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Purpose

To obtain and aliquot sputum specimens from LCBRN subjects. Only subjects with productive coughs that can produce sputum should be asked to supply these samples.

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. 60 mL screw-cap sterile specimen container (Thermo Scientific Samco, Cat.# 03-006)
2. LCBRN sputum collection package containing collection tube, four sterile 1.8 mL cryovials (Thermo Scientific – NUNC, Cat.# 377267) with green caps (Thermo Scientific – NUNC, Cat.# 355018), 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. Positive displacement pipettor (Gilson Microman M1000) and sterile 100-1000 uL positive displacement pipette tips (Gilson, Cat.# F148506).

Procedure

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

1. From the LCBRN subject enrollment package, obtain duplicate sputum 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 collection container.
3. Provide the subject with the labeled specimen cup. Ask the subject to take several deep breaths, cough deeply from the lungs, and spit any produced sputum into the specimen cup.
4. Record time of sputum collection on Biofluid Collection Form.
5. Transport sealed container, Biofluid Collection Form, cryovials and aliquot labels to specimen processing lab. Use appropriate biohazard labeling and outer packaging.
6. Using a disposable pipet, transfer approximately 0.5 mL sputum aliquots to labeled 1 mL cryovials (up to 4 aliquots). Observe sterile technique during transfer and discard pipets into appropriate biohazard waste container.
7. Transfer biospecimens to -80oC freezer or in vapor phase of a liquid nitrogen freezer.
8. Record time of aliquot freezing on Biofluid Collection Form.
9. 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.
10. 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 | 12/1/2010 |
| 2 | Equipment/reagents updated. Specification for positive displacement pipettor given. | Moskaluk | 8/15/2011 |