

APPROVAL

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1. **PURPOSE**

- 1.1 To standardize obtaining nasal biopsies by non-invasive curettage for evaluation of electron microscopy for abnormalities of epithelial morphology. The ultrastructure evaluation of ciliated epithelium in ultra-thin (70-90nm) sections is essential for diagnosis of Primary Ciliary Dyskinesia (PCD).

2. **SCOPE**

- 2.1 *This procedure applies to sites performing collection of nasal biopsy for diagnosis of respiratory epithelial cells for MCC clinical evaluation and research.*
- 2.2 *This SOP starts when individuals from participating sites are trained in Obtaining Nasal Ciliary Biopsy for Transmission Electron Microscopy (TEM) procedure.*

3. **DEFINITIONS**

- 3.1 NONE

4. **EQUIPMENT**

- 4.1 Rhino-Probe curette (Synbiotics Corporation, Part 2-01103)
- 4.2 Clinical Otoscope
- 4.3 Scissors
- 4.4 Fixative Component Kit containing:
 - a) 4% glutaraldehyde + 4% paraformaldehyde in 0.2 M PBS, 2ml, glass vial
 - b) 1% tannic acid in distilled water, 2ml, glass vial
 - c) Detailed instructions '*Procedure for receipt and storage of fixative components*'
- 4.5 Shipping Component Kit containing:
 - a) Parafilm, absorbent material, 3 liquid tight bags, refrigerant packs, refrigerated shipper
 - b) Dangerous Goods in Excepted Quantities label, Fed Ex USA or domestic airway bill, Ship To label.
 - c) *Nasal Biopsy Collection Form*
 - d) Detailed instructions '*Procedure for Shipping Specimens in the fixative 2% glutaraldehyde _ 2% paraformaldehyde +0.5% tannic acid*'

5. **PROCEDURE**

- 5.1 Approximately 15 minutes prior to collection of the biopsy remove the fixative components, a vial of frozen 4% glutaraldehyde + 4% paraformaldehyde and a vial of frozen 1% tannic acid, from the freezer. One vial of each per sample.

- 5.2 Allow vials to thaw at room temperature, but not to warm to room temperature. Keep fixative cool by placing it in a container of ice.
 - 5.3 Pipette the 1% tannic acid into the vial of 4% glutaraldehyde + 4% paraformaldehyde and mix to produce 2% glutaraldehyde + 2% paraformaldehyde + 0.5% tannic acid. This will leave the vial containing the fixative properly labeled with a formaldehyde warning.
 - 5.4 Nasal biopsies containing superficial epithelial cells are obtained under direct vision through the aperture of a clinical otoscope with the patient sitting with head tilted back or lying in a supine position on an examination table. The curette is inserted through the otoscope aperture and drawn gently over the inferior surface of the inferior nasal turbinate several times.
 - 5.5 Upon retraction of the nasal curette IMMEDIATELY immerse the biopsy in cold fixative. The curette is then cut with scissors to allow curette to fit into vial of fixative when the lid is secured.
 - 5.6 Gently agitate the biopsy in the fixative, ensuring that the biopsy does not stick to the lid of the vial.
 - 5.7 Label the vial with participant's unique identifier number
 - 5.8 Store the vial of fixative containing the biopsy in a refrigerator. DO NOT FREEZE.
 - 5.9 For shipping follow directions '*Procedure for shipping specimens in the fixative 2% gluteraldehyde + 2 % paraformaldehyde + 0.5% tannic acid*' included in Shipping Component Kit.
6. **OPERATIONAL NOTES (Quality Control)**
- 6.1 Failure to follow the fixation procedure may result in poor quality images of the cilia.
 - 6.2 Do not freeze the vial of fixative containing the biopsy.
 - 6.3 Do not allow the vial of fixative containing the biopsy to sit at room temperature.
 - 6.4 Do not delay in the immediate immersion of the biopsy in the fixative upon its removal from the participant.
 - 6.5 Do not allow the biopsy to dry or place it on gauze, sponge or a paper towel.
 - 6.6 Do not immerse the biopsy in saline, water or any other solution besides the fixative.
 - 6.7 All personnel handling the fixative or fixative components must complete Formaldehyde Standard Training. Training can be completed at: <http://ehs.unc.edu>
 - 6.8 Ensure that the fixative components are not expired by checking the expiration date. Expired components must be discarded in accordance to local regulations regarding hazardous waste.
 - 6.9 If there is a delay in collecting the biopsy, thawed fixative components and/or the fixative may be stored in the refrigerator for a maximum of 24 hours. After 24 hours discard the fixative and/or fixative components according to local regulations regarding hazardous waste.