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

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.

EDTA PLASMA COLLECTION

Supplies

- EDTA Blood Collection Tubes (for example, BD vacutainers catalog # 366450)
- 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

Plasma Separation Procedure

1. After collection, gently mix the blood by inverting the tube 8 to 10 times. Store vacutainer tubes upright at 4ºC until centrifugation. Blood samples should be centrifuged within four hours of blood collection.
2. Centrifuge blood samples in a horizontal rotor (swing-out head) for 10 to 20 minutes at 1100-1300 g at room temperature.

   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. After centrifugation, plasma layer will be at the top of the tube. Mononuclear cells and platelets will be in a whitish layer, called the “buffy coat”, just under the plasma and above the red blood cells (additional processing of these cell fractions is optional).
4. Carefully collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. If more than one tube is collected, pool the plasma samples from both tubes into a 15 ml conical tube and mix. Pipette the plasma into appropriate sized aliquots in labeled cryovials. Aliquot volume is recommended to be 100 µl or 250 µl; however, some sites may determine that 1 ml aliquot sizes are needed. Close the caps tightly and place on ice. This process should be completed within 1 hour of centrifugation.
5. Check that all aliquot vial caps are secure and that all vials are labeled.
6. Place all aliquots upright in a specimen box or rack in an -80ºC or colder freezer. All specimens should remain at -80ºC or colder prior to shipping. The samples should not be thawed prior to shipping. (Plasma will be shipped on dry ice. Refer to SOP for “Shipping” instructions.)

Data points

1. Date and time of blood collection
2. Number and volume of aliquots prepared
3. Date and time into -80ºC
4. Date and time of shipping
5. Any freeze-thaw that occurs with a sample for any reason
6. 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.
- Plasma should not under go 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.