

Standard Operating Procedures Clinical Protocol I: Blood Sample Collection - PLASMA

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**** 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 following randomization.

The collection of blood should be obtained from an existing arterial or venous line, or by venipuncture (with a needle 20 gauge or larger), and should be performed by someone experienced in the technique and familiar with infectious precautions.

The use of pneumatic tube system for transfer of the collected blood to the laboratory is not recommended since it can activate white blood cells and promote unwanted release of mediators.

Processing of the blood should be done as soon as possible after collection in order to prevent the unwanted released of mediators caused by dying cells.

Specimen Collection & Handling

1. Label all citrated plasma (CPT) tubes, with matching a small sterile polypropylene tube and 6 cryogenic freezing tubes per patient/ sample collection.
2. A minimum of 4mL of whole blood is collected into a CPT tube. Inverted several times to mix the blood with the contained anticoagulant.
3. Place the tube immediately on ice.
4. In laboratory, invert the CPT tube once to mix. Centrifuge at 1700 x g (calculate the speed in rpm taking into account the size of your rotor) for 10 minutes at 4 C with brake off, in a horizontal, swinging bucket centrifuge.

5. Carefully collect the plasma using a disposable plastic pipette or sterile glass pipette from the CPT tube and transfer into a new labeled 15mL conical tube being sure not to withdraw any of the white interfacial layer.

6. Discard the pellet.

7. Aliquot plasma into 2mL cryogenic freezing tubes by placing 250 μ L of plasma per tube. Store at $\leq -70^{\circ}\text{C}$ until processing.

Special Note 1.1: only collect the plasma fraction to within 0.2mL of the interface layer. Note that after centrifugation you should have approximately 2mL of supernatant per tube so transfer only approximately 1.5mL of plasma.

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)

Supplies

- CPT tubes containing sodium citrate (Becton-Dickinson, #362760) – will accommodate 4.0mL.
- 15 ml sterile polypropylene conical tubes (Falcon/Becton-Dickinson, #35-2097)
- 2ml cryogenic freezing tubes (Corning, #430289) Internal thread
- Sterile, endotoxin-free 10ml serological pipets (VWR, #53283- 740)
- Sterile glass pasteur pipets (VWR, #14672-410)
- Rubber bulbs for pasteur pipets (VWR, #56311-062)
- P1000 pre-sterilized, pipet tips (VWR, # 53508-830)
- Insulated Styrofoam box (VWR, #15713-5~9)
- Ice bucket

Equipment

- Clinical Centrifuge with swinging-bucket rotor.
 - Sterile tissue-culture hood, BSL-2 or equivalent
 - Laboratory pipetter capable of delivering 1.0mL of liquid (eg., Rainin P-I 000)
 - Ultra low temperature -70°C freezer
 - Access to a sterilizing autoclave
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