical tube being sure not to withdraw any of the white interfacial layer.
7. Transfer 250 to 500 µl of the buffy coat (will contain some plasma as well as erythrocytes) to the labeled vial. Record the amount transferred.
8. Discard the remaining pellet.
9. Aliquot plasma into 2 ml cryogenic freezing vials by placing 250 µl of plasma per vial.
10. Place vials on ice and transport to freezer. Store at ≤-70°C.
11. Record placement of vials and enter in TRC database.

Special Note 1.1: only collect the plasma fraction to within 0.1 ml of the interface layer.

Special Note 1.2: Processing and handling of Blood for analysis – please remember endotoxin is ubiquitous and can change expression of all mediators being assayed. Endotoxin free precautions should be taken for handling of all procedures (sterile precautions and using endotoxin free solutions should suffice).

Special Note 1.3: Any blood sample believed to be contaminated with a level 2 biohazard must be handled in a biological safety cabinet (available in Room A5-126). See user manual for proper usage.

Translational Research Centre



Supplies

- Sodium citrate vacutainers (Becton-Dickson, #369714 – glass 4.5 ml, # 363083 – plastic 2.7 ml or # 363080 – plastic 1.8 ml).
- 15 ml sterile polypropylene conical tubes (Falcon/Becton-Dickinson, #35-2097)
- 2ml cryogenic freezing tubes (Corning, #430289)
- P1000 pre-sterilized, filter pipet tips (VWR, # 53508-830)
- Insulated transportation vessel

Equipment

- Centrifuge with swinging-bucket rotor.
- Sterile tissue-culture hood, BSL-2 or equivalent
- Laboratory pipetter capable of delivering 1.0 ml of liquid (eg., Rainin P-I 000)
- Ultra low temperature -80°C freezer

UNCONTROLLED SAMPLE