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Purpose

To obtain and aliquot bronchial lavage fluid specimens from LCBRN subjects. This pertains only to subjects who are undergoing bronchoscopy for purposes of their clinical care, and for whom bronchial lavage fluid is available from the procedure that would otherwise be discarded.

Responsibility

Personnel associated with the LCBRN Biospecimen Resource Sites are responsible for carrying out the sample collection, 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. 70 cc sterile mucous specimen trap (Owens & Minor, Cat. # 3583MST7000).
2. LCBRN bronchial lavage collection package containing eight sterile 1.8 mL cryovials (Thermo Scientific – NUNC, Cat.# 377267) with blue caps (Thermo Scientific – NUNC, Cat.# 354879), one sterile 1.8 mL cryovial with gray cap (Thermo Scientific – NUNC, Cat.# 375906), duplicate strips of labels and a blank copy of the LCBRN Biofluid Collection Form.
3. Protective gear (biosafety cabinet, eye/faceshield, disposable gloves, appropriate lab attire).
4. Sterile disposable polyethylene 50 mL conical tubes (BD, Cat.# **352070).**
5. Clinical centrifuge capable of delivering 1300 x g centrifugal force, with appropriate rotors and adaptors to fit the tubes.
6. Sterile disposable pipets capable of transferring 1.5 mL volumes.

Procedure

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

1. From the LCBRN subject enrollment package, obtain duplicate bronchial lavage 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 specimen container with the lavage fluid that will be transported to the specimen processing lab. This will link the subject identity to the lavage fluid sample.
3. Obtain bronchial lavage fluid from bronchoscopy procedure using 20 cc of normal saline on the affected lung side. Lavage will be collected in a leak-proof specimen trap.
4. Record time that the procedure ended on Biofluid Collection Form as the specimen collection time.
5. Transport sealed container, Biofluid Collection Form, cryovials and aliquot labels to specimen processing lab. Use appropriate biohazard labeling and outer packaging.
6. Transfer up to 40 mL of fluid to a sterile 50 ml conical tube.
7. Centrifuge at 1300 g for 25 minutes at ambient room temperature (range 68-82 oF, 20-28 oC).
8. Transfer 1.0 mL fluid supernatant aliquots to labeled 1.8 mL cryovials with the blue caps (up to 8 aliquots). Do not disturb pellet during pipeting. Leave at least 0.5 ml air space in the cryovials for expansion during freezing. Observe sterile technique during transfer and discard pipets into appropriate biohazard waste container.
9. Carefully decant the majority of remaining fluid, leaving less than 1 mL. Resuspend cell pellet and transfer to the 1.8 mL cryovial with the gray cap. Leave at least 0.5 ml air space in the cryovials for expansion during freezing.
10. Label the cryovials with the LCBRN serum aliquot labels. **Affix the duplicate labels onto the Biofluid Collection Form.** Be sure to use the “pellet” labels for the cell pellet aliquot.
11. Transfer biospecimens to -80oC freezer or in vapor phase of a liquid nitrogen freezer.
12. Record time of aliquot freezing on Biofluid Collection Form.
13. 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.
14. Store the Biofluid Collection Form with other subject study data paper documents in a secured location.

**Change History**

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| Version # | Significant change(s) | Author | Effective Date |
| 1 |  | Moskaluk | 12/1/2011 |
| 2 | Amount of saline (20cc) used added to SOP | J. Phillips | 07/15/2011 |
| 3 | Minor additions | Moskaluk | 8/15/2011 |