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Purpose

To obtain and aliquot serum samples from LCBRN subjects.

Responsibility

Personnel associated with the LCBRN Biospecimen Resource Sites who are trained in venipuncture are responsible for obtaining blood specimens competently and safely. Laboratory personnel associated with the LCBRN Biospecimen Resource Sites are responsible for carrying out the sample processing and aliquoting procedures competently and safely. Data entry into the LCBRN online database may be carried out by different personnel than those entering data onto the LCBRN Biofluid Collection Form at the time of procurement.

All personnel handling human biosamples must have training in, and adhere to, universal biohazard precautions and human subject research ethics/confidentiality principles.

Equipment/Reagents

1. LCBRN serum collection package containing collection tube, 12 sterile 1.8 mL cryovials (Thermo Scientific – NUNC, Cat.# 377267) with red caps (Thermo Scientific – NUNC, Cat.# 354968), duplicate strips of labels and a blank copy of the LCBRN Biofluid Collection Form. Tubes used for serum collection are 16 x 100 mm (10 mL) vacuum glass tubes with no additives (red top) (Becton Dickenson Cat # 366430).
2. Blood collection system (sterile needles and vacutainer holder or butterfly needle and syringe)

Note: Permissible needle gauges for blood draw: 22 gauge or smaller (following WHO Best Practices in Phlebotomy)

1. Protective gear (biosafety cabinet, eye/faceshield, disposable gloves, appropriate lab attire).
2. Clinical centrifuge capable of delivering 1300 x g centrifugal force, with appropriate rotors and adaptors to fit the tubes.
3. Pipettors and sterile disposable pipette tips capable of transferring 0.5 mL volumes. Pipette tips must be purchased with sterile certification or steam autoclaved (121oC x 30 minutes).

Note: Pipettors must be manufactured to have performance characteristics of no more than 1% systemic error (e.g. for 500 uL, dispensing error must be within +/-5 uL of this volume). Pipettors require annual calibration, with maintenance records kept on file.

Procedure

*Samples must be processed within 4 hours after collection.*

1. From the LCBRN subject enrollment package, obtain duplicate serum sample identification adhesive labels for the subject and affix one to the Biofluid Collection Form. Enter date, subject status and sample type on the form.
2. Place the duplicate label on the blood tube.
3. Obtain blood sample in red top glass vacutainer tube as per venipuncture protocol (LCBRN SOP # 10). The 10 mL-capacity tube should contain at a minimum 7 mL blood.
4. Record time of blood draw on Biofluid Collection Form.
5. Transport blood tube, Biofluid Collection Form, the cryovials and the aliquot labels to the specimen processing lab. Use appropriate biohazard labeling and outer packaging.
6. Blood must be allowed to sit upright in tube for at ambient room temperature to allow clot to form for at least 30 minutes from blood draw.
7. Centrifuge tubes at 1300 x g for 10 minutes at ambient room temperature (range 68-82 oF, 20-28 oC).
8. Transfer the tube(s) to a stable tube rack.
9. Carefully remove the vacutainer rubber stopper. Do not disrupt serum (light straw color), interface (white cloudy layer) and packed red blood cells.
10. If the serum accidentally becomes contaminated with white cells or red cells, transfer the plasma into a secondary centrifuge tube, and centrifuge a second time at 1300 g for 10 minutes to remove all potentially remaining cells
11. Aliquot serum into labeled cryovials (0.5 mL aliquots) using a pipettor and sterile-filter pipette tips (up to 12 aliquots). Do not pipette within 2 mm of interface and never allow pipette tip to drop into the interface. Observe sterile technique during transfer and discard pipette tip(s) into appropriate biohazard waste container.
12. Label the cryovials with the LCBRN serum aliquot labels. **Affix the duplicate labels onto the Biofluid Collection Form.**
13. Transfer biospecimens to -80oC freezer or in vapor phase of a liquid nitrogen freezer.
14. Record time of aliquot freezing on Biofluid Collection Form.
15. Enter data from the Biofluid Collection Form into the online LCBRN database (see separate procedure). A barcode reader should be used to enter sample container identification using the duplicate labels affixed to the Biofluid Collection Form.
16. Store the Biofluid Collection Form with other subject study data paper documents in a secured location.

References

Blood tube selection based on data presented in:

Drake SK et al (2004) Clinical Chemistry 50: 2398-2401

WHO guidelines for drawing blood: Best practices in phlebotomy (WHO Press, Geneva) (2010)

**Change History**

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| --- | --- | --- | --- |
| Version # | Significant change(s) | Author | Effective Date |
| 1 |  | Moskaluk | 12/01/2010 |
| 2 | Needle gauge specified. Manufacture information added for cryovials. Sterile specifications added for pipette tips. | Moskaluk | 8/15/2011 |