1.0 STANDARD OPERATING PROCEDURE (SOP): BRONCHOSCOPY

1.1 Endobronchial Brushings (optional)

During the bronchoscopy obtain 4 airway brushings following the procedure below:

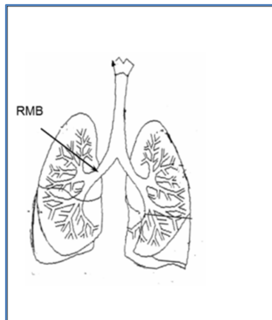
1.2 Materials and Equipment

- Cellebrity endoscopic cytology brushes (Boston Scientific #1601)
- RNaseZap (Ambion 9780; 9782)
- RNAprotect Cell Reagent (Qiagen #76526)
- Sterile PBS
- Eppendorf tubes 1.5mL (two for RNA Later & one for Saline) and 1mL for FISH DNA
- Wire cutter
- 70% isopropyl alcohol wipes
- -80ºC freezer

1.3 Procedure

Tube	Eppendorf tube containing
A	1mL of RNA protect Cell Reagent
B	1mL of 1X PBS solution for proteomic analysis
C	1mL of 1X PBS solution for DNA extraction
D	1mL of RNA protect Cell Reagent

1. Label eppendorf tubes with appropriate solution names and collection date and place in a bucket of ice.
2. Treat wire cutter with a few sprays of RNase Zap. Wipe with paper towels and then wipe twice with alcohol pads.
3. Post bronchoscopy procedure and sample collection but prior to removing the bronchoscope, endobronchial airway brushings will be obtained.
4. Proceed to the right mainstem bronchus (RMB), at the level of the Right Upper Lobe (RUL) takeoff to obtain endobronchial samples. Each brush should be obtained at a site adjacent to the previous brushing within the right mainstem area.



RMB=Right mainstem bronchus

5. Four separate brushings are taken from this area. If this area is grossly involved with a disease process (like tumor), brushings are taken from contralateral side.
6. Rub each cytology brush against the mucosa for 10-20 vigorous back and forth strokes. Rotate the brush along its long axis to obtain cells from the 360 degree surface of the brush. An abrasion will be visible at the site if good contact was made between the brush and mucosa.
7. The brush is then retracted into the sheath and removed from the bronchoscope.
8. Post brushing, the brush is brought out of the plastic sheath. Using the treated wire cutters, cut the brush where the plastic sheath ends and place into the eppendorf tube containing the appropriate reagents.
9. Shake each tube vigorously.

10. Tube A, Tube B, and Tube D are stored at -80°C. For Tube C, additional processing must occur.
11. Vortex Tube C with the brush tip for about 10 seconds.
12. Centrifuge Tube C with the brush inside the tube at 1500g for 10 minutes.
13. Leaving the brush inside the tube and not disturbing cells, carefully remove supernatant from the Tube C. A small amount of saline (<20µl) may be retained inside the tube. This is very important to keep the cells in low volume without losing the cells. Pellet may not be visible.
14. Store in -80ºC freezer.
15. Document the data point information appropriately on the Worksheets
    - Date and time collection
    - Number of brushings prepared
    - Date and time placed into -80ºC freezer
    - Any freeze-thaw that occurs with a sample for any reason
    - Additional information as specified in the Worksheets

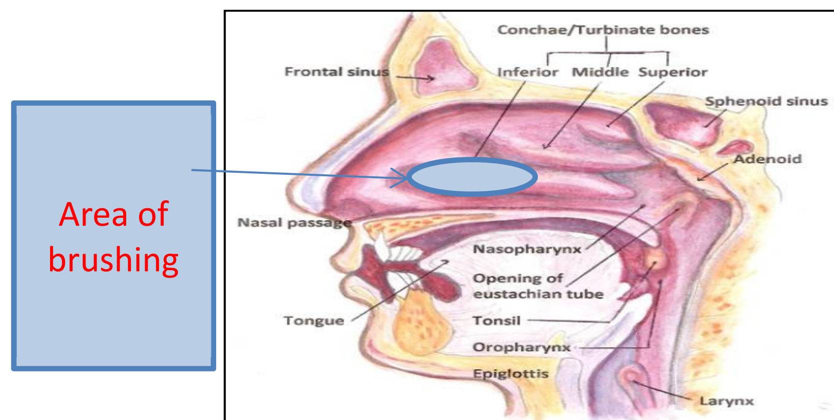
1.4 Nasal Brushings (may be done at time of blood collection)

1.5 Materials and Equipment

- Cytopak soft brushes
- RNAprotect Cell Reagent (Qiagen #76526)
- RNaseZap (Ambion 9780; 9782)
- 1% Lidocaine (either syringe with removable needle or spray bottle for administration)
- Wire cutter
- 70% isopropyl alcohol wipes
- Nasal speculum
- -80ºC freezer

1.6 Procedure

1. Tube of RNAprotect (1mL) labeled with solution name and collection date are placed in a tube box. Sterilize the wire cutter with a few sprays of RNase Zap. Wipe dry with a sterile gauze pad and then wipe with an alcohol pad two times.
2. Participant is asked to blow his/her nose to remove any debris.
3. OPTIONAL: The option is given to use lidocaine to numb the nostril prior to collection. Please note procedure below
4. Using a speculum to widen the left nostril, locate the inferior turbinate. A pen light may be useful.
5. With speculum still widening nostril, brush is inserted into the nostril just past the inferior turbinate and pressed against the outside of nostril and opposite the septum while being rotated. This should take approximately 3 seconds.



6. The brush is then removed from the nostril and the brush head is immediately placed in the tube with RNAprotect Cell Reagent.
7. Cut the brush cut off from the shaft with wire cutters.
8. Repeat steps 5-7 with the second brush (in the same nostril) if your institution is obtaining a 2nd brush to keep locally.
9. Vortex samples to ensure that all cells come into contact with Cell Protect.
10. Nasal epithelium samples should be labeled appropriately and stored in a -80°C freezer.
11. At the end of the procedure check that all aliquot vial caps are secure and that all vials are labeled appropriately.
12. Store vials upright in a labeled specimen box in a -80ºC freezer. All specimens should remain at -80ºC.
13. Document the data point information appropriately on the Case Report Forms
    - Date and time of collection
    - Number of nasal brushings prepared
    - Date and time placed into -80ºC freezer
    - Any freeze-thaw that occurs with a sample for any reason
    - Additional information as specified in the Worksheets

Kit Label Here		**Participant ID Label Here**

Staff ID: ⌴⌴⌴⌴

Date Collected: ⌴⌴ - ⌴⌴ - ⌴⌴⌴⌴

Time Collected (Airway): ⌴⌴:⌴⌴ ☐AM ☐PM
Time Collected (Nasal): ⌴⌴:⌴⌴ ☐AM ☐PM

Time Placed in Freezer (Airway) ⌴⌴:⌴⌴ ☐AM ☐PM (Exact Time)
Time Placed in Freezer (Nasal) ⌴⌴:⌴⌴ ☐AM ☐PM (Exact Time)

Freezer Temperature -80^{0}C or colder ☐Yes ☐No

X *if collected*		Freezer #	9x9 Box#	Row#	Column#	SOP followed?
☐	3541516622 AB-RNA01					Y / N
☐	3541516704 AB-PBS02					Y / N
☐	354151688 4AB-PBS-DNA03					Y / N
☐	354151696 6AB-RNA04					Y / N
☐	3541517048 NB01					Y / N