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Purpose

To obtain and aliquot plasma samples from LCBRN subjects.

To obtain a sample of white blood cells (buffy coat) 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 plasma collection package containing collection tube, nine sterile 1.8 mL cryovials (Thermo Scientific – NUNC, Cat.# 377267): 8 with purple caps for the plasma aliquots (Cat.# 375922), 1 with a purple cap for the buffy coat (Bioexpress Corporation- Cat.# C-3354-1P), duplicate strips of labels and a blank copy of the LCBRN Biofluid Collection Form. Tubes used for serum collection are 13 x 100 mm (7 mL) vacuum glass tubes with K3EDTA (lavender top) (Becton Dickenson Cat # 366450).
2. A blank LCBRN Biofluid Collection Form.
3. 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.

1. 5.8 ml sterile disposable transfer pipettes (Fisher Scientific, Cat. # 13-711-9A).
2. 5 PRIME RBC lysis solution (available from Fisher Scientific, Cat.# FP2301310 for 1000 ml).

Procedure

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

1. From the LCBRN subject enrollment package, obtain duplicate plasma 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 lavender top glass vacutainer tube as per venipuncture protocol (LCBRN SOP # 10).
4. Record time of blood draw on Biofluid Collection Form.
5. Invert the tube 8–10 times immediately after collection. This helps to prevent the formation of fibrin.
6. Transport blood tube, Biofluid Collection Form, the cryovials and the aliquot labels to the specimen processing lab. Use appropriate biohazard labeling and outer packaging.
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 disturb plasma/buffy coat interface.
10. If the plasma 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 **plasma** into labeled **purple-cap** cryovials (0.5 mL aliquots) using a pipettor and sterile-filter pipette tips (up to 8 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 plasma 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. Next, using a transfer pipet, collect the buffy coat (white cellular layer between the plasma and red cells) from the specimen (approximately 2ml from each tube) and transfer to a new 15mL conical tube.
16. Add 5 Prime RBC lysis solution to the buffy coat specimen recovered bringing the total volume to 12 mls. Mix contents by inverting tube 5 times.
17. Incubate tube for 10 minutes at ambient temperature, inverting at least one more time during the incubation period.
18. Centrifuge tubes at 1300 x g for 5 minutes at ambient room temperature. (range 68-82 oF, 20-28 oC).
19. Pour off all of red cell lysate into liquid biohazard container. Add 0.5 mL PBS to the white blood cell pellet.
20. Using a disposable transfer pipette, **gently** resuspend white cells by pipetting up and down several times.
21. Aliquot 1 mL of the cell suspension into the **pink-top** cryovial.
22. Spin tube at 1300 x g for 5 minutes (at 4°C) to pellet cells.
23. **Completely** remove supernatant from cell pellet with a pipette. **Do not disturb cell pellet.**
24. Transfer vial to -80oC freezer or in vapor phase of a liquid nitrogen freezer.
25. Record time of aliquot freezing on Biofluid Collection Form.
26. 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.
27. 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 | 12/1/2010 |
| 2 | Needle gauge specified. Manufacture information added for cryovials. Sterile specifications added for pipette tips. | Moskaluk | 8/15/2011 |
| 3 | Buffy coat isolation procedure added to protocol (starting from step 15) | Moskaluk | 10/15/2011 |