

PREPARATION OF “LUNG JUICE” FROM LUNG SPECIMEN

- By Mary Brummet

EXPERIMENTAL PROCEDURE:

1. Receive Lung specimen after bronchi have been removed. Using forceps and scissors remove small pieces of airway and place into a petri dish containing RPMI media + 1% pen-strep (P/S).
 - a. Airway pieces are usually 0.5-1cm wide by 0.5-3cm long.
2. Clean airway:
 - a. Using forceps and scissors, cut away remaining parenchyma attached to the outside of the bronchi. Perform all steps in the sterile hood and use universal precautions when handling human tissue.
3. Incubate:
 - a. After airway has been cleaned, they are placed in a 50ml conical tube with 40ml RPMI + 1% P/S and incubated overnight at 4°C.
4. Sample prep:
 - a. After incubation, the airway pieces are placed in a petri dish and using a sterile transfer pipet, any mucus that has been produced is removed from the airway and transferred to a new 50ml conical. The airway pieces are then washed with media using the transfer pipet and combined with the mucus already collected.
 - b. The viscous sample is then sonicated for 10 sec. – 2 pulses x 5 seconds each
5. Concentration:
 - a. The sonicated sample is then concentrated 1:5 using a 10,000mwco centricon.
 - b. The sample is then stored at -80°C in 1ml aliquots.

