

Standard Operating Procedure III: Blood Sample Collection and Processing- SERUM

**** 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, and should be performed by someone experienced in the technique and familiar with infectious precautions.

Specimen Collection & Handling

1. 4 mls of whole blood is collected into a red top vacutainer, or transferred from a syringe if collected from a line. Completely invert 5 times.
2. Label vacutainer with PIN and time of collection.
3. Place the tube immediately on ice in upright position.
4. Allow to clot on ice for minimum of 30 minutes, maximum 2 hours.
5. Centrifuge at 1300 x g for 20 minutes at 4°C with brake off, in a horizontal, swinging bucket centrifuge (centrifuge program 2).
6. While centrifuging, for each vacutainer specimen, label approx. 6 to 10 cryovials for serum and 1 for the pellet (SOP I). Also label a 15 ml conical tube for each patient.
7. Carefully collect the serum using a disposable plastic pipette from the vacutainer and transfer into a new, labeled 15 ml conical tube.
8. Transfer the pellet into the labeled cryotube using a sterile plastic transfer pipet. Record the volume.
9. Aliquot serum into cryovials by placing 250 microliters of serum per tube. Store at $\leq -70^{\circ}\text{C}$ until processing.

Special Note 1.1: only collect the serum fraction to within 0.1 ml of the clot.

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).

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Special Note 1.3: DO NOT re-centrifuge vacutainers. If there is red cell contamination, transfer to a new 15 mL conical tube before centrifuging to remove contaminants. Centrifuge at 1300 x g for 10 minutes and make note.

Special Note 1.4: Some investigators specifically request the use of gold-top serum separator tubes (SST). Use the same protocol, but discard the gel barrier and pellet.

Special Note 1.5: 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.

Supplies

- Red Top Vacutainer (NOT SST tubes) (for example, BD vacutainers catalog#366430)
- 15 ml sterile polypropylene conical tubes (Falcon/Becton-Dickinson, #35-2097)
- 2ml cryogenic freezing tubes (Corning, #430289)
- 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. Must accommodate 15ml conical tubes
- 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
- Access to a sterilizing autoclave