

The Early Detection Research Network (EDRN) Standard Operating Procedure (SOP) For Collection of Serum

GENERAL REQUIREMENTS

- Gloves must be worn at all times when handling specimens. This includes during removal of the rubber stopper from the blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and clean up of any spills. Tubes, needles, and pipets must be properly disposed of in biohazard containers, in accordance with institutional requirements.
- Universal precautions and OSHA (Occupational Safety and Health Administration) and institutional requirements (<http://www.osha.gov/SLTC/biologicalagents/index.html>) should be followed, including gloves, eye protection or working in a biosafety cabinet for blood processing.
- All equipment (storage, shipping, and centrifuge) must be labeled as biohazard.
- It is important to take steps to prevent hemolysis in these samples. A vacutainer is recommended. If a needle is used, a 21 gauge needle is recommended.

SERUM COLLECTION

Supplies

- Red Top Vacutainer (NOT SST tubes) (for example, BD vacutainers catalog#366430)
- Centrifuge with swinging bucket rotor
- 15 ml polypropylene conical tubes (for example, Corning 430052, Fisher cat #05-538-53D)
- Sterile cryovials with writing surface (for example, Simport T311-2 or Fisher #05-669-57)
- 2ml, 5ml and 10ml pipettes (for example, Fisher cat #13-678-11C, 13-678-11D, 13-678-11E)
- Disposable transfer pipettes (for example, Fisher cat #13-711-20)
- Automatic pipet aid
- Small ice bucket

Serum Separation Procedure

1. Filled red top blood collection tubes ("vacutainers") should sit upright after the blood is drawn at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form.
Note: Use red top (serum) tubes (silicon-coated)—no additives and not SST (serum separator tubes). These tubes, without additives, allow the red blood cells to form a clot. The clot also includes white blood cells, platelets etc. After centrifuging, the clot is at the bottom of the tube, and the serum is on top of the clot). The red top tubes do not have to be full to be used.
2. Centrifuge the blood sample at the end of the clotting time (30-60 minutes) in a horizontal rotor (swing-out head) for 20 minutes at 1100-1300 g at room temperature. If the blood is not centrifuged immediately after the clotting time (30 to 60 minutes at room temperature), the tubes should be refrigerated (4°C) for no longer than 4 hours.
Warning: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated. For an on-line calculator tool, please refer to: <http://www.changbioscience.com/cell/rcf.html>
3. Use pipette to transfer the serum (Recommendation: do not pour!). If more than one tube is drawn, pull the serum from both tubes into a 15 ml conical tube and mix. Pipette serum into the labeled cryovials, filling the vials in sequential order. Aliquot volume is recommended to be 100 µl or 250 µl. Close the caps on the vials tightly. This process should be completed within 1 hour of centrifugation.
4. *Note:* Be very careful not to pick up red blood cells when aliquoting. This can be done by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube.
Check that all aliquot vial caps are secure and that all vials are labeled.
5. Place all aliquots upright in a specimen box or rack in an -80°C or colder freezer. All specimens should remain at -80°C or colder prior to shipping. The samples should not be thawed prior to shipping.

Data points

1. Is the serum **hemolyzed**? **If yes, sample cannot be used.**
2. Date and time of blood collection
3. Number and volume of aliquots prepared
4. Date and time into -80°C
5. Date and time of shipping
6. Any freeze-thaw that occurs with a sample for any reason
7. Any variations or deviations from the SOP, problems, or issues

Notes

- Sterile, disposable droppers, pipetman, pipet aid, eppendorf repeater are examples of ways to aliquot. Depends on size of aliquots, volume of plasma, and volume of aliquots.
- Serum should not undergo freeze-thaw cycles, so choose aliquot volume carefully.
- Freezers need to have a back up generator or other emergency system Options: Create emergency management plan, such as moving to a new freezer or adding dry ice in the event of a freezer failure.