1.0 Purpose

This document describes the process for the collection of human serum from whole blood collected in red top or tiger top tubes.

2.0 Scope

These guidelines apply to personnel intending to cryopreserve serum.

3.0 Requirements:

3.1. General Requirements

All specimens will be treated as potentially hazardous. Personal protective equipment (lab coats, gloves and eye protection) must be worn at all times when handling specimens. This includes during the removal of the rubber stopper from blood tubes, centrifugation, pipetting, disposal of contaminated tubes, and cleanup of any spills. Tubes, needles and pipets must be properly disposed of in biohazard containers in accordance with institutional requirements.

It is important to take steps to prevent hemolysis in samples. The BD Vacutainer system is recommended for venous blood draws. If a needle is used, a 21-gauge needle is recommended.

3.2. Equipment:

3.2.1 Centrifuge with swinging bucket rotor (times and rcf will need to be adjusted if a fixed angle rotor is used)

3.2.2 -80°C Freezer

3.2.3 Biosafety Cabinet

3.2.4 Pipette Aid

3.3. Materials: (see supply list at end for additional details)

3.3.1 1.2ml, self-standing cryovials and caps

3.3.2 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes

3.3.3 Sterile pipettes and/or transfer pipets.

4.0 Method:

4.1 Venous blood will be drawn into red top blood collection tubes that should be gently inverted 5 times immediately upon blood draw. Keep tube(s) upright at room temperature for at least

30 to no more than 60 minutes after the blood is drawn to allow the clot to form. These tubes typically contain clot activator and may have a gel to separate the clotted blood from the serum after a spin. The clot will contain red blood cells and white blood cells, platelets etc. If the blood is not centrifuged immediately after the clotting time, the tubes should be refrigerated or placed on ice for no longer than 4 hours.

4.2 Centrifuge the blood sample at the end of the clotting time. Spin in a swinging bucket rotor for 10-20 minutes at 1300-1750 rcf at 4°C (pre-cool the centrifuge). 1300 rcf for 15 min will be sufficient for most applications. Check manufacturer's guidelines for the specific collection tubes used in your study. Note: use ≤1300 rcf if spinning glass tubes in a fixed angle rotor.

4.3 Use a pipette to transfer the serum into labeled cryovials. If serum is collected in multiple tubes for one subject, collect all tubes into a 15 ml or 50 ml conical tube; mix gently; then aliquot. Aliquot 250µl – 500µl serum (insert applicable volume) into cryovials. This process should be completed within 1 hour of centrifugation. (Note: If collection tubes do not have a gel separating the serum from the red blood cells, then be very careful not to pick up red blood cells when aliquoting. Transferring the serum to a separate tube, to avoid repeatedly going back into the collection tube, is helpful).

4.4 Check that all aliquot vial caps are secure and vials are labeled. Place all aliquots upright in a specimen box or rack in -80°C or colder freezer. All specimens should remain at -80°C or colder and will be transported frozen on dry ice only.

Record/Data Points (Use Barcode if possible to facilitate sample tracking)

1. Hemolysis (red blood cell lysis) or lipemia (cloudiness) in the specimen
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 (if applicable)
6. Any freeze-thaw that occurs with a sample for any reason
7. Any variations or deviations from the SOP, problems, or issues

Label Cryovials

1. Subject ID
2. Subject initials (if appropriate; may be an identifier)
3. Date of collection
4. Visit date (if applicable; may be an identifier. Visit number may be desired instead.)

Supplies

1. Red Top Vacutainer (for example, BD Vacutainer catalog # 366430)

2. Centrifuge with swinging bucket rotor (different times and rcf will be needed for fixed angle rotors)

3. 15 ml and/or 50 ml polypropylene conical tubes (for example, Corning 430052, Fisher catalog #05-538-53D)

4. Sterile cryovials with writing surface (for example Simport T500BOS or Fisher #05-669-57)

5. 2ml, 5ml, and 10ml pipettes (for example, Fisher cat #13-678-11C, 13-678-11D, 13-678-11E)

6. Disposable transfer pipettes (for example, Fisher cat #13-711-20)

7. Small ice bucket

8. Biohazard waste container suitable for human blood; sharps container if glass collection tubes are use

9. Appropriately sized racks and freezer storage boxes.

10. Bleach and/or 70% EtOH.

11. Paper towels or wipes

12. Gloves