Well-Differentiated Airway Cultures

I. Well-Differentiated Airway Cultures

- Cells can be plated from one of two sources:
  - 1) When cells are 70-90% confluent on plastic dishes, they can be passaged onto porous collagen coated membranes (*See HPC coating insert protocol*)
  - 2) Cells can be thawed directly from the freezer and plated on porous collagen coated membranes.
- After counting, re-suspend cells in a sufficient volume and sufficient seeding density (*See Cell Plating Density Chart*)
  Volume added to inside of 12 mm Transwell or 12 mm Millicell, 250-500 µL
  Volume added to inside of 24 mm Transwell or 30 mm Millicell, 1-1.5 mL
- Millicells: 1 insert per well in a 6 well-plate. Transwells come in a 12 well-plate format. We transfer then into a 6-well plate using special telfon rings to hold them into place.
- Media requirements: A 6 well-plate requires 2.0 - 2.5 mL of medium for Millicells, and 2.5 mL for Transwells in a 6-well plate format. If using the 12 well plate format, please follow the manufactures recommendations for media volume.
- 24 hours after seeding, aspirate off the apical fluid, rinse with PBS and add 1-2 drops of ALI on the apical side. Aspirate the basolateral media and add fresh ALI.
- Place cells on a MWF feeding schedule and wash the apical side at each feeding until the cells become confluent.
- Once the cultures reach confluence, wash the apical side with PBS, aspirate, and leave the apical side dry. Change the basolateral medium. Feed all inserts MWF with a PBS rinse of the apical side once a week.
  - Note: If establishing ALI is questionable, make inserts ALI but feed basolateral with 1.5-2mLs of media (enough media to touch bottom of insert)

FEEDING W-D airway cultures

- Turn on the blower and UV the hood for 10 minutes. Spray surface with 70% ETOH.
- Retrieve plates from incubator and inspect with eye and microscope for contamination (i.e. cloudy media).

- Place plates in hood along with ALI medium and 1xPBS.
- Aspirate all medium from each plate, using a sterile glass Pasteur pipet, tilting the plate away from your body, and placing the pipet at the edge of the well.
- With a sterile pipet, add a few drops of 1xPBS to each insert.
- Aspirate the PBS from each individual insert and either add a few drops of ALI on top if cells are not confluent, or leave dry.
- Add 2.5 mL of ALI to each well on a 6 well-plate, or recommended volume for vessel being used.

II. Typical observations:

1-7 days: The cells will form monolayers and achieve ALI

7-14 days: The cells will begin to secrete mucous

14-28 days: The cells will further differentiate, ciliate and secrete mucous

III. Hints to Avoid Contamination:

**Do not put pipets back into media bottles; a sterile pipet should be used each time. To avoid wasting pipets, take out as much media as is required for an entire stack of dishes.
**Be careful not to reach over open dishes. Dishes should only be opened while adding or removing liquid.
**Only bring one code of cells into the hood at one time. Clean and sterilize hood in between feeding different codes.